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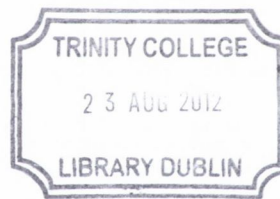
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**The effect of exercise on leg vascular
conductance & oxygen uptake kinetics, and
endothelial & inflammatory function in
women with type 2 diabetes**



**Thesis submitted for the degree of Doctorate in Philosophy
University of Dublin, Trinity College
2012**

Catherine Kiely



Thesis 9530

I Declaration

I declare that this thesis is entirely my own work with the exception of the reliability study carried out on mean arterial pressure responses between the Colin and Finapres (chapter 3), which was carried out by Ms Heather Reilly. I declare that this thesis has not been submitted as an exercise for a degree at this or any other university.

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II Abstract

Individuals with type 2 diabetes (T2DM) show a reduced maximal oxygen uptake ($\dot{V}O_{2\text{ peak}}$) in comparison to age, BMI, and activity matched non-diabetic (ND) controls. In addition, the dynamic responses of $\dot{V}O_2$ ($\dot{V}O_2$ kinetics) and leg vascular conductance (LVC) are slowed in combined groups of pre and postmenopausal women with T2DM. As yet, $\dot{V}O_2$ and LVC kinetic responses have not been investigated in postmenopausal women with T2DM. The mechanisms underpinning the impaired $\dot{V}O_2$ kinetic responses have not been well elucidated but it is thought that central factors affecting cardiac function and/or peripheral factors affecting oxygen delivery and oxygen extraction may be responsible. Exercise training has been shown to have beneficial effects on $\dot{V}O_{2\text{ peak}}$ and $\dot{V}O_2$ kinetics in premenopausal women and in a combined group of men and women with T2DM, but again, no studies have looked at the effect of exercise training on these parameters in postmenopausal women and furthermore, the mechanistic bases for this exercise improvements are not known.

The aims of the present study were to investigate the effects of a 12-week supervised exercise (combined aerobic and resistance) followed by a 16-week unsupervised exercise on $\dot{V}O_{2\text{ peak}}$ and $\dot{V}O_2$ kinetic responses during moderate intensity (80% ventilatory threshold, VT) cycle exercise and LVC kinetic responses during plantar flexion exercise. In addition, blood samples at each point of assessment were collected for analysis of measures of glycaemic control, as well as determination of levels of known inflammatory and endothelial markers.

Initially, at baseline postmenopausal women with T2DM ($n = 22$) and age and activity matched non-diabetic women ($n = 22$) underwent testing on two separate days. On day one, LVC kinetic responses were assessed using venous occlusion plethysmography averaging 3 bouts of 6 min constant load plantar-flexion exercise in the supine position at low (30% of maximum voluntary contraction (MVC)) and high (70% MVC) intensities. Additionally LVC responses to an incremental plantar-flexion test were assessed, as well as forearm vascular conductance responses to reactive hyperaemia. Subjects then completed a graded cycle test to exhaustion. On day two, $\dot{V}O_2$ kinetic responses were assessed

averaging 4 bouts of 6 min constant load cycling exercise at 80%VT on a breath-by-breath basis. Subjects then 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 assigned to either an exercise (T2DM Ex, n= 8; ND Ex, n= 10) or control (T2DM Con, n=8; ND Con, n=9) condition and at the end of the 12-week and 16-week training periods subjects returned to the laboratory to repeat the testing protocol undertaken at baseline. Maximum voluntary contraction and VT were reassessed at each visit, with $\dot{V}O_2$ and LVC kinetic responses assessed in relation to each visit. The control groups were asked to maintain their normal daily routine.

At baseline, $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) was significantly lower and $\dot{V}O_2$ kinetics at 80% VT were slowed (slower mean response time) in women with T2DM in comparison to ND women. In contrast, simultaneous measurements of CO or the rate of adaptation in CO from rest to steady-state exercise were not different between women with and without T2DM confirming that the slowed $\dot{V}O_2$ kinetic responses were not related to cardiac dysfunction. Also at baseline, LVC kinetic responses were slowed in women with T2DM during high intensity (70% MVC) (but not low intensity) plantar flexion exercise, suggesting that the slowed LVC kinetic responses could contribute to the impaired $\dot{V}O_2$ kinetic responses observed during moderate cycling in diabetes. While supervised exercise improved $\dot{V}O_{2\text{ peak}}$ and peak workload in previously sedentary women with and without T2DM, it did not enhance LVC kinetics or $\dot{V}O_2$ kinetics at loads specific to each training phase. However, the greater workloads achieved during both cycle exercise and plantar flexion exercise following supervised exercise may have obscured any improvements, which have taken place with respect to either parameter. The performance improvements observed during the supervised phase were not maintained during a subsequent period of unsupervised exercise, most likely due to a lower adherence to exercise. Indices of glycaemic control were unchanged in all groups following the intervention. On the other hand, at baseline, plasma levels of interleukin-6 (IL-6), IL-10, C-reactive protein (CRP), and visfatin mRNA expression were increased in women with T2DM (who had a higher mean BMI) than ND controls, although when groups were matched for BMI these

differences were abolished. Furthermore, exercise intervention did not result in any changes in markers of inflammation or endothelial activation in either exercise group, indicating that a more intensive exercise intervention and/or a combination of diet and exercise may be required in reducing inflammation in women with T2DM.

In conclusion this study shows that $\dot{V}O_2$ kinetics are slowed in postmenopausal women with T2DM effect that might be mediated by a slowed adaptation of leg blood flow (rather than cardiac dysfunction). In addition, performing exercise at the same relative workload post-intervention does not result in the speeding of either $\dot{V}O_2$ or LVC kinetic responses to exercise although the larger workloads obtained might have obscured these potential positive adaptations.

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V	List of Abbreviations
A	Amplitude
<i>a</i>	Baseline amplitude
a + A	Predicted peak forearm vascular conductance
ABI	Ankle brachial index
ACE	Angiotensin-converting enzyme
ACh	Acetylcholine
ACSM	American College of Sports Medicine
AGEs	Advanced glycation end products
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
AUC	Area under the curve
a-vO ₂ diff	Arteriovenous oxygen difference
BbB	Breath-by-breath
β-blocker	Beta blockers
β-cells	Beta cells
beats.min ⁻¹	Beats per minute
BMI	Body mass index
BS	Baseline
C	Celsius
c	Decay constant
CAPN10	calpain-10
cDNA	Complementary deoxyribonucleic acid
CHD	Coronary heart disease
CHF	Chronic heart failure
CI	Cardiac index
cm	Centimetre
CO	Cardiac output
CO ₂	Carbon dioxide
CO _{ffm}	Cardiac output normalised to fat free mass
COPD	Chronic obstructive pulmonary disease
counts.min ⁻¹	Counts per minute

CRP	C-reactive protein
CT	Cycle number
CVD	Cardiovascular disease
d	Day
D2S125	Microsatellite marker on chromosome 2
Δ CO	Change in cardiac output
$\Delta\Delta$ CT	Comparative CT method
<i>df</i>	Degrees of freedom
DFI	Diabetes Federation of Ireland
DNA	Deoxyribonucleic acid
$\Delta \dot{V}O_2$	Change in $\dot{V}O_2$
ΔW	Change in workload
d.wk ⁻¹	Day per week
<i>e</i>	Exponential
ECG	Electrocardiogram
EDV	End-diastolic volume
EMG	Electromyographic
End A	End amplitude
eNOS	Endothelial nitric oxide synthase
F	Conditional expression
FAM	6-carboxyfluorescein
FBF	Forearm blood flow
FMD	Flow-mediated dilation
FPG	Fasting plasma glucose
FVC	Forearm vascular conductance
g	Gram
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GK	Goto-Kaizaki
GLUT-4	Glucose transporter type 4
GP	General Practitioner
GTN	Glyceryl trinitrate
h	Hour
H ⁺	Hydrogen

H ₂ O	Water
H ₂ SO ₄	Sulphuric acid
HbA _{1C}	Glycosylated hemoglobin
h.day ⁻¹	Hours per day
HDL	High-density lipoprotein cholesterol
[HHb]	Deoxygenated hemoglobin/myoglobin
HNF4A	Hepatocyte nuclear factor-4α
HOMA-IR	Homeostatic model assessment for insulin resistance
HR	Heart rate
HRP	Horseradish peroxidase
hs-CRP	high-sensitivity C-reactive protein
h.wk ⁻¹	Hour per week
Hz	Hertz
ICAM-1	Intercellular adhesion molecule-1
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired Glucose Tolerance
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-18	Interleukin-18
K ⁺	Potassium
kcal.d ⁻¹	Kilocalorie per day
kg	Kilogram
kg.wk ⁻¹	Kilogram per week
LBF	Leg blood flow
LDL	Low-density lipoprotein cholesterol
L.min ⁻¹	Litre per minute
LOPAR	Low-level physical activity recall
LVC	Leg vascular conductance
LVDD	Left ventricular diastolic dysfunction
M	Molar concentration
m	Metre

MAP	Mean arterial pressure
MET	Metabolic equivalent
MET h.wk ⁻¹	Metabolic equivalent hour per week
MGB	Minor groove binder
mg.dL ⁻¹	Milligrams per deciliter
MI	Myocardial infarction
min	Minute
miRNA	microRNA
ml	Millilitre
ml.kg ⁻¹ .min ⁻¹	Millilitre per kilogram per minute
mmHg	Millimetre of mercury
mmol.L ⁻¹	Millimole per litre
mmol.mol ⁻¹	Milimole per mole
mRNA	Messenger Ribonucleic acid
MRT	Mean response time
mV	Millivolt
MVC	Maximum voluntary contraction
N	Newton
NCBI	National Centre for Biotechnology Information
ND Con	Control non-diabetics
ND Ex	Exercising non-diabetics
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng/ml	Nanogram per millilitre
NIDDM1	Non-insulin dependent diabetes mellitus (common, type 2) 1
NIRS	Near-infrared spectroscopy
nm	Nanometre
NO	Nitric Oxide
N ₂ O	Nitrous oxide
NOS	Nitric oxide synthase
O ₂	Oxygen
O ₂ D	Oxygen deficit
OD	Optical density
1-RM	One repetition maximum

p	Parameter
PAD	Peripheral artery disease
PAI-1	Plasminogen activator inhibitor-1
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered Saline
PCr	Phosphocreatine
PCWP	Pulmonary capillary wedge pressure
Peak HR	Peak heart rate
pg/ml	Picogram per millilitre
pmol.L ⁻¹	Picomole per litre
P_{O_2}	Partial pressure of oxygen
RER	Respiratory exchange ratio
rev.min ⁻¹	Revolutions per minute
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Revolutions per minute
RSS	Residual sums of squares
RT-PCR	Real-time polymerase chain reaction
s	Second
SAA	Serum amyloid A
SD	Standard deviation
SE	Supervised exercise
SF ₆	Sulphur hexafluoride
sICAM-1	Soluble intercellular adhesion molecule-1
SNP	Sodium nitroprusside
STPD	Standard temperature and pressure, dry
SV	Stroke volume
sVCAM-1	Soluble vascular adhesion molecule-1
SV _{ffm}	Stroke volume normalised to fat free mass
SVI	Stroke volume index
t	Time
τ	Time constant
T1DM	Type 1 diabetes

T2DM	Type II Diabetes
T2DM Con	Control diabetics
T2DM Ex	Exercising diabetics
TCL	Total cholesterol
TD	Time delay
TNF- α	Tumor necrosis factor- α
TPR	Total peripheral resistance
Trp64Arg	Arginine
TTF	Time to failure
TZD	Thiazolidinedione
UCP	Uncoupling protein
UE	Unsupervised exercise
UK	United Kingdom
μ l	Microlitre
US	United States
V	Volt
VCAM	Vascular adhesion molecule-1
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Pulmonary ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2\text{ peak}}$	Peak oxygen uptake
VOP	Venous occlusion plethysmography
$Vs(tot)$	Total systemic volume
VT	Ventilatory threshold
W	Watt
WHO	World Health Organisation
WHR	Waist to hip ratio
yr	Year

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Chapter 1: General introduction

1.1 Prevalence of type 2 diabetes (T2DM)

The prevalence of type 2 diabetes (T2DM) is increasing in rapid proportions on a global basis due to increases in population, an increasing ageing population, urbanisation and more sedentary lifestyles. T2DM accounts for 90-95% of all diabetic cases; other forms of diabetes include type 1 diabetes (T1DM), a condition caused by auto-immune destruction of the pancreatic β -cells and gestational diabetes, which develops during pregnancy and may precede the development of T2DM. Profound changes in the quality and quantity of dietary intake as well as decreased levels of physical activity have contributed to increases in the prevalence of T2DM and its complications. Worldwide, it is predicted that the number of diabetic cases will more than double by the year 2030 affecting 366 million people (Wild *et al.*, 2004). The greatest part of this increase will occur in developing countries where it is expected there will be 170% increase in the number of people with diabetes and thus by the year 2025, developing countries will account for 75% of diabetic cases (King *et al.*, 1998). However, these predictions are based on current obesity levels remaining stable.

Obesity is at the core of the diabetes epidemic, with approximately two-thirds of adults in the United States (US) being classified as overweight or obese (Flegal *et al.*, 2010). A recent investigation suggests that the prevalence of obesity has been on the increase in the US from 1976 to 2006, with obesity levels increasing in adults with and without T2DM by 58% and 136% respectively over a 20-year period, a finding that will have important implications for the increasing prevalence of T2DM and diabetes related co-morbid conditions (Kramer *et al.*, 2010).

Although the true prevalence of T2DM in Ireland is unknown, conservative estimates from the International Diabetes Federation (IDF) (IDF Diabetes Atlas, 2009) suggest that as many as 5.7% of individuals aged 20-79 are affected by diabetes (Type 1 and 2 combined). In 2002, the Diabetes Federation of Ireland (DFI) estimated that there were 200,000 people with diabetes (type 1 and 2

combined) and a further 200,000 undiagnosed cases of diabetes (DFI, 2002). In addition, they estimated that a further 250,000 people had impaired glucose tolerance, with a 50% progression rate to T2DM if lifestyle changes were not made (DFI, 2002). Although there have been limited attempts to quantify the prevalence of diabetes within the Irish context, a study conducted in general practitioner (GP) surgeries in Ireland found that from a total of 3821 people surveyed, 9.2% of individuals had T2DM (23.5% were previously undiagnosed) and a further 3.9% had pre-diabetes (Smith *et al.*, 2003).

As well as having health implications, the increasing prevalence of T2DM also has financial implications and is costing billions of euro on a worldwide basis. In the US, it was estimated that \$132 billion was spent on health care costs for diabetes in 2002 (Hogan *et al.*, 2003). In Ireland over a 12-month period in 1999/2000, the total health care costs for treating T2DM were estimated at €580.2 million for diagnosed and undiagnosed cases of T2DM, which was based on a conservative estimate of 6% prevalence (diagnosed and undiagnosed) of T2DM (Nolan *et al.*, 2006). Currently, the cost of treating T2DM in Ireland is approximately 10% of the total health expenditure but it is expected that this figure will increase to 25% by 2040 (Diabetes Expert Advisory Group, First Report, 2008).

Exercise is recognised as an important factor in the treatment and management of T2DM. Despite extensive research highlighting the importance of physical activity and exercise, 60-80% of individuals with T2DM do not exercise sufficiently and adherence to exercise programmes is low in diabetic populations (Krug *et al.*, 1991; Morrato *et al.*, 2007). However, exercise performance is impaired in individuals with T2DM (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007), a factor that may limit their participation in regular exercise. This present introduction will review the mechanistic basis for the impairment in T2DM as well as the effects of exercise training on the cardiovascular system and inflammation in individuals with this disease.

1.2 T2DM and cardiovascular disease (CVD)

Cardiovascular disease is the leading cause of death in individuals with T2DM accounting for approximately 80% of all mortality in diabetics. The risk of myocardial infarction (MI) in a diabetic individual who has not previously had an MI is as great as a non-diabetic individual who has previously experienced a MI (Haffner *et al.*, 1998). Death from CVD is three times greater in men with T2DM than non-diabetic men even in the presence of similar risk factors for CVD such as elevated cholesterol, elevated systolic blood pressure, and smoking (Stamler *et al.*, 1993). This is consistent with the lower life expectancy (8 years) observed in diabetics aged 55-64 years than their aged matched non-diabetic counterparts (Gu *et al.*, 1998).

Research suggests that there exists a 'diagnosis gap' of approximately 7 years between the development of asymptomatic hyperglycaemia and clinical diagnosis of T2DM (Harris *et al.*, 1992). It is possible that the delay in the diagnosis of T2DM may account for the fact that as many as 50% of individuals with T2DM have some manifestation of CVD at the time of diagnosis (Turner *et al.*, 1996). Furthermore, it has been estimated that the risk of developing CVD begins to increase as early as 15 years before the clinical diagnosis of T2DM (Hu *et al.*, 2002). Women who were diabetic at baseline or who developed T2DM over the course of a follow up had an increased risk of developing CVD in comparison to non-diabetic women (2.8-fold and 5.0-fold respectively) (Hu *et al.*, 2002).

Historically it was thought that T2DM was a prerequisite for the development of CVD. However, it would appear that the presence of hypertension, dyslipidemia, hyperglycaemia, and obesity are common elements to both T2DM and CVD (Haffner *et al.*, 2000; Lorenzo *et al.*, 2006; Janssen *et al.*, 2007). The presence of these cardiovascular risk factors results from an underlying insulin resistance syndrome better known as the metabolic syndrome, that precedes the development of T2DM. Evidence suggests that compared with women who remained non-diabetic, women who developed T2DM had a greater body mass index (BMI) and increased presence of hypertension and hypercholesterolemia prior to diagnosis (Hu *et al.*, 2002).

Insulin resistance is a metabolic abnormality linking the development of T2DM and CVD. The mechanistic basis by which CVD risk increases in individuals who are insulin resistant include hypertension, pro-inflammatory and prothrombotic states, and dyslipidemia. The presence of certain risk factors for CVD such as hypertension (Turner *et al.*, 1998), dyslipidemia (Sivitz *et al.*, 2007), and insulin resistance (Steinberg *et al.*, 1996) can lead to vascular damage.

1.3 Risk factors for T2DM

Factors identified as being linked to an increased risk of developing T2DM include those with a family history of T2DM (Pradhan *et al.*, 2001), obesity (Mizuno *et al.*, 2007), gestational diabetes (Pimenta *et al.*, 2004), ethnicity (Knowler *et al.*, 1978), hypertension (Turner *et al.*, 1998), dyslipidemia (Azuma *et al.*, 2007), impaired glucose tolerance (IGT), and impaired fasting glucose (IFG) (Butler *et al.*, 2003). Environmental factors have also been implicated in the aetiology of T2DM. Inactive lifestyles together with excessive intake of high calorie foods led to imbalances between energy intake and expenditure.

1.3.1 Inactivity and excessive calorie intake

The increasing prevalence of T2DM can be partially attributed to an ageing population. However, lifestyle change has also played a major role in the increased incidence of T2DM. The link between T2DM and lifestyle can be seen in the increasing prevalence of T2DM among certain populations e.g. South Asians living in urbanised settings in the UK (Riste *et al.*, 2001) and India (Ramachandran *et al.*, 2001). Indeed, longitudinal studies have indicated that inactivity together with poor dietary choices are risk factors for the development of T2DM (Perry *et al.*, 1995; Salmerón *et al.*, 1997; Salmerón *et al.*, 2001).

The majority of individuals with T2DM do not engage in sufficient regular physical activity and adherence to exercise regimes is low among those individuals who do engage in physical activity (Krug *et al.*, 1991; Morrato *et al.*, 2007). Furthermore, excessive intake of high calorie foods when combined with inactivity further exacerbates the situation. The accumulating evidence linking both physical and diet to T2DM led to the belief that it may be possible to prevent T2DM with lifestyle change. Long-term studies have been conducted to examine

the impact of making lifestyle changes on the progression from IGT to T2DM where the main lifestyle intervention targets were body mass, diet, and physical activity.

The Da Qing IGT and Diabetes Study used large population based screening to identify individuals with IGT (Pan *et al.*, 1997). The effect of diet and exercise in preventing the development of T2DM was examined over a 6-yr period. Individuals with IGT (n=577) were randomised to one of four groups; control, diet alone, exercise alone, or diet plus exercise. The dietary intervention consisted of a high-carbohydrate and low-fat diet. The groups participating in physical activity were encourage to increase their physical activity levels by 1-2 units per day where one unit was defined as 30 min walking, 10 min running, or 5 min swimming. The annual risk of progressing from IGT to T2DM was reduced from 15.7% in the control group to 8% in the intervention groups (Pan *et al.*, 1997). Furthermore, the cumulative 6-yr incidence of T2DM was 41-46% in the intervention groups versus 68% in the control group (Pan *et al.*, 1997).

In a study conducted over a similar period (5 yr), individuals with IGT were randomly assigned to an intervention or control group (Tuomilehto *et al.*, 2001). Targets for the intervention group included a reduction in body mass (>5%), reduction in total fat and saturated fat intake (<30 and 10%), fibre intake of at least 15g/1,000 kcal, and moderate exercise of at least 30 min.d⁻¹. The cumulative incidence of T2DM was 11% for the intervention group and 23% for the control group (Tuomilehto *et al.*, 2001). Furthermore, the reduction of 58% in risk of T2DM in the intervention group was directly associated with changes in lifestyle (Tuomilehto *et al.*, 2001). These results are confirmed by a large study (n=2,161) conducted in the US, which looked at the effect of lifestyle intervention on the prevention of T2DM in an ethnically diverse population (Knowler *et al.*, 2002). The lifestyle intervention consisted of reducing fat intake with the intent to achieve weight loss of at least 7% and 150 min of moderate physical activity per week. With lifestyle intervention, there was a 58% relative risk reduction in progression to T2DM, which was effective across different ages, BMI, and ethnic groups (Knowler *et al.*, 2002).

The evidence indicates that the risk of developing T2DM is greatly reduced with lifestyle changes that include reducing total calorie intake and increasing physical activity.

1.3.2 Obesity

Over a 20 year period the prevalence of obesity in adults with T2DM increased by 58% with the result that approximately two thirds of US adults with T2DM are now obese (Kramer *et al.*, 2010). The increase in obesity in individuals with T2DM has important health implications as highlighted by a recent investigation where it was reported that the 10-year cardiovascular risk of mortality increases by 40% in men and 300% in women who are both obese and diabetic (Fox *et al.*, 2008).

In addition to the presence of obesity, the distribution of body fat is also important. A predominantly upper body fat distribution results in increased rates of lipolysis and free fatty acid turnover in diabetic women (Jensen *et al.*, 1989). Obese individuals with T2DM are typically more insulin resistant than obese non-diabetics due to greater proportions of fat mass in the trunk and hepatic regions (Azuma *et al.*, 2007). It has been suggested that there are differences in metabolic activity between subcutaneous and visceral adipose tissue (Kelley & Mandarino, 2000) with the latter being associated with greater insulin resistance (Miyazaki *et al.*, 2002).

Numerous factors have been implicated in the association of obesity and impaired glucose homeostasis including: 1) the release of cytokines such as tumor necrosis factor-alpha (TNF- α) (Wood *et al.*, 2005), interleukin-6 (IL-6) (Muller *et al.*, 2002), C-reactive protein (CRP) (Okita *et al.*, 2004), and resistin (Verma *et al.*, 2003) that promote inflammation and consequently insulin resistance in muscle and liver tissues; and 2) ectopic fat distribution in liver (Gastaldelli *et al.*, 2007) and muscle (Azuma *et al.*, 2007).

1.3.3 Ethnicity and genetics

Certain ethnic groups are at increased risk of developing T2DM; some of these include African-Americans, Pima Indians, Australian Aborigines, and Hispanics as well as the increasing prevalence of T2DM among Chinese and Indian populations. In a national health study conducted in the US in 2001 in which a total of 176,807 people participated, prevalence rates for T2DM among white Americans were lower than those occurring among blacks, Hispanics, and native Americans (McNeely & Boyko, 2004). In an earlier study, prevalence rates of T2DM among Pima Indians were found to be 19 times that of white Americans and incident rates increased from childhood to adulthood where they reached 40-50% in adults over 35 years (Knowler *et al.*, 1978).

Some research indicates that T2DM is an inherited disease although the mechanism by which this may occur has yet to be established. It has been shown that first-degree relatives of patients with T2DM are insulin resistant and have increased postprandial lipid intolerance (Axelsen *et al.*, 1999). The higher prevalence of T2DM among certain families and the higher incidence of diabetes among monozygotic than dizygotic twins suggest that there may be a genetic component to the development of T2DM. The degree of expression of diabetes-related genes in an individual as well as influence from external factors in the environment may implicate more than one specific gene in causing diabetes. It has been suggested that β_3 -adrenergic receptors, which are expressed in adipose tissue play a role in controlling resting metabolic rate and lipolysis among Pima Indians (Walston *et al.*, 2000). Association has been found between various genetic variants located near hepatocyte nuclear factor-4 α (HNF4A) and diabetes status (Silander *et al.*, 2004). HNF4A controls the expression of genes involved in glucose homeostasis and insulin secretion as well as regulating gluconeogenesis in the liver.

1.4 Characteristics of T2DM

Glucose homeostasis is maintained by closely matching the balance between glucose production and glucose disposal such that fasting plasma glucose (FPG) levels are maintained within a narrow range. This is achieved by the release of insulin from the pancreatic β -cells and the subsequent action of insulin on the muscle, liver, and adipose tissues. T2DM is characterised by reduced glucose uptake and increased hepatic glucose production, both of which are related to impaired insulin secretion and reduced insulin sensitivity (Michishita *et al.*, 2008). Increased liver fat content and visceral adipose tissue in individuals with T2DM contribute to abnormalities in both glucose and lipid metabolism (Gastaldelli *et al.*, 2007). T2DM is a disease that progresses over time with patients' glucose tolerance continuously being impaired. Increased insulin resistance and impaired β -cell function are important in the progression of IGT to T2DM (Li *et al.*, 2005). Increased insulin is secreted in subjects who are insulin resistant in order to compensate for reduced insulin sensitivity. Over time this contributes to hyperglycaemia due to defective β -cell secretory capacity. Acute hyperglycaemia that occurs after consuming glucose may initiate the production of reactive oxygen species (ROS), which can impair β -cell function.

In normoglycaemic subjects, insulin secretion in the postprandial state is characterised by a biphasic pattern, with a rapid initial phase that lasts 1-3 min and peaks after 10 min, and a slower more progressive release of insulin in the second phase that lasts as long as the glucose load (DeFronzo *et al.*, 1985). The early or first phase is followed by a decrease in insulin concentrations similar to that observed at baseline. This phase is important in suppressing hepatic glucose production, lipolysis, and priming target tissues for insulin action (Groop *et al.*, 1989). In the initial stages of T2DM, the loss of early phase insulin secretion results in postprandial hyperglycaemia which over time progresses to chronic hyperglycaemia (Michishita *et al.*, 2008). During the second phase, the continued presence of glucose leads to increased insulin secretion that typically begins 10-20 min after first exposure to glucose and lasts several hours (Michishita *et al.*, 2008). This phase is reduced in T2DM patients and occurs later (Mizuno *et al.*, 2007).

According to the WHO, T2DM is diagnosed in an individual when fasting plasma glucose $>7 \text{ mmol.L}^{-1}$ (126 mg.dL^{-1}) or when 2-h plasma glucose $>11.1 \text{ mmol.L}^{-1}$ (200 mg.dL^{-1}) (WHO, 2006). In addition, glycosylated haemoglobin (HbA_{1c}), which measures the degree of glycaemic exposure over time, is also used in clinical practice. The International Expert Committee report (2009) on the role of the A1C assay in the diagnosis of diabetes has indicated that an elevated HbA_{1c} ($>6.5\%$) is associated with a substantial increase in the prevalence of microvascular complications and as such, should be used as the cut off-point for diagnosing T2DM. Data from the United Kingdom (UK) Prospective Diabetes Study (1998) indicate that for every 1% reduction in HbA_{1c} , there is a 35% reduction in microvascular complications. In contrast, a 1% increase in HbA_{1c} is associated with an increased risk of mortality (28%) independent of age, blood pressure, BMI, and smoking (Khaw *et al.*, 2001).

1.5 Acute peak exercise performance in subjects with T2DM

Maximum oxygen utilisation ($\dot{V}\text{O}_{2 \text{ peak}}$) is a measure of the capacity of the body to utilize oxygen during exercise. It represents the maximal rate that adenosine triphosphate (ATP) can be synthesized aerobically and is dependent on such factors as efficient delivery and extraction of oxygen, size of muscle mass engaged, environment, and subject characteristics. Exercise tolerance in an individual will be influenced by such factors as $\dot{V}\text{O}_{2 \text{ peak}}$, the $\dot{V}\text{O}_2$ required to perform sub-maximal workloads, and the rate at which $\dot{V}\text{O}_2$ increases ($\dot{V}\text{O}_2$ kinetics) during exercise. Oxygen uptake in the tissues can be described by the Fick equation:

$$\dot{V}\text{O}_2 = \text{CO} \times \text{a-vO}_2 \text{ diff}$$

where CO represents tissue blood flow and a-vO₂ diff represents the difference between arterial and venous oxygen contents. Blood flow to the tissues is affected by cardiac output, the ability of vascular tissues to vasodilate as well as the pressure differential acting across the vascular bed. In disease states such as T2DM, maximal exercise performance is impaired in comparison with normal

healthy individuals matched for BMI, age, and activity levels (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Colberg *et al.*, 2006). This impaired maximal exercise performance is characterised by a reduced $\dot{V}O_{2\text{ peak}}$ and peak workload during graded exercise (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Colberg *et al.*, 2006; MacAnaney *et al.*, 2011a; Wilkerson *et al.*, 2011).

For instance, exercise performance was assessed during a graded treadmill test in a group of men and women with T2DM matched with a non-diabetic group of similar age and activity level (Regensteiner *et al.*, 1995). At exhaustion, exercise time was shorter (24%) and $\dot{V}O_{2\text{ peak}}$ was lower (20%) in the group with T2DM than in the control group (21.5 ± 5.8 and 27.2 ± 5.3 ml.kg⁻¹.min⁻¹ respectively). Oxygen consumption during sub-maximal workloads was lower (16%) in the group with T2DM between 5 and 8 min (Regensteiner *et al.*, 1995). The authors suggested that the reduced rate of oxygen consumption during sub-maximal exercise in the group with T2DM may have impaired exercise performance in this population. Similarly, but during graded cycling exercise, it has been reported that $\dot{V}O_{2\text{ peak}}$ is lower at volitional exhaustion in men and women with T2DM than in non-diabetics (16.2 ± 0.1 and 22.3 ± 2.7 ml.kg⁻¹.min⁻¹ respectively) (Colberg *et al.*, 2005). These findings have also been confirmed in older men (65 ± 5 yr) with long-term diabetes (> 5 yr) (Wilkerson *et al.*, 2011). In men matched in age and BMI, $\dot{V}O_{2\text{ peak}}$ was lower in men with T2DM (1.98 ± 0.43 L.min⁻¹) than in non-diabetic men (2.72 ± 0.40 L.min⁻¹) at the end of graded exercise (Wilkerson *et al.*, 2011).

In a different study involving cycle exercise, ten premenopausal women with uncomplicated T2DM, 10 overweight non-diabetic women, and 10 lean non-diabetic women completed a graded cycle test to volitional exhaustion (Regensteiner *et al.*, 1998). Physical activity scores were similar between the groups as determined by the use of a low-level physical activity recall (LOPAR) questionnaire. Maximal aerobic capacity was lower in women with T2DM (17.1 ± 3.8 ml.kg⁻¹.min⁻¹) than either the overweight non-diabetics (22.0 ± 2.3 ml.kg⁻¹.min⁻¹) or lean non-diabetics (25.7 ± 4.9 ml.kg⁻¹.min⁻¹). A similar finding was

observed for the maximum workload achieved during the graded cycle test with again the group with T2DM reporting lower workloads (104 ± 16 W) than either the overweight (130 ± 17 W) or lean groups (121 ± 19 W) (Regensteiner *et al.*, 1998). Respiratory exchange ratio (RER) and maximum heart were similar between the groups at exhaustion (Regensteiner *et al.*, 1998).

Moreover, in a recent investigation, maximal exercise performance was assessed in 9 pre and postmenopausal women with uncomplicated T2DM, 9 overweight and 10 lean controls (Mac Ananey *et al.*, 2011a). The groups were matched for age and all individuals were classified as being sedentary (LOPAR). Women with T2DM had a lower $\dot{V}O_{2\text{ peak}}$ (15.5 ± 4.4 ml.kg⁻¹.min⁻¹) in comparison to overweight (22.7 ± 2.6 ml.kg⁻¹.min⁻¹) and lean (27.1 ± 4.3 ml.kg⁻¹.min⁻¹) controls. In addition, peak workload was lower in women with T2DM (91 ± 23 W) than either overweight (124 ± 28 W) and lean (133 ± 34 W) controls (Mac Ananey *et al.*, 2011a).

As illustrated, maximum exercise performance is consistently impaired in populations with T2DM, however, the exact cause of this impairment is unknown. It has been suggested that compromised myocardial function and/or oxygen delivery may impair exercise performance in subjects with T2DM (Regensteiner *et al.*, 1998).

1.6 Causes of exercise impairment

1.6.1 Rate of increase in oxygen uptake ($\dot{V}O_2$ Kinetics)

The $\dot{V}O_2$ response to exercise is typically characterised by two or sometimes three distinct phases (Fig. 1.1). The first phase (phase I) occurs rapidly at the beginning of exercise (10-15 s) and represents the oxygen exchange associated with the initial increase in cardiac output (CO) and pulmonary blood flow. This is followed by a rapid exponential increase in $\dot{V}O_2$ (phase II) that drives $\dot{V}O_2$ towards a steady state when exercise is performed below lactic or ventilatory threshold. Each phase has its own distinctive time constant (rate of adaptation of the response), time delay (time from the onset of the stimulus to the onset of the response), and amplitude (increase in the response). In healthy individuals, phase

II has a time constant (τ) of 20-45 s. Phase II, also described as the fast component or primary component, reflects the arrival of venous blood at the lung from the exercising muscles. This phase is reflective of oxygen consumption at muscle level during various exercise intensities. Reduced oxygen content occurs in the venous blood due to an increase in tissue oxidation. During moderate intensity exercise below lactate threshold, the onset of phase III corresponds to the point where cardiac output plateaus and venous oxygen content reaches a nadir. In healthy subjects, steady state is usually achieved within 3 min with little or no sustained increase in blood lactate. Up to this point $\dot{V}O_2$ increases in a linear fashion with work rate. In contrast, when exercising above lactate threshold phase III is either delayed (heavy exercise) or absent (severe exercise) due the presence of a slow component (Casaburi *et al.*, 1987). The magnitude of the slow component has been approximated as the increase in $\dot{V}O_2$ from minute 3 until the end of exercise. The slow component elevates $\dot{V}O_2$ above that predicted for the work rate.

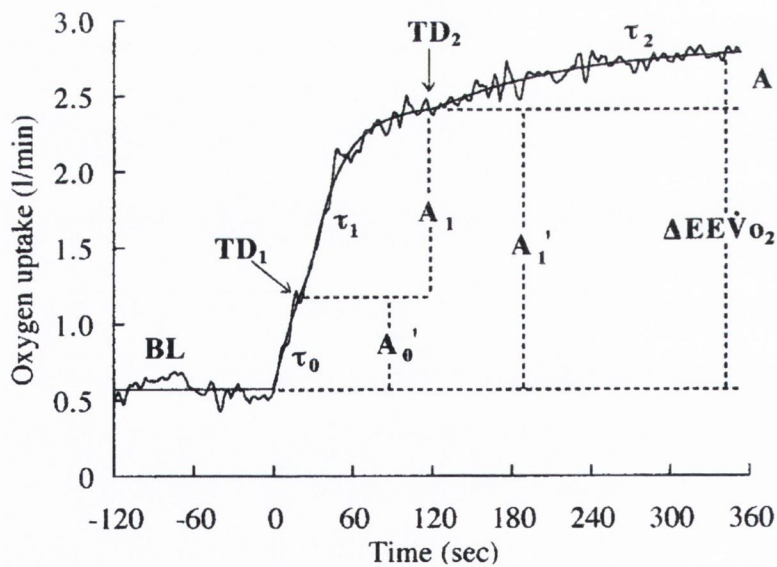


Fig. 1.1. Schematic representation of the $\dot{V}O_2$ response during heavy or very heavy intensity exercise showing three distinct phases. A_0 and τ_0 represent the amplitude and time constant for phase I; A_1 , TD_1 , and τ_1 represent the amplitude, time delay, and time constant for phase II; A_2 , TD_2 , and τ_2 represent the amplitude, time delay, and time constant for phase III (Koga *et al.*, 2005).

1.6.2 Oxygen deficit

In the transition from rest or unloaded exercise to an exercise intensity below lactate threshold, the vertical distance between the $\dot{V}O_2$ at any time point and the $\dot{V}O_2$ required during steady state represents the energy demand that must be met from energy stores within the muscle. This energy demand is largely met through phosphocreatine hydrolysis and anaerobic glycolysis. Oxygen deficit is a product of the change in $\dot{V}O_2$ ($\Delta \dot{V}O_2$) and the speed of the $\dot{V}O_2$ response denoted by the time constant (τ) of the $\dot{V}O_2$ response from exercise onset (Fig. 1.2):

$$O_2 \text{ deficit: } \Delta \dot{V}O_2 \times \tau$$

where $\Delta \dot{V}O_2$ is given in $L \cdot min^{-1}$ and τ in fractions of a minute. A faster $\dot{V}O_2$ response (smaller τ) will result in a reduced oxygen deficit. In various states such as T2DM, chronic heart failure (CHF), and chronic obstructive pulmonary disease (COPD), the time constant seems to be larger and thus these individuals will incur a larger oxygen deficit than normal healthy individuals. Slower $\dot{V}O_2$ kinetics result in increased depletion of phosphocreatine stores (PCr) and increased glycogenolysis leading to an increase in lactic acid production and reduced availability of intramuscular glycogen. This ultimately results in reduced exercise tolerance in these individuals.

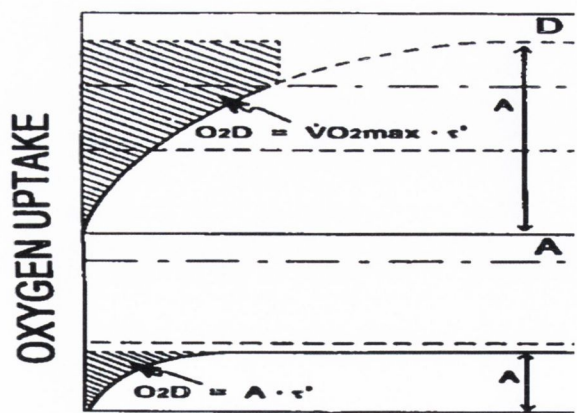


Fig. 1.2. Oxygen deficit (O_2D) for moderate exercise (below lactic threshold; bottom) and heavy exercise (above lactic threshold; top) (Barstow, 1994).

1.6.3 T2DM & $\dot{V}O_2$ kinetics

The current body of research on the dynamic response of $\dot{V}O_2$ during the initial period of exercise has been conducted on combined groups of men and women, combined groups of pre and postmenopausal women, premenopausal women, and older men (Table 1.1). As yet $\dot{V}O_2$ kinetic responses during exercise onset have not been investigated in postmenopausal women.

Table 1.1 $\dot{V}O_2$ kinetic responses in individuals with and without T2DM

Study	n	Subjects	Age (yr)	BMI (kg.m ⁻²)	Protocol	Findings
Bauer <i>et al.</i> , (2007)	T2DM: 11 ND: 11	Men & women	T2DM: 47±4 ND: 47±6	T2DM: 30.8±4.3 ND: 28.0±3.0	2 x 6 min cycling at 85% lactate threshold	$\dot{V}O_2$ kinetic responses slowed in T2DM
Brandenburg <i>et al.</i> , (1999)	T2DM: 8 Overweight: 9 Lean: 10	Pre- menopausal women	T2DM: 49.1±5.7 Overweight: 42.5±12.6 Lean: 44.1±8.2	T2DM: 31.8±6.5 Overweight: 30.3±3.5 Lean: 23.5±2.3	3 x 7 min cycling at: 20W & 30W 2 x 7 min at 80 W	$\dot{V}O_2$ kinetic responses slowed in T2DM at all intensities at baseline
Mac Ananey <i>et al.</i> , (2011)	T2DM: 9 Heavy: 9 Lean: 11	Pre & post menopausal women	T2DM: 49.1±5.7 Heavy: 42.5±12.6 Lean: 44.1±8.2	T2DM: 34.4±7.4 Heavy: 29.0±8.7 Lean: 22.7±1.2	4 x 7 min cycling at: 50% VT, 80% VT, & 50% Δ	$\dot{V}O_2$ kinetic responses slowed in T2DM at 50% VT & 80% VT
Regensteiner <i>et al.</i> , (1998)	T2DM: 10 Heavy: 10 Lean: 10	Pre- menopausal women	T2DM: 42±7 Heavy: 37±6 Lean: 36±6	T2DM: 33.1±6.3 Heavy: 30.8±3.6 Lean: 23.5±2.3	3 x 7 min cycling at: 20W & 30W 2 x 7 min at 80 W	$\dot{V}O_2$ kinetic responses slowed in T2DM at 20 W & 30 W
Wilkerson <i>et al.</i> , (2011)	T2DM: 12 ND: 12	Older men	T2DM: 65±5 ND: 62±6	T2DM: 29.2±2.5 ND: 28.4±1.2	2 x 6 min cycling at 50% maximum power	$\dot{V}O_2$ kinetic responses slowed in T2DM

Earlier investigations conducted on premenopausal women have shown that the $\dot{V}O_2$ kinetic response is slowed in premenopausal women with T2DM during cycle exercise performed below lactate threshold (Regensteiner *et al.*, 1998). Subjects performed 3 separate exercise bouts of 7-min duration (one above and two below) lactate threshold for the determination of $\dot{V}O_2$ kinetics. At workloads below lactate threshold, the $\dot{V}O_2$ kinetic response was slower in women with T2DM than either lean or overweight women (Regensteiner *et al.*, 1998). However, the exercise intensities selected in this study were based on absolute workloads (20 W, 30 W, and 80 W) rather than relative intensities that resulted in the women with T2DM working at higher percentages of their $\dot{V}O_{2\text{ peak}}$ both at 20 W ($54 \pm 10\%$) and 30 W ($60 \pm 9\%$) than either overweight (20 W: $39 \pm 9\%$, 30 W: $47 \pm 9\%$) or lean women (20 W: $37 \pm 7\%$, 30 W: $44 \pm 11\%$) (Regensteiner *et al.*, 1998).

In a separate study conducted by the same research group, the same workloads (20 W, 30 W, and 80 W) were used to assess the $\dot{V}O_2$ response in premenopausal women with T2DM, overweight and lean non-diabetics (Brandenburg *et al.*, 1999). The exercise protocol required each subject to complete 3 bouts of 7-min cycling. A mono-exponential function was used to fit the $\dot{V}O_2$ data from the onset of exercise until minute 6 (for low and moderate intensities of 20 W and 30 W) or minute 3 (for high intensity of 80 W). The time constant for the $\dot{V}O_2$ response was significantly slower in the group with T2DM at each workload than either non-diabetic group (Brandenburg *et al.*, 1999). As in the study of Regensteiner *et al.*, (1998), maximal exercise performance was impaired in the individuals with T2DM and as such, using absolute workloads resulted in subjects with T2DM exercising at a greater percentage of their $\dot{V}O_{2\text{ peak}}$ than their non-diabetic counterparts. Thus, it may have been more appropriate if the authors selected workloads based on relative exercise intensities as this might provide a better insight on $\dot{V}O_2$ kinetics between individuals with and without T2DM.

Overcoming this limitation, others examined the effect of using relative exercise intensities as a means to compare $\dot{V}O_2$ kinetics in men and women with and without T2DM (Bauer *et al.*, 2007). In this study subjects completed 2 bouts of 6-min cycling at 85% of lactate threshold that was preceded by 4-min of unloaded cycling. The time constant for the $\dot{V}O_2$ kinetic response was slower in the group with T2DM (43.8 ± 9.6 s) than in the non-diabetic group (34.2 ± 8.2 s). The results were presented as an overall time constant for the $\dot{V}O_2$ response. It is worth noting that each subject completed just 2 bouts of square wave exercise and the $\dot{V}O_2$ data time aligned and averaged to provide a single kinetic $\dot{V}O_2$ response. Previously it has been suggested that at least 3 bouts of constant load exercise are necessary to reduce variability of results between $\dot{V}O_2$ data (Regensteiner *et al.*, 1998; Gusso *et al.*, 2008).

A more recent study investigating $\dot{V}O_2$ kinetic responses in pre and postmenopausal women with and without T2DM, subjects performed three 7-min bouts of constant load cycling above (50% Δ (midpoint between VT and $\dot{V}O_{2\text{ peak}}$)) and below ventilatory threshold (50% and 80% of VT) (Mac Ananey *et al.*, 2011a). Kinetic parameters were determined by fitting a bi-exponential curve to the $\dot{V}O_2$ data for exercise intensities below ventilatory threshold and a tri-exponential curve was used to fit $\dot{V}O_2$ data at exercise intensities above ventilatory threshold. Women with T2DM displayed a slowing of the time constant of the $\dot{V}O_2$ response both at 50% VT (34.2 ± 15.7 s) and 80% VT (39.1 ± 9.0 s) in comparison to overweight (15.4 ± 7.3 and 24.8 ± 8.8 s respectively) and lean controls (20.2 ± 9.7 and 26.8 ± 10.4 s respectively) (Mac Ananey *et al.*, 2011a).

In contrast to previous investigations, one former study failed to find any differences in $\dot{V}O_2$ kinetic responses between an older group of men (65 ± 5 yr) with long-term T2DM (9.3 ± 3.8 yr) and their non-diabetic counterparts (Wilkerson *et al.*, 2011). In this study, subjects completed 2 bouts of 6-min cycle exercise at a power output equivalent to 50% of maximum. Prior to fitting the $\dot{V}O_2$ data, the first 20 s of data were deleted and a mono-exponential function was used to fit the $\dot{V}O_2$ data. In contrast to previous investigations, the $\dot{V}O_2$ kinetic response was not different between men with and without T2DM (43 ± 17 vs.

41±12 s respectively) despite a reduced $\dot{V}O_{2\text{ peak}}$ in men with T2DM ($1.98\pm0.43\text{ L}\cdot\text{min}^{-1}$) in comparison to non-diabetic men ($2.72\pm0.40\text{ L}\cdot\text{min}^{-1}$) (Wilkerson *et al.*, 2011). The time constant of the $\dot{V}O_2$ kinetic response in men with T2DM is somewhat similar to that seen in previous investigations while in contrast, the non-diabetic group evidenced a slowing of the $\dot{V}O_2$ kinetic response in comparison to healthy individuals in former investigations (Brandenburg *et al.*, 1999; Bauer *et al.*, 2007). It would therefore appear that the non-diabetic group might have experienced an age-related slowing of the $\dot{V}O_2$ kinetic response, while there was no further deterioration in the $\dot{V}O_2$ kinetic response in men with T2DM (Wilkerson *et al.*, 2011).

In summary, the dynamic response of $\dot{V}O_2$ during sub-maximal exercise is slowed during both absolute (Regensteiner *et al.*, 1998; Brandenburg *et al.* 1999) and relative (Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a) exercise intensities in premenopausal women, pre and postmenopausal women, and combined groups of men and women; although it seems that $\dot{V}O_2$ kinetic responses are not slowed in older men with T2DM (Wilkerson *et al.*, 2011). However, previous investigations that focused solely on women have included a combined group of both pre and postmenopausal women or a group of premenopausal women only. It is currently not known if the $\dot{V}O_2$ kinetic response is affected by postmenopausal status in women with T2DM.

1.6.4 Cardiac function

The research on cardiac function in diabetic populations is quite limited, in particular regarding to cardiac output and stroke volume, and the relative contributions of central and peripheral cardiovascular limitations on exercise performance in this population remain unclear.

Indirect evidence suggests that cardiac output during exercise may be affected by the presence of T2DM. For instance, in a study by Poirier *et al.*, (2001), as many as 60% of individuals with T2DM showed impaired left ventricular diastolic dysfunction (LVDD). In this study, individuals with T2DM did not have any evidence of diabetic complications, hypertension, or chronic heart failure (CHF)

and performed a graded exercise test showing no ischemia. Of the 60% who had LVDD evaluated using Doppler echocardiography, 28% had a pseudo normal pattern of ventricular filling and 32% had impaired relaxation at rest (Poirier *et al.*, 2001). A pseudo normal filling pattern is significant as it indicates an intermediary stage between impaired relaxation and restrictive filling, and thus, is a more advanced stage of LVDD. However, these measurements were performed at rest and thus the results need to be interpreted with caution.

During graded cycling exercise, premenopausal women recently diagnosed with T2DM but with no evidence of cardiac dysfunction at rest, demonstrated a disproportionate increase in pulmonary capillary wedge pressure (PCWP) in comparison to non-diabetic individuals (Regensteiner *et al.*, 2009). In addition, increased PCWP was inversely correlated with a reduction in myocardial perfusion (Regensteiner *et al.*, 2009). An elevated PCWP is significant as it suggests an elevated filling pressure for a given ventricular volume and more importantly, may precede the development of cardiac dysfunction at rest and during exercise in more advanced T2DM. However, no differences were observed in peak CO or cardiac index (CI) responses between individuals with and without T2DM (Regensteiner *et al.*, 2009).

Confirming these results, others have reported reduced early diastolic relaxation and greater left ventricular filling pressures at rest in overweight men and women with T2DM in comparison to age, weight, and activity matched controls (Baldi *et al.*, 2006). Furthermore, maximal treadmill performance was reduced in normotensive men with T2DM who showed LVDD at rest in comparison to individuals with normal diastolic function (Poirier *et al.*, 2000); though apart from assessing maximal treadmill performance in the former investigation, the authors did not perform any measurements on cardiac function during exercise (Poirier *et al.*, 2000).

Recently during sub-maximal exercise, CO measured using a CO₂ rebreathing method using the Fick equation was lower in adolescent females with T2DM than in obese and non-obese non-diabetics (Gusso *et al.*, 2008). CO was measured at rest and when subjects had achieved heart rates of 100 and 200 beats.min⁻¹. This

was to prevent the confounding influence that heart rate may have on stroke volume. When cardiac output and stroke volume were normalised to fat free mass (CO_{ffm} and SV_{ffm}), both CO_{ffm} and SV_{ffm} were 30-40% lower in individuals with T2DM than non-diabetics (Gusso *et al.*, 2008). In addition, subjects with T2DM showed a lower aerobic capacity than their non-diabetic counterparts (Gusso *et al.*, 2008).

In contrast, others have shown similar maximal and sub-maximal cardiac output in men and women with T2DM in comparison to non-diabetic men and women during cycle exercise (Baldi *et al.*, 2003). Sub-maximal CO was determined using a rebreathing technique at 60 and 70% of $\dot{V}O_{2 \text{ peak}}$ when subjects had reached steady state. While CO was numerically lower in the T2DM group at both intensities, the results did not reach statistical significance. Stroke volume was not different between the groups at either exercise intensity. CO at maximal exercise intensity was estimated rather than measured by multiplying the maximum heart rate achieved during an incremental test by the stroke volume achieved at 70% of $\dot{V}O_{2 \text{ peak}}$. Again, CO was lower in subjects with T2DM but the result was not statistically significant (10.0 ± 1.7 v 11.2 ± 2.0 L.min⁻¹) (Baldi *et al.*, 2003). The authors concluded that CO is not responsible for the reduction in $\dot{V}O_{2 \text{ peak}}$ observed in the group with T2DM. However, the $\dot{V}O_{2 \text{ peak}}$ achieved in the control group (23.8 ± 6.4 ml.kg⁻¹.min⁻¹) suggests that these subjects represented an aerobically unfit population and may have accounted for the lack of significance in CO between individuals with and without T2DM.

In a recent study, impedance cardiography was used to determine cardiac function during cycling exercise in individuals with and without T2DM (Joshi *et al.*, 2010). Subjects completed one bout of 10-min cycling at 50 W. The capacity to maximally increase CO during exercise at 50 W, a measure of cardiac reserve, was lower in the group with T2DM and was attributed to a smaller rise in stroke volume observed during exercise (Joshi *et al.*, 2010). A limitation to the study was the omission of an incremental test to determine maximal aerobic capacity and consequently determine work rates relative to each individual's $\dot{V}O_{2 \text{ peak}}$ rather than working with absolute workloads.

Another recent study has shown that left ventricular diastolic elastance is impaired during supine cycling exercise in men and women with T2DM free from overt heart disease (Ha *et al.*, 2007). However, stroke volumes were similar between individuals with and without T2DM at rest and exercise (Ha *et al.*, 2007). A different study by the same group showed that left ventricular diastolic elastance was greater during exercise in women with T2DM than their male counterparts (Ha *et al.*, 2008). The authors suggested that the smaller ventricle size in women together with underlying myocardial abnormalities might lead to increased ventricular stiffness during exercise.

Using an inert gas rebreathing technique based on the Fick equation, a recent investigation indicates that the slowed $\dot{V}O_2$ kinetic response observed in pre and postmenopausal women with T2DM at the onset of sub-maximal exercise was not related to reduced CO responses (Mac Ananey *et al.*, 2011a). Both CO and CI responses measured at rest and during light and moderate intensity exercise were not different in women with and without T2DM (Mac Ananey *et al.*, 2011a).

It would appear that both maximal and sub-maximal CO responses are not reduced in adult individuals with T2DM who are free from overt CVD (Baldi *et al.*, 2003; Regensteiner *et al.*, 2009; Mac Ananey *et al.*, 2011a). However, LVDD is a common feature of T2DM (Poirier *et al.*, 2001) and as such, may contribute to the exercise intolerance frequently observed in T2DM in addition to preceding the cardiac dysfunction observed in more advanced cases of T2DM.

1.6.5 A-vO₂ difference

Evidence suggests that the lower $\dot{V}O_{2\text{ peak}}$ observed in T2DM subjects might be at least in part caused by a reduction in arteriovenous oxygen difference (a-vO₂ diff) (Baldi *et al.*, 2003). Men and women with T2DM matched in age and body composition to a non-diabetic group reported lower $\dot{V}O_{2\text{ peak}}$ on exhaustion following graded cycling exercise, which was accompanied with a 19% lower a-vO₂ diff (13.54 ± 2.73 v 16.75 ± 2.96 ml.100ml⁻¹ blood) estimated at maximum exercise (Baldi *et al.*, 2003). A significant relationship was found between $\dot{V}O_{2\text{ peak}}$ and a-vO₂ diff but not CO suggesting that the lower $\dot{V}O_{2\text{ peak}}$ observed in

individuals with T2DM can be attributed to a limitation in maximal oxygen extraction. However, a-vO₂ diff measured at 60% and 70% of $\dot{V}O_{2\text{ peak}}$ was not different between individuals with and without T2DM (Baldi *et al.*, 2003). In agreement with this finding, a-vO₂ diff measured at rest and during sub-maximal cycle exercise was similar between women with T2DM and non-diabetic overweight and lean controls during light, moderate and heavy intensity exercise (Mac Ananey *et al.*, 2011a). Thus it would appear that the mechanisms underpinning the slowed $\dot{V}O_2$ kinetic response in individuals with T2DM are not caused by impairments in oxygen extraction as least in uncomplicated T2DM. As yet, the impact of the menopause on a-vO₂ diff in women with T2DM has not been investigated.

While the research on oxygen extraction during exercise in individuals with T2DM is limited, there is a large body of evidence indicating that mitochondrial function is impaired in individuals with T2DM and thus may contribute to the exercise intolerance observed in T2DM. Research carried out on mitochondrial function in the skeletal muscle of individuals with T2DM indicates that both mitochondrial structural and functional capacity are reduced in individuals with T2DM (Kelley *et al.*, 2002). In addition to possessing smaller mitochondria, individuals with T2DM had a reduced citrate synthase activity in comparison to lean non-diabetics (Kelley *et al.*, 2002). Indeed, lower oxidative enzyme capacity appears to persist across different muscle fibre types as reported in a study by He *et al.*, (2001), where within each fibre type, oxidative enzyme activity was lower and lipid content was greater in individuals with T2DM than lean controls.

There is evidence to suggest that fibre type distribution in the vastus lateralis muscle of the leg is altered in individuals with T2DM in comparison to obese and lean controls (Larsen *et al.*, 2009). Individuals with T2DM had a greater percentage of type IIX fibres and a lower percentage of type I fibres than controls (Larsen *et al.*, 2009). In addition to the reduced capillary density and reduced oxidative enzyme activity in the group with T2DM, carbohydrate oxidation was also lower in these individuals during exercise intensities above 50% of $\dot{V}O_{2\text{ peak}}$ (Larsen *et al.*, 2009). The reduced oxidative enzyme activity in T2DM is

consistent with a reduction in mitochondrial function or content in the skeletal muscle of individuals with T2DM.

Consistent with these results, others have also found a reduction in oxidative enzyme activity in individuals with T2DM (Simoneau & Kelley, 1997). The ratio between glycolytic and oxidative enzyme activities is indicative of the balance between cytosolic and mitochondrial capacities for ATP resynthesis. However, this balance appears to be disturbed in individuals with T2DM as reflected in the ratio of glycolytic to oxidative enzyme activity (Simoneau & Kelley, 1997). Furthermore, the imbalance between oxidative and glycolytic enzyme capacities within the skeletal muscle of individuals with T2DM could contribute to the impaired exercise performance frequently observed elsewhere in individuals with T2DM through increased reliance on ATP generation via anaerobic glycolysis.

However, as oxygen extraction during exercise was not measured in the aforementioned studies it is difficult to determine if these results can be extrapolated to a wider population with T2DM.

1.6.6 Dynamics of blood flow

At the onset of dynamic exercise blood must be delivered rapidly to the working muscles to facilitate the delivery of oxygen and substrates and to enable the removal of carbon dioxide and waste products. To achieve this end CO increases, blood flow to the visceral regions and skin is reduced while blood flow to the working muscles is increased. As mean arterial pressure (MAP) remains relatively constant during exercise the increase in blood flow is achieved through reductions in vascular resistance. During times of maximum perfusion, relaxation of vascular smooth muscle cells increases vessel diameter and thus, allows for greater blood flow. The rhythmic cycle of muscle contraction produces variations in blood flow with the inflow of blood occurring during the relaxation phase. This pattern is prevalent at both low and high intensity exercise (Radegran & Saltin, 1998).

Quantification of the dynamic response characteristics of any physiological regulatory system provides insight into the underlying mechanisms responsible

for the non-steady adaptations at exercise onset. Intrinsic to this approach is 1) identification of the number of discrete parameters, namely the time delay, time constant, and amplitude of the response; and 2) quantifying the number of distinct phases of the response (Tschakovsky *et al.*, 2006). Extensive research across a wide range of exercises including repeated hand grip (Shoemaker *et al.*, 1997), plantar flexion (Quaresima *et al.*, 2001), and knee extension (Shoemaker *et al.*, 1994) indicates that the blood response to exercise is biphasic in nature and increases rapidly at the onset of dynamic exercise (Fig. 1.3). Some studies suggest that in addition to the first two phases, a third phase may be observed during periods of intense exercise (Saunders & Tschakovsky, 2004). The first phase is characterised by a rapid incline in blood flow lasting approximately 5-7 s (Shoemaker *et al.*, 1997; Radegran & Saltin, 1998) before reaching a plateau followed by a second phase beginning approximately 15-20 s into the exercise bout (MacDonald *et al.*, 1998) that slowly reaches a new steady state. At higher exercise intensities the onset of the third phase occurs approximately 90-130 s into exercise (Saunders *et al.*, 2005).

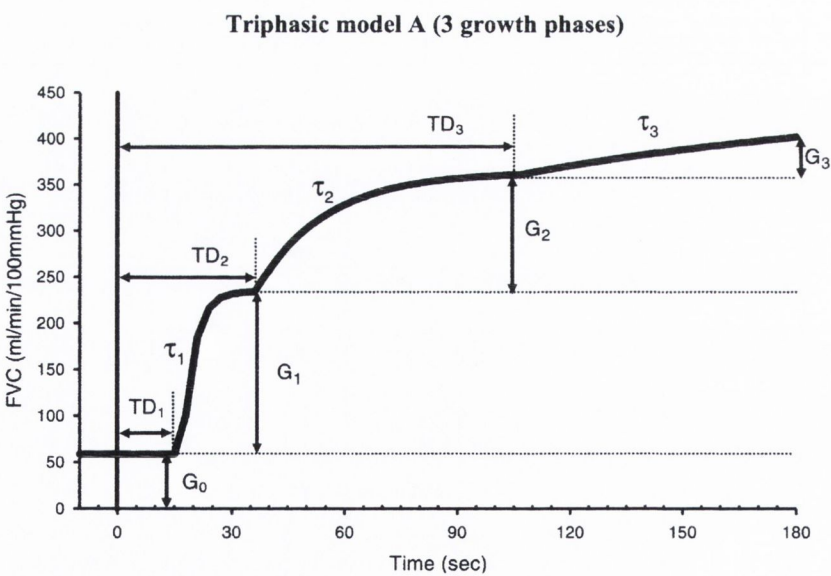


Fig. 1.3. Schematic representation of the exponential increase in blood flow following the onset of exercise. G_0 , G_1 , and G_2 are the amplitudes; TD_1 , TD_2 , and TD_3 are the time delays; τ_1 , τ_2 , and τ_3 are the time constants for phases I, II, and III respectively (Saunders *et al.*, 2005).

However, a fast decay following the initial first phase has been observed in leg blood flow responses during low intensity knee extension exercise in supine (MacDonald *et al.*, 1998) and upright positions (Shoemaker *et al.*, 1996) which suggests that the structure that defines the hyperaemic response is not specifically characterised by 'growth-only' phases. A recent study investigated the structure of the hyperaemic response (measured using venous occlusion plethysmography (VOP)) during plantar flexion exercise by fitting six 'growth only' and 'growth and decay' models to leg vascular conductance (LVC) responses during rest-exercise and exercise-exercise transitions (Reeder and Green, unpublished observations). The goodness of fit was determined on statistical grounds by performing pairwise comparisons between the different models (Reeder & Green, unpublished observations). Across a range of different exercise intensities (30, 60, and 90% MVC) 'growth and decay' models were the best models of fit during rest-exercise transitions (Reeder & Green, unpublished observations). In the majority of responses, a rapid and fast decay was superimposed on the basic growth structure and in some cases a second slower decay was observed at a later stage, the onset of which coincided with a decline in electromyographic (EMG) activity of the triceps surae complex (Reeder & Green, unpublished observations). In fact, leg vascular conductance responses recorded in the current study at light intensities (30% MVC) clearly showed 2 growth phases and 1 decay phase (Fig. 1.4), whereas responses at high intensities (70% MVC) were biphasic (Fig. 1.5).

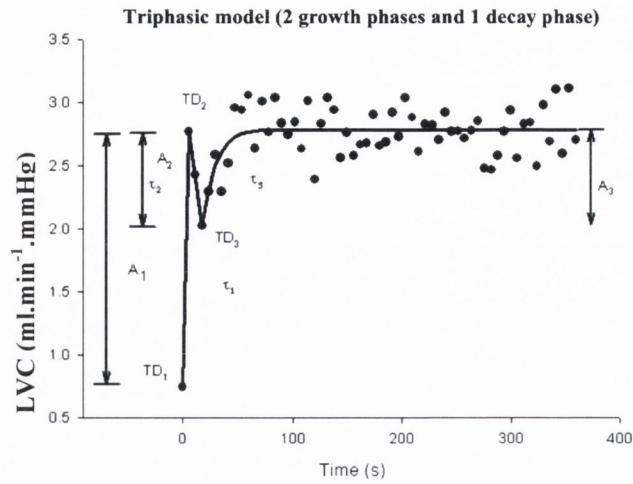


Fig. 1.4. Schematic representation of a triphasic ‘growth and decay’ response following the onset of plantar flexion exercise. A represents the amplitude, TD represents the time delay, and τ represents the time constant of the growth (phase I and phase III) and decay (phase II) phases.

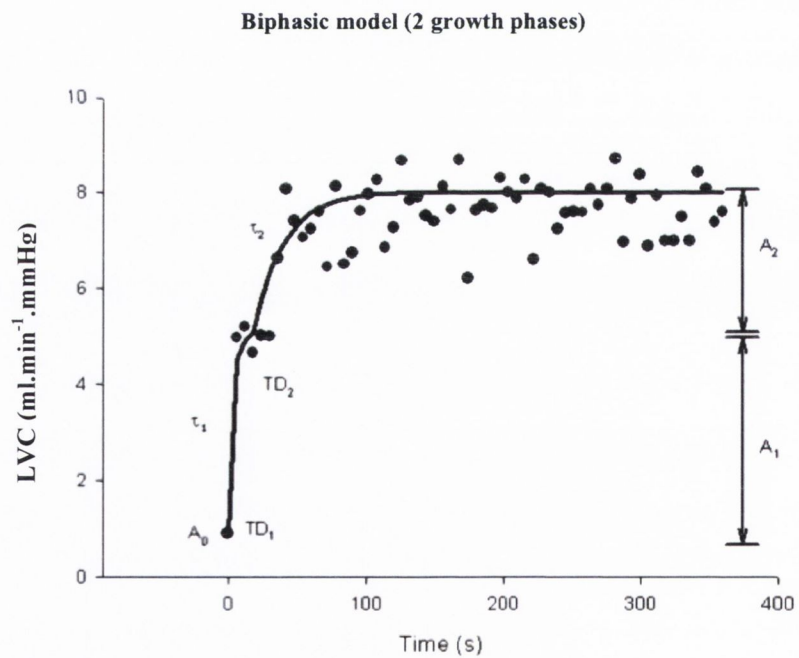


Fig. 1.5. Schematic representation of a biphasic ‘growth only’ response following the onset of plantar flexion exercise. A represents the amplitude, TD represents the time delay, and τ represents the time constant of the growth phases.

There are conflicting reports regarding the mechanism behind the initial hyperaemic response to exercise. Some suggest that the rapid blood flow response observed during phase I (5-7 s) may implicate muscle pump action as a determinant of the initial blood flow response rather than vasodilation (Gotshall *et al.*, 1996; Sheriff & Hakeman, 2001). Others have suggested that the first phase is influenced principally by vasodilatory factors (Saunders & Tschakovsky, 2004) and that during the second growth phase metabolic and endothelial-derived vasodilatory factors may contribute to the regulation of blood flow. Various metabolites have been implicated in the blood flow response to exercise and some include potassium (K^+), hydrogen (H^+), acetylcholine (ACh), and adenosine. Their effects in blood flow regulation may be elicited through action on vascular tone or inhibition of vasoconstriction.

1.6.7 Blood Flow and T2DM

The lower sub-maximal exercise performances observed in T2DM subjects as demonstrated by slowed oxygen uptake kinetics may be related to impairments in skeletal muscle perfusion even in the absence of cardiovascular complications. However, the exact mechanisms responsible for limitations in oxygen delivery in this population remain unclear.

Investigations in rodent models have illustrated abnormal oxygen delivery within muscle microcirculation at rest in diabetic rats (Padilla *et al.*, 2006) and impaired microvascular P_{O_2} responses during exercise in diabetic rats (Padilla *et al.*, 2007). This evidence suggests a limitation in oxygen transport relative to oxygen usage in animal models. It is possible that the observed limitation in maximal aerobic capacity and impaired exercise performance in humans could be attributed to impaired oxygen delivery and exchange.

Leg blood flow (LBF) responses using thermodilution were assessed in men with and without T2DM matched in weight and $\dot{V}O_{2\text{ peak}}$ (Kingwell *et al.*, 2003). Subjects completed 25 min of supine cycling at 60% of $\dot{V}O_{2\text{ peak}}$. During exercise LBF was significantly lower in T2DM subjects between 10 and 25 min ($2.78 \pm 0.19 \text{ L}\cdot\text{min}^{-1}$ v $3.73 \pm 0.38 \text{ L}\cdot\text{min}^{-1}$) and together with higher blood pressure

responses in subjects with T2DM contributed to increased vascular resistance in these subjects (Kingwell *et al.*, 2003). ACh contributes to vasodilation by its interaction with endothelial M₃ muscarinic receptor stimulating it to release nitric oxide (NO) and prostanoids. As LBF responses to endothelium-dependent vasodilation (ACh) were attenuated in the T2DM group, it is possible that either the ACh stimulated release of NO, prostanoids or the reaction between smooth muscle and these substances is blunted in T2DM (Kingwell *et al.*, 2003). Furthermore, a significant relationship was found between LBF during exercise and fasting plasma glucose indicating that chronic hyperglycaemia may be in part responsible for the blunted LBF response observed in T2DM (Kingwell *et al.*, 2003).

Near-infrared spectroscopy (NIRS) is a non-invasive technique used to determine changes in concentrations of oxygenated and deoxygenated haemoglobin/myoglobin ([HHb]) in the small vessels and skeletal muscle. Using this technique it was reported that the initial skeletal muscle deoxygenation response at the beginning of sub-maximal exercise was greater in men and women with T2DM highlighting an imbalance between oxygen transport and oxygen consumption (Bauer *et al.*, 2007). This is possibly due to impaired blood flow responses in these subjects as indicated by the greater mean response time (47.7 ± 14.3 v 35.8 ± 10.7 s) and slower phase II time constant (43.6 ± 12.9 v 32.0 ± 9.7 s) in microvascular blood flow kinetics estimated from $\dot{V}O_2$ and [HHb] data (Bauer *et al.*, 2007).

Using VOP, the dynamic response of LVC was determined during plantar flexion exercise at 70% MVC in pre and postmenopausal women with T2DM, overweight and lean controls (Mac Ananey *et al.*, 2011b). The time constant of the second phase was reduced in women with T2DM (66.4 ± 29.2 s) in comparison to overweight (22.2 ± 13.4 s) and lean controls (21.8 ± 9.3 s) and resulted in a greater mean response time (MRT) in women with T2DM (Fig. 1.6) (Mac Ananey *et al.*, 2011b). There were no differences between the groups in either the End amplitude (End A) or amplitudes of each phase indicating that the capacity to vasodilate might be preserved in T2DM. Furthermore, MAP responses were

similar between women with T2DM and their non-diabetic counterparts. Therefore, the slowing of the hyperaemic response in women with T2DM, as indicated by a greater MRT, was related to the slowed rate of increase in LVC during the second phase (Mac Ananey *et al.*, 2011b).

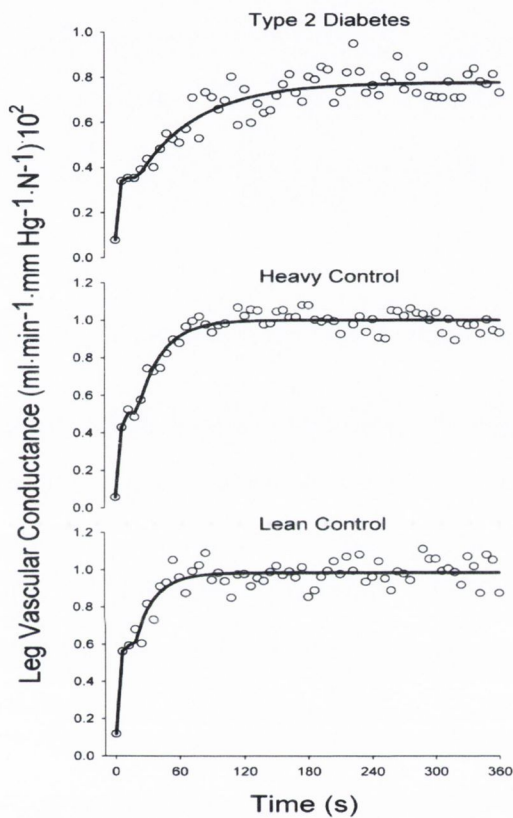


Fig. 1.6. LVC responses (open circles) during calf exercise in an individual with type 2 diabetes, a heavy control, and a lean control. The continuous lines of best fit illustrate the biphasic response of LVC. Note the relatively slow response of the second hyperaemic phase in the diabetic subject (Mac Ananey *et al.*, 2011b).

The slowed rate of increase in $\dot{V}O_2$ kinetics observed at the onset of sub-maximal exercise (Regensteiner *et al.*, 1998; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a) together with the estimated slowed microvascular blood flow kinetics (Bauer *et al.*, 2007) and slowed LVC kinetic responses (Mac Ananey *et al.*, 2011b) suggest that the impairment in muscle blood flow dynamics seems to be a contributory factor to limitations in oxygen uptake dynamics, during sub-maximal exercise in T2DM.

1.7 Role of exercise training in T2DM

The benefits that patients with T2DM can acquire from regular exercise are numerous and include improved exercise performance as indicated by a greater $\dot{V}O_2$ peak and increased time to failure, reduced BMI and percentage body fat, increased insulin sensitivity, improved lipid profile, reduction in pro-inflammatory cytokines, improved endothelial function, and decreased risk of developing CVD. Research indicates that individuals with T2DM can benefit from a single bout of exercise. Glucose uptake increases in response to a single bout of exercise with the effect being greater in individuals with T2DM with greater BMI and poorer glycaemic control (Oguri *et al.*, 2009).

Studies have examined the role of different types of exercise in diabetic populations. These include aerobic (Lehmann *et al.*, 1995; Brandenburg *et al.*, 1999; Zoppini *et al.*, 2006), resistance (Fenicchia *et al.*, 2004; Cohen *et al.*, 2008), and a combination of aerobic and resistance training (Maiorana *et al.*, 2001a). The types of aerobic exercises used in these studies include walking, jogging, cycling, and rowing. With resistance training, investigators have included a range of exercises targeting upper and lower body muscle groups. Some studies have used a combination of approaches in treating T2DM including diet and exercise interventions (Eriksson & Lindgarde, 1991).

Short-term interventions have been conducted over periods of 2-3 months (Holten *et al.*, 2004; Colberg *et al.*, 2006) with longer interventions being conducted from 12 months (Cohen *et al.*, 2008) to 5 years (Eriksson & Lindgarde, 1991). In these interventions, subjects with T2DM exercised 2-3 d.wk⁻¹ for 60-90 min. Different exercise intensities have been reported in the literature for both aerobic and resistance exercise. Exercise intensities of 75% of $\dot{V}O_2$ peak (Mourier *et al.*, 1997) and 70-85% of peak heart rate (Peak HR) (Brandenburg *et al.*, 1999; Maiorana *et al.*, 2001a) have been used in aerobic exercise. In relation to resistance training, subjects with T2DM have exercised at 55-65% of MVC (Maiorana *et al.*, 2001a) and 70-80% of 1 repetition maximum (1-RM) (Holten *et al.*, 2004; Colberg *et al.*, 2006). Interventions using resistance training have included 8-12 exercises with 2-3 sets of 8-12 repetitions.

Almost all of the studies included here have shown positive results in subjects with T2DM in response to exercise intervention across a range of parameters including body composition (Eriksson & Lindgarde, 1991; Lehmann *et al.*, 1995; Mourier *et al.*, 1997), glucose control (Eriksson & Lindgarde, 1991; Fenicchia *et al.*, 2004), triglycerides (Lehmann *et al.*, 1995), and insulin sensitivity (Mourier *et al.*, 1997; Holten *et al.*, 2004). Improvements have also been found in physical fitness parameters such as $\dot{V}O_2$ kinetics (Brandenburg *et al.*, 1999), $\dot{V}O_{2\text{ peak}}$ (Eriksson & Lindgarde, 1991), and strength (Colberg *et al.*, 2006).

1.7.1 $\dot{V}O_{2\text{ peak}}$ and $\dot{V}O_2$ kinetics

Aerobic training has consistently shown to be an effective intervention to increase $\dot{V}O_{2\text{ peak}}$ in T2DM (Zanuso *et al.*, 2010). One former study investigated the effect of aerobic, resistance, or combined aerobic plus resistance training on $\dot{V}O_{2\text{ peak}}$ and muscle strength in 251 individuals with T2DM (Larose *et al.* 2011). Following a 6-month period of intervention, $\dot{V}O_{2\text{ peak}}$ increased in the aerobic and the aerobic plus resistance groups. In addition, there was an increase in strength in the leg press, bench press, and seated row in the combined aerobic plus resistance group and the resistance group (Larose *et al.* 2011). However, combined aerobic plus resistance training did not provide any additional benefits in either $\dot{V}O_{2\text{ peak}}$ or strength to either aerobic or resistance training alone (Larose *et al.* 2011).

The clinical significance of a slowed $\dot{V}O_2$ kinetics response at the onset of exercise has not been well evaluated. To date only one former study has examined the impact of exercise intervention on the $\dot{V}O_2$ kinetic response to the onset of exercise in a population with T2DM (Brandenburg *et al.*, 1999). Premenopausal women with T2DM, and non-diabetic overweight and lean women underwent a 3-month supervised exercise intervention consisting of regular aerobic exercise (3 d.wk⁻¹ for 60 min) performed at 70-85% of peak heart rate (Brandenburg *et al.*, 1999). Each individual was required to complete 36 training sessions. There were no differences between the groups with respect to the number of training sessions completed (T2DM = 35.5, overweight = 35.3, and lean = 35.2). At baseline, women with T2DM had a lower $\dot{V}O_{2\text{ peak}}$ and slower $\dot{V}O_2$ kinetic response than either overweight or lean women during cycle exercise

at 20, 30, and 80 W. Following exercise intervention, $\dot{V}O_2$ kinetics improved in women with T2DM by 39 and 22% at 20 and 30 W respectively with a tendency towards a faster response at 80 W. Furthermore, $\dot{V}O_2$ kinetic responses were not different between the groups after exercise intervention at 20, 30, or 80 W. $\dot{V}O_{2\text{ peak}}$ increased by 28% in women with T2DM and 8% in overweight non-diabetics while remaining unchanged in lean women (Brandenburg *et al.*, 1999). If the capacity to begin exercise is impaired in populations with T2DM, then these individuals may perceive exercise as being more difficult and they may therefore be less inclined to engage in even low or moderate intensity exercise. However, regular aerobic exercise appears to be important in helping individuals with T2DM overcome this limitation at exercise onset as demonstrated by a speeding of the $\dot{V}O_2$ kinetic response during low and moderate intensity cycle exercise following 3 months of regular exercise (Brandenburg *et al.*, 1999).

The majority of research has focused on the effects of aerobic exercise on glucose control and insulin sensitivity as this type of exercise engages large muscle groups and can be performed for prolonged periods of time. Resistance training is also effective in recruiting large muscle mass particularly when upper and lower body exercises are included. This form of training not only increases strength but also muscle mass, muscle endurance, and posture. Bone density is also enhanced due to the weight-bearing component of some exercises. It is possible that some subjects, particularly those who are obese, may prefer this type of training as they may find that the physical strain induced by cardiovascular exercise is absent in resistance training.

In fact, significant improvements have been observed in $\dot{V}O_{2\text{ peak}}$ in men and women with T2DM (16.2 ± 0.9 to 17.8 ± 0.9 ml.kg⁻¹.min⁻¹) following resistance training (Colberg *et al.*, 2006). Subjects completed a total of 8 exercises (both upper and lower body) and were required to perform 8-12 repetitions of 3 sets beginning initially at 50% of 1-RM and then increasing to 60 and 70% in the subsequent months. However, the $\dot{V}O_{2\text{ peak}}$ achieved post training was still lower than that achieved in a non-diabetic group (21.3 ± 1.6 ml.kg⁻¹.min⁻¹) who had undergone similar resistance training. Significant improvements were found in

percentage body fat in the group with T2DM post training (36.8 ± 1.5 v 32.6 ± 1.9 %) (Colberg *et al.*, 2006).

1.7.2 Cardiac function

The impact of exercise intervention on cardiac function in populations with T2DM has not been well investigated. A recent study reported a reduction in arterial stiffness in older (71.4 yr) individuals with T2DM who also had multiple cardiovascular risk factors following 3 months of aerobic exercise (Madden *et al.*, 2009). Subjects were assigned to aerobic (60 min at 60-75% of heart rate reserve) or non-aerobic groups (core and strength training) for 3 d.wk⁻¹. Following intervention, there was a decrease in both radial (-20.7 ± 6.3 vs. $+8.5 \pm 6.6$ %) and femoral (-13.9 ± 6.7 vs. $+4.4 \pm 3.3$ %) pulse-wave velocity in the aerobic group but not the in the non-aerobic group (Madden *et al.*, 2009). These improvements occurred in the absence of improvements in weight loss, blood pressure, FPG, and $\dot{V}O_{2 \text{ peak}}$ (Madden *et al.*, 2009). The authors speculated that pulsatile stretching of collagen fibres during aerobic exercise might break collagen crosslinks caused by non-enzymatic glycation and thereby reduce arterial stiffness (Madden *et al.*, 2009).

Individuals with T2DM (57 yr) underwent regular aerobic training over a 3-month period to assess the effects of chronic exercise on LVDD (Brassard *et al.*, 2007). Exercise intervention consisted of cycling at 60-70% of $\dot{V}O_{2 \text{ peak}}$ for 60 min 3 d.wk⁻¹. Following exercise intervention $\dot{V}O_{2 \text{ peak}}$ increased (28.6 ± 3.9 to 32.7 ± 5.7 ml.kg⁻¹.min⁻¹) and normalisation of LVDD was observed in 5 out of 11 (4 of whom had grade 1 LVDD) subjects (Brassard *et al.*, 2007). Longer intervention periods may be required to improve more severe forms of LVDD (Brassard *et al.*, 2007).

In a different study, adaptations in arterial compliance following exercise intervention were investigated in postmenopausal women with T2DM (McGavock *et al.*, 2004). Subjects underwent a combination of aerobic (30-55 min at 65-75% of heart rate reserve) and resistance (7 upper & lower body exercises, 3 sets of 10-15 repetitions at 50-65% of 1-RM) exercise 3 d.wk⁻¹ for 12

weeks. Improvements were reported in $\dot{V}O_{2\text{ peak}}$ (21.3 ± 3.3 to 24.5 ± 4.2 $\text{ml.kg}^{-1}.\text{min}^{-1}$) and large artery compliance (1.0 ± 0.4 to 1.2 ± 0.4 mL.mmHg^{-1}) despite no changes in left ventricular filling dynamics, insulin sensitivity, lipid profile, or blood pressure (McGavock *et al.*, 2004). The authors suggested that enhanced endothelium-dependent vasodilation may have resulted in increased arterial compliance in these women (McGavock *et al.*, 2004).

1.7.3 Glycaemic control

Exercise training has consistently been shown to be effective in improving glycaemic control in T2DM. However, to maximise its effectiveness it is important to consider the mode of exercise (aerobic vs. resistance), whether it is combined with dietary intervention and whether it is performed in a supervised or unsupervised setting.

Fenicchia *et al.*, (2004) investigated the effect of acute and chronic exercise on glucose control and insulin responses in women with and without T2DM where subjects completed resistance training 3 d.wk^{-1} for 6 weeks during which they performed a combination of 8 upper and lower body exercises (3 sets of 8-12 repetitions). Improvements were found in 1-RM in upper and lower body exercises in both groups and in addition, there was a reduction in fat mass in women with T2DM following 6 weeks of resistance training (Fenicchia *et al.*, 2004). Subjects performed 3 oral glucose tolerance tests: pre-training, 12-24 h after the first exercise session (acute), and 60-72 h after the final session (chronic). Integrated glucose concentration, expressed as the area under the curve (AUC), was improved after the acute bout of exercise ($3,355.0 \pm 324.6$ to $2,868.0 \pm 324.0$ mmol.L^{-1}) but was not improved after chronic training ($3,606.0 \pm 337.0$ mmol.L^{-1}) in women with T2DM (Fenicchia *et al.*, 2004). The same trend was observed in peak glucose concentrations in women with T2DM (pre-training: 20.2 ± 1.4 mmol.L^{-1} ; acute: 17.2 ± 1.7 mmol.L^{-1} ; chronic: 19.9 ± 1.7 mmol.L^{-1}). In contrast, there were no changes in insulin concentrations after either acute or chronic exercise in women with T2DM. The authors concluded that a longer period of exercise intervention may be needed to impact on insulin concentrations (Fenicchia *et al.*, 2004).

In postmenopausal women with T2DM, the effect of aerobic versus aerobic plus resistance training on insulin sensitivity was investigated following a 16-wk supervised intervention programme (Cuff *et al.*, 2003). Subjects were randomly assigned to one of 3 treatments: control, aerobic plus resistance (Ae + RT), or aerobic (Ae). The Ae + RT group performed aerobic exercise at 60-75% heart rate reserve in addition to performing 5 upper and lower body exercises for the resistant component (2 sets of 12 repetitions). The Ae group performed low-impact low intensity dynamic movement instead of resistance exercise while also completing the same aerobic exercise as the Ae + RT group. Each group trained 3 d.wk⁻¹ for 75 min. Glucose infusion rates increased in the Ae + RT group (1.82 ± 0.52 ml.kg⁻¹.min⁻¹) and were greater than the control group (0.07 ± 0.28 ml.kg⁻¹.min⁻¹) while remaining unchanged in the Ae (0.55 ± 0.36 ml.kg⁻¹.min⁻¹) (Cuff *et al.*, 2003). In addition, normal muscle density was greater in the Ae + RT group (10.5 ± 2.0 cm²) than either the Ae (2.3 ± 1.8 cm²) or control (-3.5 ± 1.7 cm²) groups following intervention (Cuff *et al.*, 2003). The improved glucose disposal was associated with a reduction in both subcutaneous and visceral adipose tissue area as well as an increase in muscle density (Cuff *et al.*, 2003). The study highlighted the advantage of combining both aerobic and resistance exercise in an intervention programme in comparison to aerobic training alone.

The effectiveness of resistance training plus weight loss versus weight loss alone on glycaemic control and body composition was examined in men and women with T2DM (60-80 yr) by Dunstan *et al.*, (2002). Subjects were randomised to receive high intensity resistance exercise plus moderate weight loss (RT & WL) or moderate weight loss plus a control programme (WL) (Dunstan *et al.*, 2002). The study was conducted over 6 months and measurements were repeated at 3-month intervals. Both groups were placed on a healthy eating plan during the first month that was designed to elicit a weight loss of 0.25 kg.wk⁻¹. The RT & WL group completed 3 sessions per week consisting of 9 upper and lower body exercises at 75-85% of 1-RM while the WL group completed 3 static stretching sessions per week. Adherence to training was not difference between the groups (RT & WL: 88%, WL: 85%). Both total body mass and fat mass were reduced in RT & WL and WL groups at 3 months and 6 months (Dunstan *et al.*, 2002). Significant reductions were found in HbA_{1C} levels in the RT & WL group only

both at 3 months (-0.6 ± 0.7 %) and 6 months (-1.2 ± 0.9 %) (Dunstan *et al.*, 2002). In contrast, no changes were observed in the WL group with respect to HbA_{1C} or glucose concentrations (Dunstan *et al.*, 2002). The authors concluded that a combined resistance plus weight loss programme is more effective in improving glycaemic control than weight loss programme alone and is independent of improvements in body composition (Dunstan *et al.*, 2002).

A follow up study was completed to investigate if the improvements acquired during the supervised exercise intervention could be maintained during home-based resistance training over 6 months (Dunstan *et al.*, 2005). The RT & WL group were provided with an instruction booklet, training, and dumbbells to follow an individualised training programme 3 d.wk⁻¹ (9 upper and lower body exercises at 60-80% of 1-RM). In contrast, the WL group were provided with a flexibility wall chart to enable them continue with the flexibility programme developed in the supervised phase. There was a significant reduction in adherence to training in both RT & WL (72.6%) and WL (78.1%) groups (Dunstan *et al.*, 2005). The improvements acquired in HbA_{1C} were not maintained during the home-based programme; a significant increase in HbA_{1C} levels was observed in both groups at 12 months but was not different from baseline (Dunstan *et al.*, 2005). Fasting glucose concentrations were not changed during the home-based phase. While both groups reported an increase in body weight and total body fat at 12 months from 6 months, body weight remained lower at 12 months than at baseline (Dunstan *et al.*, 2005). Furthermore, upper and lower body strength which were increased from baseline at 6 months, were maintained at 12 months in the RT & WL group (Dunstan *et al.*, 2005). The deterioration in glycaemic control was attributed to the reduced adherence to exercise and reduction in volume and intensity of training in the home-based phase (Dunstan *et al.*, 2005).

A more recent study by the same authors compared the effectiveness of a community centre-based versus home-based resistance training programmes on the maintenance of glycaemic control in overweight sedentary men and women with T2DM aged 40-80 yr (Dunstan *et al.*, 2006). Initially all subjects completed a 2-month supervised phase during which they performed 8 resistance exercises 2

d.wk⁻¹ at 75-85% of 1-RM. Thereafter, subjects were randomly assigned to carry out a maintenance programme in either a community and recreation centre (centre) or in their own home (home) over a 12-month period (2 d.wk⁻¹). Adherence to exercise during the supervised phase was 87±15 % while in the maintenance phase adherence was 68.1±25.0 and 67.1±27.1 % in the centre and home groups respectively (Dunstan *et al.*, 2006). Furthermore, individuals in the centre group who attended exercise sessions more frequently (75-100%) had a greater reduction on HbA_{1C} levels (-0.5±0.8 vs. 0.5±1.1%) than those who attended fewer sessions (0-59%) (Dunstan *et al.*, 2006). There were reductions in HbA_{1C} levels in the centre group at 2 months (-0.5±1.0 %) and 14 months (-0.4±1.0 %) (Dunstan *et al.*, 2006). Fasting glucose and insulin concentrations were not different in the home group but were reduced in the centre group at 14 months (-0.8±1.8 mmol.L⁻¹ and -22.2±47.6 pmol.L⁻¹ respectively). As the legs constitute a large area of muscle mass, the emphasis on whole body exercise in the centre group may have been a factor in helping to maintain glycaemic control in contrast to the home group who were not able to replicate specific leg exercises used in the supervised phase using just dumbbells (Dunstan *et al.*, 2006).

A long-term intervention programme of 5-years duration consisting of dietary intervention and supervised exercise was successful in improving body composition, glucose control, and aerobic capacity in subjects with T2DM and IGT (Eriksson & Lindgarde, 1991). The initial 12 months consisted of dietary advice and supervised exercise. Thereafter, subjects continued with the programme of diet and exercise on their own. In both groups, body mass decreased by 2.3-3.7% and $\dot{V}O_2$ peak increased by 10-14%. The accumulated incidence of T2DM was 10.6% post intervention while more than half of patients with T2DM were in remission after a follow-up period of 6 years. Thus it appears that supervised exercise is an important element of physical activity interventions.

In a large study (606 participants), individuals with the metabolic syndrome or T2DM were assigned to either supervised aerobic plus resistance training and exercise counselling (exercise) or counselling alone (control) (Balducci *et al.*, 2010). The exercise group underwent supervised exercise training over a 12-

month period. Following intervention, improvements were found in HbA_{1C}, HDL, LDL, and both systolic and diastolic blood pressure in the exercise group in comparison to the control group (Balducci *et al.*, 2010). The authors suggest that while counselling was successful in achieving the recommended amount of physical activity in the control group, larger volumes of physical activity are required to affect change in cardiovascular risk factors in these individuals (Balducci *et al.*, 2010).

Individuals with newly diagnosed T2DM (n=593) were randomly assigned to receive control, dietary intervention or dietary intervention plus a physical activity programme (Andrews *et al.*, 2011). At 6 months, glycaemic control improved in both the diet and diet plus activity groups and the improvements were maintained at 12 months (Andrews *et al.*, 2011). The addition of physical activity to the dietary intervention programme did not result in greater reductions in HbA_{1C} (Andrews *et al.*, 2011). It is possible that the physical activity intervention (30 min of brisk walking 5 d.wk⁻¹) was insufficient to exert any further reductions in HbA_{1C} (Andrews *et al.*, 2011).

A recent investigation examined the effect of supervised exercise on HbA_{1C} levels in men and women with T2DM over a 9-month period (Church *et al.*, 2010). Individuals (n=262) were randomly assigned to aerobic, resistance, combined aerobic plus resistance, or control groups. In comparison to the control group, there was a reduction in HbA_{1C} in the combined aerobic plus resistance group only (-0.34%) but not in either the aerobic or resistance groups (Church *et al.*, 2010). In addition, $\dot{V}O_{2\text{ peak}}$ improved in the combined group only (+1 ml.kg⁻¹.min⁻¹) (Church *et al.*, 2010). This study highlights the importance of including both aerobic and resistance exercise in exercise intervention programmes. A similar investigation examined the effect of aerobic, resistance, or combined aerobic plus resistance training on HbA_{1C} levels in a large group (n=251) of individuals with T2DM (39-70 yr) over a 6-month period (Segal *et al.*, 2007). While aerobic or resistance training alone improved glycaemic control in individuals with T2DM, combined aerobic plus resistance training resulted in a

greater reduction in HbA_{1C} versus aerobic (-0.46 percentage point) and resistance (-0.59 percentage point) training alone (Segal *et al.*, 2007).

Therefore, research indicates that to effectively improve glycaemic control, exercise intervention is most successful when conducted in supervised settings, when combined with weight loss/dietary intervention or when a combination of aerobic and resistance exercise is included.

1.7.4 Vascular Function

Diabetes related deaths are largely attributable to atherosclerotic macrovascular complications and therefore, the importance of preserving vascular function in T2DM cannot be underestimated. To this end, a combined aerobic and resistance training intervention was carried out to assess the effects on vascular function in individuals with T2DM (Maiorana *et al.*, 2001a). In a crossover design study, subjects were randomly assigned to trained or untrained groups for 8 weeks and this was followed by 8 weeks of training or detraining. Exercise intervention consisted of aerobic and resistance exercise performed 3 d.wk⁻¹ for 1 h. Aerobic training consisted of walking and cycling (70-80% of peak HR) while resistance training consisted of 7 exercises incorporating both upper and lower body muscle groups (55 to 65% of MVC). Resistance vessel function was determined by assessing both endothelium-dependent and endothelium-independent vasodilation using venous occlusion plethysmography and intra-arterial infusions of ACh and sodium nitroprusside (SNP) respectively. Conduit vessel function was assessed at rest, after flow mediated dilation induced by reactive hyperaemia, and following administration of glyceryl trinitrate (GTN). In response to exercise training, forearm ischemia induced an increase in flow mediated-dilation (FMD) (largely endothelium and NO dependent) from $1.7 \pm 0.5\%$ to $5.0 \pm 0.4\%$ while in contrast, exercise training did not affect endothelium-independent vasodilation to GTN (Maiorana *et al.*, 2001a). When forearm blood responses were analysed by comparing the infused arm to the non-infused arm during administration of ACh, the ratio of blood flow between the infused and non-infused arms at increasing doses of ACh were greater following training (Maiorana *et al.*, 2001a). In contrast, no effect was observed for SNP. The authors conclude that combined aerobic and resistance training enhances both resistance and conduit vessel

function in individuals with T2DM and that this adaptation appears to be predominately endothelium-dependent (Maiorana *et al.*, 2001a). It is possible that other mechanisms might include enhanced sensitivity of smooth muscle to NO or changes in the structure of the vasculature (Maiorana *et al.*, 2001a). The improvements observed in vascular function that occurred in response to training might be associated with increased flow and shear stress on the vascular walls. HbA_{1C} and fasting plasma glucose were also lower post-intervention (Maiorana *et al.*, 2001a). This might be due to the improved hemodynamic response to training as glucose uptake is dependent on blood flow to the skeletal muscle.

In both men with and without T2DM, glucose uptake, protein content of GLUT-4, and insulin signalling improved in response to one-legged strength training over a 6-wk period training 3 d.wk⁻¹ (Holten *et al.*, 2004). Leg uptake and release of glucose were calculated as arterio-venous concentrations in whole blood multiplied by blood flow. During a euglycemic-hyperinsulinemic clamp, leg blood flow was greater in the trained than untrained leg and was accompanied by increased glucose clearance in the trained leg in both groups (Holten *et al.*, 2004).

To examine the effects of resistance exercise on endothelial function, sedentary men (n = 16) and women (n = 15) with T2DM underwent 14 months of high intensity resistance training (Cohen *et al.*, 2008). The intervention programme consisted of a 2-month supervised phase where subjects trained 2 d.wk⁻¹ beginning initially at 50-60% of 1-RM and increasing to 75-85% of 1-RM after the first week. Subjects completed a combination of upper and lower body exercises (3 sets of 8 repetitions). Following the supervised phase, subjects were randomly assigned to either a community fitness and recreation centre-based programme (centre) or home-based programme (home). The centre group followed a resistance programme similar to that of the supervised phase while the home group were provided with one hand weight and encouraged to complete an upper body resistance programme. All subjects were required to complete 2/3 sessions per week for 14 months. A laser Doppler flow technique was used to assess endothelial function during which vascular responses to ACh and SNP were recorded. Exercise adherence during the unsupervised phase was comparable between the groups (centre: 97±9.3 %; home: 88±8.3 %) (Cohen *et*

al., 2008). Vascular responses to ACh or SNP were not different following 2 months of supervised exercise (Cohen *et al.*, 2008). Following unsupervised training at the 14th month, vascular responses to ACh (all doses) and SNP (most doses) increased in both centre and home groups (from baseline and 2 months) but were not different between the groups (Cohen *et al.*, 2008). The authors suggested that the association observed between endothelial function at 14 months and preceding HbA_{1C} at baseline and 2 months indicate that glycaemic control could be an important determinant in the regulation of endothelial function (Cohen *et al.*, 2008). Furthermore, the improvements in vascular responses were not solely endothelial-dependent as improvements were also found in endothelium-independent vasodilation, thereby suggesting increased responsiveness to smooth muscle vasodilators such as NO (Cohen *et al.*, 2008).

A recent study investigated the effect of aerobic and resistance exercise on endothelial function and incidence of CVD in men and women with uncomplicated T2DM (Okada *et al.*, 2010). Subjects were randomly assigned to exercise (n = 21) or control (n = 17) groups for 3 months. Exercise training consisted of 55 min of aerobic exercise and 20 min of resistance exercise performed 3-5 d.wk⁻¹. Endothelial function was determined by assessing the brachial artery response to FMD. FMD increased in the exercise group but was unchanged in the control group after 3 months (Okada *et al.*, 2010). HbA_{1C} levels were decreased in both exercise (8.5±1.8 to 7.0±1.3 %) and control (7.9±1.1 to 6.8±0.8 %) groups but were not different between the groups following intervention (Okada *et al.*, 2010). In the follow up period (24 months), 4 subjects in the control group experienced adverse cardiovascular events (1 = angina, 3 = cerebral infarction) while in contrast subjects in the exercise group did not experience any adverse cardiovascular events (Okada *et al.*, 2010). It appears that regular exercise improves endothelial function independent of glycaemic control in men and women with T2DM.

In contrast, Middlebrooke *et al.*, (2006) failed to find any improvement in microvascular function in men and women with T2DM following 6 months of aerobic exercise (Middlebrooke *et al.*, 2006). The exercise intervention consisted of 30 min of aerobic exercise (70-80% of peak HR) performed 3 d.wk⁻¹ (2/3

sessions were supervised). Microvascular function was determined from maximum skin hyperaemia to local heating and endothelium-dependent and endothelium-independent responses to iontophoretic application of ACh and SNP. Maximum hyperaemia, peak responses to ACh or SNP, and respective AUCs were not affected by exercise intervention (Middlebrooke *et al.*, 2006). These results together with the lack of improvement in $\dot{V}O_{2\text{ peak}}$ (Exercise: 19.34 ± 5.17 to 20.43 ± 5.57 ml.kg⁻¹.min⁻¹; Control: 20.11 ± 5.33 to 19.25 ± 4.08 ml.kg⁻¹.min⁻¹) would indicate that the exercise intervention was insufficient to exert any positive influence on vascular function. The authors suggested that the micro and macrovasculature might respond differently to exercise intervention (Middlebrooke *et al.*, 2006).

In summary, it would appear that combined aerobic plus resistance training is more affective in improving endothelial function in individuals with T2DM than resistance/aerobic training alone. Combined aerobic plus resistance training improves both resistance and conduit vessel function in individuals with T2DM and this is predominantly endothelium-dependent. However, some improvements have been found in endothelium-independent vasodilation following resistance training alone in diabetic populations.

1.7.5 Inflammation

Chronic low-grade inflammation which is characterised by the presence of increased circulating levels of several inflammatory cytokines such as IL-6 (Muller *et al.*, 2002), IL-8 (Bruun *et al.*, 2003), CRP (Giannopoulou *et al.*, 2005; Balducci *et al.*, 2010), TNF- α (Wood *et al.*, 2005), and resistin (Verma *et al.*, 2003), are increased in disease states such as T2DM (Pradhan *et al.*, 2001; Gleeson *et al.*, 2011). The presence of hyperglycaemia increases oxidative stress and inflammation, both of which may contribute to vascular damage and endothelial dysfunction. Consequently, this may have implications for individuals with T2DM as impaired vascular function may negatively affect exercise performance.

TNF- α is a pro-inflammatory cytokine synthesised by macrophages, lymphocytes, and adipose tissue. TNF- α is associated with impairments in insulin action and consequently, glucose uptake in muscle and adipose tissue through inhibitory effects on GLUT-4 and GLUT-4 gene expression. The effects of TNF- α on insulin action are multiple and involve several proteins such as Akt substrate 160 in skeletal muscle (Plomgaard *et al.*, 2005). In addition, it also modulates the release of free fatty acids from adipose tissue and increases lipolysis (Kern *et al.*, 2001). A 4-wk intervention programme consisting of diet and exercise has been shown to be effective in reducing TNF- α levels in obese individuals with T2DM while remaining unchanged in non-obese subjects with T2DM (Katsuki *et al.*, 1998). The dietary treatment consisted of consuming between 1,400-1,720 kcal.d⁻¹ and subjects were also required to walk 15,000 steps daily, as measured by a pedometer. At baseline obese subjects with T2DM had greater levels of visceral fat and higher concentrations of TNF- α than non-obese subjects with T2DM while in contrast HbA_{1C} levels were not different between the groups. The improvement in TNF- α concentrations in the obese subjects with T2DM is attributed to the greater degree of visceral fat loss in these subjects (Katsuki *et al.*, 1998).

CRP is a pro-inflammatory cytokine whose synthesis in liver is mostly mediated by IL-6. CRP is an important indicator of atherosclerosis, MI, stroke, and death from CVD across different age groups and ethnicity in both men and women (Ridker, 2003). Evidence exists to support the role of CRP in the upregulation of transcription factor NF- κ B, which contributes to the development of atherosclerosis (Lin *et al.*, 2005). NF- κ B activation mediates the expression of pro-inflammatory genes and inflammatory markers. Regular aerobic exercise (4 d.wk⁻¹ for 6 months) reduced concentrations of CRP and IL-18 as well as providing an anti-inflammatory effect by increasing concentrations of IL-10 in obese subjects with T2DM (Kadoglou *et al.*, 2007). Significant improvements were also observed in $\dot{V}O_2$ peak, glucose control, lipid profile, and insulin resistance (Kadoglou *et al.*, 2007).

A recent study investigated the anti-inflammatory effect exercise training over a 12-month period in individuals with T2DM and the metabolic syndrome (Balducci *et al.*, 2010). Individuals were randomly assigned to the following conditions: control, low-intensity physical activity, high-intensity aerobic exercise, or aerobic plus resistance exercise. There was a reduction in levels of hs-CRP and IL-6 in both the high-intensity aerobic group and the combined aerobic plus resistance group but TNF- α was reduced in the combined aerobic plus resistance group only (Balducci *et al.*, 2010). This study highlights the importance of exercise intensity in affecting change in pro-inflammatory cytokines and indicates that long-term high-intensity aerobic or combined aerobic plus resistance training may be required to reduce concentrations of certain pro-inflammatory cytokines.

Other adipocytokines such as resistin and leptin are elevated in cases of obesity, while in contrast, adiponectin levels are reduced (Vendrell *et al.*, 2004). Leptin is a peptide hormone found in adipocytes that is involved in the regulation of energy consumption, energy expenditure, and body weight. Adiponectin attenuates hepatic glucose production through insulin action and increases circulating levels of anti-inflammatory cytokines such as IL-10 as well as reducing the activities of TNF- α . Resistin is synthesised in inflammatory cells and it interferes with glucose uptake, impairs glucose tolerance, and insulin action. Resistin is associated with acute phase reactants and is indicative of the development of CVD in subjects with T2DM (Reilly *et al.*, 2005).

Giannopoulou *et al.*, (2005) investigated the effects of diet and/or exercise on adipocytokines and inflammatory cytokines in postmenopausal women with T2DM. Women were randomly assigned to diet alone (D), exercise alone (EX), or diet plus exercise (D + E) for 14 weeks. Exercise intervention consisted of supervised walking 3/4 d.wk⁻¹ at 65-70% of $\dot{V}O_{2\text{ peak}}$ for 60 min. The diet intervention consisted of a mono-unsaturated diet composed of 40% fat, 40% carbohydrate, and 20% protein and energy intake was determined from resting metabolic rates and daily activity levels. The D + E intervention combined these two interventions and adjusted energy intake on days that subjects exercised. The

adipocytokines, resistin and adiponectin, were not altered by any of the lifestyle treatments. In contrast, leptin levels were reduced in the D and D + E groups but not in the EX group. The inflammatory cytokines, IL-6 and TNF- α , were unchanged following intervention while CRP levels were reduced by 15% in each group with no differences observed between the treatments (Giannopoulou *et al.*, 2005). There were reductions in both visceral and subcutaneous adipose tissue and insulin resistance in both EX and D + E but not in D (Giannopoulou *et al.*, 2005). It was suggested that greater weight loss, longer intervention period, or greater improvements in insulin resistance must occur before changes in certain adipocytokines and cytokines becomes apparent (Giannopoulou *et al.*, 2005).

IL-6 exerts pro-inflammatory effects through increases in lipolysis and fat oxidation in adipose tissue as well as increasing gene transcription in subcutaneous tissue. BMI and percentage body fat are important indicators of plasma concentrations of IL-6 (Mohamed-Ali *et al.*, 1997) and in particular visceral adipose tissue produces greater quantities of IL-6 than subcutaneous adipose tissue with the effect being 4-fold greater among obese individuals in both of these tissues (Bruun *et al.*, 2003).

Aerobic exercise over 12 weeks reduced waist circumference and fasting concentrations of IL-6 in obese men with T2DM, as well as lean and obese non-diabetic men (Dekker *et al.*, 2007). The reductions in fasting IL-6 levels (52% in obese T2DM, 32% lean, and 17% in obese) were attributed to improvements in waist circumference and visceral obesity in all groups. Exercise training had no effect on CRP or insulin sensitivity suggesting that CRP maybe more responsive to changes in insulin sensitivity than adiposity. Others have shown that a longer aerobic training programme conducted over 6 months was effective in lowering P-selectin and intercellular adhesion molecule-1 (ICAM-1) in patients with T2DM (Zoppini *et al.*, 2006). Subjects completed 60 min of aerobic exercise 2 d.wk⁻¹ at 50-70% of heart rate reserve. However, as concentrations of hs-CRP and TNF- α receptors remained unchanged and body composition was similar, it is possible that the frequency or intensity of exercise was insufficient.

In fact, a more intensive short-term diet and exercise intervention was effective in reducing oxidative stress and inflammation in obese men with T2DM (Roberts *et al.*, 2006). Subjects were placed on a high fibre, low fat diet in combination with daily exercise (45-60 min at 70-80% of peak HR) during a 3-wk residential programme. Vascular endothelial cell activation was reduced following intervention as indicated by reduced serum levels of ICAM-1 and E-selectin, intercellular ICAM-1 and vascular adhesion molecule-1 (VCAM-1) expression, CRP, lipid profile, insulin, and FPG (Roberts *et al.*, 2006).

A recent study compared the effects of different exercise modalities on inflammatory markers and adipocytokines in subjects with T2DM over a 12-wk period (Jorge *et al.*, 2011). Subjects were randomly assigned to aerobic (cycling at an intensity equivalent to lactate threshold), resistance (7 upper and lower body exercises), aerobic plus resistance (50% of the volume of aerobic and resistance groups), or control groups for 60 min 3 d.wk⁻¹. There were no changes in adiponectin or resistin while in contrast, visfatin increased in all four groups following intervention (Jorge *et al.*, 2011). A significant reduction was observed in CRP but not TNF- α or IL-6 in each of the four groups (Jorge *et al.*, 2011). Improvements were also found in blood pressure, fasting plasma glucose, and lipid profile in each group (Jorge *et al.*, 2011). The authors suggested that the improvements observed in the control group could be attributed to better compliance with medication and diet (Jorge *et al.*, 2011).

Visfatin is an adipocytokine that binds to the insulin receptor and causes hypoglycaemia by reducing glucose release from liver cells and stimulating glucose utilisation in adipocytes and myocytes. It also mediates the production of TNF- α and IL-6. In a young population (15-30 yr), plasma visfatin levels were assessed before and after 3 months of exercise training in individuals with T2DM and non-diabetic obese controls (Brema *et al.*, 2008). Subjects exercised for 60 min 4 d.wk⁻¹ at 75% of $\dot{V}O_2$ peak. Following intervention, visfatin levels decreased in both individuals with T2DM (55.8 $\pm$ 17.2 to 11.6 $\pm$ 4.4 ng/ml) and obese controls (64.7 $\pm$ 10.7 to 29.5 $\pm$ 7.1 ng/ml) and were lower in individuals with T2DM at 3 months (Brema *et al.*, 2008). The mechanistic basis by which these

improvements occurred are unclear, given that with the exception of a reduction in waist circumference in individuals with T2DM, there were no significant changes in whole body insulin sensitivity, hepatic insulin sensitivity, insulin secretion, or oral glucose insulin sensitivity index (Brema *et al.*, 2008).

Apelin is an endogenous ligand for the G-protein-coupled receptor APJ, which is synthesised and secreted by adipocytes. Increased apelin levels have been found in morbidly obese non-diabetic subjects (Heinonen *et al.*, 2005) and subjects with IGT or T2DM (Li *et al.*, 2006). Diet induced weight loss reduces apelin levels in obese women (Castan-Laurell *et al.*, 2008) but not in subjects with the metabolic syndrome (Heinonen *et al.*, 2009). It would appear that obesity *per se* is not the main determinant of apelin levels. Research indicates that apelin levels are associated with TNF- α (Heinonen *et al.*, 2009) and HOMA-IR (Li *et al.*, 2006). As yet no studies have examined the effect of exercise intervention on apelin levels in subjects with T2DM.

In most studies, exercise intervention appears to exert a positive influence on reducing the effects of inflammation in individuals with T2DM. However, the lack of improvement noted in some cytokines in certain studies suggests that more intense or longer intervention programmes may be required. In addition, greater weight loss and in particular greater loss of visceral fat may be important in relation to adipocytokines.

1.7.6 Exercise prescription

From the research shown it would appear that individuals with T2DM can benefit from regular exercise training $2/3 \text{ d.wk}^{-1}$ for a minimum of 60 min at moderate exercise intensities 65-80% of maximum heart rate or 60-70% of $\dot{V}O_{2 \text{ peak}}$ for aerobic exercise and 70-80% of 1-RM for resistance exercise. Supervised exercise training and education are also key factors in ensuring compliance to training as well as providing motivation (Eriksson & Lindgarde, 1991). For individuals with T2DM, the American College of Sports Medicine (ACSM) recommends that individuals with T2DM should undertake at least 150 min.wk⁻¹ of moderate to vigorous aerobic exercise at least 3 d.wk⁻¹ (Colberg *et al.*, 2010). In addition to aerobic training, it is recommended that moderate to vigorous resistance training is performed at least $2/3 \text{ d.wk}^{-1}$ (Colberg *et al.*, 2010).

1.8 Aims

$\dot{V}O_{2\text{ peak}}$ is reduced in individuals with uncomplicated T2DM, in comparison to non-diabetic individuals of similar age, BMI, and activity level (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999). Previous research indicates that the magnitude of this exercise impairment in $\dot{V}O_{2\text{ peak}}$ can be as great as 20% (Regensteiner *et al.*, 1995; Baldi *et al.*, 2003; Regensteiner *et al.*, 1999; Regensteiner *et al.*, 2009). In addition, the dynamic response of $\dot{V}O_2$ is abnormal in premenopausal women and combined groups of pre and postmenopausal women with T2DM (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Mac Ananey *et al.*, 2011a) but it appears to be similar in older men with T2DM in comparison to non-diabetic controls (Wilkerson *et al.*, 2011). However, to date no studies have investigated $\dot{V}O_2$ kinetic responses in T2DM in postmenopausal women. The mechanisms underpinning this exercise impairment have not been well elucidated but previous studies indicate that central factors affecting cardiac function and peripheral factors affecting oxygen delivery and extraction may be responsible.

Thus, the aim of the first experiment was firstly to investigate the dynamic response of $\dot{V}O_2$ during moderate intensity cycle exercise (80% VT) in postmenopausal women with T2DM. To explore the role of central cardiac function as a possible mechanism in contributing to the impaired $\dot{V}O_2$ kinetic responses observed in individuals with T2DM, CO responses were also assessed at the same exercise intensity. To further explore the dynamic response characteristics of blood flow in postmenopausal women with T2DM, the aim of our second experiment was to investigate the dynamic response of LVC during light (30% MVC) and high intensity (70% MVC) supine plantar flexion exercise as a possible contributor to impaired oxygen delivery in this population.

Regular exercise is an important element in the treatment of T2DM. Current research indicates that a) a combined programme of aerobic and resistance exercise is more effective than aerobic/resistance exercise alone (Cuff *et al.*, 2003) and b) supervised exercise is more effective than home-based training interventions (Dunstan *et al.*, 2005; Dunstan *et al.*, 2006). The research on the

impact of chronic exercise on the physiological adaptations in $\dot{V}O_2$ and LVC kinetics is very limited. In the only published study on T2DM, Brandenburg *et al.*, (1999) showed a significant speeding of $\dot{V}O_2$ kinetics at low and moderate intensities following a 3-month supervised aerobic training intervention in premenopausal women. However, they used the same absolute intensities post-training as at baseline (30, 50, & 80 W). The aim of the third experiment of the current thesis therefore, was to examine the effect of both supervised (3 months) and unsupervised (4 months) exercise on the dynamic response of both $\dot{V}O_2$ and LVC during sub-maximal cycling ($\dot{V}O_2$ kinetics) and plantar flexion (LVC kinetics) exercise. Exercise intensities for both cycle exercise and plantar flexion exercise were recalculated based on new post-exercise $\dot{V}O_{2\text{ peak}}$ (80% VT) and MVC (30% MVC) scores.

The presence of chronic hyperglycaemia induces vascular damage and endothelial dysfunction through increases in oxidative stress and inflammation. Chronic low-grade inflammation is characterised by the presence of increased circulating levels of several inflammatory cytokines and is increased in disease states such as T2DM (Muller *et al.*, 2002; Verma *et al.*, 2003; Giannopoulou *et al.*, 2005; Wood *et al.*, 2005). As yet no study has investigated the effect of exercise on inflammation and markers of endothelial dysfunction in conjunction with $\dot{V}O_2$ and LVC kinetics in individuals with T2DM. The aim of the fourth experiment of the thesis was therefore, to investigate the role of chronic exercise over a 7-month period (3 months supervised followed by 4 months unsupervised) on markers of inflammation and endothelial dysfunction.

Chapter 2: Materials and methods

2.1 Subjects

The subjects included postmenopausal women with and without T2DM. All women were classified as sedentary ($< 1 \text{ h.wk}^{-1}$ of moderate intensity exercise for at least 6 months prior to enrolment), which was confirmed by physical activity scores using the Low Level Physical Activity Recall (LOPAR; Appendix 1) questionnaire (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999).

2.1.1 Subject recruitment

Women with T2DM were recruited from both the Diabetes Unit in St. Colmcille's Hospital, Loughlinstown, Co. Dublin and St Vincent's University Hospital, Dublin. Potential patients were identified by medical chart review in order to ascertain suitability for the study, which included confirmation of age, BMI, medication, blood pressure, and absence of co-morbid conditions. The investigator approached suitable patients and an outline of the study was given to each patient. Confirmation of details (as outlined above in the medical chart) was provided and in addition, patients gave an estimation of their physical activity pattern over the preceding six months. Contact details were obtained from interested patients and these patients were provided with an information sheet outlining the study procedure (Appendix 2). Interested patients were contacted by phone one week after the hospital visit and arrangements were made for a stress test with those willing to participate.

Non-diabetics were recruited from the general population. Poster advertisements were placed on notice boards around Trinity College Dublin and emails outlining the details of the study were sent to large companies in the vicinity of college.

Detailed information outlining the aims and study procedures (see Appendix 2) as well as the potential risks and benefits to be acquired as a result of participating in the study were provided to each subject. Written consent was obtained from each subject prior to participation in the study (see Appendix 3). The study was conducted according to the Declaration of Helsinki and was approved by the Faculty of Health Sciences Research Ethics Committee, Trinity College Dublin.

2.1.2 Subject inclusion and exclusion criteria

Individuals with T2DM were included in this study if: a) they were aged between 30 and 75 years, b) their duration of diabetes was less than 10 years, c) their HbA_{1C} was <10% on therapy, d) they were sedentary ($\leq 1 \text{ h.wk}^{-1}$ of exercise and did not participate in a continuous exercise program for the last 6 months), and e) their diabetes was treated by diet or oral agents (hypoglycaemic and anti-platelets were permitted). In addition, diabetics did not have any clinical evidence of ischemic heart disease (normal electrocardiogram (ECG) during treadmill stress test) or peripheral arterial disease (PAD).

Non-diabetics were included in the study if they were a) sedentary ($\leq 1 \text{ h.wk}^{-1}$ of exercise and did not participate in a continuous exercise program for the last 6 months), and b) had a fasting glucose level $< 7 \text{ mmol.L}^{-1}$.

Women with T2DM were deemed unsuitable for the study if: a) their diabetes condition was treated with insulin or any anti-diabetic drug from the Thiazolidinedione (TZD) family at the beginning of the study (TZD's have been shown to improve vascular function in diabetic populations, b) systolic blood pressure was $>170 \text{ mmHg}$ at rest or $>220 \text{ mmHg}$ with exercise, c) diastolic blood pressure was $>95 \text{ mmHg}$ at rest or $>105 \text{ mmHg}$ with exercise, d) persistent proteinuria (urine protein $>200 \text{ mg.dL}^{-1}$), or e) high creatinine levels ($>2 \text{ mg.dL}^{-1}$) which is indicative of kidney or liver dysfunction respectively,

Non-diabetics were excluded from the study if they were taking medication that could interfere with exercise performance or systemic/peripheral hemodynamic adaptations (β -blockers). Additional grounds for exclusion included the presence of cardiovascular disease such as PAD and CHD.

2.2 Medical assessments

All diabetic subjects underwent a full medical examination (medical history, physical examination, and stress test) carried out by a qualified medical practitioner in St. Colmcille's Hospital, Loughlinstown, Co. Dublin, not longer than one month prior to enrolment in the study. The presence/absence of

ischemic heart disease was established during a stress test (modified Bruce protocol) using a 12-lead ECG where heart rate and blood pressure were monitored continuously throughout the test. The absence of PAD was determined by the absence/presence of claudication/pain in the extremities during the stress test and by measurements of ankle brachial index (ABI) ($0.9 < \text{ABI} < 1.3$). On a separate day venous blood samples were taken from each subject in the hospital.

All non-diabetic subjects underwent a full medical examination by a qualified medical practitioner in the Cardiovascular Laboratory, Department of Physiology, Trinity College Dublin, to determine suitability for the study. Absence of cardiovascular disease and/or other medical conditions were confirmed by medical questionnaire (see Appendix 4) and physical examination. Fasting venous blood samples were taken from each subject on the same day.

2.3 Physical characteristics, activity levels, and haematology

2.3.1 Body mass

Body mass was measured using a platform beam scales (AVERY, England).

2.3.2 Height

Height was recorded using a SecaTM Stationmeter (Seca Ltd., Germany).

2.3.3 Body mass index (BMI)

Body mass index was calculated by dividing the weight (kg) by the height (m) squared ($\text{BMI} = \text{kg} \cdot \text{m}^{-2}$).

2.3.4 Waist to hip ratio (WHR)

The waist measurement at the level of the umbilicus was determined using a measuring tape. The hip measurement at the level of the greater trochanter was measured using a measuring tape. The WHR was calculated by dividing the waist measurement by the measurement at the hip: $\text{WHR} = \text{waist (cm)} / \text{hip (cm)}$.

2.3.5 Ankle brachial index (ABI)

Ankle brachial index was measured using an MD6 Bidirectional Doppler (Hokanson, USA), sphygmomanometer (Hokanson, USA), and a blood pressure

cuff (Hokanson, USA). Measurements were taken from the brachial, posterior tibialis, and dorsalis pedis arteries while the subject was placed in a supine position.

Brachial pressure: A blood pressure cuff (Hokanson, USA) was placed 2.5 cm above the antecubital fossa with the arm positioned at the level of the heart. Conducting gel was placed on the doppler and the doppler placed at a 45° angle towards the subject's head until a pulse was heard. The blood pressure cuff was then inflated using a sphygmomanometer (Hokanson, USA) to 20-30 mmHg above the point where the pulse could not be heard. The cuff was slowly deflated at a rate of 2-3 mmHg per second to the point at which the first audible pulse was heard. This was recorded as systolic blood pressure. The same procedure was repeated on the other arm with the higher of the two readings used to calculate ABI.

Ankle pressure: The blood pressure cuff was placed 2.5 cm above the medial malleolus. Conducting gel was placed on the doppler and the doppler was placed at a 45° angle towards the subject's knee until a pulse was heard. The blood pressure cuff was then inflated to 20-30 mmHg above the point where the pulse could not be heard. The cuff was slowly deflated at a rate of 2-3 mmHg per second to the point at which the first audible pulse was heard. This was recorded as systolic blood pressure. The pressure in both the dorsalis pedis and posterior tibialis arteries were taken in both legs and the higher of the two readings used to calculate ABI.

Formula: ABI is calculated by dividing the higher of the dorsalis pedis or posterior tibial systolic pressures for each ankle by the higher of the brachial systolic pressures:

$$\text{ABI} = \text{highest ankle pressure (mmHg)} / \text{highest brachial arm pressure (mmHg)}$$

2.3.6 Blood pressure

Blood pressure was measured during bouts 5 and 6 at rest and during exercise (30 s and 240 s) using a sphygmomanometer (Hokanson, USA) and stethoscope (3MTM Littmann, UK). A blood pressure cuff (Hokanson, USA) placed around

the upper right arm was inflated using a sphygmomanometer to 20-30 mmHg above the point where the pulse could not be heard. The cuff was slowly deflated at a rate of 2-3 mmHg per second until such time as the first sound was heard (systolic blood pressure). The pressure was further released until no sound could be heard (diastolic blood pressure).

Mean arterial pressure (MAP) was calculated as follows:

$$1/3 \text{ systolic blood pressure} + 2/3 \text{ diastolic blood pressure}$$

Total peripheral resistance (TPR) was calculated by dividing MAP by CO as shown:

$$\text{TPR} = \text{MAP}/\text{CO}$$

2.3.7 *Lactate*

Capillary blood samples were taken from a subgroup of subjects (n = 17) at rest prior to each bout of unloaded cycling for the estimation of blood lactate (YSI 1500 SPORT, Yellow Springs, USA) in order to confirm that the 12-min resting periods were sufficient for blood lactate levels to return to baseline levels (Appendix 5). A pinch was made on the subject's right middle finger using a blood lancet (Braun, Germany) and blood was squeezed from the finger into a capillary tube (Marienfeld, Germany). The blood was taken from the capillary tube using a syringepet (YSI 1500 SPORT, Yellow Springs, USA) and transferred to the lactate analyser for the estimation of blood lactate (mmol.L⁻¹).

2.3.8 *Leg volume*

Leg volume of the exercising leg was calculated using a regression equation for the prediction of volume and masses of tissue components (Clarys & Marfell-Jones, 1986):

$$(\text{tibial maleolar length} \times [38.20851 + \text{maximal calf girth} - (\pi \times (\text{medial calf skinfold}/10))] \times 80.24423 - 2467.9)$$

Both tibial-maleolar length (from the tibial plateau to the centre of the medial malleolus) and maximal calf girth (widest point of the calf in a relaxed state) were measured using a measuring tape. A skinfold caliper (Harpenden, West Sussex, UK) was used to determine medial calf skinfold (measured at the maximum circumference of the calf on the midline of its medial border). Bi-malleolar breadth was measured as the horizontal distance between the medial and lateral malleolus using a vernier caliper (Dual scale vernier calliper, Mitutoya).

2.3.9 Haematology

All venous blood samples were drawn from the antecubital vein of the arm into 6 ml vacutainers. A full blood count was determined for each subject from collection of whole blood samples, which were analysed using a haematological particle analyser (Coulter AcT diff; Coulter Ltd, UK) at the Department of Physiology, Trinity College Dublin. Fasting glucose, HbA_{1c}, and lipid profile for each subject were analysed at the Haematological Laboratory, St Colmcille's Hospital, Loughlinstown, Co Dublin. Whole blood samples were used to measure fasting plasma glucose (Olympus AU640, USA) and HbA_{1c} (G7 HLC-723, Tosoh Bioscience, Belgium). Fasting blood serum samples were used to measure serum lipid profile (Olympus AU640, USA).

2.3.10 Physical activity

The Low Level Physical Activity Recall questionnaire (LOPAR) was used to determine physical activity levels. Each subject had to recall their daily activity for the previous seven days, which were then divided into specific categories (work, home, and leisure). The amount of energy expenditure was then quantified in terms of metabolic equivalents (MET) for each category. Participants were asked about the number of hours spent performing very light (1.0-1.5 METs), light (2-3 METs), moderate (3-5 METs), heavy (5-7 METs), and very heavy (7+ METs) activities. Energy expenditure in MET h.wk⁻¹ was calculated for each subject by multiplying the amount of time spent performing an activity by the MET value of that activity (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999) (Appendix 1).

In addition, a subgroup of participants (T2DM n=18; non-diabetic n=19) was required to wear RT3 triaxial accelerometers for 5 days to determine energy

expenditure (Rowlands *et al.*, 2004; Perry *et al.*, 2010). The device, which was worn on the waist during all waking hours, detects acceleration in three orthogonal axes (vertical, mediolateral, and anterior-posterior) and combines these to provide a triaxial vector magnitude count. The vector magnitude count was given a specified time interval of one minute. The accelerometers were returned to the laboratory after 5 days and the data downloaded to a computer using an RT3 docking station (StayHealthy, Inc; Monrovia, California) and RT3 Stayhealthy software (StayHealthy, Inc; Monrovia, California). Previously defined cut points were used to define minutes of physical activity into categories: inactive (0 – 100 counts.min⁻¹), light (101 – 970 counts.min⁻¹), moderate (971 – 2333 counts.min⁻¹), and vigorous (2334 + counts.min⁻¹). The physical activity patterns for the 5 days were averaged giving a mean output for one day. The total time spent in each category was expressed in h.day⁻¹.

2.4 Experimental design

The thesis is divided into four experimental chapters. In experiment 1, chapter 3, $\dot{V}O_2$ kinetics and cardiac responses were assessed during constant load cycle exercise at 80% VT. In the second experiment (chapter 4), LVC kinetic responses were determined during constant load plantar flexion exercise at 30% and 70% MVC. In addition, peak responses during incremental plantar flexion exercise were also determined. Reactive hyperaemia of the forearm was performed to assess endothelial function. In experiment 3 (chapter 5), the effect of supervised (12 wk) followed by unsupervised (16 wk) exercise intervention on $\dot{V}O_2$ and LVC kinetic responses was determined. Experiment 4 (chapter 6) looked at the effect of exercise intervention (supervised (12 wk) followed by unsupervised (16 wk)) on markers of inflammation and endothelial dysfunction.

In both experiment 1 and 2, results are presented in two sections: postmenopausal women not matched by BMI and BMI matched postmenopausal women. The matching refers to BMI and not the number of subjects.

2.4.1 Experiment 1: Cycling performance

Each subject was required to visit the Cardiovascular Laboratory in the Department of Physiology, Trinity College Dublin, on two occasions separated by at least 72 h. Subjects were required to refrain from participating in strenuous physical activity 24 h prior to testing. On the first occasion subjects completed an incremental cycle test to volitional exhaustion for the determination of peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$) and ventilatory threshold (VT). During Visit 2, each subject completed 6 bouts of 6 min cycling at 80% VT. Each 6-min bout was preceded by 3-min of semi-unloaded cycling at 10 W and each bout was separated by a 12-min rest period. Bouts 1-4 were used for the determination of $\dot{V}O_2$ kinetics and bouts 5-6 were used to determine CO. CO measurements were made at rest and during exercise (30 s and 240 s) at 80% VT.

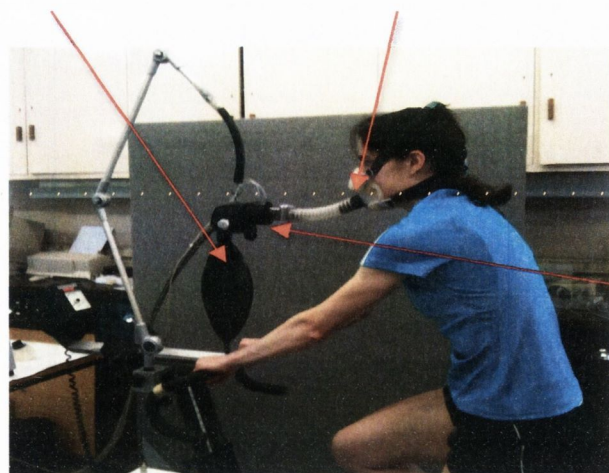
2.4.1.1 Visit 1: Incremental graded cycle exercise test

An incremental cycle test was performed on an electro-magnetically braked cycle ergometer (Excalibur Sport; LODE Ltd, Netherlands) with saddle height, handlebar height, and 'reach' measured and recorded for subsequent visits (Fig. 2.1). Subjects were fitted with a nose clip and a silicone facemask (Hans Rudolph, USA 7900). A pulse oximeter (Innocor, Innovision, Denmark) was fitted to the middle finger. Heart rate was recorded every 5 s using a polar heart rate transmitter (S610i, Polar Electro Oy, Finland). Pulmonary ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) were determined breath-by-breath (BbB) (Innocor, Innovision, Denmark).

The test commenced with a 3-min rest period after which subjects began cycling at 40 W (pedal cadence was maintained between 60-65 rev.min⁻¹). The workload was increased by 20 W every 3 min until volitional exhaustion. This was defined as the inability to maintain the required power output for more than 3 s. The peak workload achieved was defined as the highest workload that a subject could sustain for a minimum period of 1 min. $\dot{V}O_{2\text{ peak}}$ was defined as the highest 30-s average of the BbB data. VT was established using the V-slope method (Beaver *et al.*, 1986).

3-l rebreathing bag

Mouthpiece



3-way respiratory valve

Fig. 2.1. Subject performing cycle exercise

2.4.1.2 Visit 2: Constant load cycling exercise at 80% VT

Subjects completed four square-wave transitions of 6 min cycling from 3 min unloaded cycling (10 W) at a power output equivalent to 80% VT, which was established during the graded cycle test on Visit 1. Each exercise bout was separated by a 12-min recovery period where subjects were allowed to dismount the cycle ergometer and sit in a nearby chair. Heart rate was continuously monitored during recovery periods, thereby ensuring heart rates returned to resting levels prior to commencing the next bout of exercise. A pilot study carried out on a group of subjects suggested that a 12-min recovery period was sufficient for blood lactate levels to return to baseline concentrations (Appendix 5). The resting periods applied between bouts have previously been shown to be sufficient for heart rate and blood lactate levels to return to resting levels (Barstow *et al.*, 1996; Wilkerson *et al.*, 2005; Burnley *et al.*, 2006; Koppo *et al.*, 2009). During exercise BbB data and heart rate (5-s basis) were recorded continuously.

Two additional bouts of 6-min cycling at 80% VT were completed for the estimation of CO. Each bout was again preceded by 3-min semi-unloaded cycling at 10 W and a 12-min recovery period separated each bout. BbB data and heart rate were recorded continuously throughout exercise. CO was measured (inert gas rebreathing technique; Innocor, Innovision, Denmark) at rest prior to unloaded cycling and during exercise (30 s and 240 s) at 80% VT. Subjects were

familiarised with the rebreathing protocol by completing a number of practice measurements using the CO rebreath apparatus. In addition, blood pressure was measured at rest and during exercise (30 s and 240 s) using a sphygmomanometer (Hokanson, USA) and stethoscope (3M™ Littmann, UK).

Arteriovenous O₂ difference (a-vO₂ diff) was calculated by rearranging the Fick equation as follows:

$$\text{a-vO}_2 \text{ diff (ml.100ml}^{-1} \text{ blood)} = \dot{\text{V}}\text{O}_2 \text{ (L.min}^{-1}\text{)}/\text{CO (L.min}^{-1}\text{)} \times 100$$

The rate of increase of CO, CI, and a-vO₂ diff (% steady state) were estimated as follows:

$$(\text{CO at 30 s} - \text{CO at rest})/(\text{CO at 240 s} - \text{CO at rest}) \times 100$$

$$(\text{CI at 30 s} - \text{CI at rest})/(\text{CI at 240 s} - \text{CI at rest}) \times 100$$

$$(\text{a-vO}_2 \text{ diff at 30 s} - \text{a-vO}_2 \text{ diff at rest})/(\text{a-vO}_2 \text{ diff at 240 s} - \text{a-vO}_2 \text{ diff at rest}) \times 100$$

2.4.2 Experiment 2: Calf performance

Testing was carried out in the Cardiovascular Laboratory in the Department of Physiology, Trinity College Dublin. Subjects were required to refrain from participating in strenuous physical activity 24 h prior to testing. Initially, subjects were familiarised with the custom-built calf ergometer before completing 5 maximum voluntary contractions (MVC) of the calf muscle. Subjects then performed three bouts (6 min each) of low intensity (30% MVC) plantar flexion exercise. This was then followed by a plantar flexion incremental test to failure. Force output, leg blood flow (LBF), blood pressure, and heart rate were recorded during each protocol. Following the incremental test, a reactive hyperaemic test was performed on the forearm of the right arm to determine endothelial function. In addition, a subgroup of 12 subjects (T2DM n=7; non-diabetic n=5) completed a further two bouts of high intensity (70% MVC) plantar flexion exercise. All procedures were carried out in the supine position.

2.4.2.1 Subject familiarisation procedure with the calf ergometer

The familiarisation period was used to familiarise subjects with the custom-built calf ergometer, calf exercise and experimental protocol. Initially subjects completed a series of low intensity plantar flexions of the right calf muscle in the supine position while maintaining a contraction/relaxation ratio of 2 s/4 s. Subjects were encouraged to give a 'square wave' force response. This response was characterised by a rapid increase of the force output to the target force, stabilisation of the force output during contraction, and a rapid decrease in force output at the beginning of the relaxation phase.

2.4.2.2 Maximum voluntary contraction (MVC)

The MVC of each subject was determined by instructing the subjects to exert the greatest amount of possible force on the force-plate while maximally plantar flexing the right foot. Subjects performed 5/6 brief (3 s) maximum contractions with 60-s rest in between. The greatest force produced was recorded as the MVC and was used to calculate the intensity used during the constant load tests (i.e. 30% and 70% MVC). Feedback and encouragement were provided by the investigator.

2.4.2.3 Constant load plantar flexion exercise

Subjects completed 3 x 6 min bouts of plantar flexion exercise at 30% MVC and a subgroup (n = 12) completed an additional 2 x 6 min bouts at 70% MVC. A 10-min (for 30% MVC bouts) or 15-min (for 70% MVC bouts) recovery period separated each bout. Isometric contractions were performed intermittently such that the calf muscle contracted for 2 s and relaxed for 4 s. This duty cycle (2 s/4 s) has been previously shown to replicate the ratio of the period of activation of the calf muscle during walking and provides a period of relaxation long enough to measure blood flow (Egana & Green, 2005). A metronome was used to maintain a contraction/relaxation ratio of 2 s/4 s for the exercise period. A total of 60 data points were obtained for each exercise bout.

Leg vascular conductance (LVC) during exercise was calculated from simultaneous measurements of leg blood flow (LBF) and MAP ($LVC = LBF/MAP$) measured during the 4-s relaxation phase of the 6-s duty cycle. Resting LVC was

calculated from simultaneous measurements of resting LBF and MAP measured prior to each exercise bout.

2.4.2.4 Calf incremental test to failure

Subjects completed an incremental test to failure on the calf ergometer in the horizontal position. The initial force was set at 100 N and the force was increased by 150 N every 2 min until the subject failed to maintain the required force during two successive contractions. The peak force achieved was defined as the highest force that a subject could sustain for a minimum period of 60 s. As for the constant load tests, a metronome was used to maintain a contraction/relaxation ratio of 2 s to 4 s for the exercise period.

LVC (LBF/MAP) during the incremental test was again calculated during the 4-s relaxation phase of the 6-s duty cycle, and resting LVC was measured prior to the incremental test.

2.4.2.5 Reactive hyperaemia

Forearm resting vascular conductance responses were determined every 10 s over a 1-min period from the right forearm in the supine position. An occlusion period of 5 min was then applied, after which forearm vascular conductance hyperaemic responses were measured over a subsequent 10-min period. Following the occlusion, forearm vascular conductance responses were determined every 10 s for the first 5 min and then once every 60 s for the 5 min thereafter. The resting and hyperaemic forearm vascular conductance responses were determined from simultaneous measurements of forearm blood flow and MAP.

2.4.3 Experiment 3: Intervention study

2.4.3.1 Phases of the experiment and overview of the laboratory visits

Both exercise and control groups were required to visit the Cardiovascular Laboratory in Trinity College Dublin on 2 occasions separated by at least 72 h at 3 different time points: baseline (BS), post-supervised exercise intervention (SE), and post-unsupervised exercise intervention (UE) over a 7-month period (Table 2.1). Subjects were required to refrain from participating in strenuous physical activity 24 h prior to testing. Between BS and SE, both exercise groups

completed a 12-wk supervised exercise programme in Monkstown Pool and Fitness Centre, Monkstown, Co. Dublin while control groups continued with their normal life. Following the supervised exercise intervention, both exercise groups were required to complete a further 16 weeks of unsupervised exercise while again control groups continued with their normal life.

During Visit 1, subjects underwent a familiarisation period with the custom-built calf ergometer before completing 5 MVC's of the calf muscle. In addition, LVC was determined during constant load plantar flexion exercise where subjects completed 3 bouts of 6 min duration at 30% MVC. This was followed by a calf plantar flexion incremental test to failure. Forearm vascular conductance was determined at rest and during a reactive hyperaemia response following a 5-min occlusion period. All these procedures were carried out in the supine position. Following the calf plantar flexion exercises during Visit 1, subjects also underwent an incremental cycle test to volitional exhaustion for the determination of $\dot{V}O_{2\text{ peak}}$ and VT.

During Visit 2, each subject completed 6 bouts of 6 min cycling at 80% VT. Each 6-min bout was preceded by 3 min semi-unloaded cycling at 10 W and each bout was separated by a 12-min rest period. Bouts 1-4 were used for the determination of $\dot{V}O_2$ kinetics and bouts 5-6 were used to determine CO. CO measurements were made at rest and during exercise (30 s and 240 s) while cycling at 80% VT.

At each of the 3 phases of the intervention (BS, SE, and UE) subjects were required to provide fasting venous blood samples.

Table 2.1. Experimental phases

	T2DM Exercise (n=8)	Non-diabetic Exercise (n=10)	T2DM Control (n=8)	Non-diabetic Control (n=9)
Week 1-4	Baseline (BS) Initial screening and recruitment Stress test (12-lead ECG)/medical Blood sampling Assessments of activity levels: LOPAR Functional assessment (Trinity College Dublin): Visit 1: • Anthropometrical measurements • Calf MVC • Constant load plantar flexion exercise at 30% MVC (3 bouts x 6 min) • Plantar flexion incremental test • Forearm blood flow (reactive hyperaemia) • Incremental cycle test Visit 2: • Constant load cycle test at 80% VT: 6 bouts x 6 min • Bouts 1-4: $\dot{V}O_2$ kinetics, bouts 5 & 6: CO			
	Week 5-16	Supervised Exercise (SE) 12 -wk supervised exercise training		Normal life
Week 17-18	Functional and blood assessment repeated (Workshop on exercise prescription for unsupervised exercise)			
Week 19-34	Unsupervised Exercise (UE) 16-wk unsupervised exercise training		Normal life	
Week 35-36	Functional and blood assessment repeated			

2.4.3.2 Subject attrition

A schematic representation of subject attrition rates is presented in Fig. 2.2. To begin with, 132 individuals with T2DM were identified as suitable candidates by chart review and approached by the investigator. From the initial number of 132, 27 expressed an interest in taking part and later completed a stress test in St. Colmcille's Hospital, Loughlinstown, Co. Dublin. This group of 27 also completed baseline testing. A total of 38 non-diabetic women completed a medical and 33 of them completed the initial baseline testing. Five non-diabetics dropped out after completing the medical citing lack of time as a reason for

dropping out. During the supervised exercise (between BS and SE), 9 diabetics and 7 non-diabetics dropped out, citing lack of time and family commitments as reasons for dropping out. During the unsupervised phase (between SE and UE), a further 2 diabetics and 7 non-diabetics dropped out citing again family commitments, lack of time, and injury as reasons for dropping out. Only subjects who completed both exercise phases and subsequent laboratory assessments were included for statistical purposes.

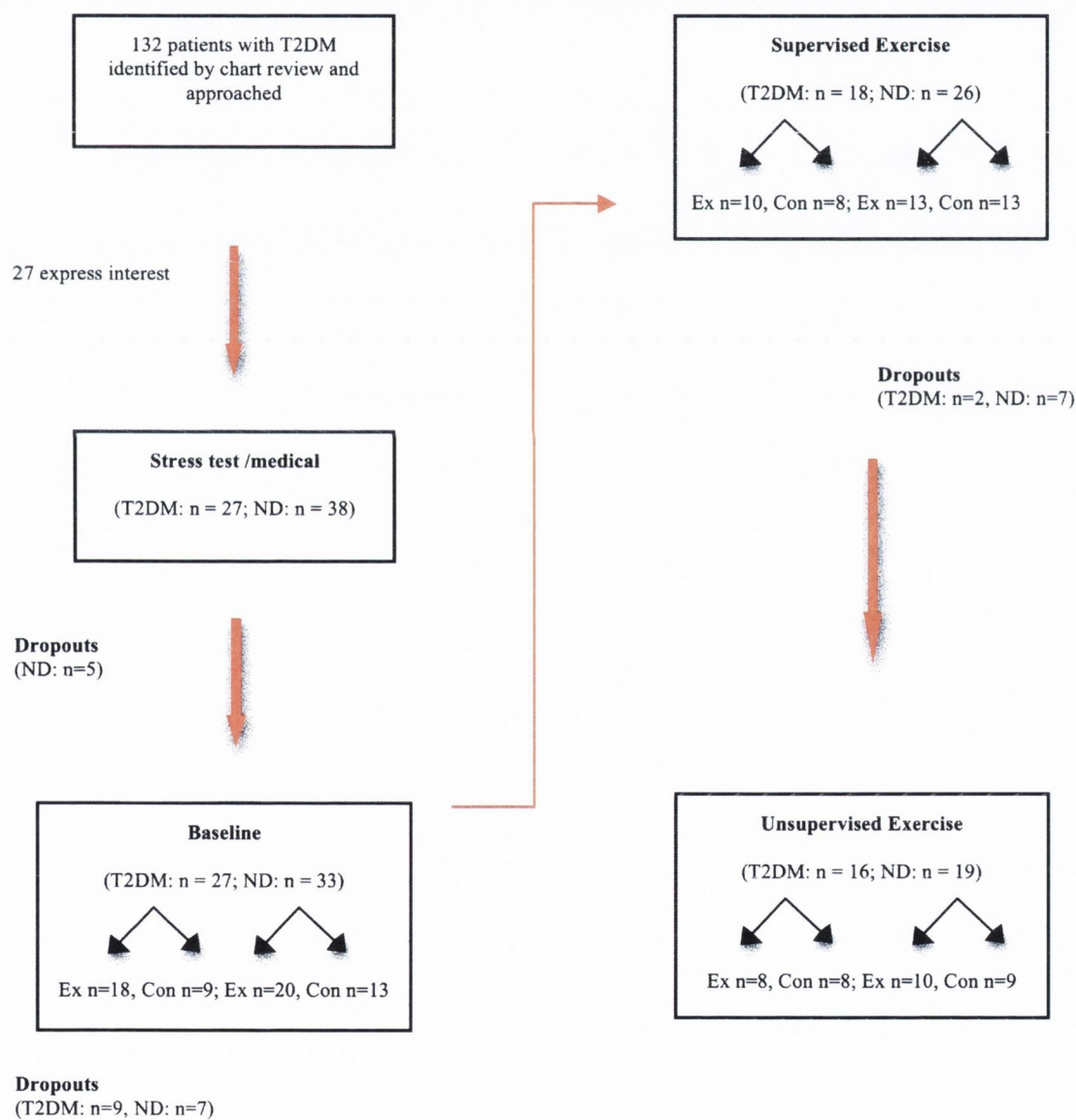


Fig. 2.2. Subject attrition rates over the course of the study

2.4.3.3 Visit 1

Anthropometrical measurements were taken from each subject prior to exercise on the custom-built calf ergometer. Following a familiarisation period, subjects completed a series of MVC's for the determination of workload during the constant load plantar flexion tests (30% MVC). Subjects then completed a graded calf plantar flexion test for the determination of peak workload. Following a 20-min passive rest period, endothelial function was assessed during reactive hyperaemia of the forearm. Finally, visit 1 was completed with a graded cycle test to volitional exhaustion for the determination of $\dot{V}O_{2\text{ peak}}$ and VT.

2.4.3.4 Visit 2: overview

During Visit 2 each subject completed 6 bouts of 6 min cycling at 80% VT. Each 6-min bout was preceded by 3-min semi-unloaded cycling at 10 W and each bout was separated by a 12-min rest period. Bouts 1-4 were used for the determination of $\dot{V}O_2$ kinetics and bouts 5-6 were used to determine CO. CO measurements were made at rest and during exercise (30 s and 240 s) at 80% VT.

2.4.3.5 Exercise interventions

(i) Overview

The intervention consisted of 2 phases, a 12-wk supervised exercise (SE) intervention phase followed by a 16-wk unsupervised exercise (UE) intervention phase. For the SE phase, both exercise groups (T2DM and non-diabetics) were required to visit Monkstown Pool and Fitness Centre, Monkstown, Co Dublin 3 d.wk⁻¹ for between 1-1.5 h over a 12-wk period. Compliance was set at a minimum of 32 sessions out of a possible 36. Supervised exercise sessions were conducted Mondays, Wednesdays, and Fridays from 5pm to 7.30pm, thereby allowing subjects some flexibility in scheduling their exercise sessions. When subjects could not make these sessions, arrangements were made to conduct supervised morning sessions from 8 am to 9.30 am on non-consecutive days. The exercise intervention consisted of cardiovascular and resistance exercises. All exercise sessions were supervised by the investigator or a qualified research assistant to ensure correct technique and appropriate work intensities in addition to monitoring attendance. Following the SE phase, subjects were required to

maintain the same frequency, intensity, and duration during the UE phase. Subjects were offered a free membership for the 16-wk period if they wanted to continue to use the facilities in Monkstown Pool and Fitness Centre but they were allowed to train at home, in their local vicinities, or make arrangements to attend a gym of their choice.

(ii) Supervised exercise (SE)

Subjects were equipped with a heart rate monitor (Cardiosport, USA), which was compatible with the cardiovascular equipment in the fitness centre. This enabled the subject and investigator to monitor training intensities using heart rate zones previously determined from the graded cycle test (60-85% peak heart rate). In addition, blood pressure measurements were taken periodically using an Omron M7 blood pressure monitor (Intelli TM Sense).

Subjects followed a prescribed workout consisting aerobic, resistance, and core strengthening exercises performed in the following order:

Component 1: cardiovascular 1

The first cardiovascular component consisted (in the first month) of 20 min cycling exercise at 70% of peak heart rate (peak HR) that included a warm up period of 5 min (60% peak HR). In the second and third months, subjects cycled for 25 min at 75 and 80% of peak HR respectively. Following the initial aerobic component, subjects performed a series of static stretching exercises that included the major muscle groups: calves, quadriceps, hamstrings, latissimus dorsi, erector spinae, biceps, triceps, and pectorals.

Component 2: core strengthening

The second component consisted of core strengthening exercises that included: calf-raises, sit-ups, v-sits, squats, plank, and back extensions. Subjects initially (month 1) performed 20 repetitions of each exercise, increasing to 30 repetitions in months 2 and 3.

Component 3: resistance

The resistance component consisted of a series of 4 upper body (bench press, row, shoulder press, and lat pull down) and 3 lower body (leg extension, leg curl, and leg press) exercises performed on compound exercise machines. The 1-repetition maximum (1-RM) for each subject was assessed at wk 0 and reassessed every 4 weeks with the training load adjusted accordingly. Subjects performed 2 sets of 10 repetitions at 60% of 1-RM (month 1) that was later increased to 70% of 1-RM for months 2 and 3. Rest periods of 2-3 min were included between each set. For the first month, subjects performed upper exercises on day one, lower body exercises on day 2, and completed both upper and lower body exercises on day 3. During months 2 and 3, subjects completed both upper and lower body exercises 3 d.wk⁻¹.

Component 4: cardiovascular 2

The second cardiovascular component consisted of 15 min exercise for the first month, increasing to 20 and 25 min for months 2 and 3 respectively, with a cool down period of 5 min at the end of each session. The exercise intensity was initially set at 70% peak HR for month 1. In month 2, the second cardiovascular session was adjusted to include 3 sets of 90-s intervals performed at 75%, 80%, and 85% of peak HR. In month 3, subjects performed 3 sets of 2-min intervals at the same exercise intensities. Aerobic exercise could be performed on a machine of the subject's choice (bike, treadmill, rowing, cross trainer, and stair masters).

During the SE phase, subjects were being continuously educated about their exercise programme (frequency, duration, and intensity). In addition, subjects learned to rate the intensity of aerobic exercise using rating of perceived exertion and associate that intensity with specific heart rate zones as displayed on the heart rate monitor. The purpose of this education component was to enable subjects to take charge of their own training in the unsupervised exercise phase.

(iii) Unsupervised exercise (UE)

Following completion of the SE phase, subjects returned to the Cardiovascular Laboratory in Trinity College Dublin to repeat the baseline measurements. During the course of these visits, the investigator designed an exercise programme

for the UE phase based on each individual's interests and time constraints. Subjects were encouraged to select activities that best replicated the intensity and duration of exercise performed during the SE phase. They were provided with free access to the facilities in Monkstown Fitness Centre for the duration of this phase. In addition, they were allowed to train in their own homes, local parks, or nearby leisure centres. Regular phone contact (once per month) was maintained with each subject and feedback/advice regarding exercise prescription was provided when needed.

2.4.4 Experiment 4: Inflammatory and endothelial markers

2.4.4.1 Blood samples

Venous blood samples were drawn from the antecubital vein in arm into 6 ml lithium heparin vacutainers (BD, Oxford, UK) for the determination of plasma levels of IL-6, IL-10, TNF- α , CRP, SAA, sICAM-1, and sVCAM-1. The vacutainers were centrifuged at 5,000 rpm for 10 min at room temperature and the plasma extracted and stored at -80°C for later use. In addition, venous blood samples were drawn from the antecubital vein in arm into 2.5 ml PAXgene Blood RNA tubes (PreAnalytix, Qiagen, UK) for the determination of mRNA levels of IL-6, TNF- α , resistin, and visfatin. The blood RNA tubes were stored at -80°C until RNA extraction was performed.

Blood samples were collected from women with and without T2DM at BS. The exercise groups completed 12 wk of supervised exercise intervention; this was then followed by 16 wk of unsupervised exercise intervention. The control groups continued with their normal lifestyle during this period. Blood samples were again taken from all subjects at SE and at UE.

2.5 Equipment used

2.5.1 $\dot{V}O_2$ kinetics and CO

2.5.1.1 Cycle ergometer

All cycle exercise protocols were performed on an electrically braked isokinetic cycle ergometer (Excalibur Sport; LODE Ltd, Netherlands) at a cadence between 60-65 rev.min⁻¹.

2.5.1.2 Cardiac output and metabolic gas analyser

Metabolic and CO measurements were carried out using a closed circuit inert gas rebreathing device (Innocor, Innovision, Denmark). Breath-by-breath expired gas allowed the determination of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and respiratory exchange ratio (RER) from simultaneous measurements of respiratory flow and expired O₂ and CO₂ concentrations by photoacoustic gas analysis. The system consisted of a three-way respiratory valve with a mouthpiece and a 3-litre rebreathing bag connected to an infrared photoacoustic gas analyser (Innocor; Innovision, Denmark). CO was measured by rebreathing in a closed system containing a gas mixture of 0.5% nitrous oxide (N₂O blood-soluble) and 0.1% sulphur hexafluoride (SF₆ blood-insoluble) diluted with atmospheric air. During inspiration the concentration of N₂O decreases at a rate that is proportional to pulmonary blood flow. Greater pulmonary blood flow facilitates a greater diffusion of N₂O from the lungs to the circulation. CO can then be determined from estimated measurements of pulmonary blood flow, $\dot{V}O_2$, and arteriovenous saturation as shown:

$$\text{Pulmonary blood flow} = -\beta \frac{Vs(tot)(C1 + C2)}{\alpha b}$$

where $Vs(tot)$ is total systemic volume (STPD), $C1$ is 760/(ambient pressure in mmHg – 47), $C2$ is the diffusion constant of N₂O, and αb is the Bunsen solubility coefficient of N₂O in the blood.

Rebreathing was performed over 5-8 breaths while subjects maintained a constant breathing frequency by breathing in synchrony with a graphical tachymeter on the Innocor screen, this was typically at a rate of 20 breaths per minute. To maintain a constant breathing volume subjects had to completely empty the rebreathing bag at the end of each inspiration. The last 2/3 breaths were used for the calculation

of pulmonary blood flow and cardiac output. Approximately 3-5 respiratory cycles were needed to obtain N₂O washout. The rate of disappearance of the N₂O from the alveolar space was proportional to the flow of blood perfusing the ventilated parts of the lungs. This is equal to cardiac output in the absence of a significant intrapulmonary shunt. From each rebreath performed various measurements were taken including; CO, heart rate (HR), stroke volume (SV), a-vO₂ diff, and oxygen consumption ($\dot{V}O_2$).

The system was calibrated on a daily basis. As part of the calibration procedure ambient data (room temperature, relative humidity, and atmospheric pressure) were inputted to the device. The oxygen sensor was calibrated using ambient room air. A 3-litre calibration syringe (Hans Rudolph Ltd., USA) was used to calibrate the flow-metre. The gas delay was calibrated by rapidly inspiring and slowly expiring over 10 repetitions.

Cardiac index (CI) and stroke volume index (SVI) were calculated by dividing both CO and SV by body surface area (BSA) as shown:

$$CI (L.min^{-1}.m^{-2}) = CO (L.min^{-1})/BSA (m^2)$$

$$SVI (ml.m^{-2}) = SV (ml)/BSA (m^2)$$

2.5.2 LVC kinetics and peak calf performance

2.5.2.1 Calf ergometer

Single leg plantar flexion exercise (right foot) was performed on a custom-built calf ergometer while subjects were in a supine and fully extended position (Fig. 2.3). The ergometer was fitted with an immobile force-plate connected to a load cell and against which force could be applied during plantar flexion of the calf muscle (within a restricted range of ~10° or less). The right foot was strapped into a leather boot, which was attached to the force-plate while the left foot was immobilised on a padded platform. In order to prevent backward displacement and postural changes during exercise, subjects were fitted with a body harness, which was secured to the tilt table. An adjustable saddle, which was fitted to the table, was positioned so as to prevent knee flexion during exercise and thereby preventing additional muscles from acting on the force-plate. The saddle height

was recorded for each subject. The force detected by the strain gauge was amplified (RS Stock no. 846-147) and then sampled at 40 Hz before being processed by a PowerLab analog-digital converter (ML 795, AD Instruments). The forces exerted on the force-plate could be seen as traces on a visual monitor. Subjects used this visual display to control their muscular effort and to conform to specific instructions given by the investigator.

The calf ergometer was calibrated manually at the beginning of each testing day in the upright position. An unloaded force-plate was set to 0 V and a series of weights (range: 0-80 kg) were placed in ascending order of mass on the force-plate. The corresponding voltage produced by each weight was recorded from the computer trace. Each weight was removed in reverse order and the corresponding voltage was again recorded from the computer trace. The forces in V were then converted to mV. Linear relationships between weight (g) and force (mV) (both ascending and descending forces) were constructed in a spreadsheet (Excel, Windows 2000). The mV at 20 kg and 80 kg were used to convert mV readings in PowerLab (ML 795, AD Instruments) to newton's (N). The mean values for at 20 kg and 80 kg (both ascending and descending) can be seen in Appendix 6.

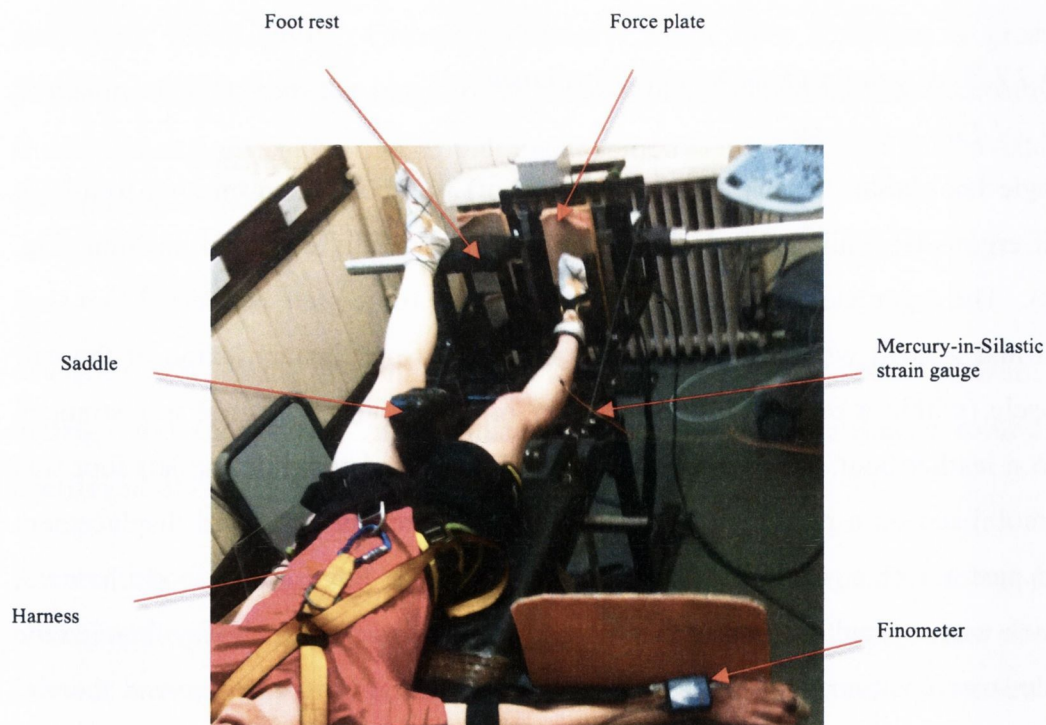


Fig. 2.3. Subject performing plantar flexion exercise on the calf ergometer

2.5.2.2 Leg blood flow at rest and during exercise

Blood flow was measured non-invasively using venous occlusion plethysmography (VOP) while subjects were in a supine position. Changes in limb volume were measured using a mercury-in-Silastic strain gauge (Hokanson, USA), placed around the widest circumference of the calf muscle, and a plethysmograph (Hokanson EC-6, USA). The strain gauge was connected to a plethysmograph and the signal from the plethysmograph transmitted through a PowerLab analog-digital converter (ML 795, AD Instruments) to a computer with Chart software (LabChart 5, AD Instruments, Australia) for Windows. A cuff (Hokanson, USA) was placed around the right thigh and fixed in position using velcro fasteners.

Resting blood flow measurements were made at one minute intervals prior to exercise by measuring the average rate of change in leg volume during the brief period (10 s) when the cuff was inflated to 50 mmHg. For the duration of the exercise, the cuff was also inflated to 50 mmHg immediately prior to the first contraction and was maintained throughout the exercise period. During exercise LBF was measured during the 4-s relaxation period by assessing changes in limb volume detected by the strain gauge. The change in limb volume was only assessed when the force had returned within 10 N of a stable minimal value, and only if this stable force was maintained for at least 3 s. As blood flow was measured across 2-3 cardiac cycles, each blood flow measurement represents an average blood flow during the relaxation phase and not an average of the entire duty cycle (Egana & Green, 2005). During the relaxation phase blood volume in the calf muscle increased due to increased arterial inflow and occluded venous outflow. This resulted in an increase in calf girth during relaxation, which was detected by reductions in the resistance of the strain gauge. The accumulated blood in the calf muscle was squeezed back into circulation during the contraction phase. The increase in girth over time during the 4-s relaxation phase was used to determine blood flow (Fig. 2.4).

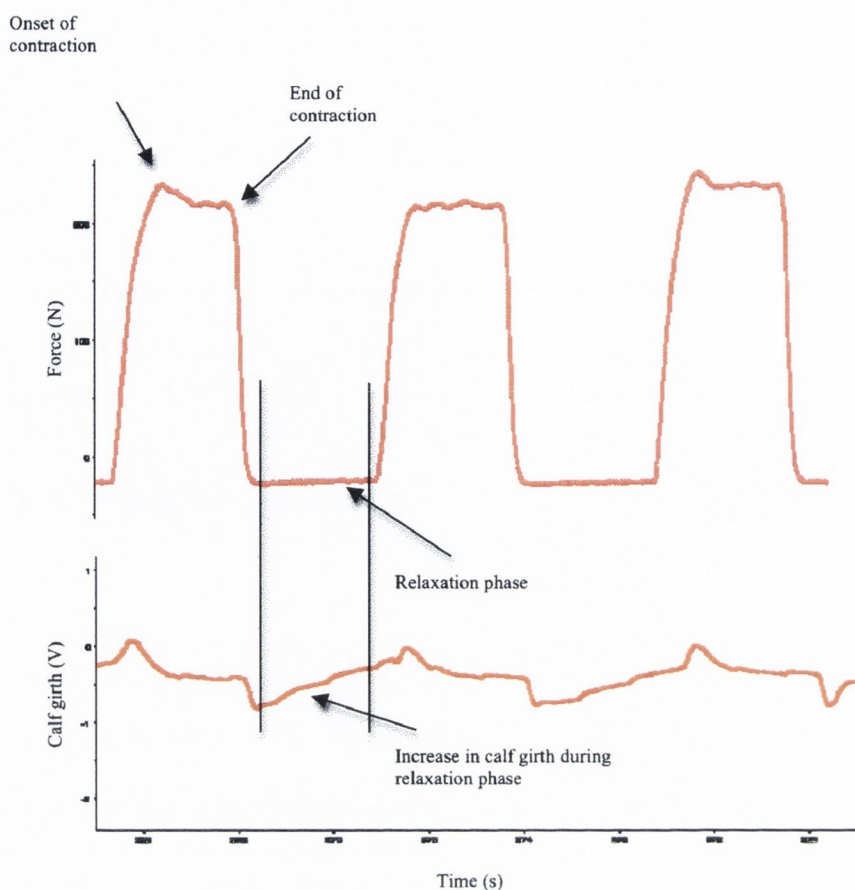


Fig. 2.4. Force output and calf girth traces during plantar flexion exercise. Blood flow is determined during the relaxation phase of the duty cycle. See text for further details.

2.5.2.3 Forearm blood flow

Forearm blood flow was measured at rest and during reactive hyperaemia using VOP. A mercury-in-Silastic strain gauge (Hokanson, USA) was positioned around the right forearm and secured in position using adhesive tape (Fig. 2.5). The strain gauge measured 2 cm less than the widest girth of the forearm. The strain gauge was connected to a plethysmograph (Hokanson EC-6) and the signal from the plethysmograph transmitted through a PowerLab analog-digital converter (ML 795, AD Instruments) to a computer with Chart software (LabChart 5; ADInstruments, Australia) for Windows. An arm pneumatic cuff (Hokanson, USA) was fitted around the right arm, which was fully extended at the level of the heart and fixed in position using velcro fasteners (Fig. 2.5). An applanation tonometry device (Colin CBM700, Japan) was used to determine beat-by-beat blood pressure and heart rate throughout the procedure by fixing a tonometric blood pressure sensor to the opposite left wrist (Fig. 2.6). A second

cuff (Hokanson, USA) was placed around the left upper arm while the right arm was also fully extended at the level of the heart (Fig. 2.6).

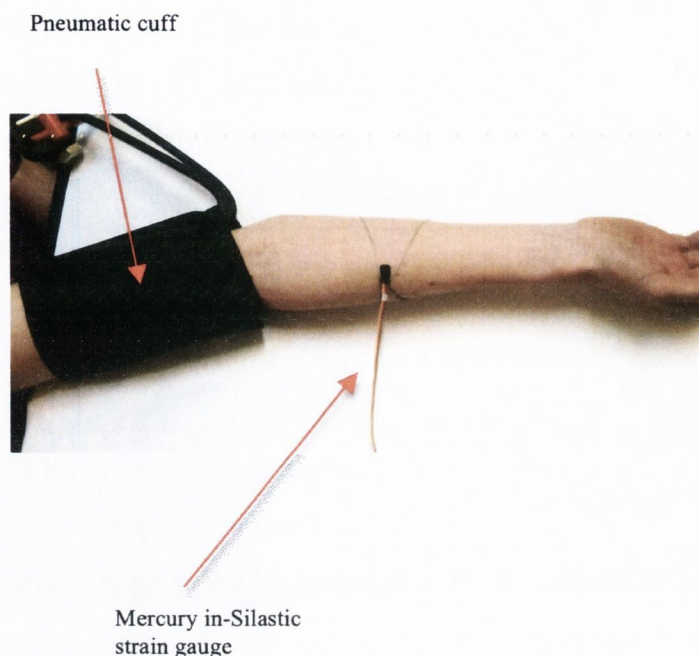


Fig. 2.5. Subject undergoing a reactive hyperaemic test of the forearm

Forearm blood flow at rest and post occlusion (cuff inflated to 200 mmHg for 5 min) was determined by rapidly inflating (5 s) and deflating (5 s) the cuff on the right arm to 50 mmHg and 0 mmHg respectively. The 5 min occlusion period resulted in complete ischemia where both venous and arterial blood flow were restricted. During the rapid cuff inflation (50 mmHg) arterial inflow is maintained while venous outflow is occluded. This results in an increase in forearm volume and consequently the strain gauge is stretched. Stretching of the strain gauge results in an increase in voltage, which is displayed as a computer trace. Forearm blood flow was determined during the second cardiac cycle of the venous occlusion period. The second cardiac cycle was chosen as inflation of the cuff may cause an artefact, which may be incorrectly interpreted as part of the cardiac cycle. Forearm blood flow (FBF) was calculated from the rate of change in forearm volume over time and expressed as $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$. Forearm vascular conductance (FVC) was calculated by dividing FBF by MAP ($\text{FVC} = \text{FBF}/\text{MAP}$) and values were expressed as $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} \cdot 10^{-1}$. Values were multiplied by 10 for better clarity.

2.5.2.4 Blood pressure

Brachial blood pressure was measured on a beat-to-beat basis at rest and during calf plantar flexion exercise using either applanation tonometry (COLIN CBM7000, Japan) (Fig. 2.6) or using a ‘volume clamp’ method (Finometer Midi, Finapres Medical Systems, Amsterdam, The Netherlands) (Fig. 2.7) of the right radial artery. The research team acquired the Finometer unit during the second year of this thesis, so ~50% of data were analysed using it. A pilot study showed that the correlation of both units from simultaneously recorded data collected during incremental plantar flexion exercise was very high (0.8) (Appendix 7).

For the applanation tonometry, an oscillometric cuff (Hokanson, USA) was placed around the upper right arm. A tonometric blood pressure sensor (COLIN, Japan) was placed over the radial artery on the right wrist and held in place with a wristband (COLIN, Japan) that prevented any movement of the subject’s wrist. The arm was abducted at 90° at the level of the heart.

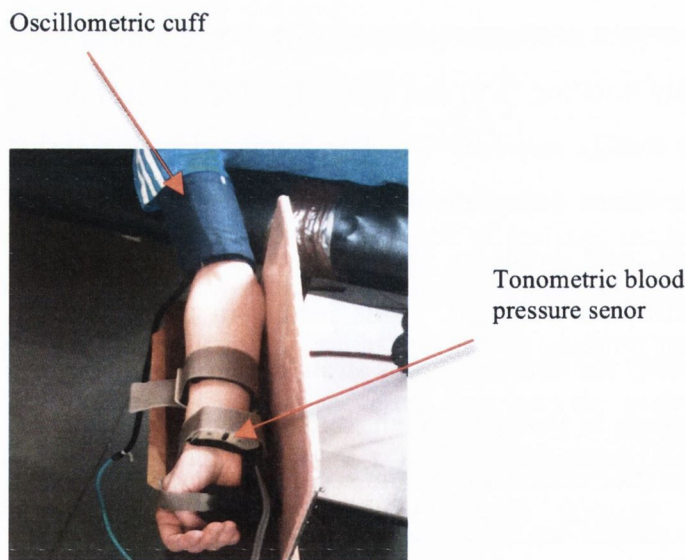


Fig. 2.6. Oscillometric cuff and tonometric blood pressure sensor

In the remainder of subjects, blood pressure was measured continuously and non-invasively at the fingertip using photoplethysmography and a ‘volume-clamp’ method (Finometer Midi, Finapres Medical Systems, Amsterdam, The Netherlands). The hand was positioned at the side of the subject at heart level.

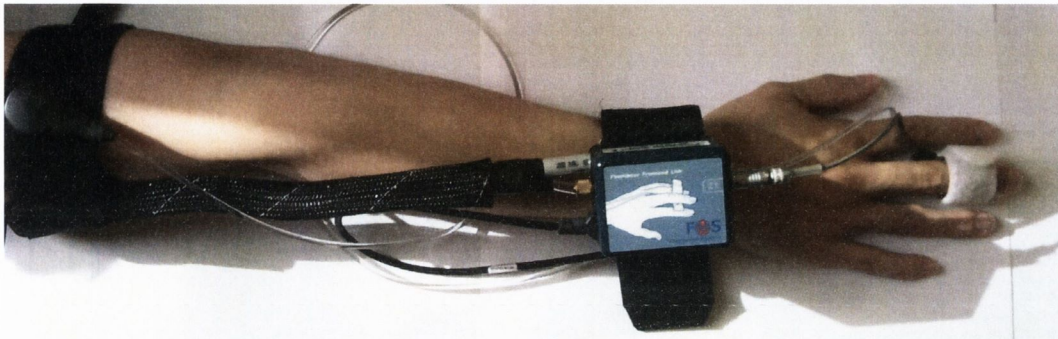


Fig. 2.7. Subject wearing a Finometer unit for the determination of blood pressure

Mean arterial pressure (mmHg) was calculated as follows:
 $\frac{1}{3}$ systolic blood pressure + $\frac{2}{3}$ diastolic pressure

Measurements of MAP during repeated trials of the same exercise (30% and 70%) were averaged to yield a single set of time series data.

2.5.2.5 Heart rate

Heart rate was measured from the R-R interval at rest and during exercise at 30 s, 180 s, and 360 s.

2.5.3 Inflammatory and endothelial markers

2.5.3.1 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

2.5.3.2 Vascular injury kit

MSD 96-well Multi-spot Vascular Injury Panel II Assay (CRP, SAA, sICAM-1, and sVCAM-1)	MSD, USA
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2.5.3.3 General laboratory materials

Tween-20	Sigma, Wicklow, Ireland
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2.5.3.4 General laboratory products

Biosphere filter pipette tips	Sarstedt, Nümbrecht, Germany
Falcon tubes (15 ml, 50 ml)	Sarstedt, Nümbrecht, Germany
Microtubes (0.5 ml, 1.5 ml)	Sarstedt, Nümbrecht, Germany
Optical adhesive covers UK	Applied Biosystems, Warrington, UK
96-well optical reaction plates UK	Applied Biosystems, Warrington, UK
PCR tubes	Sarstedt, Nümbrecht, Germany
Pipette tips	Sarstedt, Nümbrecht, Germany

2.5.3.5 Molecular reagents

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

2.5.3.6 Enzyme linked immunosorbent assay (ELISA)

Plasma levels of IL-6, IL-10, and TNF- α were determined using commercially available ELISA kits (BioLegend, Cambridge, UK).

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

A 96-well plate (BioLegend, Cambridge, UK) was coated with capture antibody (1:200 dilution with coating buffer (5x coating buffer diluted to 1x with deionised water) and incubated overnight at 4°C. The plate was washed with wash buffer ((Phosphate-buffered saline) 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; 200 μ l/well), sealed and incubated for 1 h at room temperature with shaking at 200 rpm. After washing, standards (appropriately diluted in reagent diluent) and samples were added (50 μ l/well). The plate was sealed and allowed to incubate at room temperature for 2 h with shaking at 200 rpm. After washing the plate, biotinylated detection anti-body (1:200 dilution in reagent diluent; 50 μ l/well) was added and the plate sealed and incubated at room temperature for 1 h with shaking at 200 rpm. The plate was again washed and reacted with Avidin-Horseradish Peroxidase (HRP) (1:1000 dilution in reagent diluent; 50 μ l/well) for 30 min at room temperature with shaking at 200 rpm. After washing, TMB substrate solution (1:1 mixture of colour reagent A and colour reagent B) was added (50 μ l/well) and the plate incubated in the dark for a minimum of 15 min or until such time as sufficient colour development had taken place. The reaction was stopped using 1M H₂SO₄ (50 μ l/well). The optical density (OD) was measured at 450 nm 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.

2.5.3.8 RNA extraction

The blood sample was incubated in the RNA tube at room temperature for 2 hours to achieve lysis of blood cells and then centrifuged for 10 minutes at 3,000-5,000 x g. Following decanting of the supernatant blood RNA tube by decanting, RNase-free water (4 ml) 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 3,000-5,000 x g. Following this, the supernatant was completely removed. Resuspension buffer (350 µl) was added to the RNA tube and the tube was vortexed until the pellet was visibly dissolved. The sample was pipetted into a 1.5 ml microcentrifuge tube, into which binding buffer (300 µl) and proteinase K (40 µl) were added. The microcentrifuge tube was vortexed for 5 s and incubated for 10 min at 55°C in a shaker-incubator at 400-1,400 rpm. The lysate from the microcentrifuge tube was pipetted into a shredder spin column placed in a 2 ml processing tube and then centrifuged for 3 min at a speed of 14,000 x g. The supernatant of the flow-through fraction was pipetted to a new 1.5 ml microcentrifuge tube without disturbing the pellet in the processing tube and ethanol (350 µl) added to the sample, which was then vortexed. Some of the sample (700 µl) was pipetted into a RNA spin column placed in a 2 ml processing tube and centrifuged for 1 min at 8,000-20,000 x g. The spin column was placed in a new 2 ml processing tube and the remaining sample pipetted into the RNA spin column and centrifuged for 1 min at 8,000-20,000 x g. The spin column was placed in a new 2 ml processing tube into which BR3 wash buffer was added (350 µl) and the RNA spin column centrifuged for 1 min at 8,000-20,000 x g. The spin column was again placed in a new 2 ml processing tube. DNase reaction mixture (80 µl; 1:8 dilution of reconstituted rDNase in DNA digestion buffer) was pipetted directly onto the centre of the silica membrane of each column. Samples were incubated with this solution at room temperature for 15 min to digest DNA. BR3 wash buffer was added (350 µl) to the spin column and the spin column was centrifuged for 1 min at 8,000-20,000 x g. The spin column was placed in a new 2 ml processing tube; BR4 wash buffer (1:4 dilution with ethanol) was added (500 µl) the spin column was centrifuged for 1 min at 8,000-20,000 x g. After placing the spin column in a new 2 ml processing tube, BR4 wash buffer was added (500 µl) and the spin column was centrifuged for 3 min at 8,000-20,000 x g. The spin column was placed in a new 2 ml processing tube and centrifuged for 1 min at 8,000-20,000 x g. Following this, the spin column was placed in a 1.5 ml microcentrifuge tube and elution buffer (40 µl) was pipetted directly onto the spin column membrane. The spin column was centrifuged for 1 min at 8,000-20,000 x g to elute the RNA.

This step was again repeated. The eluate was incubated for 5 min 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.

2.5.3.9 RNA quantification and reverse transcription

The 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 due to the ability it has to absorb light. An OD of 1.0 at 260 nm represents an RNA concentration of 44 µg.ml⁻¹. This allows the RNA concentration in each sample of extracted RNA to be determined using the following equation:

$$\text{RNA} = \text{OD}_{260} \times \text{dilution factor} \times 44 \text{ } \mu\text{g.ml}^{-1}$$

In addition, the absorbance is measured at a wavelength of 280 nm, which allows the purity of RNA to be determined. A ratio of OD₂₆₀:OD₂₈₀ of approximately 1.8-2.0 indicates that the RNA is pure. RNA concentrations were equalised with RNase-free H₂O 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 (17 µl) was mixed with 2x master mix (17 µl, 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 H₂O) 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 10 min, then at 37°C for 120 min. The cDNA was frozen at -20°C for real-time polymerase chain reaction (RT-PCR) analysis.

2.5.3.10 Real-time polymerase chain reaction (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 2.2).

Table 2.2 Gene reference

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

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>

2.5.3.11 Multi-target (multiplex) RT-PCR

Initially cDNA was diluted with RNase-free H₂O (1:4 dilution) and 4 μ l of the dilute was added to each well on a PCR plate. Following this, target primers (0.5 μ l), GAPDH (0.5 μ l), and Taqman master mix (5 μ l) were mixed and added to each well (10 μ l reaction volume). The solution were pipetted using an electronic pipette (EDP3 10-100 μ l) to enable 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: step 1: 50°C for 2 min, step 2: 95°C for 20 s, step 3: 95°C for 1 s, and step 4: 60°C for 20 s. Steps 3 and 4 were repeated 40 times.

2.5.3.12 RT-PCR analysis

The comparative CT ($\Delta\Delta$ CT) 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 between groups with T2DM and control 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.

2.5.3.13 Vascular injury

A multi-spot 96-well 4 spot vascular plate was coated with 5% blocking solution (150 μ /well) and incubated at room temperature at 1 h 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 a reagent diluent (40 μ /well). Following this, standards (appropriately diluted in reagent diluent) and samples (1:200 dilution with 1% blocker A solution (5% blocker A solution diluted to 1% with PBS) were added (10 μ l/well). The plate was sealed and allowed to incubate at room temperature for 2 h with shaking. After washing the plate, detection anti-body (1:50 dilution in reagent diluent; 25 μ l/well) was added and the plate sealed and incubated at room temperature for 1 h with shaking. The plate was again washed and reacted with 1X Read Buffer T (4X Read Buffer T diluted to 1X with distilled water; 150 μ l/well). The plates were read immediately using a Sector Imager 2400 (MSD, USA) and analysed using Discovery Workbench 3.0 software (MSD, USA).

2.6 Data analysis

2.6.1 $\dot{V}O_2$ kinetic analysis

The BbB data were examined to exclude any errant breaths caused by coughing, sighing, or swallowing, which are considered not to be reflective of the underlying kinetics. For each bout the BbB data were subsequently interpolated to give 1-s values. The four bouts were time aligned to the start of exercise, superimposed, and ensemble averaged to reduce BbB noise (Barstow & Mole, 1991; Ozyener *et al.*, 2001; Pringle *et al.*, 2003; Koppo *et al.*, 2009). To further enhance the underlying physiological response characteristics each average response was smoothed using a 5-s moving filter (Barstow & Mole, 1991). The

baseline $\dot{V}O_2$ was defined as the average $\dot{V}O_2$ measured during cycling between 150 s and 30 s prior to starting cycling at 80% VT.

The time course of the $\dot{V}O_2$ response was described in terms of a two component exponential function (MacDonald *et al.*, 1998) and was curve fit using a bi-exponential function (Sigma Tablecurve 2D v5.01, USA). Each exponential curve was used to describe one phase of the $\dot{V}O_2$ response. The first phase coincided with the onset of exercise and the second term began after an independent time delay:

$$\dot{V}O_2(t) = A_0 - A_1(1 - e^{-(t - TD_1)/\tau_1})F_1 + A_2(1 - e^{-(t - TD_2)/\tau_2})F_2$$

In the case of 7 women (T2DM n=4; non-diabetic n=3) a tri-exponential function was used to fit the $\dot{V}O_2$ data. For these individuals, only the parameter estimates from the first two phases were included in the analysis.

$$\dot{V}O_2(t) = A_0 - A_1(1 - e^{-(t - TD_1)/\tau_1})F_1 + A_2(1 - e^{-(t - TD_2)/\tau_2})F_2 + A_3(1 - e^{-(t - TD_3)/\tau_3})F_3$$

$$\begin{array}{lll} \text{where:} & F_1 = 0 \text{ for } t < TD_1 & \text{and} \quad F_1 = 1 \text{ for } t \geq TD_1 \\ & F_2 = 0 \text{ for } t < TD_2 & \text{and} \quad F_2 = 1 \text{ for } t \geq TD_2 \\ & F_3 = 0 \text{ for } t < TD_3 & \text{and} \quad F_3 = 1 \text{ for } t \geq TD_3 \end{array}$$

$\dot{V}O_2(t)$ is the $\dot{V}O_2$ at any given time point; A_0 is the baseline $\dot{V}O_2$ measured during the 3-min cycling at 10 W; A_1 , A_2 , and A_3 are the asymptotic amplitudes for the exponential terms for phase I, II, and III; τ_1 , τ_2 , and τ_3 are the time constants for phase I, II, and III; and TD_1 , TD_2 , and TD_3 are the time delays for phase I, II, and III. The parameters F_1 , F_2 , and F_3 are conditional expressions that limit the fitting of a particular phase to the period at and beyond the time delay associated with that phase. Data that exceeded the 95% prediction intervals during an initial fit of a model were excluded. The model was fitted to the data using a weighted least-squares non-linear regression procedure.

An indicator of the rate of change for the total response for each subject was obtained by calculating the mean response time (MRT). The MRT is the time required to achieve approximately 63% of the baseline and the exercise plateau (MacDonald *et al.*, 1998).

Biphasic:
$$\text{MRT} = [A_1/(A_1 + A_2)(\text{TD}_1 + \tau_1)] + [A_2/(A_1 + A_2)(\text{TD}_2 + \tau_2)]$$

Triphasic:
$$\text{MRT} = [A_1/(A_1 + A_2 + A_3)(\text{TD}_1 + \tau_1)] + [A_2/(A_1 + A_2 + A_3)(\text{TD}_2 + \tau_2)] + [A_3/(A_1 + A_2 + A_3)(\text{TD}_3 + \tau_3)]$$

The total amplitude (End A) of the $\dot{V}\text{O}_2$ response, which represents the change in $\dot{V}\text{O}_2$ from rest to the end of exercise, was also calculated for each subject:

Biphasic:
$$\text{End A} = A_0 + (A_1(1 - e^{-(360 - \text{TD}_1)/\tau_1})) + (A_2(1 - e^{-(360 - \text{TD}_2)/\tau_2}))$$

Triphasic:
$$\text{End A} = A_0 + (A_1(1 - e^{-(360 - \text{TD}_1)/\tau_1})) + (A_2(1 - e^{-(360 - \text{TD}_2)/\tau_2})) + (A_3(1 - e^{-(360 - \text{TD}_3)/\tau_3}))$$

The $\dot{V}\text{O}_2$ gain ($\Delta \dot{V}\text{O}_2 / \Delta W$) was calculated as follows:

$$\Delta \dot{V}\text{O}_2 / \Delta W = (\text{End A} - \text{Baseline } \dot{V}\text{O}_2) / (\text{Workload at 80\% VT} - 10 \text{ W})$$

2.6.2 Heart rate kinetic analysis

In a subgroup of women (T2DM $n=15$; non-diabetic $n=14$), heart rate responses from the first four bouts were averaged to yield a single time-series of heart rate data for each subject and were then fitted using a mono-exponential function as shown:

$$\text{Heart rate } (t) = A_0 + A(1 - e^{-(t - \text{TD})/\tau})$$

where heart rate (t) is the heart rate at any time point; A_0 is the initial heart rate during the 3-min cycling at 10 W; A is the amplitude of the response; TD is the

time delay; and τ is the time constant. Fitting procedures were identical to those described for oxygen uptake.

The End A of the heart rate response, which represents the change in heart rate from rest to the end of exercise, was also calculated for each subject:

$$\text{End A} = A_0 + (A(1 - e^{-(360 - \text{TD})/\tau}))$$

2.6.3 Leg vascular conductance kinetics

The plethysmographic estimates of LBF ($\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$) were converted to $\text{ml} \cdot \text{min}^{-1}$ using an estimate of each subject's leg volume obtained from anthropometric measurements of the leg (section 2.3.8). LBF ($\text{ml} \cdot \text{min}^{-1}$) was then converted to LVC ($\text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) by dividing LBF ($\text{ml} \cdot \text{min}^{-1}$) by MAP (mmHg). LVC responses from the three 6 min exercise bouts were averaged to yield a temporal profile of LVC based on 60 average estimates for each subject. The temporal response profile for each subject were curve-fit with a biphasic 'growth only' model (70% MVC) or triphasic 'growth and decay' model (30% MVC) (Sigma Tablecurve 2D v5.01, USA) on the basis that a biphasic or triphasic response was more apparent during 70% and 30% MVC plantar flexion respectively.

Biphasic 'growth only' model:

$$y(t) = A_0 + A_1(1 - e^{-(t-\text{TD1})/\tau_1})F1 + A_2(1 - e^{-(t-\text{TD2})/\tau_2})F2$$

$$\begin{aligned} \text{where:} \quad & F1 = 0 \text{ for } t < \text{TD1} \text{ and } F1 = 1 \text{ for } t \geq \text{TD1} \\ & F2 = 0 \text{ for } t < \text{TD2} \text{ and } F2 = 1 \text{ for } t \geq \text{TD2} \end{aligned}$$

$y(t)$ is vascular conductance at time t , A_0 is the baseline amplitude, A_1 , and A_2 are the amplitudes, TD_1 and TD_2 are the time delays, and τ_1 and τ_2 are the time constants of the first (rapid growth) and second (slow growth) phases respectively. The parameters $F1 - F2$ are conditional expressions that limit the fitting of a particular phase to the period at and beyond the time delay associated with that phase.

Triphasic ‘growth and decay’ model:

$$y(t) = A_0 + A_1(1 - e^{-(t-TD1)/\tau1})F1 - A_2(1 - e^{-(t-TD2)/\tau2})F2 + A_3(1 - e^{-(t-TD3)/\tau3})F3$$

where:

$$F1 = 0 \text{ for } t < TD1 \text{ and } F1 = 1 \text{ for } t \geq TD1$$

$$F2 = 0 \text{ for } t < TD2 \text{ and } F2 = 1 \text{ for } t \geq TD2$$

$$F3 = 0 \text{ for } t < TD3 \text{ and } F3 = 1 \text{ for } t \geq TD3$$

$y(t)$ is vascular conductance at time t , A_0 is the baseline amplitude, A_1 , A_2 , and A_3 are the amplitudes, TD_1 , TD_2 , and TD_3 are the time delays and τ_1 , τ_2 , and τ_3 are the time constants of the first (rapid growth), second (rapid decay), and third (slow growth) phases respectively. The parameters $F1 - F3$ are conditional expressions that limit the fitting of a particular phase to the period at and beyond the time delay associated with that phase.

For both biphasic and triphasic models, data that exceeded the 95% prediction intervals during an initial fit of a model were excluded. No more than four data points were removed from the original time-series of data. The model was fitted to the data using a weighted least-squares nonlinear regression procedure (Sigma Tablecurve 2D v5.01, USA).

The mean response time (MRT), which represents the time taken to reach 63% of the overall amplitude of the response from the baseline value, was calculated as a weighted sum of the time delay and time constant of each phase for the biphasic ‘growth only’ model:

$$MRT = (A_1/(A_1 + A_2))(TD_1 + \tau_1) + (A_2/(A_1 + A_2))(TD_2 + \tau_2)$$

The End A for both biphasic ‘growth only’ and triphasic ‘growth & decay’ models was calculated as follows:

Biphasic ‘growth only’ model:

$$\text{End A} = A_0 + (A_1(1 - e^{-(360 + TD1)/\tau1})) + (A_2(1 - e^{-(360 + TD2)/\tau2}))$$

Triphasic ‘growth & decay’ model:

$$\text{End A} = A_0 + (A_1(1 - e^{(-360 + \text{TD1})/\tau_1})) - (A_2(1 - e^{(-360 + \text{TD2})/\tau_2})) + (A_3(1 - e^{(-360 + \text{TD3})/\tau_3}))$$

2.6.4 Biphasic ‘growth only’ and triphasic ‘growth and decay’ models

The goodness of fit of either biphasic ‘growth only’ or triphasic ‘growth and decay’ models was established on statistical grounds. The difference between the two models (significance $P < 0.05$) was determined by calculating the F-test statistic for nested models as follows:

$$F = [(RSS_1 - RSS_2)/(p_2 - p_1)]/[RSS_2/(n - p_2)]$$

where RSS_1 and RSS_2 are the residual sums of squares and p_1 and p_2 are the parameters in the biphasic ‘growth only’ or triphasic ‘growth and decay’ models respectively, and n is the number of observations in the time series. If an F-test value exceeded the critical F value ($df = p_2 - p_1, n - p_2$), where df represents the degrees of freedom, then the triphasic ‘growth and decay’ model was chosen as the best model of fit.

2.6.5 Reactive hyperaemia responses

Vascular conductance hyperaemic responses were curve-fit using a mono-exponential function: $y = a + be^{-cx}$ (Sigma Tablecurve 2D v5.01, USA), where a represents the baseline vascular conductance, b is the amplitude of the curve, and c is the decay constant. Data that exceeded the 95% prediction intervals during an initial fit of a model were excluded. No more than two data points were removed from the original time-series of data.

2.6.6 Vasodilatory and hyperaemic slopes during incremental plantar flexion exercise

To determine both the hyperaemic (LBF ($\text{ml} \cdot \text{min}^{-1}$) vs. % of maximum force and LBF ($\text{ml} \cdot \text{min}^{-1}$) vs. force (N)) and vasodilatory (LVC ($\text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) vs. % of maximum force and LVC ($\text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) vs. force (N)) responses during

incremental plantar flexion exercise, each individual's LBF/LVC response was plotted against the corresponding force output (for LBF/LVC expressed in relation to peak force) or percentage of maximum force (for LBF/LVC expressed as % of peak force). From the corresponding regression line, the slope for LBF/LVC was determined for each subject.

2.7 Statistical analysis

For the baseline studies, an unpaired Student's t-test (PRISM, Version 5.03, GraphPad Software Inc, USA) was used to compare effects between women with and without T2DM. Data that were not normally distributed were analysed using the Mann-Whitney U test. The level of significance was set as $P \leq 0.05$. All values are expressed as mean $\pm$ SD.

For the intervention study, a three-way repeated measures ANOVA (exercise vs. control, diabetic status, and time) was used to compare effects within and between groups. Bonferroni post hoc tests were carried out to detect differences between groups. The level of significance was set as $P \leq 0.05$. The data were analysed using Datadesk (Version 6.2.1) and values are expressed as means $\pm$ SD.

Chapter 3: Effect of T2DM on the dynamic response characteristics of $\dot{V}O_2$ in postmenopausal women during acute exercise

3.1 Aims

- (a) Firstly, to investigate if $\dot{V}O_2$ kinetic responses are slowed in postmenopausal women with T2DM matched in age but not BMI
- (b) Secondly, to investigate if the findings observed in postmenopausal women persist in BMI and aged matched women with T2DM
- (c) Explore if CO responses are related to impairments in $\dot{V}O_2$ kinetic responses in any of these women with T2DM

3.2 Experimental protocol overview

Subjects were required to visit the Cardiovascular Laboratory in the Department of Physiology, Trinity College Dublin, on two occasions separated by at least 72 h. On the first visit, $\dot{V}O_{2\text{ peak}}$ and VT were determined during an incremental cycle test to volitional exhaustion. On the second visit, subjects performed 6 bouts of 6 min constant load cycling at 80% VT. Bouts 1-4 were used for the determination of $\dot{V}O_2$ kinetics while bouts 5 & 6 were used to determine CO. CO was measured at rest and during exercise (30 s and 240 s) at 80% VT.

3.3 Subjects

A total of forty-one postmenopausal women were recruited to participate in the study: 20 with T2DM and 21 healthy controls. All women were classified as sedentary ($< 1 \text{ h} \cdot \text{wk}^{-1}$ of moderate intensity exercise for at least 6 months prior to enrolment), which was confirmed by physical activity scores using the Low Level Physical Activity Recall (LOPAR; Appendix 1) questionnaire (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999) and the use of RT3 triaxial accelerometers (Stay Healthy, Inc; Monrovia, California) (Rowlands *et al.*, 2004; Perry *et al.*, 2010).

3.3.1 Medications

Of the 20 women with T2DM, 5 were undergoing dietary intervention and 15 were receiving oral hypoglycaemic agents (metformin n=11; metformin & gliclazide n=3; gliclazide n=1). In addition, 11 diabetic women were on anti-hypertensive medication (lisinopril n=2; ramipril n=1; candesartan cilexetil n=1; perindopril arginine n=2; telmisartan n=1; angiotensin II antagonists (losartan n=1); ACE inhibitors (ramapril n=1); calcium channel blocker (amlodipine n=1); perindopril arginine & amlodipine n=1). A further 3 women with T2DM were taking β -blockers (metoprolol tartrate n=1; sotalol n=1; bisoprolol fumarate n=1). None of the non-diabetic women were receiving anti-hypertensive medication. A further 13 diabetic women and 1 non-diabetic woman were receiving lipid-lowering therapy (T2DM: rosuvastatin n=3; atorvastatin n=8; simvastatin n=1; pravastatin n=1 and non-diabetic: atorvastatin n=1). A total of three women were undergoing hormone replacement therapy (T2DM: n=1, estradiol; non-diabetic: n=2, raloxifene).

3.4 Results

Following on and to be consistent with the aims of the study, the results for women with and without T2DM are presented in two sections: age-matched postmenopausal women, and age and BMI matched postmenopausal women.

3.4.1 Subjects

3.4.1.1 Physical characteristics

Physical characteristics and habitual physical activity levels in women with and without T2DM are presented in Table 3.1. Individual subject characteristics can be seen in Appendix 8.

In postmenopausal women, significant differences were observed in BMI, body mass ($P<0.0001$), and WHR ($P<0.01$) between groups with and without T2DM.

In BMI matched postmenopausal women, there were no differences between women with and without T2DM with respect to age, physical activity levels, BMI, weight, height, WHR, or ABI.

Table 3.1. Physical characteristics and activity levels

	T2DM (n = 20)	Non-diabetic (n = 21)	T2DM (n = 12)	Non-diabetic (n = 9)
	Postmenopausal		BMI matched postmenopausal	
Age (yr)	56.7±4.0	56.0±5.5	57.2±4.8	56.2±3.4
BMI (kg.m ⁻²)	34.1±5.5***	26.4±4.0	30.8±2.9	30.2±2.3
Weight (kg)	85.6±13.8***	67.3±11.5	78.6±9.5	77.3±8.2
Height (m)	1.58±0.05	1.60±0.07	1.60±0.05	1.60±0.03
WHR	0.96±0.05**	0.90±0.07	0.94±0.05	0.91±0.07
ABI	1.06±0.10	1.09±0.10	1.04±0.10	1.10±0.09
LOPAR (MET h.wk ⁻¹)	104±46	111±48	94±43	119±58

Significantly different from non-diabetic ** $P<0.01$, *** $P<0.0001$.

RT3 accelerometers were used to determine physical activity levels in a subgroup of combined pre and postmenopausal women with and without T2DM (T2DM n = 18; non-diabetic n = 19) (Fig. 3.1). There were no differences between women with and without T2DM in the time spent in inactive, light, moderate, or vigorous activity categories.

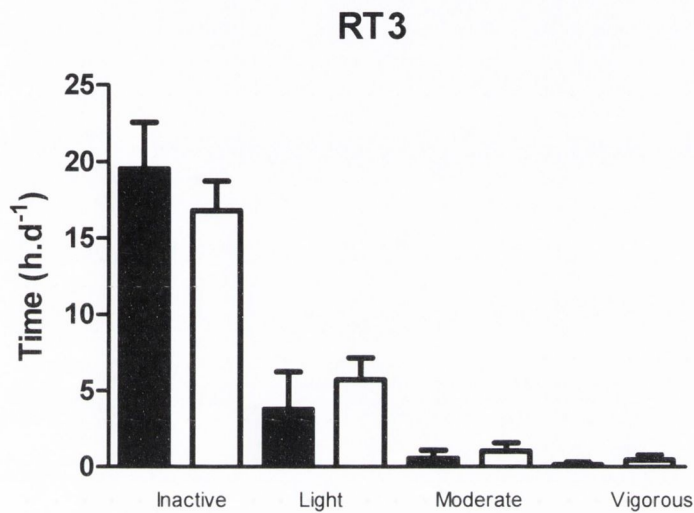


Fig. 3.1. Time spent in inactive, light, moderate, and vigorous activity categories in a subgroup of combined pre and postmenopausal women with and without T2DM.

3.4.1.2 Haematology

Glycosylated haemoglobin (HbA_{1C}), fasting plasma glucose (FPG), total cholesterol (TCL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides in women with and without T2DM are presented in Table 3.2. Individual results can be seen in Appendix 9.

For all group comparisons, FPG was greater in women with T2DM than non-diabetic women ($P<0.0001$).

In postmenopausal women, women with T2DM had greater levels of HbA_{1C} (expressed as a percentage and in mmol.mol⁻¹) ($P<0.0001$) and triglycerides ($P<0.0001$) and lower levels of TCL ($P<0.0001$), HDL ($P<0.0001$), and LDL ($P<0.05$) than their non-diabetic counterparts.

In BMI matched postmenopausal women, HbA_{1C} (expressed as a percentage and in mmol.mol⁻¹) ($P<0.01$) was greater and TCL ($P<0.01$), HDL ($P<0.0001$), LDL ($P<0.05$) were lower in women with T2DM than non-diabetic women.

Table 3.2. Haematological parameters

	T2DM	Non-diabetic	T2DM	Non-diabetic
	Postmenopausal		BMI matched postmenopausal	
HbA _{1C} (%)	6.7±1.0***	5.5±0.2	6.7±0.7**	5.5±0.1
HbA _{1C} (mmol.mol ⁻¹)	50.2±10.9***	36.4±2.3	49.4±7.8**	36.7±1.7
FPG (mmol.L ⁻¹)	8.3±2.5***	4.6±0.6	8.6±2.3***	4.7±0.5
TCL (mmol.L ⁻¹)	4.5±0.8***	5.8±1.4	4.7±0.8**	6.1±1.1
HDL (mmol.L ⁻¹)	1.3±0.3***	1.9±0.4	1.4±0.2***	2.1±0.4
LDL (mmol.L ⁻¹)	2.9±0.7*	3.7±1.2	3.0±0.7*	4.0±1.0
Triglycerides (mmol.L ⁻¹)	1.6±0.6***	1.0±0.4	1.6±0.6	1.0±0.4

Significantly different from non-diabetic * $P<0.05$, ** $P<0.01$, *** $P<0.0001$.

3.4.2 Peak cycling performance

Peak physiological responses of the graded cycle test in women with and without T2DM are presented in Table 3.3 and Fig. 3.2. Individual peak cycling performance data can be seen in Appendix 10.

In postmenopausal women, relative $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$), $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT ($P<0.0001$), peak workload (W), and workload (W) at VT ($P<0.05$) were lower in women with T2DM than non-diabetic controls. There were no differences in absolute $\dot{V}O_{2\text{ peak}}$ (L.min^{-1}) between women with and without T2DM.

In BMI matched postmenopausal women, relative $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$), peak workload (W), workload at VT (W), and time to failure (TTF) (min) were lower in women with T2DM ($P<0.05$). In contrast, there were no differences in absolute $\dot{V}O_{2\text{ peak}}$ (L.min^{-1}) or $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT between women with or without T2DM.

Table 3.3. Peak cycling performance parameters

	T2DM (n = 20)	Non-diabetic (n = 21)	T2DM (n = 12)	Non-diabetic (n = 9)
	Postmenopausal		BMI matched postmenopausal	
Peak HR (beats.min ⁻¹)	149±20	156±12	155±15	146±7
Age predicted peak HR (%)	91.0±12.1	95.5±8.3	95.5±9.3	89.2±4.4
Peak $\dot{V}_E$ (L.min ⁻¹)	59.5±14.2	57.0±12.2	57.7±9.6	56.6±15.4
TTF (min)	11.9±2.4	13.3±3.3	11.9±2.6*	14.7±2.9
Workload at VT (W)	82.0±14.4*	98.1±24.4	81.7±15.9*	106.7±24.9
RER	1.10±0.05	1.10±0.06	1.11±0.14	1.09±0.07
$\dot{V}O_2$ at VT (% $\dot{V}O_{2\text{ peak}}$)	76.0±20.7	73.1±8.2	76.0±20.7	71.1±9.5

Significantly different from non-diabetic * $P<0.05$.

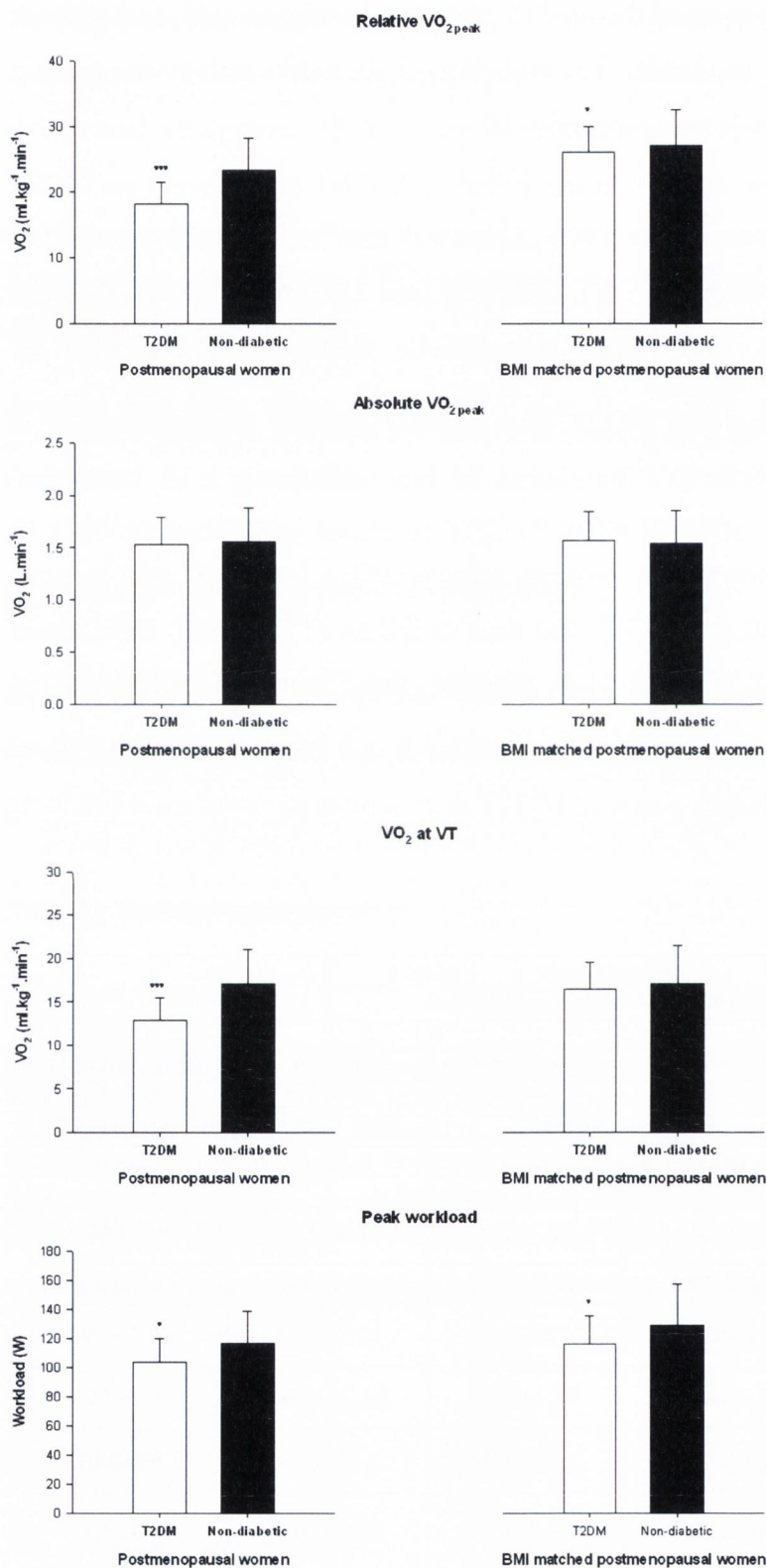


Fig. 3.2. $\dot{V}O_{2\text{peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$ and L.min^{-1}), $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT, and peak workload (W) in combined groups of postmenopausal women and groups of BMI matched postmenopausal women with and without T2DM. Significantly different from non-diabetic * $P < 0.05$, *** $P < 0.0001$.

3.4.3 $\dot{V}O_2$ kinetic responses during cycle exercise at 80% VT

Mean $\dot{V}O_2$ kinetic parameters during cycle exercise at 80% VT from women with and without T2DM are presented in Table 3.4. Representative $\dot{V}O_2$ kinetic responses from postmenopausal women with and without T2DM (matched by BMI) as well as the predicted responses from the bi-exponential curve fits are depicted in Fig. 3.3. Individual $\dot{V}O_2$ kinetic parameters can be seen in Appendix 11.

In postmenopausal women, A_0 was greater ($P<0.01$), A_1 was lower ($P<0.05$), and both τ_2 and MRT were slowed in women with T2DM in contrast to non-diabetic women ($P<0.01$).

When postmenopausal women were matched by BMI, both τ_2 and MRT remained slower in individuals with T2DM than non-diabetics ($P<0.05$). In addition, the End A was lower in women with T2DM than non-diabetic women ($P<0.05$).

Table 3.4. Mean $\dot{V}O_2$ kinetic parameters during cycle exercise at 80% VT

	T2DM (n = 20)	Non-diabetic (n = 21)	T2DM (n = 12)	Non-diabetic (n = 9)
	Postmenopausal		BMI-matched postmenopausal	
A_0 (L.min ⁻¹)	0.60±0.13**	0.50±0.06	0.57±0.10	0.53±0.07
A_1 (L.min ⁻¹)	0.21±0.06*	0.30±0.15	0.22±0.06	0.35±0.16
TD ₁ (s)	5.9±4.3	4.6±2.6	6.4±4.4	3.6±2.5
τ_1 (s)	11.4±3.8	11.3±4.7	12.7±3.7	11.8±4.5
A_2 (L.min ⁻¹)	0.40±0.12	0.46±0.21	0.41±0.14	0.52±0.21
TD ₂ (s)	29.0±7.1	30.3±7.1	30.3±8.3	30.9±7.8
τ_2 (s)	49.8±11**	37.1±9.2	49.6±11.6*	36.1±8.3
End A (L.min ⁻¹)	1.24±0.23	1.28±0.31	1.22±0.22*	1.42±0.26
MRT (s)	57.9±12.2**	47.6±9.9	58.6±12.7*	46.8±10.7
$\dot{V}O_2$ gain (ml.min ⁻¹ .W ⁻¹)	10.8±2.6	11.0±3.4	11.0±2.5	11.6±1.3

Significantly different from non-diabetic * $P<0.05$, ** $P<0.01$.

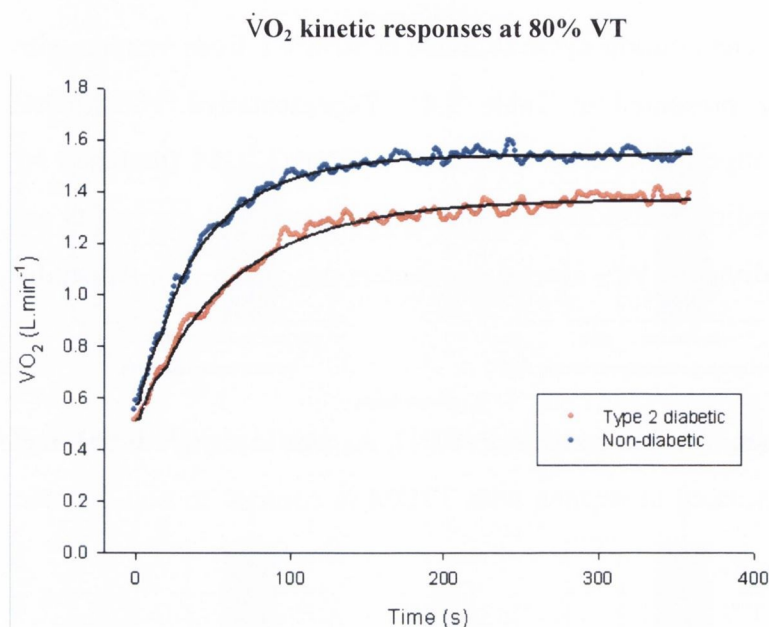


Fig. 3.3. $\dot{V}O_2$ kinetic responses and the predicted responses from the bi-exponential curve fits during cycle exercise at 80% VT from representative postmenopausal BMI matched women with and without T2DM.

3.4.4 Heart rate kinetic responses during cycle exercise at 80% VT

Mean heart rate kinetic parameters from women with and without T2DM are presented in Table 3.5. Representative heart rate kinetic responses from postmenopausal women with and without T2DM as well as the predicted responses from the mono-exponential curve fits are depicted in Fig. 3.4. Individual heart rate kinetic parameters can be seen in Appendix 12.

In the groups of postmenopausal women, τ was slower in subjects with T2DM compared to non-diabetic controls ($P < 0.05$). In women with T2DM, A_0 was greater ($P < 0.01$) and A was lower ($P < 0.0001$) than non-diabetic women.

In BMI matched postmenopausal women with T2DM, τ was slower ($P < 0.05$) than non-diabetic women. In the subgroups of BMI matched postmenopausal women, both A_0 and End A were greater in women with T2DM than non-diabetic women ($P < 0.01$).

Table 3.5. Heart rate kinetic parameters for cycle exercise at 80% VT

	T2DM (n = 15)	Non-diabetic (n = 14)	T2DM (n = 8)	Non-diabetic (n = 6)
	Postmenopausal		BMI matched postmenopausal	
Baseline A ₀ (beats.min ⁻¹)	105±16**	88±8	109±15**	86±4
A (beats.min ⁻¹)	25±9***	39±7	29±9	34±6
TD (s)	2.9±3.4	5.6±4.1	2.1±3.1	2.7±1.0
τ (s)	75.2±23.2*	59.9±10.5	85.9±25.7*	59.2±11.0
End A (beats.min ⁻¹)	129±18	127±13	137±7**	120±10

Significantly different from non-diabetic **P*<0.05, ***P*<0.01, ****P*<0.0001.

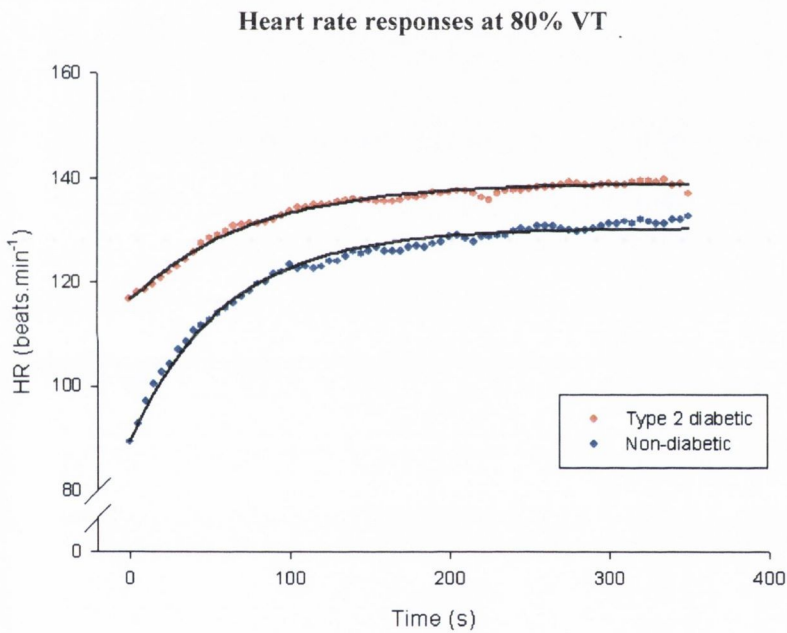


Fig. 3.4. Heart rate kinetic responses and the predicted responses from the mono-exponential curve fits during cycle exercise at 80% VT from representative postmenopausal women with and without T2DM.

3.4.5 Cardiovascular responses during cycle exercise at 80% VT

Mean cardiovascular responses (CO, CI, SV, stroke volume index (SVI), and HR), a-vO₂ diff, TPR, and MAP at rest and during cycle exercise (30 s and 240 s) at 80% VT in women with and without T2DM are presented in Figs 3.5 and 3.6. Individual responses can be seen in Appendix 13.

For all group comparisons, both CO and a-vO₂ diff were not different between women with and without T2DM either at rest or during exercise, while in contrast, resting SVI was lower in women with T2DM than non-diabetic controls ($P<0.0001$).

In the groups of postmenopausal women, resting HR ($P<0.0001$) and MAP ($P<0.01$) were greater and MAP was greater during exercise at both 30 s ($P<0.01$) and 240 s ($P<0.05$) in women with T2DM than non-diabetic controls.

In the subgroups of BMI matched postmenopausal women, HR at rest ($P<0.01$) and during exercise both at 30 s ($P<0.01$) and 240 s ($P<0.05$) was greater while resting SV was lower ($P<0.01$) in women with T2DM than non-diabetic controls.

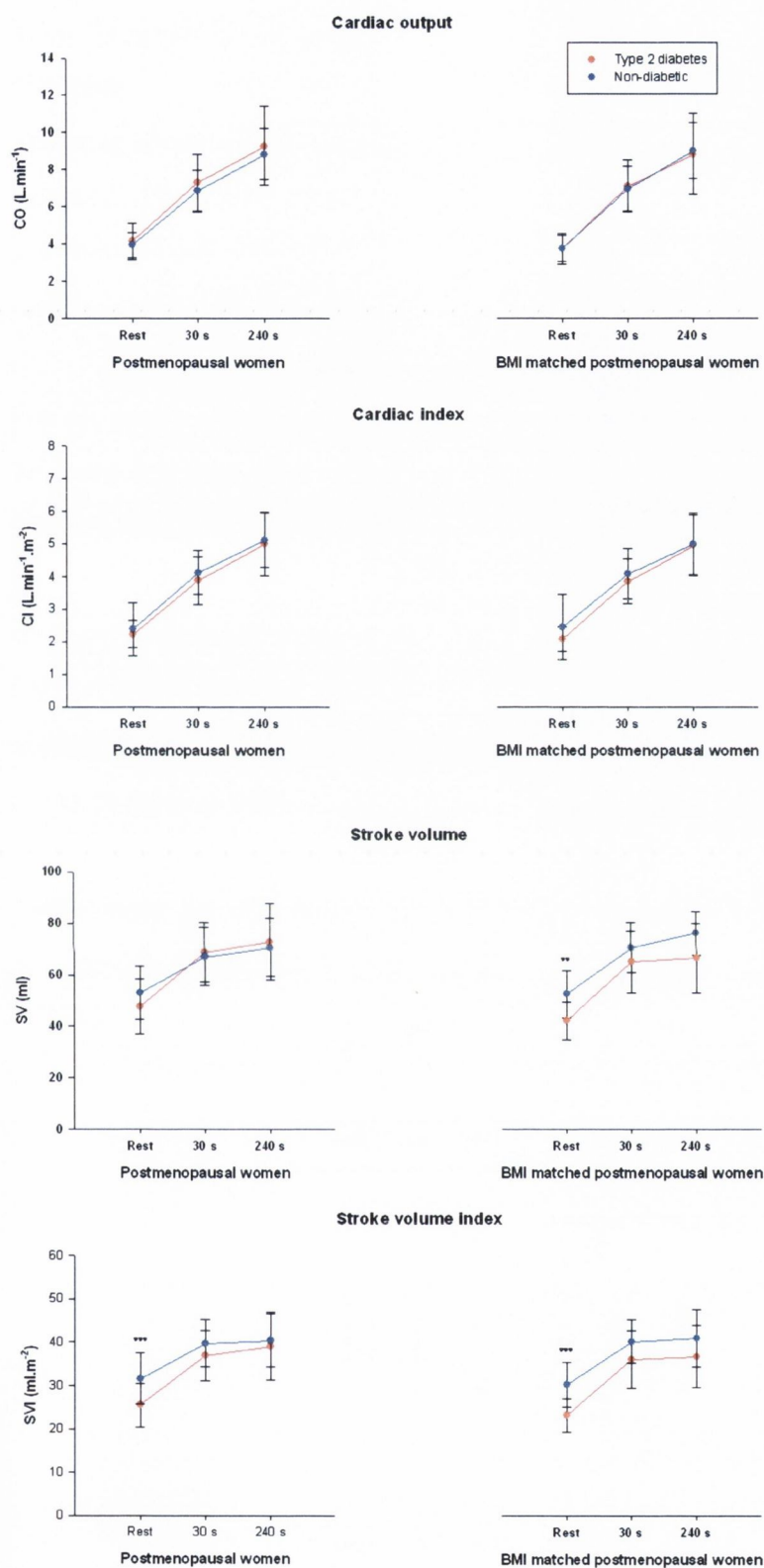


Fig. 3.5. Mean cardiovascular responses for women with and without T2DM. Significantly different from non-diabetic *** $P<0.0001$.

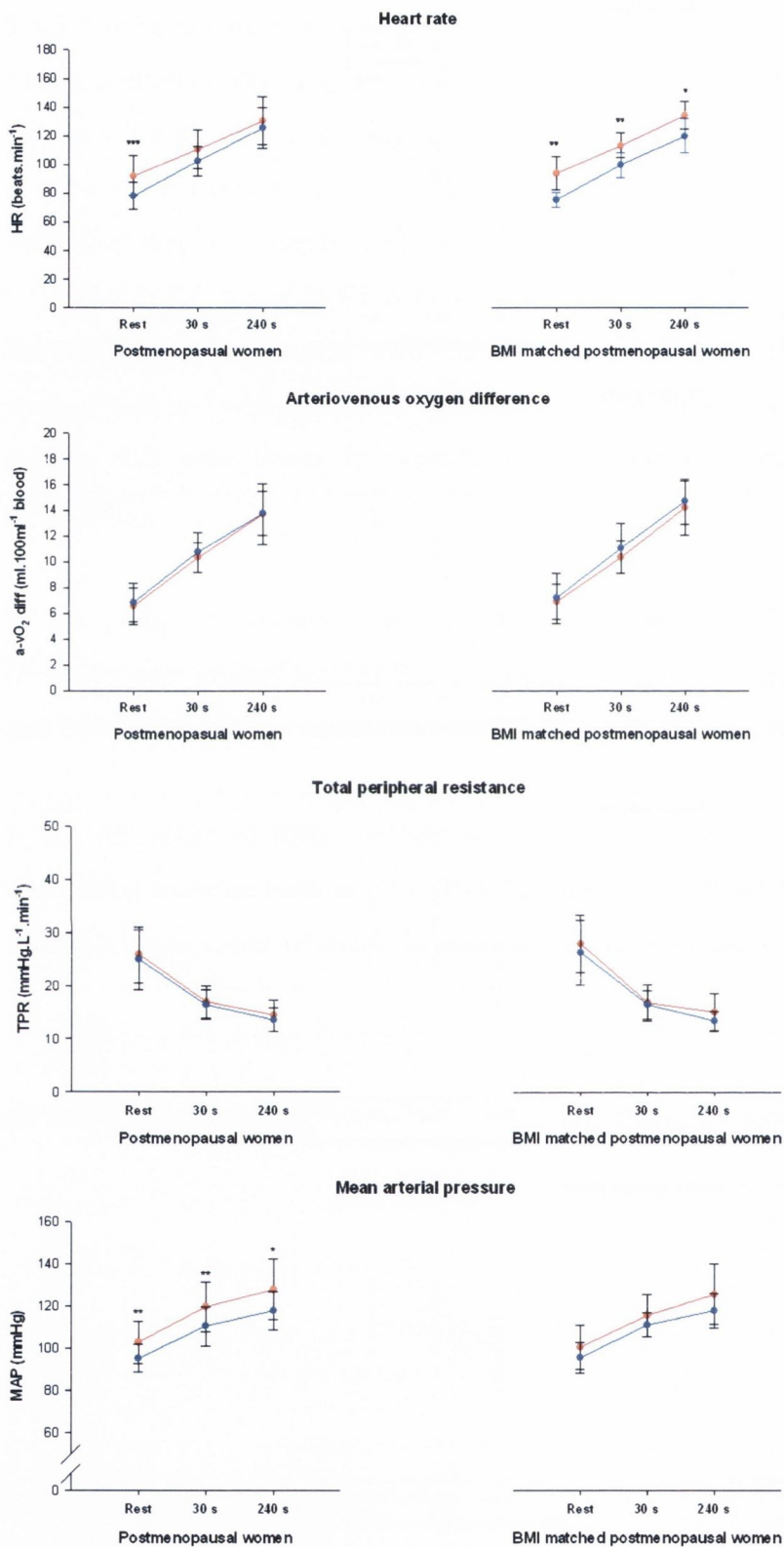


Fig. 3.6. Mean HR, a-vO₂ diff, TPR, and MAP responses for women with and without T2DM. Significantly different from non-diabetic * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

3.4.6 Magnitude of increase in cardiovascular responses between rest and exercise

The magnitude of increase in CO, CI, and a-vO₂ diff between rest and 30 s and between 30 s and 240 s were similar in the combined groups of pre and postmenopausal women with and without T2DM (Fig.s 3.5 and 3.6). Individual results can be seen in Appendix 13.

For all group comparisons, the magnitude of increase in CO, CI, and a-vO₂ diff between rest and 30 s and between 30 s and 240 s was not different between women with and without T2DM.

In postmenopausal women, the magnitude of increase in both SV ($P<0.01$) and SVI ($P<0.05$) between rest and 30 s was greater in women with T2DM than non-diabetic women while the magnitude of increase in HR between rest and 30 s was lower in women with T2DM than non-diabetic women ($P<0.01$).

There were no differences in the magnitude of increase in SV, SVI, or HR between the subgroups of BMI matched postmenopausal women with and without T2DM.

3.4.7 Rates of adaptation of cardiovascular responses from rest to steady state exercise

The rates of adaptation of CO, CI, and a-vO₂ diff from rest to steady state exercise (% steady state) in women with and without T2DM are presented in Table 3.6. Individual results can be seen in Appendix 13.

There were no differences between the groups of postmenopausal women with and without T2DM, and the subgroup of BMI matched postmenopausal women with and without T2DM in % steady state CO, CI, or a-vO₂ diff.

Table 3.6. Rate of increase in CO, CI, and a-vO₂ diff

	T2DM (n = 20)	Non- diabetic (n = 21)	T2DM (n = 12)	Non- diabetic (n = 9)
% Steady state	Postmenopausal		BMI matched postmenopausal	
CO	66.1±43.2	59.4±13.7	74.1±50.0	60.8±13.9
CI	61.7±26.0	80.0±106.0	64.9±24.6	92.0±140.3
a-vO ₂ diff	52.4±27.5	58.9±18.4	52.4±27.5	58.9±13.4

3.5 Discussion

The principal findings of the present study are that for all group comparisons, maximal aerobic capacity was reduced in women with uncomplicated T2DM in comparison to non-diabetic women. Secondly, a slower rate of adjustment of pulmonary $\dot{V}O_2$ (longer MRT) was also evident during moderate intensity constant load cycle exercise in women with T2DM. Thirdly, the simultaneous measurements of CO during exercise onset (30 s) and steady-state moderate exercise (240 s) or the magnitude of increase in CO from 30 s to 240 s (closely associated with phase 2 of the $\dot{V}O_2$ kinetic response) were not different between women with and without T2DM. These findings suggest that the impaired $\dot{V}O_2$ kinetic responses observed in women with T2DM are not related to cardiac dysfunction.

Peak cycle performance

Previous investigations have reported reduced $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) in individuals with T2DM in comparison to age, weight, and activity matched non-diabetics, the magnitude of which is approximately 20% (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Baldi *et al.*, 2003). Consistent with previous findings, the results of the present study indicate that for postmenopausal women (not matched by BMI), $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) is reduced (approximately 20%) in women with T2DM in comparison to non-diabetic women of a similar age and activity level. While $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) was lower in BMI matched postmenopausal women with T2DM than non-diabetic controls, the difference in $\dot{V}O_{2\text{ peak}}$ between women with and without T2DM was somewhat lower (approximately 12%) than that reported previously in age and BMI matched women (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Baldi *et al.*, 2003).

Consistent with the findings of a previous investigation (Mac Ananey *et al.*, 2011a), $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT was lower in postmenopausal women with T2DM (not matched by BMI) than non-diabetic controls. It could be argued that the lower workload at VT in both the group of postmenopausal women with T2DM in comparison to non-diabetic women could have contributed to the

reduced $\dot{V}O_2$ at VT in these women. Despite a reduced workload at VT in BMI matched women with T2DM in comparison to non-diabetic women, $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT was comparable between both groups of women. In agreement with our findings in relation to BMI matched postmenopausal women, Regensteiner *et al.*, (1998) failed to find any differences in $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT in premenopausal women with T2DM and lean & overweight controls. In addition, in the present study, the $\dot{V}O_2$ at VT relative to $\% \dot{V}O_{2 \text{ peak}}$ was not different between any of the groups of women with and without T2DM (approximately 76% in T2DM and 73% in non-diabetics); and while these values might seem high in these population groups, they are similar to those reported in a number of studies (approximately 67-78% in T2DM) (Regensteiner *et al.*, 1998; Mac Ananey *et al.*, 2011a; Sales *et al.*, 2011) while employing the V-slope method and using similar increments as in the present study.

$\dot{V}O_2$ kinetics

In the present study, a slowing in the adjustment of the $\dot{V}O_2$ response (longer MRT) was evident in women with T2DM during cycle exercise below VT and was associated with a significantly longer time constant of phase 2 of the $\dot{V}O_2$ response. This is indicative of a diabetes-induced impairment in the efficiency of the $\dot{V}O_2$ response to exercise and is associated with a faster onset of fatigue (Hughson & Tschakovsky, 1999). This finding has been previously observed during moderate intensity cycle exercise in premenopausal women, combined groups of pre and postmenopausal women, and combined groups of men and women with T2DM (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a) but not in older men with T2DM (Wilkerson *et al.*, 2011).

Successful modelling of a physiological system allows for a more accurate prediction of the integrated response of the physiological system being studied in response to a particular stimuli (Lamarra, 1990). Intrinsic to this approach is identification of the number of distinct phases of the response as well as the parameters that define each of the phases (Tschakovsky *et al.*, 2006). Therefore, to enhance the physiological response and to enable more accurate estimates of

the parameters defining each phase, a bi-exponential function was used to fit the $\dot{V}O_2$ data in the current study. This approach has been used elsewhere in both cycling and knee extension exercise (MacDonald *et al.*, 1998; Bauer *et al.*, 2007).

In agreement with the findings of prior investigations (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a) where slowed $\dot{V}O_2$ kinetic responses have been reported in premenopausal women, combined groups of pre and postmenopausal women, and combined groups of men and women with T2DM, both the time constant of the second phase (τ_2) and MRT were slowed in women with T2DM in the current investigation. However, one former investigation failed to find any differences in $\dot{V}O_2$ kinetic responses between older men with and without T2DM (Wilkerson *et al.*, 2011). It is worth noting that in the former investigation (Wilkerson *et al.*, 2011), the rate of adaptation in the $\dot{V}O_2$ kinetic response in non-diabetic men was somewhat slower (41 s) than that of younger non-diabetic individuals in prior investigations (approximately 28–34 s) (Regensteiner *et al.*, 1998; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a); which could be indicative of an age-related slowing in the $\dot{V}O_2$ kinetic response in non-diabetic men in the study by Wilkerson *et al.*, (2011).

However, previous investigations have used groups of participants including a combination of male and female subjects (Bauer *et al.*, 2007), premenopausal women only (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999) or a combination of pre and postmenopausal women (Mac Ananey *et al.*, 2011a). The present study is the first to report a slowed rate of adaptation of the $\dot{V}O_2$ kinetic response at exercise onset in a group comprising only of postmenopausal women with T2DM.

It has been well documented that oestrogen exerts protective effects on vascular function in premenopausal women in comparison to postmenopausal women and older men (Celermajer *et al.*, 1994; Sorensen *et al.*, 1995; Perregaux *et al.*, 1999). However, the beneficial effects that oestrogen exerts on vascular function are diminished if not abolished in the presence of T2DM, as impaired endothelium-dependent vasodilation has been reported in premenopausal women with T2DM (Steinberg *et al.*, 2000), a finding that is consistent with the impaired $\dot{V}O_2$ kinetic

responses in premenopausal women (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999). However, as we also observed impaired $\dot{V}O_2$ kinetic responses in groups of postmenopausal and BMI matched postmenopausal women with T2DM compared with non-diabetic controls, this indicates a diabetes-associated defect in rate of adjustment in the $\dot{V}O_2$ kinetic response in women in which the protective effects of oestrogen are not present.

As the second phase is reflective of oxygen consumption by the exercising muscle, the results indicate that diabetes contributes to an impaired muscle $\dot{V}O_2$ response during the initial stages of exercise. The impaired sub-maximal exercise performance observed in women with T2DM in the current study is clinically relevant as it suggests individuals with T2DM will encounter difficulties carrying out routine activities and may explain why women with T2DM perceive exercise to be harder than non-diabetic women (Huebschmann *et al.*, 2009). Furthermore, this exercise intolerance apparent in individuals with T2DM may discourage them from becoming involved in regular exercise even at low and moderate intensities.

Mechanisms underpinning the slowed $\dot{V}O_2$ kinetics

In healthy individuals, the control of $\dot{V}O_2$ is dependent on both oxygen delivery (which is in turn dependent on CO and blood flow) and the capacity to extract and utilise oxygen at the level of the contracting myocyte. As diastolic dysfunction (measured at rest) is common in T2DM (Poirier *et al.*, 2001; Baldi *et al.*, 2006) and has previously been linked with impaired exercise performance in individuals with T2DM (Poirier *et al.*, 2000; Ha *et al.*, 2008), it is not unreasonable to suggest that central abnormalities may underline the impaired oxygen uptake responses in T2DM individuals during exercise observed elsewhere (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a).

In an effort to address if the impaired of the slowed $\dot{V}O_2$ kinetic responses in individuals with T2DM are related to the impaired pumping capacity of the heart, CO was measured at rest and during exercise (30 s and 240 s). The 30-s measurement was designed to correspond with the onset of phase 2 while the 240-s measurement corresponded to steady state. CO responses were not different

either at rest or during exercise between women with and without T2DM for all group comparisons. Our results are in agreement with those of previous investigations, where similarities have been reported in CO responses during exercise (30 and 240 s) at low and moderate intensities (Mac Ananey *et al.*, 2011a) and during steady state sub-maximal exercise (Baldi *et al.*, 2003) between individuals with T2DM and non-diabetic controls.

Furthermore, both the magnitude of increase in CO (Δ CO 30 s to 240 s) and the rate of increase in CO during the initial period of exercise (t=30 s) in relation to the steady state levels of exercise (t=240 s) were comparable in women with and without T2DM. In fact, others have reported a greater change in CO from 30 s to 240 s in women with T2DM in comparison to lean and overweight non-diabetics at 80% VT (Mac Ananey *et al.*, 2011a). As this period (from 30 to 240 s) is closely aligned to phase 2 of the $\dot{V}O_2$ kinetic response, it appears that the slowed $\dot{V}O_2$ kinetic responses observed in women with T2DM in the current study are not due to a slowed rate of increase in CO.

In contrast, others have reported that cardiac reserve, which is a measure of the capacity to maximally increase CO during exercise is reduced in individuals with T2DM (Joshi *et al.*, 2010). In addition, pulmonary capillary wedge pressure (PCWP) was greater in individuals with T2DM during peak exercise despite the absence of cardiac abnormalities at rest and similar peak CO responses (Regensteiner *et al.*, 2009). As such, these results by Regensteiner *et al.*, (2009) may indicate the presence of subclinical cardiac dysfunction in the former study, which may precede the cardiac abnormalities observed at rest and during exercise in longer established T2DM.

Chronic hyperglycaemia affects myocardial structure through various mechanisms that leads to altered gene expression and protein function and ultimately results in abnormal blood flow, contractility, and cardiomyopathy (Sheetz & King, 2002). Previous research indicates that left LVDD limits exercise performance in normotensive subjects with T2DM that could indicate a reduction in preload due to reduced left ventricular compliance (Poirier *et al.*, 2000). Others have

suggested that a reduced blood volume and higher heart rate may affect the lower end-diastolic volume (EDV) and SV in men with T2DM (Lalande *et al.*, 2010). In the present study, in the groups of BMI matched postmenopausal women, resting SV was lower in women with T2DM than non-diabetic controls. Furthermore, resting SVI was lower in both groups with T2DM and due to the compensatory increase in resting heart rate, CI responses between diabetics and non-diabetics were similar in both the groups of postmenopausal women and BMI matched postmenopausal women. The lower resting SVI could be an indication of a reduced EDV and as many individuals with T2DM have diastolic dysfunction and pseudo normal filling patterns (Baldi *et al.*, 2006), it is therefore possible that some element of myocardial dysfunction exists among the women with T2DM in the current study.

Alternatively, properties within skeletal muscle may affect $\dot{V}O_2$ at sub-maximal exercise intensities. Studies have shown that individuals with T2DM have a higher type IIb to type I fibre ratio and a reduced oxidative enzyme activity (Saltin *et al.*, 1979; Simoneau & Kelley, 1997), reduced capillary density (Larsen *et al.*, 2009), and lower mitochondrial content (Kelley *et al.*, 2002). A decrease in the oxidative capacity of skeletal muscle in T2DM could lead to a reduction in oxygen extraction from the capillary to the myocyte and therefore lead to reductions in the amount of oxygen consumed by contracting muscles during exercise. This is consistent with the finding of a reduced a- $\dot{V}O_2$ diff in individuals with T2DM during peak exercise but not sub-maximal exercise (Baldi *et al.*, 2003). However, a limitation exists with the former study as peak a- $\dot{V}O_2$ diff was estimated rather than measured by the Fick equation using $\dot{V}O_2$ and CO where sub-maximal SV and peak HR were used to determine CO (Baldi *et al.*, 2003). Furthermore, as only two individuals achieved their age-predicted maximum heart rate (Baldi *et al.*, 2003) it is possible that the CO responses at peak exercise could be underestimated, which would result in inaccurate estimations of a- $\dot{V}O_2$ diff.

In the current investigation, oxygen extraction during exercise was not different between women with and without T2DM. In addition, the magnitude of change in a- $\dot{V}O_2$ diff between rest to 30 s and from 30 s to 240 s was comparable between all

groups with and without T2DM. Our findings are supported by those of Mac Ananey *et al.*, (2011a) who reported similarities in a-vO₂ diff between women with T2DM and non-diabetic lean and overweight controls during exercise (30 s and 240 s) above or below VT. The transient overshoot in muscle deoxygenation responses (reflecting tissue oxygen extraction) observed elsewhere in most subjects with T2DM above that required during steady state exercise (Bauer *et al.*, 2007), indicates a greater reliance on oxygen extraction during the initial 90-100 s of moderate intensity exercise. It would therefore appear that oxygen extraction is not a limiting step during the initial period of exercise and that the ‘overshoot’ observed in the former study (Bauer *et al.*, 2007) may be a compensatory mechanism to cope with the slowed rate of increase in blood flow at exercise onset. In the former study by Bauer *et al.*, (2007), microvascular blood flow kinetic responses (estimated using near-infrared spectroscopy) were slowed in individuals with T2DM, which suggests that impaired oxygen delivery may be in part responsible for the exercise intolerance in diabetic populations. This notion is consistent with the finding of the greater decline in microvascular P_{O_2} across the rest-exercise transition in diabetic rats (Behnke *et al.*, 2002). Furthermore, recent evidence indicates that vascular conductance kinetic responses during calf exercise are impaired in women with T2DM (Mac Ananey *et al.*, 2011a).

In support of a diabetes-associated limitation in oxygen delivery, we found a slowed rate of increase in heart rate kinetic responses in women with T2DM. However, the mechanisms responsible for the increase in heart rate during exercise (increased sympathetic input, reduced parasympathetic input, and increased hormonal influence - epinephrine) are different to those that regulate blood flow (mechanical compression of resistance vessels, interaction of neural vasoconstrictor activity, and vasodilation), so caution needs to be applied when interpreting these results. Slowed heart rate kinetic responses have previously been reported in premenopausal women with T2DM (Regensteiner *et al.*, 1998) but not combined groups of pre and postmenopausal women with T2DM (Mac Ananey *et al.*, 2011a). The disparities between the current study and that of Mac Ananey *et al.*, (2011a) might be explained by the fact that most individuals with T2DM in the current study were taking anti-hypertensive medication whereas all participants in the study by Mac Ananey *et al.*, (2011a) were normotensive.

While none of the women in the study by Regensteiner *et al.*, (1998) were taking anti-hypertensive medication, the HbA_{1C} levels suggests a more advanced form of T2DM and might explain the slowed rate of increase in heart rate responses in these women. Further evidence for impairment in oxygen delivery to the musculature in the present study comes from the presence of a diabetes effect on MAP as MAP responses were greater in postmenopausal women with T2DM (not matched by BMI) both at rest and during exercise at 30 and 240 s.

Limitations

Due to the limited temporal resolution of the inert gas rebreathing technique, we were unable to continuously measure CO responses during exercise. Continuous measurement of CO during the initial period of exercise could provide valuable insight as to the possible mechanisms associated with the slowed $\dot{V}O_2$ kinetic responses observed in women with T2DM.

While some women with T2DM were taking β -blockers ($n=3$), the overall number is small and as such is unlikely to have affected the results. Furthermore, $\dot{V}O_2$ kinetic responses were slowed in the subgroup of BMI matched postmenopausal women with T2DM, yet this analysis did not include women who were taking β -blockers. As the cut-off points for both systolic and diastolic blood pressure in the initial screening were greater for women with T2DM than non-diabetics, this is likely to explain the greater MAP responses in postmenopausal women with T2DM (not matched by BMI) both at rest and during exercise. While the cut-off point for serum creatinine in the initial screening at baseline were greater for women with T2DM than non-diabetics, all individuals with T2DM were screened medically and absent of any manifestation of nephropathy. The cut-off point in relation to serum creatinine screening represent the outside limits and is not reflective of the serum creatinine concentrations of individuals with T2DM in the current investigation. The majority of women with T2DM were receiving lipid-lowering therapy in contrast to one non-diabetic woman, which is likely to have contributed lower total cholesterol levels in women with T2DM.

Conclusions

In the absence of any cardiac abnormalities and similarities in oxygen extraction, it is probable that the slowed rate of adaptation of $\dot{V}O_2$ during the initial period of exercise is associated with a maldistribution and/or slowed rate of adaptation in blood flow. To further explore this concept, LVC kinetic responses were studied during low and high intensity plantar flexion exercise. This will be discussed in the next section.

Chapter 4: Effect of T2DM on the dynamic response characteristics of LVC in postmenopausal women during acute exercise

4.1 Aims

- (a) To investigate if LVC kinetic responses are slowed in postmenopausal women with T2DM matched in age but not BMI
- (b) To investigate if the findings observed in postmenopausal women persist in BMI and age matched women with T2DM
- (c) To investigate both the vasodilatory and hyperaemic responses during incremental supine plantar flexion exercise in all group comparisons; and
- (d) To investigate if forearm reactive hyperaemic responses are impaired in women with T2DM

4.2 Experimental protocol overview

Subjects were required to visit the Cardiovascular Laboratory in the Department of Physiology, Trinity College Dublin on one occasion. Following a familiarisation period with the calf ergometer, subjects completed 5 MVC's of the calf muscle. In addition, a subgroup of 12 subjects (T2DM $n=7$; non-diabetic $n=5$) completed a further 2 bouts plantar flexion exercise at 70% MVC. This was followed by 3 bouts of plantar flexion exercise at 30% MVC. Subjects then completed an incremental plantar flexion exercise test to failure. Following the incremental test, reactive hyperaemia of the forearm was performed to assess endothelial function.

4.3 Subjects

A total of forty-four postmenopausal women were recruited to participate in the study: 22 with T2DM and 22 healthy controls. All women were classified as sedentary ($< 1 \text{ h} \cdot \text{wk}^{-1}$ of moderate intensity exercise for at least 6 months prior to enrolment), which was confirmed by physical activity scores using the Low Level Physical Activity Recall (LOPAR; Appendix 1) questionnaire (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999).

4.3.1 Medications

Of the 22 women with T2DM, 5 were undergoing dietary intervention and 17 were receiving oral hypoglycaemic agents (metformin n=13; metformin & gliclazide n=3; gliclazide n=1). In addition, 11 diabetic women were on anti-hypertensive medication (lisinopril n=2; ramipril n=1; candesartan cilexetil n=1; perindopril arginine n=2; telmisartan n=1; angiotensin II antagonists (losartan n=1); ACE inhibitors (ramapril n=1); calcium channel blocker (amlodipine n=1); perindopril arginine & amlodipine n=1). A further 3 women with T2DM were taking β -blockers (metoprolol tartrate n=1; sotalol n=1; bisoprolol fumarate n=1). None of the non-diabetic women were receiving anti-hypertensive medication. A further 12 diabetic women and 1 non-diabetic woman were receiving lipid-lowering therapy (T2DM: rosuvastatin n=3; atorvastatin n=7; simvastatin n=1; pravastatin n=1 and non-diabetic: atorvastatin n=1). A total of three women were undergoing hormone replacement therapy (T2DM: n=1, estradiol; non-diabetic: n=2, raloxifene).

4.4 Results

As for the previous experimental chapter, the results for women with and without T2DM are presented in two sections: initially including subgroups of postmenopausal women, and then including subgroups of BMI matched postmenopausal women.

4.4.1 Subjects

4.4.1.1 Physical characteristics

Physical characteristics and physical activity levels in women with and without T2DM are presented in Table 4.1. Individual results can be seen in Appendix 14. There were no differences between groups of postmenopausal women with and without T2DM with respect to age, height, ABI, or physical activity levels. BMI ($P<0.0001$), body mass ($P<0.0001$), and WHR ($P<0.05$) were greater in women with T2DM than non-diabetics.

For BMI matched postmenopausal women, there were no differences between women with and without T2DM with respect to physical characteristics or physical activity levels.

Table 4.1. Physical characteristics and activity levels

	T2DM (n = 22)	Non-diabetic (n = 22)	T2DM (n =12)	Non-diabetic (n = 10)
	Postmenopausal		BMI matched postmenopausal	
Age (yr)	57.1±4.2	56.1±5.4	57.2±4.8	56.2±3.4
BMI (kg.m ⁻²)	34.6±5.5***	26.4±3.9	30.8±2.9	30.2±2.3
Weight (kg)	86.8±13.8***	67.3±11.2	78.6±9.5	77.3±8.2
Height (m)	1.58±0.05	1.59±0.06	1.60±0.05	1.60±0.03
WHR	0.96±0.05*	0.90±0.08	0.94±0.05	0.91±0.07
ABI	1.08±0.10	1.09±0.10	1.04±0.1	1.10±0.09
LOPAR (MET h.wk ⁻¹)	104±46	111±48	94±43	119±58

Significantly different from non-diabetic * $P<0.05$, *** $P<0.0001$.

4.4.1.2 Haematology

Glycosylated haemoglobin (HbA_{1C}), fasting plasma glucose (FPG), total cholesterol (TCL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides in women with and without T2DM are presented in Table 4.2. Individual results can be seen in Appendix 15.

In the combined groups of postmenopausal women, both HbA_{1C} (expressed as a percentage and in mmol.mol⁻¹) and FPG were greater in women with T2DM than non-diabetic women (*P*<0.0001). In the subgroups of BMI matched postmenopausal women, both HbA_{1C} (expressed as a percentage and in mmol.mol⁻¹) (*P*<0.01) and FPG were greater in women with T2DM than their non-diabetic counterparts (*P*<0.0001).

In postmenopausal women, TCL (*P*<0.0001), HDL (*P*<0.0001), and LDL (*P*<0.05) were lower and triglycerides (*P*<0.0001) were greater in women with T2DM than non-diabetic women. In BMI matched postmenopausal women, TCL (*P*<0.01), HDL and LDL (*P*<0.05) were lower in women with T2DM than non-diabetic women.

Table 4.2. Haematology

	T2DM	Non-diabetic	T2DM	Non-diabetic
	Postmenopausal		BMI matched postmenopausal	
HbA _{1C} (%)	6.7±1.0***	5.5±0.2	6.7±0.7**	5.5±0.1
HbA _{1C} (mmol.mol ⁻¹)	50.2±10.9***	36.4±2.3	49.4±7.8**	36.7±1.7
FPG (mmol.L ⁻¹)	8.3±2.5***	4.6±0.6	8.6±2.3***	4.7±0.5
TCL (mmol.L ⁻¹)	4.5±0.8***	5.8±1.4	4.7±0.8**	6.1±1.1
HDL (mmol.L ⁻¹)	1.3±0.3***	1.9±0.4	1.4±0.2*	2.1±0.4
LDL (mmol.L ⁻¹)	2.9±0.7*	3.7±1.2	3.0±0.7*	4.0±1.0
Triglycerides (mmol.L ⁻¹)	1.6±0.6***	1.0±0.4	1.6±0.6	1.0±0.4

Significantly different from non-diabetic **P*<0.05, ***P*<0.01, ****P*<0.0001.

4.4.2 Maximum voluntary contraction (MVC)

Calf MVC results in women with and without T2DM can be seen in Fig. 4.1 and individual results can be seen in Appendix 16. There were no differences in MVC between groups of postmenopausal women with and without T2DM, or the subgroups of BMI matched postmenopausal women with and without T2DM.

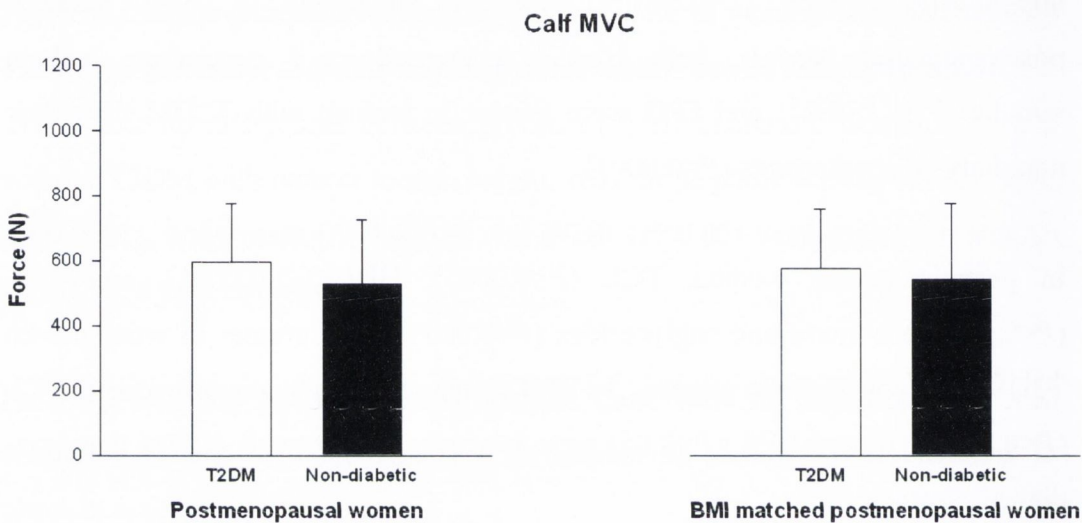


Fig. 4.1. Calf MVC responses

4.4.3 Incremental plantar flexion exercise

4.4.3.1 Resting calf LBF and LVC prior to incremental plantar flexion exercise
Resting LBF, LVC, and leg volumes in women with and without T2DM can be seen in Table 4.3 and individual results can be seen in Appendix 17.

Similarities were observed between the subgroup of postmenopausal women with and without T2DM for resting LVC and resting LBF. Both resting LBF and resting LVC were lower in the subgroup of BMI matched postmenopausal women with T2DM in comparison to their non-diabetic counterparts ($P<0.05$). Leg volume was greater in both the group of postmenopausal women with T2DM than non-diabetic women ($P<0.05$), while in contrast, leg volume was not different between the subgroup of BMI matched postmenopausal women with and without T2DM.

Table 4.3. Resting LBF, LVC, and leg volumes

	T2DM (n = 22)	Non-diabetic (n = 22)	T2DM (n =12)	Non-diabetic (n = 10)
	Postmenopausal		BMI matched postmenopausal	
Resting LBF (ml.min ⁻¹)	53.6±26.9	53.3±20.3	40.9±9.7*	62.0±24.4
Resting LVC (ml.min ⁻¹ .mmHg ⁻¹)	0.54±0.25	0.56±0.27	0.40±0.08*	0.62±0.29
Leg volume (ml)	2789±507*	2418±441	2520±330	2625±372

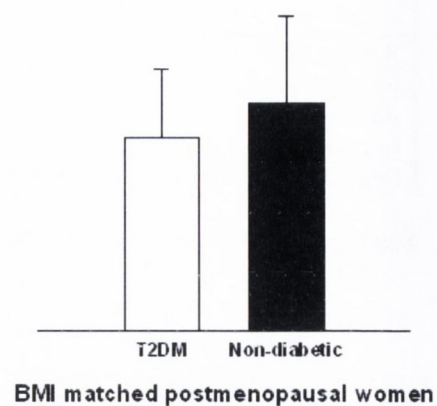
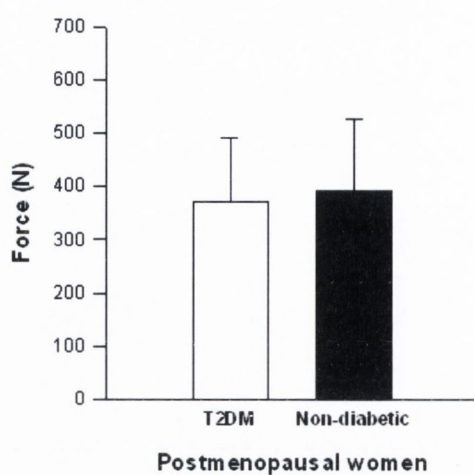
Significantly different from non-diabetic **P*<0.05.

4.4.3.2 Peak calf performance during incremental plantar flexion exercise

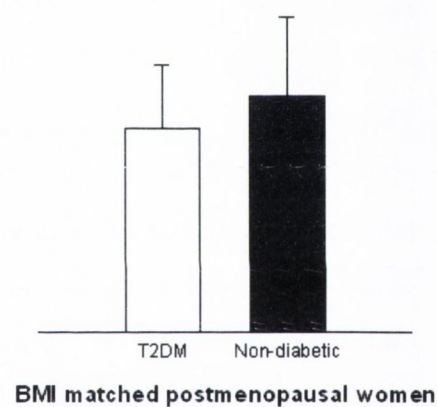
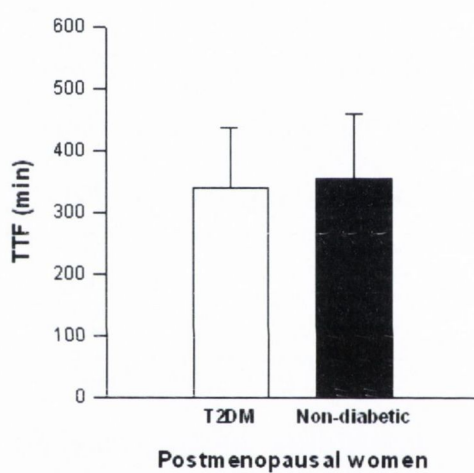
Peak calf performance during incremental plantar flexion exercise in women with and without T2DM can be seen in Fig. 4.2. Individual results can be seen in Appendix 16.

There were no differences in peak force or time to failure (TTF) between the group of postmenopausal women or the subgroup of BMI matched postmenopausal women with and without T2DM. In contrast, peak force/MVC was lower in the group of postmenopausal women and the subgroup of BMI matched postmenopausal women with T2DM than non-diabetic women (*P*<0.05).

Peak force



Time to failure



Peak force/MVC

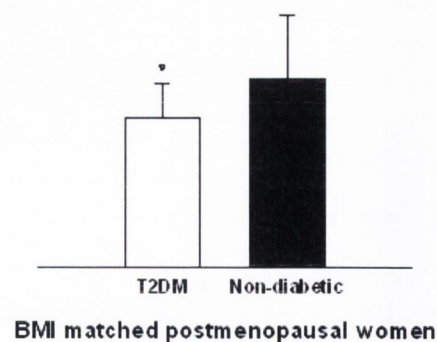
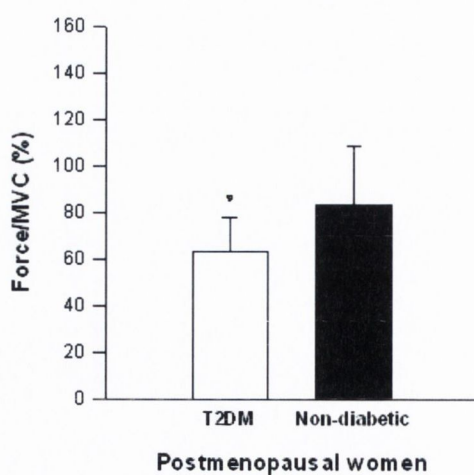


Fig. 4.2. Peak force, time to failure, and peak force/MVC. Significantly different from non-diabetic * $P < 0.05$.

4.4.3.3 LVC and LBF during incremental plantar flexion exercise

LBF and LVC responses during incremental plantar flexion exercise in women with and without T2DM can be seen in Fig. 4.3. Individual results can be seen in Appendix 17.

Peak LVC and LBF responses were not different between the groups of postmenopausal women with and without T2DM or between the subgroups of BMI matched postmenopausal women with and without T2DM.

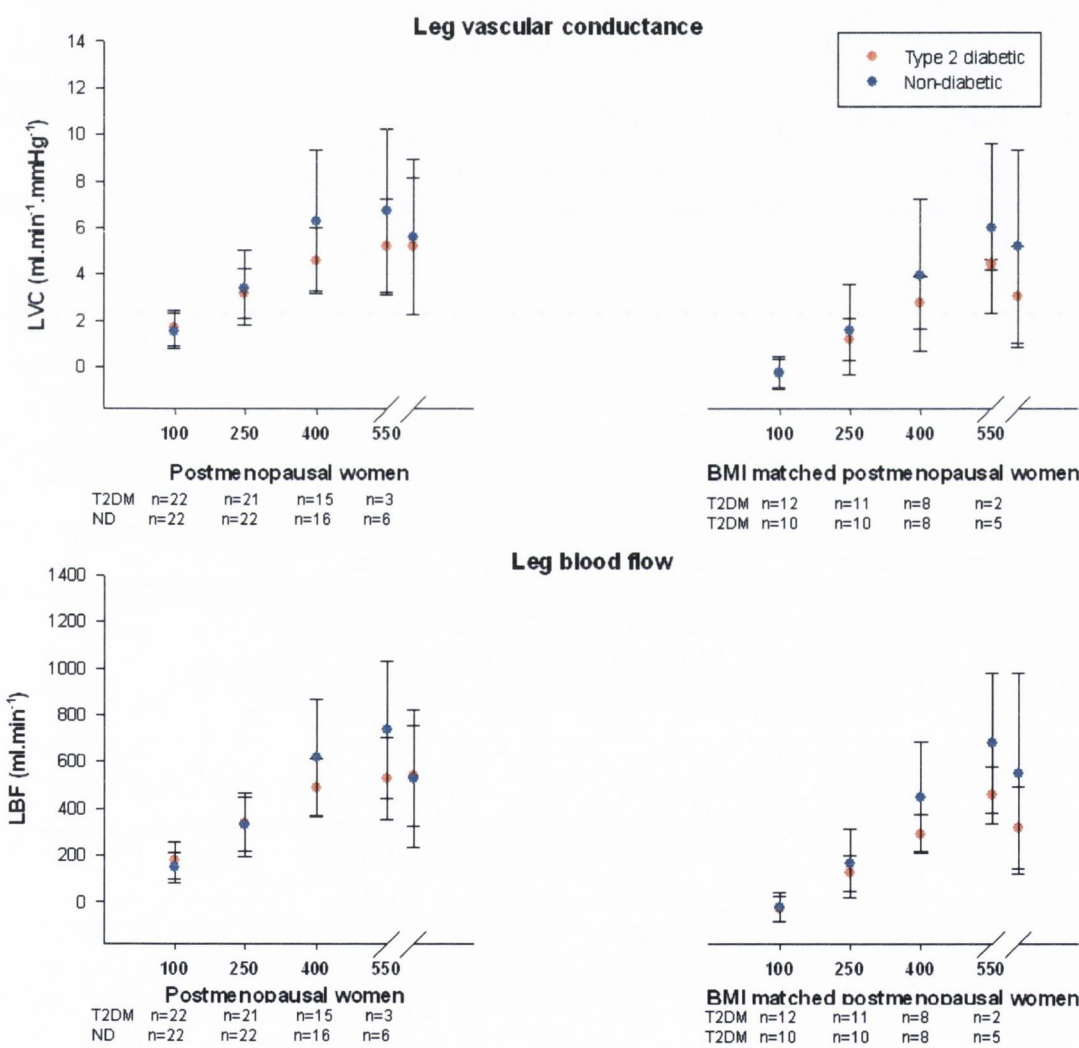


Fig. 4.3. LVC and LBF responses during incremental plantar flexion exercise.

4.4.3.4 Hyperaemic and vasodilatory responses during incremental plantar flexion exercise

The hyperaemic (slope of LBF (ml.min^{-1}) vs. % of maximum force and slope of LBF (ml.min^{-1}) vs. force (N)) and vasodilatory (slope of LVC ($\text{ml.min}^{-1}.\text{mmHg}^{-1}$) vs. % of maximum force and slope of LVC ($\text{ml.min}^{-1}.\text{mmHg}^{-1}$) vs. force (N)) responses during incremental plantar flexion exercise in women with and without T2DM are shown in Table 3.4. Results for each individual can be seen in Appendix 17.

There were no differences in the hyperaemic (slope of LBF (ml.min^{-1}) vs. % of maximum force) or vasodilatory responses (slope of LVC ($\text{ml.min}^{-1}.\text{mmHg}^{-1}$) vs. % of maximum force) between postmenopausal women with and without T2DM or between BMI matched postmenopausal women with and without T2DM.

Table 4.4. LVC and LBF slopes expressed as a percentage of the maximum force (% max) and absolute force (N)

	T2DM (n = 22)	Non-diabetic (n = 22)	T2DM (n = 12)	Non-diabetic (n = 10)
	Postmenopausal		BMI matched postmenopausal	
Slope LBF (ml.min^{-1}) vs. % max force	376±201	517±306	336±158	570±332
Slope LVC ($\text{ml.min}^{-1}.\text{mmHg}^{-1}$) vs. % max force	3.46±1.9	5.11±3.2	3.19±1.5	5.0±3.4
Slope LBF (ml.min^{-1}) vs. force (N)	0.96±0.38	1.43±1.15	0.86±0.23	1.27±0.54
Slope LVC ($\text{ml.min}^{-1}.\text{mmHg}^{-1}$) vs. force (N)	0.008±0.003	0.013±0.008	0.008±0.003	0.012±0.009

4.4.4 LVC kinetics responses during plantar flexion exercise at 30% MVC and 70% MVC

4.4.4.1 LVC kinetics responses during plantar flexion exercise at 30% MVC

Mean LVC kinetic responses during plantar flexion exercise at 30% MVC can be seen in Table 4.5. Representative LVC kinetic responses from women with and without T2DM as well as the predicted responses from the tri-exponential curve fits are depicted in Fig. 4.4. Individual results can be seen in Appendix 18.

There were no differences in the time constant of the second growth phase (τ_3), or overall End A between the group of postmenopausal women with and without T2DM or the subgroup of BMI matched postmenopausal women with and without T2DM.

In the group of postmenopausal women, the time constant of the first growth phase (τ_1) was faster ($P<0.05$) and the time delay of the decay phase (TD_2) occurred earlier ($P<0.05$) in women with T2DM than non-diabetic controls. In BMI matched postmenopausal women, both TD_1 ($P<0.05$) and TD_2 ($P<0.0001$) occurred earlier in women with T2DM than non-diabetic women.

Table 4.5. Mean LVC kinetic responses during plantar flexion exercise at 30% MVC

	T2DM (n = 22)	Non-diabetic (n = 22)	T2DM (n =12)	Non-diabetic (n = 10)
	Postmenopausal		BMI matched postmenopausal	
A ₀ (ml.min ⁻¹ .mmHg ⁻¹)	0.58±0.23	0.63±0.42	0.47±0.14	0.76±0.58
A ₁ (ml.min ⁻¹ .mmHg ⁻¹)	2.31±1.20	1.71±1.21	2.19±1.19	1.52±1.31
TD ₁ (s)	1.1±1.0	1.5±0.8	1.0±0.9*	1.9±0.9
τ_1 (s)	3.3±2.3*	5.1±3.3	3.3±2.4	4.7±3.7
A ₂ (ml.min ⁻¹ .mmHg ⁻¹)	0.84±0.81	0.54±0.70	0.71±0.35	0.40±0.48
TD ₂ (s)	7.7±4.6*	12.6±9.6	7.8±5.4***	18.0±6.9
τ_2 (s)	8.1±7.1	8.4±8.9	6.3±2.3	9.9±12.7
A ₃ (ml.min ⁻¹ .mmHg ⁻¹)	1.06±0.78	0.88±0.69	0.89±0.56	0.71±0.47
TD ₃ (s)	20.2±6.0	23.3±10.3	20.4±6.1	27.0±10.2
τ_3 (s)	14.8±8.9	13.9±7.9	13.1±6.8	14.8±10.4
End A (ml.min ⁻¹ .mmHg ⁻¹)	3.97±1.80	3.21±1.70	3.48±1.59	3.00±1.70

Significantly different from non-diabetic * $P<0.05$, *** $P<0.0001$.

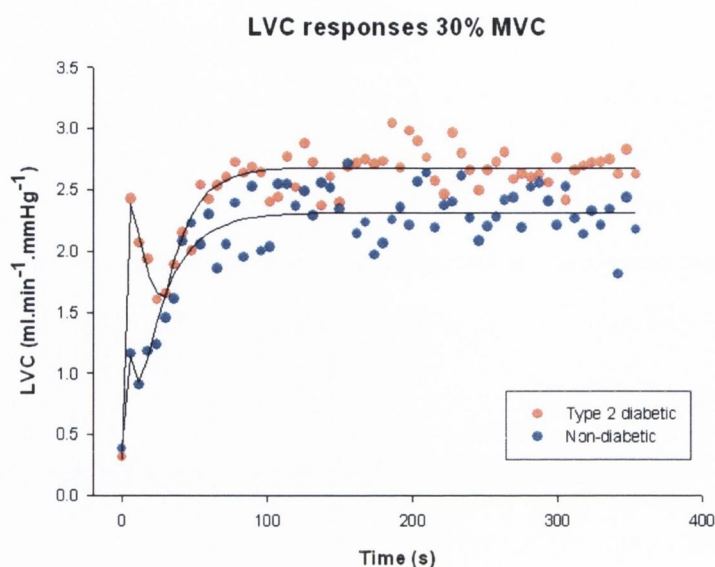


Fig. 4.4. LVC kinetic responses and tri-exponential curve fits during plantar flexion exercise at 30% MVC from representative subjects from postmenopausal women with and without T2DM.

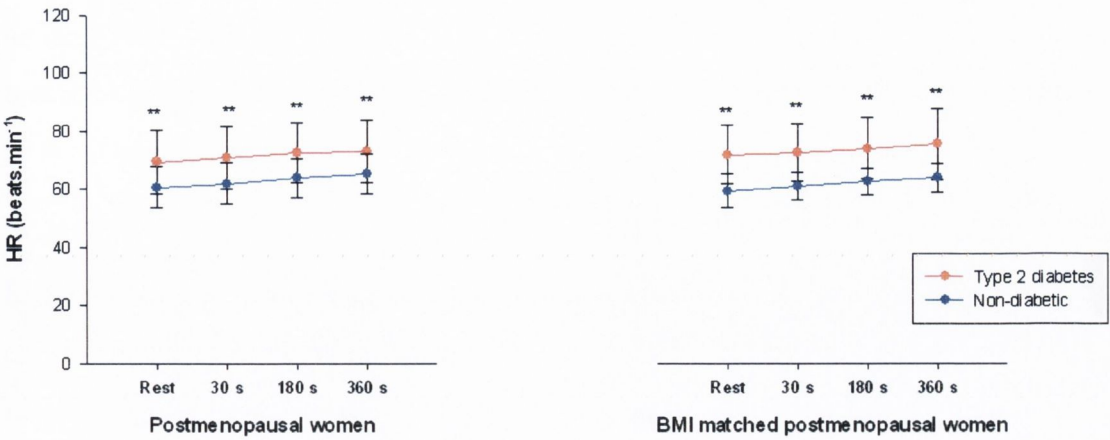
4.4.4.2 Heart rate and MAP responses during plantar flexion exercise at 30% MVC

Heart rate and MAP responses at rest and during plantar flexion exercise at 30% MVC in women with and without T2DM are presented in Fig. 4.5. Individual results can be seen in Appendix 19.

In the groups of postmenopausal women, heart rate responses were greater in women with T2DM than non-diabetic women both at rest ($P<0.01$) and during exercise at 30 s, 180 s, and 360 s ($P<0.01$). Resting MAP was greater in women with T2DM than non-diabetic women ($P<0.05$). In contrast, during exercise, MAP was not different between postmenopausal women with and without T2DM.

In the subgroup of BMI matched postmenopausal women, heart rate was greater in women with T2DM than non-diabetics both at rest and during exercise at 30 s, 180 s, and 360 s ($P<0.01$). MAP was similar in BMI matched postmenopausal women with and without T2DM both at rest and during exercise.

Heart rate responses 30% MVC



Mean arterial pressure responses 30% MVC

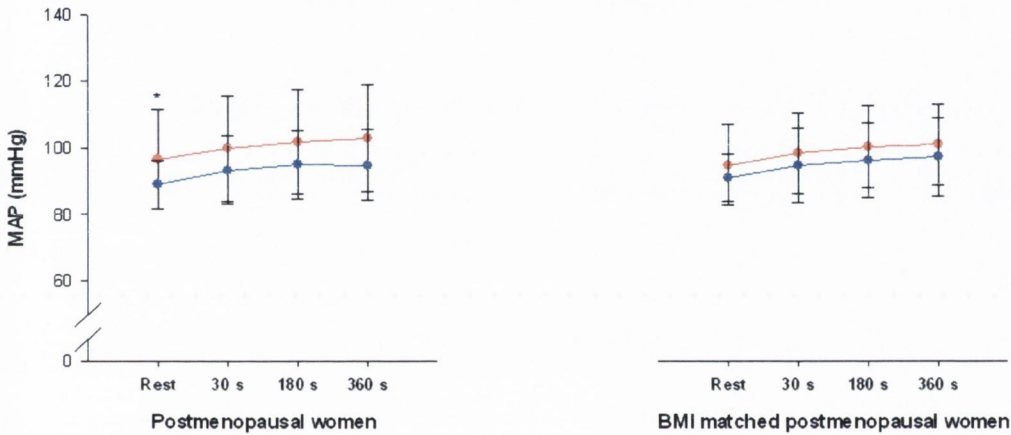


Fig. 4.5. HR and MAP responses during plantar flexion exercise at 30% MVC. Significantly different from non-diabetic * $P<0.05$, ** $P<0.01$.

4.4.4.3 LVC kinetics responses during plantar flexion exercise at 70% MVC

Mean LVC kinetic responses during plantar flexion exercise at 70% MVC can be seen in Table 4.6. Representative LVC kinetic responses from women with and without T2DM as well as the predicted responses from the bi-exponential curve fits are depicted in Fig. 4.6. Individual results can be seen in Appendix 20.

The results for the groups of postmenopausal and subgroups of BMI-matched postmenopausal women are identical so only one set of results are shown. In the subgroup of BMI matched postmenopausal women, τ_2 was slowed in women with T2DM in comparison to non-diabetic controls ($P<0.05$).

Table 4.6. Mean LVC kinetic responses during plantar flexion exercise at 70% MVC

	T2DM (n = 7)	Non-diabetic (n = 5)
	BMI matched postmenopausal	
A₀ (ml.min⁻¹.mmHg⁻¹)	0.60±0.23	0.49±0.20
A₁ (ml.min⁻¹.mmHg⁻¹)	2.57±0.98	2.90±2.56
TD₁ (s)	0.9±0.7	0.6±0.8
τ₁ (s)	4.1±1.8	3.1±1.4
A₂ (ml.min⁻¹.mmHg⁻¹)	2.44±0.93	1.93±1.34
TD₂ (s)	17.4±4.6	14.4±4.2
τ₂ (s)	38.0±17.8*	17.6±9.1
End A (ml.min⁻¹.mmHg⁻¹)	5.61±1.77	5.33±2.57
MRT (s)	30.5±12.7	17.9±10.8

Significantly different from non-diabetic * $P<0.05$.

LVC responses 70% MVC

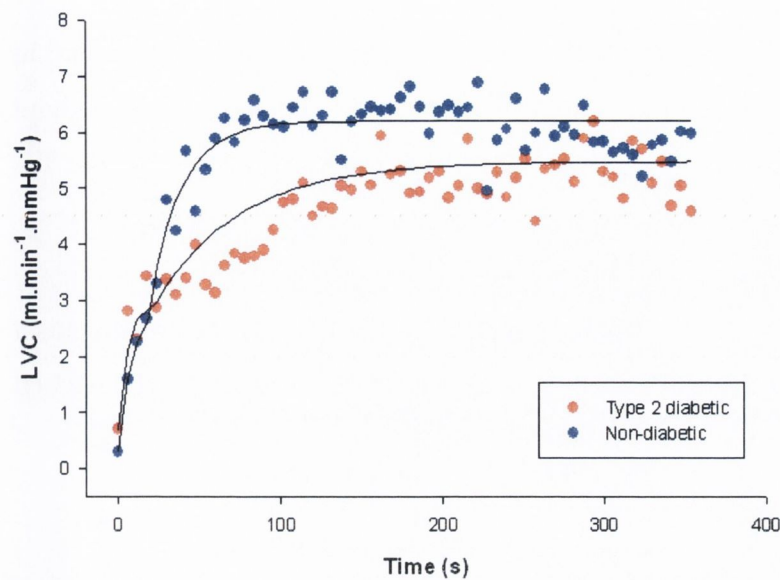


Fig. 4.6. LVC kinetic responses and bi-exponential curve fits during plantar flexion exercise at 70% MVC from representative subjects from postmenopausal women with and without T2DM.

4.4.4.4 Heart rate and MAP responses during plantar flexion exercise at 70% MVC

HR and MAP responses during plantar flexion at 70% in women with and without T2DM are presented in Fig. 4.7. Individual results can be seen in Appendix 21.

For all group comparisons, there were no differences in HR or MAP responses both at rest and during exercise between women with and without T2DM.

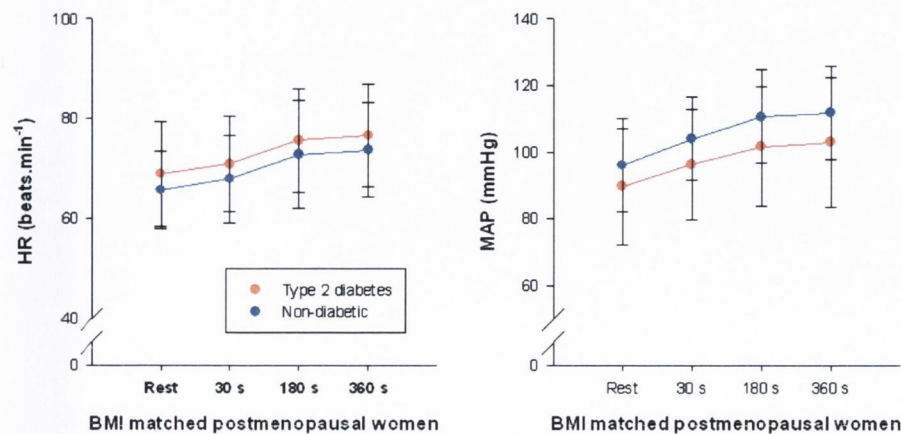


Fig. 4.7. HR and MAP responses during plantar flexion exercise at 70% MVC

4.4.5 Forearm vascular conductance (FVC) kinetic responses during reactive hyperaemia of the forearm

FVC kinetic responses during reactive hyperaemia of the forearm are presented in Table 4.7. Representative FVC kinetic responses from women with and without T2DM as well as the predicted responses from the mono-exponential curve fits are depicted in Fig. 4.9. Individual results can be seen in Appendix 22.

FVC kinetic responses were similar between the groups of postmenopausal women with and without T2DM and between the subgroups of BMI matched postmenopausal women with and without T2DM.

Table 4.7. Mean FVC kinetic responses during reactive hyperaemia of the forearm

	T2DM (n = 19)	Non- diabetic (n = 19)	T2DM (n = 11)	Non- diabetic (n = 8)
	Postmenopausal		BMI matched postmenopausal	
Resting FVC (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	2.0±1.1	2.2±1.9	1.8±0.6	1.5±0.6
a (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	1.9±0.9	1.8±1.1	1.7±0.5	2.2±1.0
A (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	16.1±4.9	22.0±8.9	17.6±4.9	22.2±7.1
c	0.05±0.03	0.05±0.04	0.05±0.02	0.07±0.05
Predicted peak FVC (a + A) (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	17.9±4.8	21.8±8.9	19.1±4.6	24.4±7.6
AUC (arbitrary units)	1007±367	1059±519	917±187	1080±346
Peak FVC (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	17.8±5.2	22.2±9.5	19.2±4.9	25.0±8.5

Reactive hyperemia responses

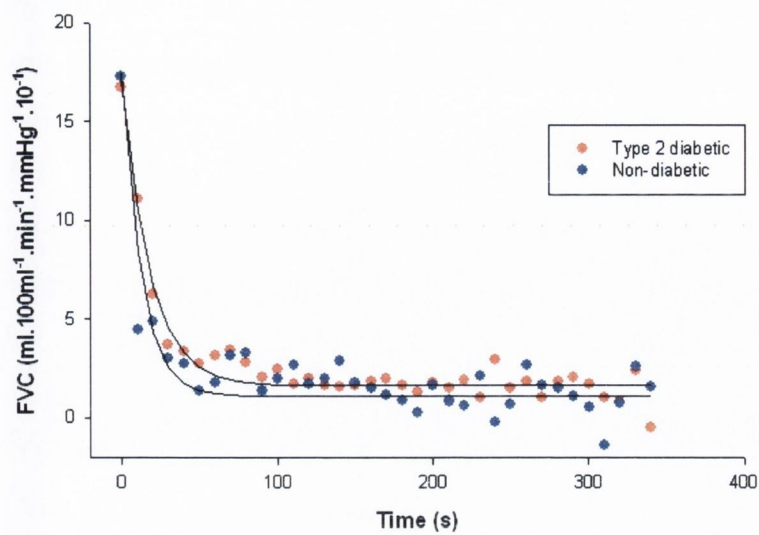


Fig. 4.8. FVC responses during reactive hyperaemia of the forearm from individual subjects with and without T2DM.

4.5 Discussion

The principle findings of this experiment are firstly that the dynamic response of LVC is slowed in women with T2DM during high intensity but not low intensity plantar flexion exercise in comparison to non-diabetic women. Secondly, reactive hyperaemia vasodilatory responses are not different between women with and without T2DM indicating that endothelial function is preserved in the forearm of women with T2DM. Thirdly, both the vasodilatory and hyperaemic responses (expressed in relation to maximum force and expressed in relation to % of maximum force) during incremental plantar flexion exercise are similar in women with and without T2DM. For all group comparisons, MVC, peak force, and TTF were not different while in contrast peak force/MVC was lower in women with T2DM than non-diabetic women.

Historically the hyperaemic response was thought to consist of two growth phases during moderate intensity exercise (Shoemaker *et al.*, 1997; MacDonald *et al.*, 1998; Radegran & Saltin, 1998) and possibly a third growth phase during high intensity exercise (Saunders *et al.*, 2005). However, following the initial exponential increase in blood flow during phase 1, a fast decay phase has been observed during low intensity knee extension exercise in supine (MacDonald *et al.*, 1998) and upright positions (Shoemaker *et al.*, 1996) indicating that the structure that characterises the hyperaemic response is not always defined by 'growth-only' phases.

Recent evidence indicates that 'growth and decay' models might best describe the structure of the hyperaemic response during rest-exercise transitions across a wide range of exercise intensities during calf plantar flexion exercise. Qualitative analysis of the hyperaemic response measured using VOP revealed that a rapid and fast decay was superimposed on the basic growth structure in the majority of cases (Reeder & Green, unpublished observations). In the current investigation, a triphasic response (two growth phases and one decay phase) was prevalent in 32 of 44 subjects while the remaining 12 subjects demonstrated a biphasic response (2 growth phases) during exercise at 30% MVC. Therefore, to enhance the physiological response and to enable more accurate estimates of the parameters defining each phase, a triphasic 'growth and decay' model was used to fit the data

at 30% MVC. A biphasic ‘growth only’ model was used to fit the data at 70% MVC as a biphasic response was prevalent in 10 of 12 subjects.

First growth phase

The rapid increase in blood flow in the first 5-7 s of exercise which is closely linked to the pattern of muscle activation has led to the suggestion that the increase in blood flow during phase 1 is influenced principally by the mechanical action of the muscle pump (Gotshall *et al.*, 1996; Sheriff & Hakeman, 2001). More recent evidence provides unequivocal support for rapid vasodilation following the release of muscle contraction in humans during handgrip exercise in both in rest-exercise transitions and exercise-exercise transitions (Saunders & Tschakovsky, 2004). Irrespective of the mechanistic basis responsible for the increase in blood flow in phase 1, it appears that the LVC kinetic response during phase 1 remains intact in women with T2DM during low and high intensity exercise as the rate of adaptation of the time constant of phase 1 was similar in women with and without T2DM during exercise at 70% MVC and similar among the BMI matched postmenopausal women with and without T2DM during exercise at 30% MVC, a finding that has been observed elsewhere across different exercise modalities and intensities (Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011b). In fact τ_1 was faster (and not slower) in the group of postmenopausal women with T2DM than non-diabetic controls during exercise at 30% MVC.

Second growth phase

Recent evidence obtained during plantar flexion exercise indicates that EMG activity of the triceps surae complex does not increase during phase 2, which suggests that the mechanistic basis underpinning this phase is not directly linked to muscle activation (Reeder & Green, unpublished observations). It has been suggested that phase 2 is a flow-mediated, endothelium-dependent phase that is possibly linked to the decline in myocyte P_{O_2} (Gorczynski & Duling, 1978) and the production of vasoactive metabolites such as adenosine (Marshall, 2007), NO and prostaglandins (Mortensen *et al.*, 2009), although lack of data on the time course of the release of these substances into the interstitial fluid limits the

understanding on the role they play in relation to regulating the hyperaemic response during this phase.

In the current investigation, we failed to find any differences in LVC kinetic responses between women with and without T2DM during low intensity plantar flexion exercise. The time constant of the second growth phase indicated that the adjustment in the vasodilatory responses in both groups was similar. It is possible that the magnitude of the exercise intensity (30% MVC) was insufficient to emphasise any differences in vasodilatory responses between women with and without T2DM. In contrast, we found that the dynamic response of LVC was slowed in women with T2DM during high intensity plantar flexion exercise in comparison to non-diabetic women. The slowing of the hyperaemic response during plantar flexion exercise at 70% MVC in women with T2DM was characterised by a slowed rate of increase of the hyperaemic response during phase 2 (longer time constant) and is consistent with a diabetes-associated impairment in the efficiency of the endothelial-dependent vasodilatory response to high intensity exercise.

The finding of a slowed hyperaemic response during high intensity plantar flexion exercise confirms earlier research where microvascular blood flow kinetics, estimated from $\dot{V}O_2$ and deoxygenated haemoglobin responses, were slowed at the onset of moderate intensity cycle exercise (Bauer *et al.*, 2007). To date, only one former study has investigated the dynamic response characteristics of LVC during upright plantar flexion exercise, the findings of which indicated that LVC kinetic responses are slowed in women with T2DM during high intensity exercise (Mac Ananey *et al.*, 2011b). However, in the study by Mac Ananey *et al.*, (2011b), subjects were tilted from the supine to upright posture immediately prior to the onset of the exercise, and as such, differences in the cardiovascular reflexes involved in tilting the body to upright posture between T2DM and non-diabetics might have affected the LVC kinetic responses. However, the current investigation is first to determine LVC kinetic responses in postmenopausal women during low and high intensity plantar flexion exercise in the supine position. Together the findings of the current investigation and those by Mac

Ananey *et al.*, (2011b) indicate that the efficiency with which the vasodilatory response can react to an exercise load is impaired in women with T2DM at least during high intensity exercise, both during upright and supine postures.

As the phasic amplitudes and overall End A were comparable between women with and without T2DM during both 30% and 70% MVC plantar flexion exercise, this indicates that the capacity to vasodilate remains intact during both low and high intensity exercise in individuals with T2DM. In contrast, reduced leg blood flow responses have been reported in men with T2DM during moderate steady state cycling exercise (Kingwell *et al.*, 2003) and low intensity leg extension exercise (Lalande *et al.*, 2008). The disparities between the current study and those of previous investigations (Kingwell *et al.*, 2003; Lalande *et al.*, 2008) might be explained by such differences as the muscle mass studied, exercise modality, and/or method of determining blood flow. In support of our findings, Mac Ananey *et al.*, (2011b) did not find any differences in phasic amplitudes or overall End A between women with T2DM and their lean and overweight non-diabetic counterparts during upright plantar flexion exercise.

As certain diseased states such as T2DM are often associated with increases in blood pressure and blood flow is determined by perfusion pressure, leg blood flow is presented as LVC (LBF/MAP). We did not find any differences in MAP between women with and without T2DM during exercise at either 30% or 70% MVC, a finding that has been observed elsewhere during high intensity exercise at 70% MVC (Mac Ananey *et al.*, 2011b). This observation in the current study might be due to the fact that almost half of the women with T2DM were receiving hypertensive medication and as such, their hypertension was well controlled. While resting MAP was greater in the group of postmenopausal women with T2DM than non-diabetic women at 30% MVC, this finding was eliminated in the subgroup of BMI matched postmenopausal women with T2DM, thereby suggesting that greater BMI may contribute to hypertension in T2DM. In support of this theory, it has been shown that blood pressure is greater among individuals with greater BMI and this effect is greater in individuals with T2DM (Janssen *et al.*, 2007). Exercising heart rates were greater in women with T2DM than their non-diabetic counterparts during exercise at 30% MVC while in contrast, heart

rates were not different between women with and without T2DM either at rest or during exercise at 70% MVC. The later findings are in agreement with those reported by Mac Ananey *et al.*, (2011b), who in addition, reported similarities in heart rate kinetic responses during high intensity plantar flexion exercise between women with T2DM and their lean and overweight counterparts. It is possible that the higher heart rates observed during low intensity exercise in the current study in the presence of similar MAP responses may have been an attempt to maintain similar blood flow responses and hence, LVC responses to exercise.

Mechanisms responsible for impaired blood flow responses in T2DM

While cardiac dysfunction is common in T2DM, it is unlikely to explain the impaired LVC kinetic responses in women with T2DM during high intensity exercise in the current investigation based on the following evidence: firstly, LVC reflects vasodilation in the exercising muscle, secondly, the differences in LVC kinetic responses are a reflection of impaired blood flow responses as MAP responses were similar in both women with and without T2DM during exercise, and thirdly, exercising heart rates were not different between groups with and without T2DM. Furthermore, as ankle-brachial measurements were not different between women with and without T2DM it is unlikely that the presence of PAD contributed to the slowed rate of increase in LVC kinetic responses in women with T2DM.

A more likely candidate is the presence of chronic hyperglycaemia, which may contribute to impaired vasodilation in T2DM through multiple mechanisms including activation of protein kinase C, increased activity of the polyol pathway, increased production of oxygen-derived free radicals, and advanced glycosylation end products. Associations have been found between leg blood flow during exercise and fasting plasma glucose (Kingwell *et al.*, 2003), and between HbA_{1c} and maximal exercise capacity (Demir *et al.*, 2001) indicating that hyperglycaemia may be in part responsible for the blunted LBF response observed in individuals with T2DM (Kingwell *et al.*, 2003). It is possible that the blunted LBF responses to endothelium-dependent vasodilation (ACh) in individuals with T2DM (Kingwell *et al.*, 2003) might be due to increased inactivation of nitric

oxide or decreased reactivity of the vascular smooth muscle cells to nitric oxide (Williams *et al.*, 1996).

Reactive hyperaemia

We assessed endothelial function during reactive hyperaemia of the forearm using VOP in an attempt to establish if endothelial function was impaired in our women with T2DM. Abnormalities in peak blood flow following reactive hyperaemia are indicative of structural defects in the resistance vessels. However, peak reactive hyperaemia responses were not different between women with and without T2DM in the current investigation indicating that maximal vasodilation remains intact in T2DM. These findings are consistent with a previous investigation where similarities have been reported in peak post-ischemic blood flow responses in individuals with T2DM following reactive hyperaemia of the forearm (McVeigh *et al.*, 1992; Williams *et al.*, 1998). Similarly, in the present study, the decay constant and area under the curve (AUC) were not different between women with and without T2DM indicating that the rate of recovery post-ischemia was not impaired in women with T2DM.

Vascular structure differs significantly between arms and legs in terms of cross-sectional area and therefore differences may exist between the limbs with respect to blood flow and shear stimuli. In support of limb-specific vascular function, blunted vascular responses have been reported in the leg but not in the arm in response to both endothelial-dependent and endothelial-independent vasodilation in healthy young non-diabetic men (Newcomer *et al.*, 2004). Therefore, caution is required when extrapolating the findings of a specific limb to the entire body. As we did not assess endothelial function in the lower limbs it is possible that vascular function may be impaired in the lower limbs of individuals with T2DM and may contribute to the slowed rate of adjustment in LVC kinetics in the present study.

Incremental plantar flexion exercise

While both peak force and time to failure were not different between women with and without T2DM, peak force expressed as a percentage of MVC was lower in women with T2DM. As individuals with T2DM display a greater type IIb to type

I fibre ratio and reduced capillary density (Larsen *et al.*, 2009), it seems plausible to suggest that the reduced blood supply as a consequence of reduced capillary supply and greater proportion of glycolytic fibres would lead to earlier onset of fatigue. Evidence supporting this argument can be found in a study conducted on Goto-Kaizaki (GK) diabetic rats where exercising steady-state blood flow was not impaired despite a greater proportion of blood flow being directed to fast-twitch glycolytic fibres (Copp *et al.*, 2010). The increased blood flow to glycolytic muscle regions during exercise may reflect an altered distribution of oxygen delivery relative to oxygen utilisation within the muscle and may explain why we found a lower peak force to MVC ratio in the current investigation.

Analysis of calf hemodynamic responses to plantar flexion exercise indicates that both the hyperaemic (slope of LBF vs. force) and vasodilatory (slope of LVC vs. force) responses are preserved in women with T2DM. Furthermore, as peak vasodilatory responses were not different between women with and without T2DM, this indicates that the capacity to maximally vasodilate is preserved in T2DM. In support of our findings, it has previously been reported that capillary blood flow is similar during an incremental handgrip protocol between individuals with and without T2DM (Womack *et al.*, 2009). However, abnormal capillary recruitment patterns in diabetic individuals with microvascular complications resulted in impaired blood flow responses during the same protocol (Womack *et al.*, 2009). It would therefore appear that the impaired hemodynamic responses observed in the former study (Womack *et al.*, 2009) are a function of microvascular disease rather than diabetes *per se*.

The similarities observed in relation to the both the hyperaemic (slope of LBF vs. force) and vasodilatory (slope of LVC vs. force) responses during incremental exercise appear to be in conflict with our earlier findings of a slowed rate of adjustment in LVC kinetics in women with T2DM during high intensity plantar flexion exercise. It is important to note that hemodynamic responses are presented as an average of the last 30 s of each increment to allow subjects reach steady state conditions and as such, this analysis does not give any information on the rate of adaptation of the hyperaemic response during plantar flexion exercise. In fact, it is possible that the LVC kinetic responses at the highest changes in

workloads towards the end of the incremental test were slowed in T2DM (as observed during the constant load intensity of 70% MVC), an effect that could have reduced the % peak force/MVC in women with T2DM.

Limitations

The accuracy of venous occlusion plethysmography (VOP) as a means to determine blood flow has previously been questioned (Radegran, 1999). However, VOP provides a means by which to non-invasively measure blood flow derived from conduit as well as feed arteries. In T2DM, it is possible that the main arteries could be affected by vascular disease and thus, VOP provides a means by which to overcome this problem. Furthermore, blood flow measurements by VOP have been shown to closely resemble those made by Doppler ultrasound during plantar flexion exercise (Green *et al.*, 2011).

Previous attempts to characterise the vascular conductance response to exercise have identified a biphasic pattern during low to moderate intensity exercise (Shoemaker *et al.*, 1997; Radegran & Saltin, 1998). However, we noticed an initial fast decay present after the first growth phase during low intensity (30%) plantar flexion exercise. A triphasic ‘growth and decay’ model was fitted to each individual response during low intensity plantar flexion exercise on the basis that this particular model provided the best model of fit (established statistically) and thus, we are confident that this model provides a more accurate determination of the parameter estimates that define each phase than the traditional biphasic model, at least during plantar flexion at 30% MVC.

A large number of women with T2DM were receiving anti-hypertensive medication and it is possible that this might have contributed to the similarities in MAP responses between women with and without T2DM during plantar flexion exercise at both 30% and 70% MVC. Furthermore, the lower total cholesterol levels in women with T2DM might be due to the fact that the majority of women with T2DM were receiving lipid-lowering therapy. It is possible that both lipid-lowering and anti-hypertensive medications could affect vascular function and thus it is possible that the similarities in LVC kinetic responses between women

with and without T2DM during low-intensity plantar flexion exercise could be attributed to the effect of medication (lipid-lowering and anti-hypertensive). While hormone replacement therapy has been known to augment blood flow in postmenopausal women, this is unlikely to have affected the results in the current investigation as the overall number of women receiving hormone replacement therapy was very low (T2DM: n=1; ND: n=2).

Conclusions

The slowing of the $\dot{V}O_2$ kinetic response observed during sub-maximal exercise in individuals with T2DM in the first experiment and in previous investigations (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a) as evidenced by a longer rate of adaptation of the time constant of the second phase, results in reduced efficiency of the $\dot{V}O_2$ response in adapting to exercise and consequently earlier onset of fatigue leading to reduced exercise performance. The findings from the first experiment suggest that CO and oxygen extraction do not contribute to the impaired $\dot{V}O_2$ kinetic responses observed in women with T2DM during moderate intensity cycle exercise. It is therefore tempting to suggest that the impaired hyperaemic response observed in current investigation at the beginning of high intensity plantar flexion exercise might contribute to the slowed rate of adaptation in $\dot{V}O_2$ during this period.

Chapter 5: Effect of exercise training on the dynamic response of $\dot{V}O_2$ and LVC in women with T2DM

5.1 Aims

The aims of this experiment were to:

- (a) Investigate the effect of supervised exercise on $\dot{V}O_2$ kinetic responses performed at intensities relative to each individual's current VT and $\dot{V}O_{2\text{ peak}}$ (80% VT) in women with T2DM
- (b) Explore the role played by CO as a possible mechanism underlying the potential improvement in $\dot{V}O_2$ kinetic responses
- (c) Investigate the effect of supervised exercise on LVC kinetic responses in women with T2DM during low intensity plantar flexion exercise at intensities relative to each individual's current MVC
- (d) Examine if the potential benefits acquired in these variables (aims a to c) during the supervised phase can be maintained during an unsupervised period of exercise, again assessing all intensities based on post-unsupervised exercise peak parameters ($\dot{V}O_{2\text{ peak}}$ and MVC)

5.2 Experimental protocol overview

Both exercise and control groups were required to visit the Cardiovascular Laboratory in Trinity College Dublin on 2 occasions at baseline (BS), post-supervised exercise intervention (SE), and post-unsupervised exercise intervention (UE) over a 7-month period. During the period between BS and SE, both exercise groups completed a 12-wk supervised exercise programme while control groups continued with their normal life. The supervised phase was followed by unsupervised exercise intervention over a 16 wk period.

Following an initial familiarisation period with the calf ergometer during visit 1, subjects completed 5 MVC's of the calf muscle. This was followed by constant load plantar flexion exercise (3 x 6 min) at 30% MVC. In addition, subjects completed an incremental plantar flexion exercise test to failure. Endothelial function was determined during a reactive hyperaemic test of the forearm.

Subjects also completed an incremental cycle test to volitional exhaustion for the determination of $\dot{V}O_{2\text{ peak}}$ and VT.

During Visit 2, subjects completed 6 bouts of 6 min cycling at 80% VT. Bouts 1-4 were used for the determination of $\dot{V}O_2$ kinetics and bouts 5-6 were used to determine CO. CO measurements were made at rest and during exercise (30 s and 240 s) while cycling at 80% VT.

5.3 Subjects

The study included a total of 35 women, 16 with T2DM and 19 non-diabetics. They were both premenopausal (n=5) and postmenopausal (n=30). The women were divided into 4 groups: T2DM exercise n = 8 (premenopausal n=1), T2DM control n = 8 (premenopausal n=1), non-diabetic exercise n = 10 (premenopausal n=1), and non-diabetic control n = 9 (premenopausal n=2). All women were classified as sedentary ($< 1 \text{ h.wk}^{-1}$ of moderate intensity exercise) for at least 6 months prior to enrolment, which was confirmed by physical activity scores using the Low Level Physical Activity Recall (LOPAR; Appendix 1) questionnaire (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999).

5.3.1 Medications

Of the 16 women with T2DM, 3 were undergoing dietary intervention and 13 were receiving oral hypoglycaemic agents (metformin n=11; metformin & gliclazide n=1; gliclazide n=1). In addition, 7 diabetic women were on anti-hypertensive medication (lisinopril n=2; ramipril n=1; candesartan cilexetil n=1; perindopril arginine n=2; telmisartan n=1; angiotensin II antagonists (telmisartan n=1; losartan n=1); ACE inhibitors (ramapril n=1); calcium channel blocker (amlodipine n=1); perindopril arginine & amlodipine n=1). One woman was taking a β -blocker (sotalol n=1). One non-diabetic woman was receiving anti-hypertensive medication (olmesartan medoxomil). A further 9 diabetic women and 2 non-diabetic women were receiving lipid-lowering therapy (T2DM: rosuvastatin n=1; atorvastatin n=6; simvastatin n=1; pravastatin n=1 and non-diabetic: atorvastatin n=2). A total of three women were undergoing hormone replacement therapy (T2DM: n=1, estradiol; non-diabetic: n=2, raloxifene).

5.4 Results

5.4.1 Subjects

5.4.1.1 Adherence to training

A total of 18 individuals (T2DM n = 8; non-diabetic n = 10) completed both the SE and UE intervention phases. A further 17 non-exercising individuals (T2DM n = 8; non-diabetic n = 9) completed exercise testing post-supervised exercise and post-unsupervised exercise. Training compliance was not different between the exercise groups during the SE phase with T2DM Ex group completing 99±3% and the ND Ex group completing 95±6% of the total 36 exercise sessions. During the UE phase, there was a reduction in the number of exercise sessions ($P<0.05$) completed by both exercise groups with the T2DM Ex and ND Ex groups completing 84±34% and 77±28% of the total exercise sessions respectively (48). However, these adherence rates of the UE phase were similar between the two training groups.

5.4.1.2 Baseline characteristics

Baseline physical characteristics and haematological results in women with and without T2DM are presented in Table 5.1. Individual results can be seen in Appendices 23 and 24.

There were no differences between the four groups with respect to age, height, or leg volume. HbA_{1C} levels (expressed as a percentage and in mmol.mol⁻¹) were greater in the diabetic groups than both non-diabetic groups ($P<0.0001$).

Table 5.1. Baseline physical and haematological characteristics

	T2DM		Non-diabetic		Diabetes effect
	Exercise (n = 8)	Control (n = 8)	Exercise (n = 10)	Control (n = 9)	
Age (yr)	57.3±4.5	56.0±5.8	55.5±9.0	52.1±7.5	
Height (m)	1.58±0.06	1.60±0.04	1.58±0.08	1.62±0.03	
Leg volume (ml)	2576±383	2722±491	2407±397	2475±615	
HbA _{1C} (%)	6.7±0.5	6.3±0.9	5.4±0.2	5.4±0.5	$P<0.0001$
HbA _{1C} (mmol.mol ⁻¹)	49.6±6.0	45.2±9.9	35.9±1.9	35.3±5.4	$P<0.0001$

5.4.1.3 Physical characteristics and activity levels following the intervention

The effect of the exercise interventions on physical characteristics and activity levels for women with and without T2DM in the exercise and control groups at baseline (BS), supervised exercise (SE), and unsupervised exercise (UE) are presented in Table 5.2. Individual physical characteristics and activity levels can be seen in Appendix 23.

There was a main effect of diabetes status for body mass, BMI, and WHR at BS, SE, and UE ($P<0.01$), so that they were significantly greater in women with T2DM than non-diabetics.

Table 5.2. Physical characteristics and activity levels

		T2DM		Non-diabetic		Diabetes effect
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=9)	
Body mass (kg)	BS	78.1±10.7	86.7±14.2	65.3±10.4	69.7±14.8	$P<0.01$
	SE	76.5±9.7	85.9±14.2	64.3±9.8	69.3±14.8	
	UE	76.1±9.6	86.6±15.4	64.5±9.8	69.3±14.2	
BMI (kg.m ⁻²)	BS	31.3±4.2	34.0±5.6	26.2±3.4	26.6±5.0	$P<0.01$
	SE	30.6±3.9	33.7±5.5	25.7±2.8	26.4±5.0	
	UE	30.6±3.8	34.0±5.9	25.8±2.9	26.5±4.9	
WHR	BS	0.95±0.05	0.95±0.05	0.89±0.07	0.88±0.08	$P<0.01$
	SE	0.96±0.05	0.95±0.05	0.89±0.07	0.89±0.09	
	UE	0.95±0.05	0.95±0.05	0.88±0.08	0.89±0.09	
ABI	BS	1.08±0.08	1.11±0.10	1.07±0.12	1.10±0.15	
	SE	1.06±0.09	1.06±0.09	1.11±0.06	1.09±0.15	
	UE	1.13±0.09	1.03±0.05	1.05±0.08	1.05±0.08	
LOPAR (Met h.wk ⁻¹)	BS	98±43	125±65	101±56	126±24	
	SE	149±52	122±48	109±50	137±35	
	UE	126±49	166±84	119±51	123±53	

5.4.1.4 Hematological characteristics

The effect of exercise intervention on haematological parameters for women with and without T2DM in the exercise and control groups at BS, SE, and UE are presented in Table 5.3. Individual results can be seen in Appendix 24.

Significant differences were observed between women with and without T2DM (main effect = diabetes status) with respect to FPG ($P<0.0001$), TCL ($P<0.05$), HDL ($P<0.01$), and triglycerides ($P<0.05$).

Table 5.3 Hematological characteristics

		T2DM		Non-diabetic		Diabetes effect
		Exercise	Control	Exercise	Control	
FPG (mmol.L ⁻¹)	BS	7.5±0.6	8.4±2.6	4.5±0.7	4.6±0.5	$P<0.0001$
	SE	7.4±1.1	7.3±2.2	4.8±0.5	4.9±0.4	
	UE	7.6±0.8	7.2±1.7	4.9±0.5	4.8±0.4	
TCL (mmol.L ⁻¹)	BS	4.4±0.6	4.6±0.6	5.9±1.5	5.3±0.9	$P<0.05$
	SE	4.1±0.6	4.6±0.8	5.6±0.8	5.4±0.7	
	UE	4.1±0.6	4.4±0.9	5.4±1.1	5.5±1.1	
HDL (mmol.L ⁻¹)	BS	1.5±0.2	1.2±0.2	2.1±0.4	1.7±0.5	$P<0.01$
	SE	1.5±0.2	1.2±0.3	2.0±0.3	1.7±0.4	
	UE	1.5±0.3	1.3±0.2	1.9±0.4	1.9±0.3	
LDL (mmol.L ⁻¹)	BS	2.7±0.5	3.2±0.5	3.8±0.4	3.3±0.6	
	SE	2.3±0.3	3.1±0.7	3.7±0.8	3.3±0.7	
	UE	2.3±0.4	2.9±1.0	3.5±0.9	3.3±0.8	
Triglycerides (mmol.L ⁻¹)	BS	1.4±0.6	1.7±0.6	1.0±0.5	1.0±0.4	$P<0.05$
	SE	1.6±0.3	1.7±0.4	0.9±0.3	1.3±0.6	
	UE	1.4±0.3	1.5±0.7	1.1±0.7	0.9±0.2	

5.4.2 Peak and sub-maximal cycling performance

5.4.2.1 Peak cycling performance parameters

The effect of the exercise intervention on peak cycling performance parameters in women with and without T2DM in both exercise and control groups are presented in Table 5.4 and Fig. 5.1. Individual peak cycling performance results can be seen in Appendix 25.

Peak $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) was significantly lower in women with T2DM in comparison to non-diabetic women ($P<0.01$) at all time points (Fig. 4.2). Exercise intervention resulted in an increase in $\dot{V}O_{2\text{ peak}}$ in both T2DM Ex and ND Ex at SE ($P<0.0001$) and UE ($P<0.05$) from BS, while in contrast, there was a reduction in $\dot{V}O_{2\text{ peak}}$ in both T2DM Ex and ND Ex at UE from SE ($P<0.05$). Significant treatment (exercise vs. control) x time interactions were observed for $\dot{V}O_{2\text{ peak}}$ with exercising groups having a greater $\dot{V}O_{2\text{ peak}}$ than control groups at BS ($P<0.05$), SE and UE ($P<0.0001$).

The percentage increase in $\dot{V}O_{2\text{ peak}}$ was not different between women with and without T2DM (Fig. 4.3). In both exercise groups, the percentage increase in $\dot{V}O_{2\text{ peak}}$ was greater at SE than at BS ($P<0.0001$) and greater than in control groups at SE ($P<0.05$). In contrast, the increase in $\dot{V}O_{2\text{ peak}}$ was lower at UE than at SE in both the T2DM Ex and ND Ex groups ($P<0.0001$).

There was no difference in $\dot{V}O_{2\text{ peak}}$ (L.min^{-1}) between women with and without T2DM (Fig. 4.2). Exercising groups had a greater $\dot{V}O_{2\text{ peak}}$ at SE than at BS ($P<0.0001$) and a greater $\dot{V}O_{2\text{ peak}}$ than control groups at SE ($P<0.0001$). There was a reduction in $\dot{V}O_{2\text{ peak}}$ from SE to UE in both the exercising groups ($P<0.01$).

$\dot{V}O_2$ at VT was lower in women with T2DM than non-diabetic women ($P<0.01$) (Fig. 4.2). Training resulted in an increase in $\dot{V}O_2$ at VT in both exercise groups at SE from BS ($P<0.0001$) and a decrease from SE to UE ($P<0.05$). Furthermore, $\dot{V}O_2$ at VT was greater in exercise than control conditions at BS ($P<0.01$), SE ($P<0.0001$), and UE ($P<0.01$).

Peak workload was greater in both exercise groups at SE ($P<0.0001$) and at UE ($P<0.05$) but decreased in both exercise groups from SE to UE ($P<0.01$) (Fig. 4.2). In addition, peak workload was greater in exercising groups than control groups at SE ($P<0.0001$) and UE ($P<0.01$).

Workload at VT was lower in women with T2DM than non-diabetic women ($P<0.05$). Exercise intervention resulted in an increase in the load at VT in both exercise groups at SE from BS ($P<0.01$) and a decrease from SE to UE ($P<0.01$) (Fig. 5.2). Load at VT was greater in exercise than control groups at SE ($P<0.0001$) and UE ($P<0.05$).

A treatment x time interaction was observed for time to failure (TTF) with exercising groups increasing TTF from BS to SE ($P<0.0001$) and UE ($P<0.01$), while in contrast, TTF decreased from SE to UE in exercising groups ($P<0.05$) (Table 4.5). Furthermore, TTF was greater in exercising groups than non-exercising groups at SE ($P<0.0001$) and UE ($P<0.01$).

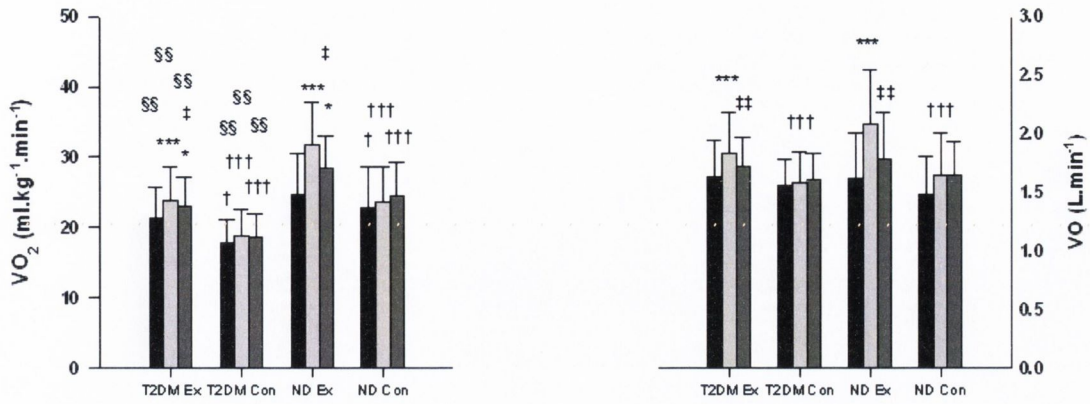
Other parameters including peak HR (beats.min⁻¹ & % predicted maximum), peak $\dot{V}_E$ (L.min⁻¹), peak RER, and $\dot{V}O_2$ at VT (% $\dot{V}O_{2\text{ peak}}$) are shown in Table 4.5.

Table 5.4. Peak cycling performance responses

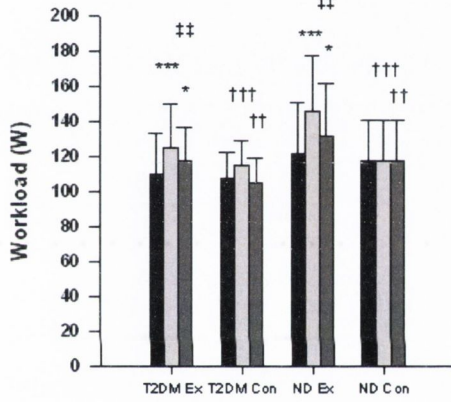
		T2DM		Non-diabetic	
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=8)
Peak HR (beats.min ⁻¹)	BS	152±18 ^a	153±13	156±10	161±12
	SE	151±19	158±16	162±11	160±12
	UE	150±15 ^{ζζ}	153±15	161±9	160±13
Peak HR (% predicted maximum)	BS	93±11	93±8	95±7	95±7
	SE	92±11 ^{ζζ}	96±8	99±8	95±6
	UE	92±9 ^{ζζ}	93±8 ^ζ	98±6	95±6
Peak RER	BS	1.11±0.06	1.10±0.05	1.11±0.06	1.07±0.06
	SE	1.09±0.06	1.08±0.04	1.10±0.05	1.07±0.02
	UE	1.10±0.04	1.07±0.04	1.08±0.04	1.06±0.05
$\dot{V}_E$ (L.min ⁻¹)	BS	60.8±9.4	57.8±9.4	57.8±16.1	55.5±9.7
	SE	63.1±7.7	58.9±9.3	72.0±17.4	64.8±20.5
	UE	57.7±10.5	61.6±6.1	66.7±14.3	62.9±15.4
TTF (min)	BS	12.4±3.5	12.6±1.9	14.2±4.0	14.0±3.3
	SE	15.4±3.4 ^{***}	12.5±1.9 ^{†††}	17.7±4.2 ^{***}	14.1±3.2 ^{†††}
	UE	13.6±2.9 ^{**‡}	12.1±2.4 ^{††}	16.2±4.1 ^{**‡}	14.0±3.0 ^{††}
$\dot{V}O_2$ at VT/ (% $\dot{V}O_{2\text{ peak}}$)	BS	73.2±6.9	69.8±3.8	71.9±8.5	70.7±11.0
	SE	71.4±5.0	66.9±6.6	72.2±8.4	71.5±7.6
	UE	70.1±7.5	71.0±5.3	72.5±7.8	73.1±5.6

Significantly different from BS ^{**}*P*<0.01, ^{***}*P*<0.0001; significantly different from SE [‡]*P*<0.05; significantly different from exercise ^{††}*P*<0.01, ^{†††}*P*<0.0001; significantly different from ND Ex ^ζ*P*<0.05, ^{ζζ}*P*<0.01; significantly different from ND Con ^a*P*<0.05.

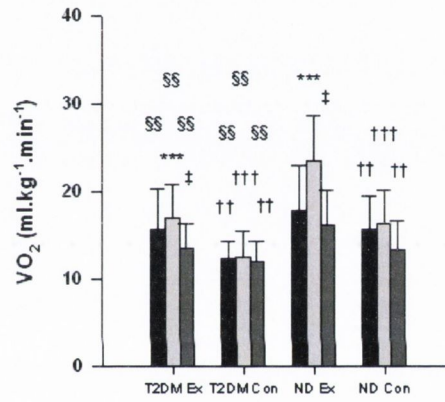
Peak $\dot{V}O_2$



Peak workload



$\dot{V}O_2$ at VT



Workload at VT

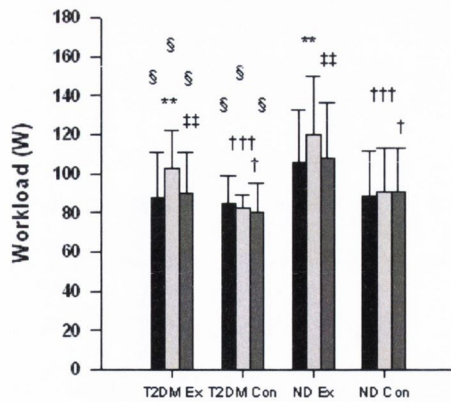


Fig. 5.1. Peak $\dot{V}O_2$ & workload and $\dot{V}O_2$ & workload at VT obtained during the graded cycling test. Significantly different from non-diabetic § $P<0.05$, §§ $P<0.01$; significantly different from BS * $P<0.05$, ** $P<0.01$, *** $P<0.0001$; significantly different from SE ‡ $P<0.05$, ‡‡ $P<0.01$; significantly different from exercise † $P<0.05$, †† $P<0.01$, ††† $P<0.0001$.

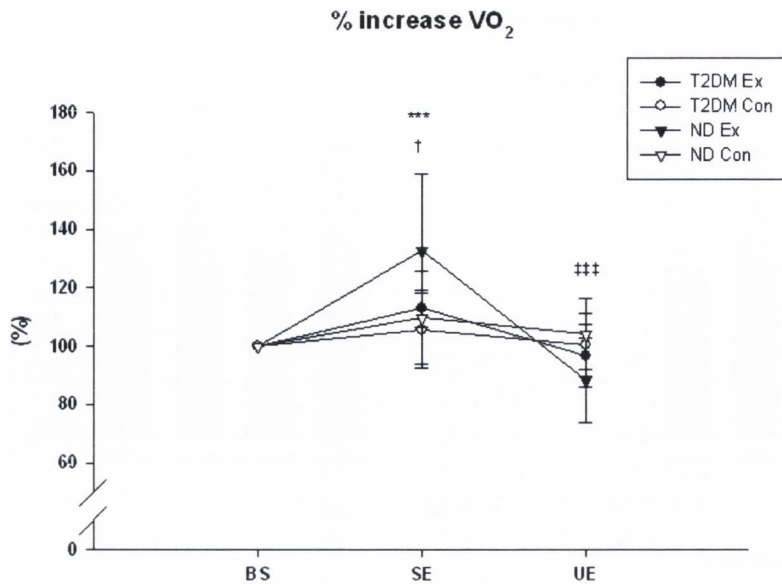


Fig. 5.2. Percentage increase in $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$). Significantly different from BS within each exercise group $***P<0.0001$; significantly different from SE within each exercise group $†††P<0.0001$; significantly different from exercise groups $†P<0.01$

5.4.2.2 Mean $\dot{V}O_2$ kinetic parameters during cycle exercise at 80% VT

The effect of exercise intervention on the mean $\dot{V}O_2$ kinetic parameters during cycle exercise at 80% VT for women with and without T2DM in the exercise and control groups are presented in Table 5.5. Representative $\dot{V}O_2$ kinetic responses from each group as well as the predicted responses from the bi-exponential curve fits are depicted in Fig. 5.3. Individual $\dot{V}O_2$ kinetic responses can be seen in Appendix 26.

The time constant of the first phase (τ_1) was unaffected by condition, treatment, or time. In contrast, the time constant of the second phase (τ_2) was slowed in diabetic groups ($P<0.0001$). A significant effect of treatment was observed for τ_2 with exercise groups reporting a faster τ_2 than control groups ($P<0.01$). The overall mean response time (MRT) was slower in women with T2DM than non-diabetic women ($P<0.05$) and was slower in control than exercise conditions ($P<0.05$).

In regard to the amplitudes, the baseline amplitude (A_0) was greater in women with T2DM than non-diabetic women ($P<0.05$). In contrast, the amplitude of the

first phase (A_1) was lower in women with T2DM than non-diabetic women ($P<0.05$). At BS, A_1 was greater in ND Ex than T2DM Con ($P<0.01$), T2DM Ex and ND Con ($P<0.0001$). In addition, A_1 was greater in ND Ex than ND Con ($P<0.01$) and T2DM Con ($P<0.0001$) at SE. In the ND Ex group, A_1 was lower at UE than BS and SE ($P<0.01$). The amplitude of the second phase A_2 or End A were unaffected by condition, treatment, or time.

The time delay of the first phase (TD_1) increased from SE to UE in T2DM Con ($P<0.05$). The time delay of the second phase (TD_2) was not different either within or between groups.

There were no differences in the $\dot{V}O_2$ gain either within or between groups.

Table 5.5. Mean $\dot{V}O_2$ kinetic parameters during cycle exercise at 80% VT

		T2DM		Non-diabetic		Diabetes effect	Treatment effect
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=8)		
Workload at VT (W)	BS	87.5±23.8	85.0±14.1	106.0±26.7	92.5±21.2	$P<0.05$	
	SE	102.5±19.8 **	82.5±7.1 †††	120.0±29.8 **	95.0±20.7 †††		
	UE	90.0±21.4 ‡‡	80.0±15.1 †	108.0±28.6 ‡‡	91.4±19.5 †		
A_0 (L.min ⁻¹)	BS	0.58±0.11	0.59±0.10	0.51±0.04	0.50±0.11	$P<0.05$	
	SE	0.60±0.07	0.63±0.13	0.52±0.09	0.50±0.10		
	UE	0.60±0.07	0.63±0.10	0.55±0.06	0.55±0.08		
A_1 (L.min ⁻¹)	BS	0.21±0.07 ζζζ	0.22±0.07 ζζ	0.42±0.15	0.19±0.08 ζζζ	$P<0.05$	
	SE	0.28±0.13	0.17±0.10 ζζζ	0.42±0.12	0.20±0.08 ζζ		
	UE	0.25±0.06	0.18±0.07	0.24±0.09 ***‡‡	0.19±0.07		
TD ₁ (s)	BS	7.1±3.8	4.8±3.8	4.3±3.2	4.7±2.3		
	SE	9.0±3.7	2.9±4.7	5.6±2.3	5.6±4.0		
	UE	6.9±3.4	10.3±4.0‡	6.5±3.8	6.0±3.3		
τ_1 (s)	BS	12.5±4.8	12.1±3.8	11.4±4.2	12.5±4.0		
	SE	12.7±4.6	10.8±3.5	14.4±3.1	14.0±4.3		
	UE	15.5±3.5	13.5±4.1	10.4±4.0	15.7±3.3		
A_2 (L.min ⁻¹)	BS	0.50±0.25	0.40±0.17	0.43±0.28	0.49±0.15		
	SE	0.53±0.14	0.46±0.16	0.59±0.24	0.50±0.16		
	UE	0.42±0.18	0.47±0.16	0.68±0.31	0.49±0.16		
TD ₂ (s)	BS	29.7±8.1	28.5±7.6	34.3±7.0	30.5±6.4		
	SE	29.7±5.6	22.8±7.4	28.2±4.9	31.7±4.4		
	UE	34.2±7.9	30.5±5.2	29.2±5.4	31.4±5.2		
τ_2 (s)	BS	47.2±5.8	50.5±5.3	36.9±11.0	37.9±5.6	$P<0.0001$	$P<0.01$
	SE	43.4±7.8	50.9±10.8	39.9±13.1	42.0±6.3		
	UE	38.1±10.5	54.3±8.2	37.1±7.1	39.2±4.2		
End A (L.min ⁻¹)	BS	1.29±0.27	1.23±0.23	1.38±0.32	1.17±0.25		
	SE	1.40±0.20	1.25±0.26	1.61±0.40	1.20±0.29		
	UE	1.26±0.18	1.28±0.23	1.46±0.41	1.22±0.25		
MRT (s)	BS	58.3±7.3	56.0±7.6	42.1±11.3	54.6±5.9	$P<0.05$	$P<0.05$
	SE	54.9±5.0	57.3±13.8	50.6±14.5	58.3±6.5		
	UE	53.6±9.9	67.6±7.2	53.3±7.2	56.9±4.0		
$\dot{V}O_2$ gain (ml.min ⁻¹ .W ⁻¹)	BS	11.2±2.4	10.8±2.4	11.5±1.3	9.4±1.5		
	SE	11.2±0.6	11.3±2.7	13.2±4.9	10.2±1.5		
	UE	10.7±1.5	12.0±2.7	11.7±2.0	9.0±1.0		

Significantly different from BS ** $P<0.01$; significantly different from SE ‡‡ $P<0.01$; significantly different from exercise † $P<0.05$, ††† $P<0.0001$; significantly different from ND Ex ζζζ $P<0.01$ ζζζζ $P<0.0001$.

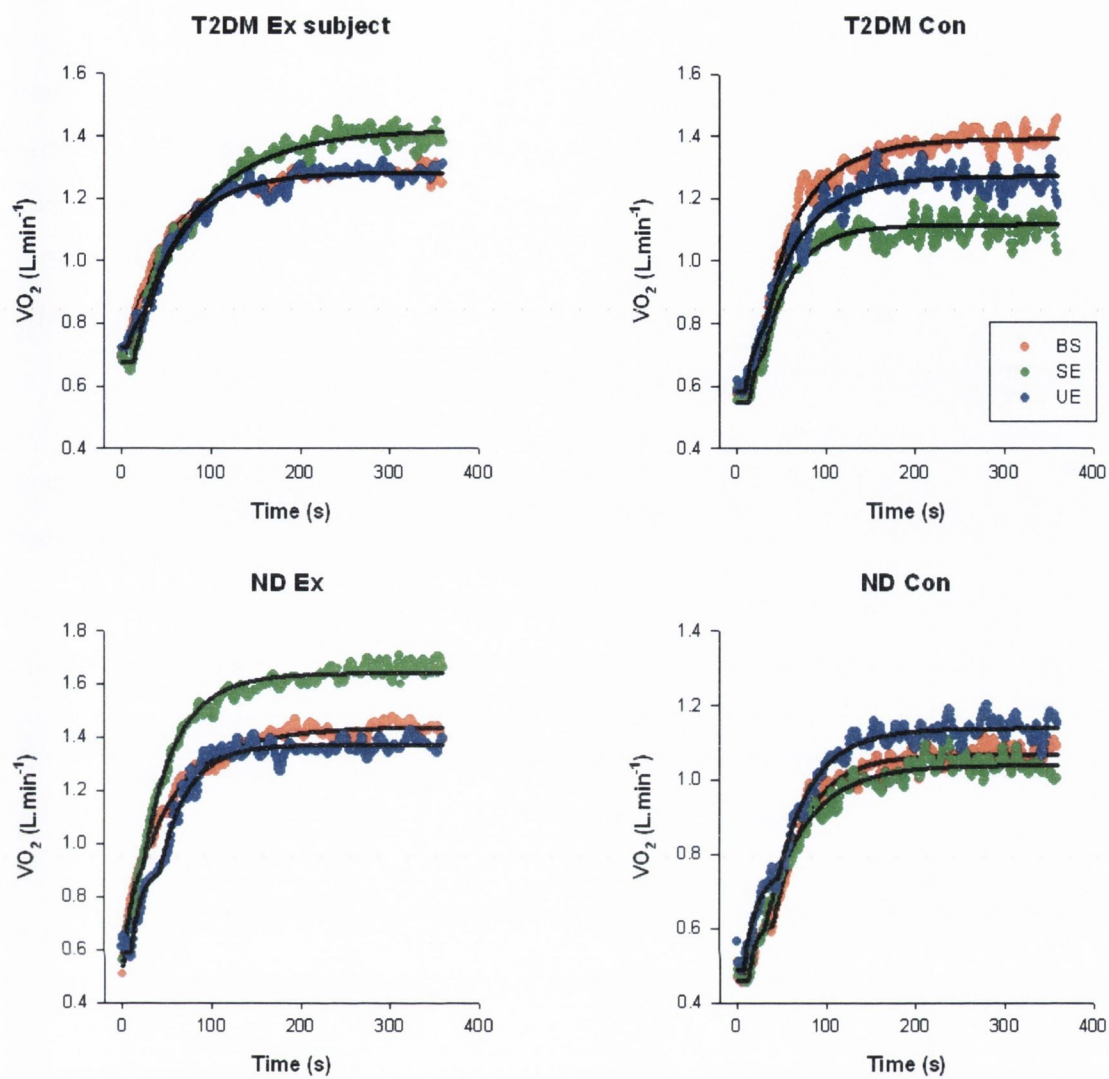


Fig. 5.3. $\dot{V}O_2$ kinetic responses and predicted responses from the bi-exponential curve fits during cycle exercise at 80% VT from representative subjects from each group at BS, SE, and UE.

5.4.2.3 Mean heart rate kinetic responses during cycle exercise at 80% VT

The effect of exercise intervention on the mean the heart rate kinetic parameters during cycle exercise at 80% VT for women with and without T2DM in exercise and control groups are presented in Table 5.6. Representative heart rate kinetic responses from each group as well as the predicted responses from the mono-exponential curve fits are depicted in Fig. 5.4. Individual heart rate kinetic responses can be seen in Appendix 27.

The baseline amplitude (A_0), amplitude of the first phase (A_1), the time delay (TD), time constant (τ), and mean response time (MRT) were not different either within or between groups.

Table 5.6. Mean heart rate kinetic responses during cycle exercise at 80% VT

		T2DM		Non-diabetic	
		Exercise (n = 8)	Control (n = 8)	Exercise (n = 9)	Control (n = 7)
Baseline A_0 (beats.min ⁻¹)	BS	105±15	111±20	84±5	90±8
	SE	98±13	113±19	89±10	89±10
	UE	97±18	108±9	89±14	93±12
A_1 (beats.min ⁻¹)	BS	29±7	24±11	37±8	37±9
	SE	27±12	17±6	44±13	45±15
	UE	31±7	24±9	41±14	37±16
TD (s)	BS	4.8±4.7	1.6±1.6	5.8±3.8	5.3±5.8
	SE	5.6±5.4	1.7±3.3	4.7±4.6	7.7±3.0
	UE	7.4±7.2	6.2±8.2	4.4±6.2	9.3±12.0
τ (s)	BS	81.9±37.2	71.2±14.4	59.6±9.1	62.7±11.4
	SE	68.5±28.7	95.5±43.2	55.9±12.3	56.4±21.0
	UE	47.9±22.4	108.7±46.2	60.4±6.4	57.6±20.4
End A (beats.min ⁻¹)	BS	133±9	135±19	121±9	127±14
	SE	124±15	130±15	132±14	133±12
	UE	127±17	131±15	130±10	130±14

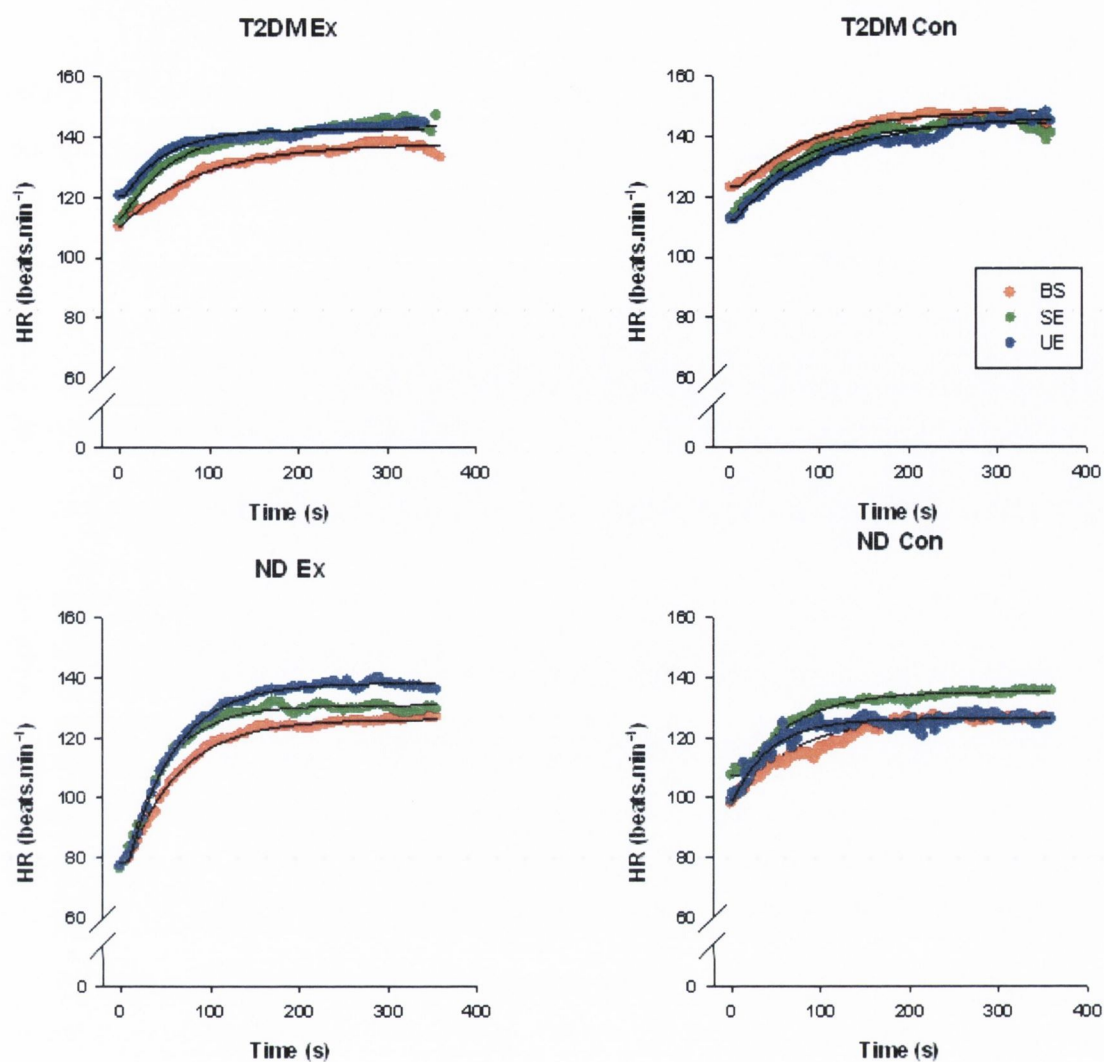


Fig. 5.4. Heart rate kinetic responses and predicted responses from the mono-exponential curve fits during cycle exercise at 80% VT from representative subjects from each group at BS, SE, and UE.

5.4.2.4 Cardiac responses during constant load cycle exercise at 80% VT

The effect of exercise training on cardiac responses during constant load cycle exercise at 80% VT for women with and without T2DM in exercise and control groups can be seen in Fig. 5.5 (CO and CI), Fig. 5.6 (SV & SVI), Fig. 5.7 (HR and a-vO₂ diff), and Fig. 5.8 (MAP and TPR). Individual responses can be seen in Appendix 28.

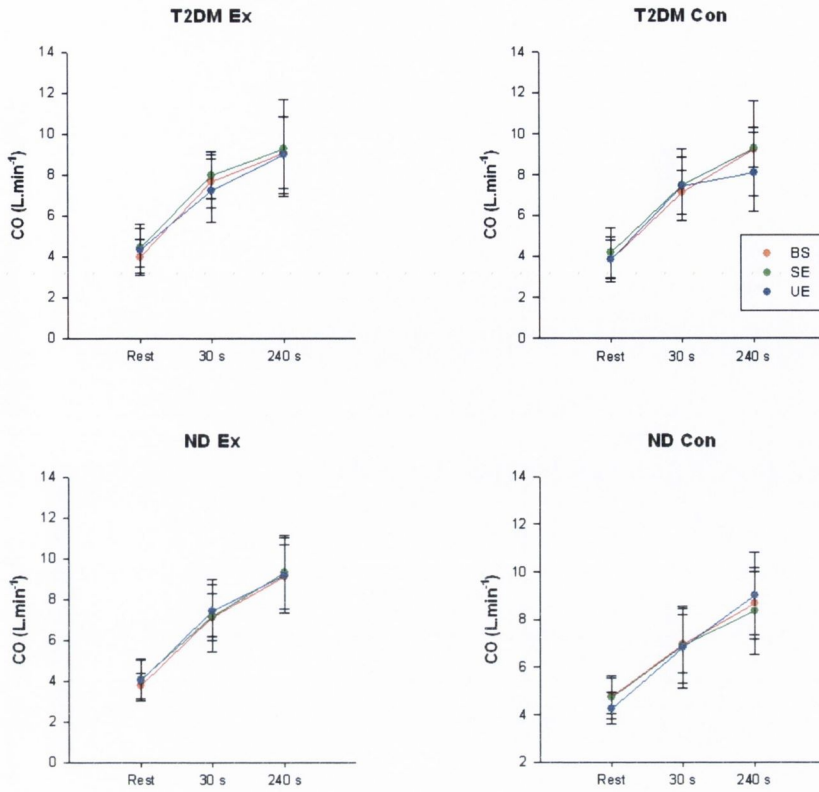
CO, SV, SVI, a-vO₂ diff, and TPR responses were not different at rest or during exercise (30 s and 240 s) either within or between groups.

CI responses were not different at rest or during exercise at 240 s either within or between groups. In contrast, CI at 30 s was greater in exercise than control groups ($P<0.05$).

HR responses both at rest and during exercise at 240 s were not different either within or between groups. During exercise at 30 s, HR was lower in non-diabetics at UE than SE ($P<0.05$).

MAP responses were greater in women with T2DM than non-diabetic women both at rest ($P<0.01$) and during exercise at 30 s and 240 s ($P<0.05$).

Cardiac output



Cardiac index

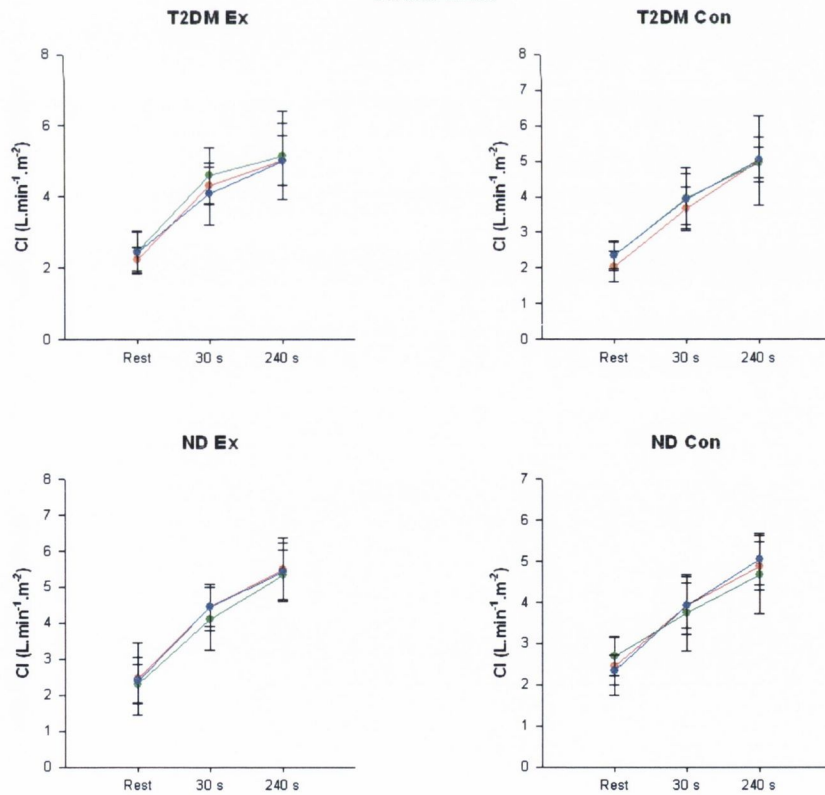


Fig. 5.5. Mean CO and CI responses at rest and during cycle exercise (30 s and 240 s) at 80% VT at BS, SE, and UE in each group. No training effect was observed for CO or CI. For between group comparisons see written text.

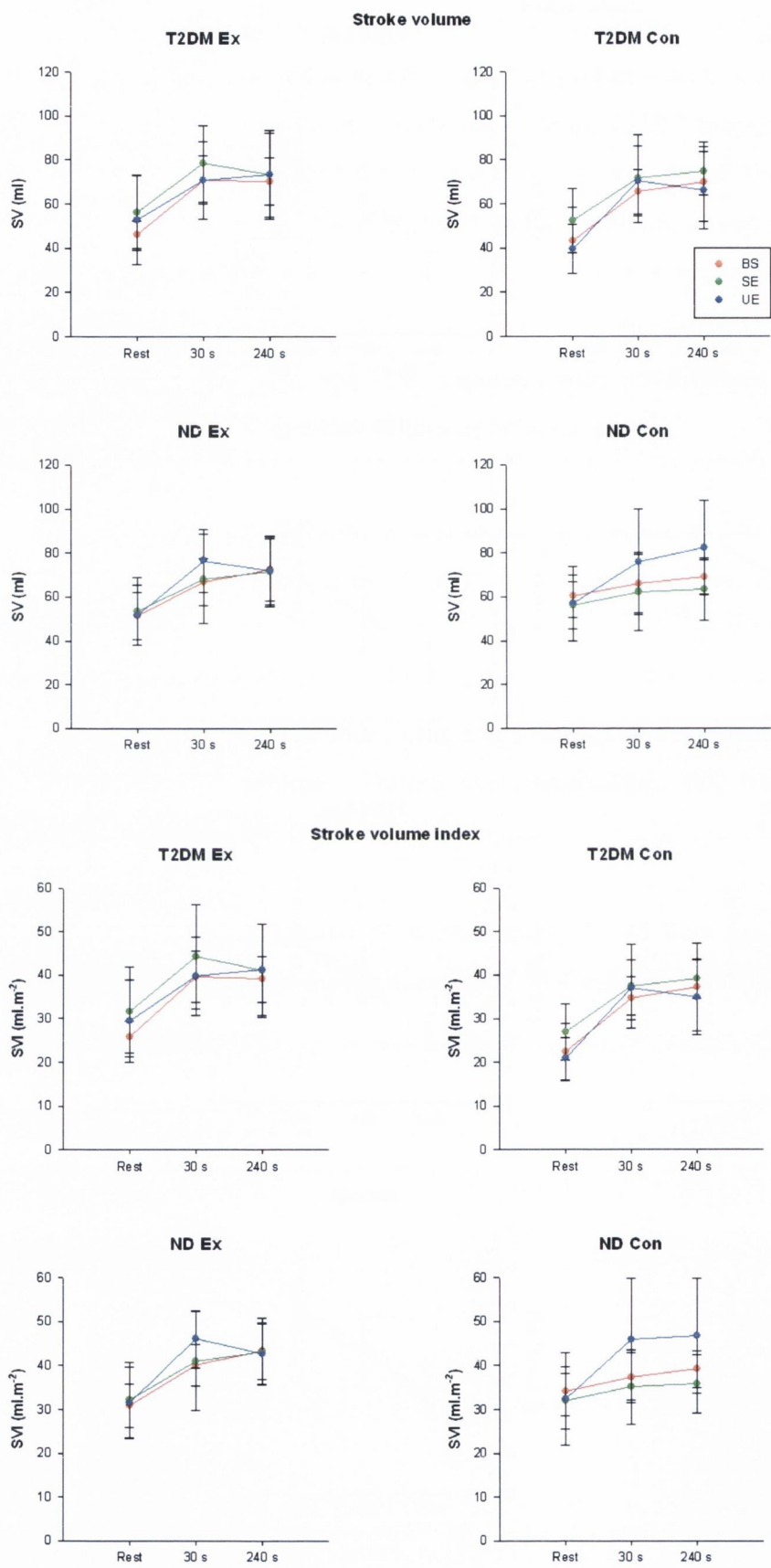


Fig. 5.6. Mean SV and SVI responses at rest and during cycle exercise (30 s and 240 s) at 80% VT at BS, SE, and UE in each group. No training effect was observed for SV or SVI.

Heart rate

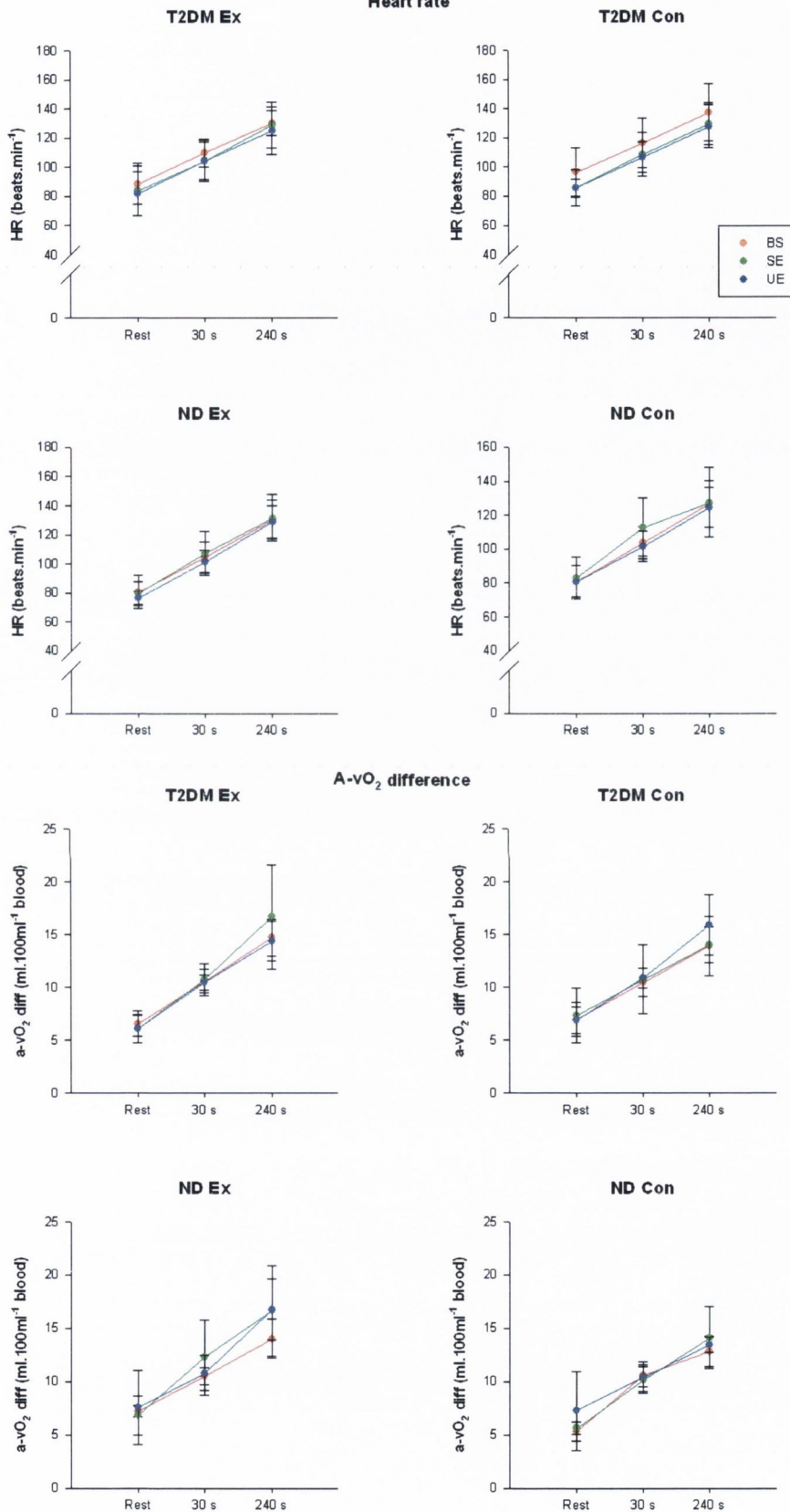


Fig. 5.7. Mean HR and a-vO₂ diff responses at rest and during cycle exercise (30 s and 240 s) at 80% VT at BS, SE, and UE in each group. No training effect was observed for HR or a-vO₂ diff responses. For between group comparisons see written text.

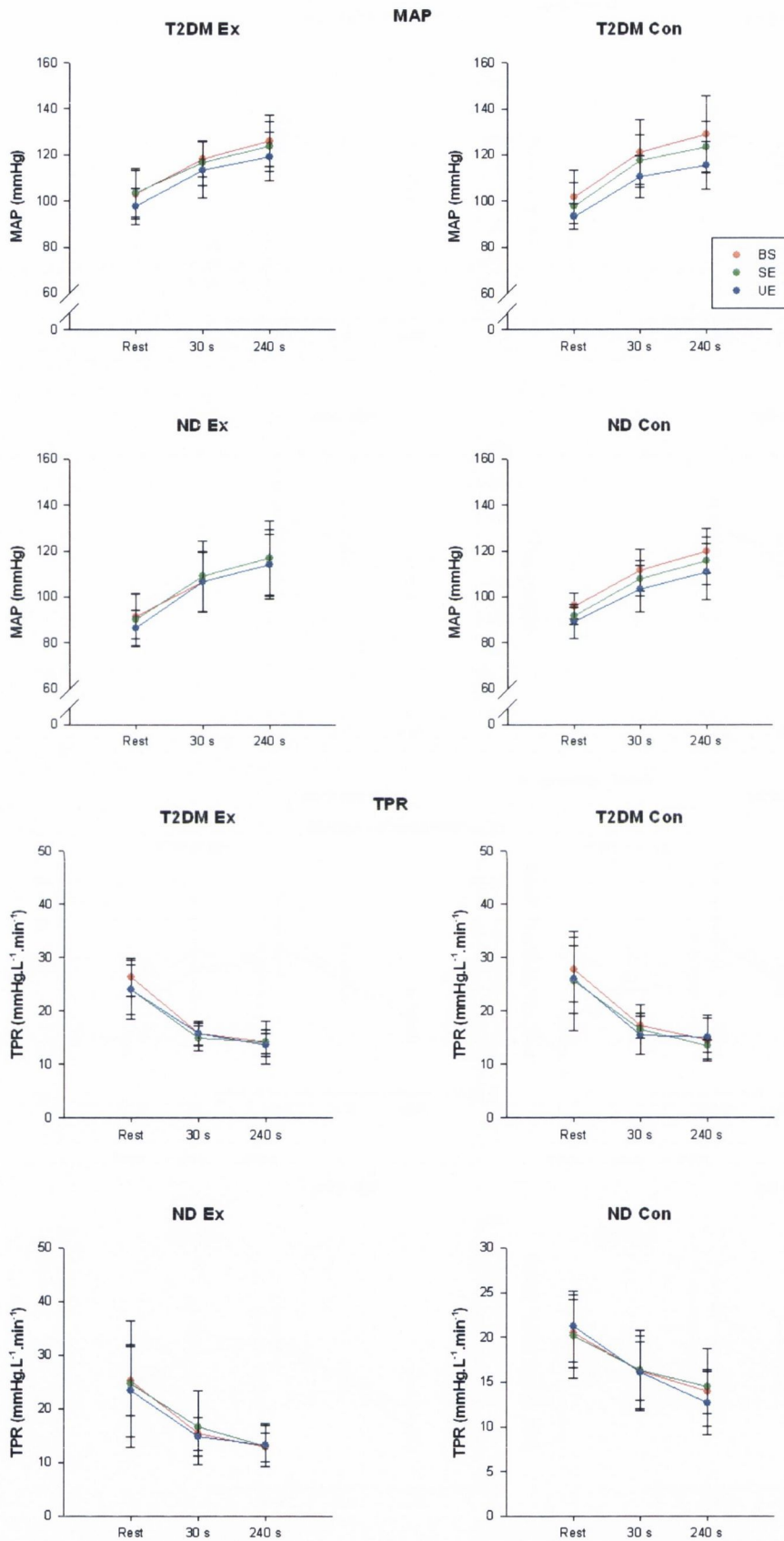


Fig. 5.8. Mean MAP and TPR responses at rest and during cycle exercise (30 s and 240 s) at 80% VT at BS, SE, and UE in each group. No training effect was observed for MAP or TPR responses. For between group comparisons see written text.

5.4.2.5 Percentage steady state CO, CI, and a-vO₂ diff

The effect of exercise intervention on the dynamic response of CO, CI, and a-vO₂ diff for women with and without T2DM in exercise and control groups is presented in Table 5.7. Individual results can be seen in Appendix 29.

The rate of increase of CI, and a-vO₂ diff relative to the % steady state level were not different either within or between groups. The increase of CO at 30 s in relation to 240 s was greater in women with T2DM than non-diabetic women ($P<0.05$).

Table 5.7. Percentage steady state CO, CI, and a-vO₂ diff

% Steady state		T2DM		Non-diabetic		Diabetes effect
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=8)	
CO	BS	73.9±14.9	74.9±63.4	64.1±17.6	55.3±15.2	$P<0.05$
	SE	82.9±48.4	63.4±26.7	55.3±18.7	60.8±25.6	
	UE	61.7±20.8	89.0±22.1	70.0±10.4	52.6±28.9	
CI	BS	75.8±16.2	61.4±29.5	55.1±40.8	56.5±20.4	
	SE	88.9±48.6	62.4±27.4	54.1±20.4	52.3±56.8	
	UE	64.2±26.8	81.1±13.8	70.6±11.2	53.9±29.2	
a-vO ₂ diff	BS	51.6±19.4	55.5±28.6	51.9±21.4	70.4±10.1	
	SE	47.9±24.9	55.3±50.4	57.7±17.8	56.6±17.3	
	UE	50.3±12.8	44.6±11.6	41.9±14.5	62.2±18.1	

5.4.3 Peak and sub-maximal calf performance during plantar flexion exercise

5.4.3.1 Peak calf performance during incremental plantar flexion exercise

The effect of exercise intervention on peak calf performance during incremental plantar flexion exercise for women with and without T2DM in exercise and control groups can be seen in Fig. 5.9. Individual peak calf performance responses can be seen in Appendix 30.

Calf MVC increased in the exercise groups from BS to SE ($P<0.0001$) and UE ($P<0.05$) and was greater in exercise than control groups at SE ($P<0.05$).

Training intervention resulted in an increase in time to failure (TTF) at SE ($P<0.0001$) and UE ($P<0.05$) in both exercise groups and in addition, TTF was greater in exercise than control groups at SE ($P<0.01$).

In both exercise groups, peak force was greater at SE ($P<0.0001$) and UE ($P<0.01$) than at BS and was greater in exercise than control groups at SE ($P<0.01$).

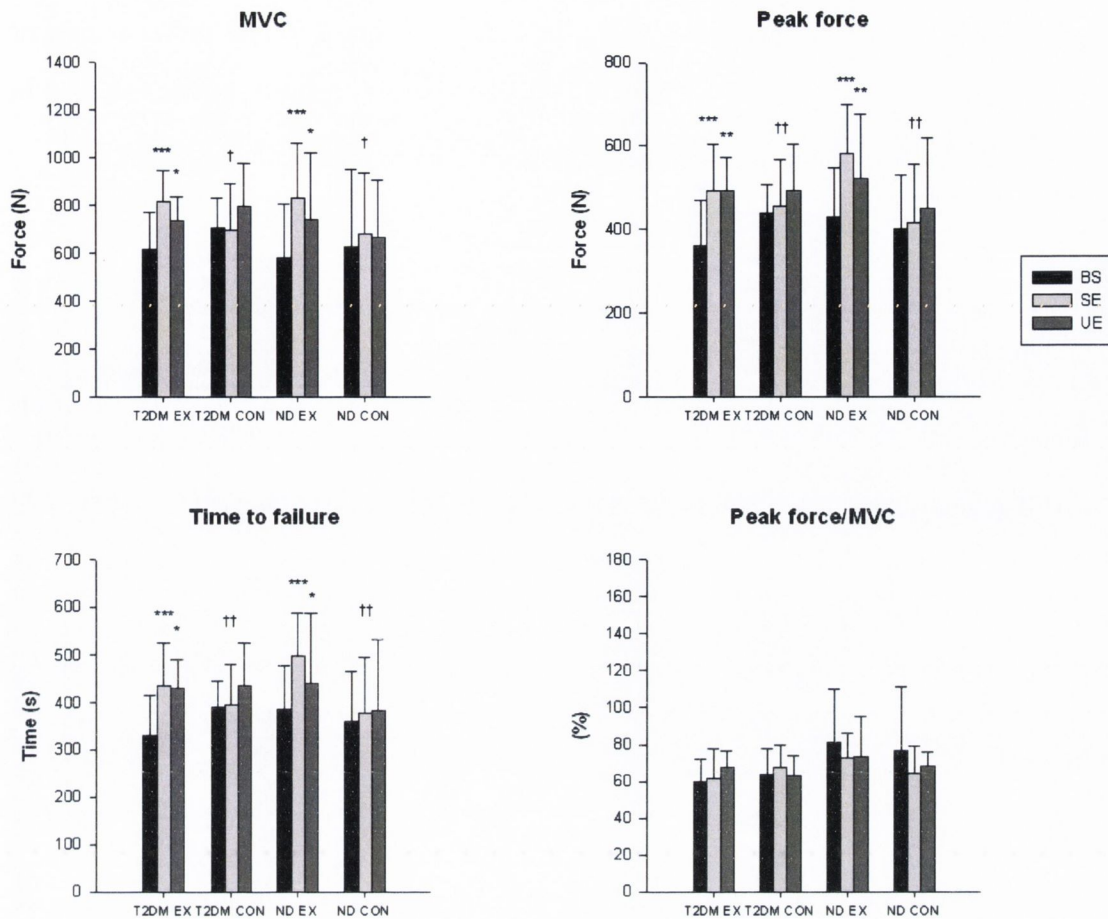


Fig. 5.9. Peak calf performance data. Significantly different from BS * $P<0.05$, ** $P<0.01$, *** $P<0.0001$; significantly different from exercise † $P<0.05$, †† $P<0.01$.

5.4.3.2 LVC kinetic responses during plantar flexion exercise at 30% MVC

The effect of exercise intervention on LVC kinetic responses during plantar flexion exercise at 30% MVC for women with and without T2DM in exercise and control groups can be seen in Table 5.8. Representative LVC kinetic responses from women with and without T2DM in exercise and control groups as well as the predicted responses from the tri-exponential curve fits are depicted in Fig. 5.10. Individual LVC kinetic responses can be seen in Appendix 31.

The amplitudes and time delays of the first and second growth phases, as well as the time delay of the decay phase (TD_2) were unaffected by condition, treatment, or time.

The time constant of the first growth phase (τ_1) was not different either within or between groups. The time constant of the second growth phase (τ_3) was slowed in T2DM Con in comparison to ND Con and T2DM Ex ($P<0.05$).

Table 5.8. Mean LVC kinetic responses during plantar flexion exercise at 30% MVC

		T2DM		Non-diabetic	
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=9)
A_0 (ml.min ⁻¹ .mmHg ⁻¹)	BS	0.47±0.13	0.59±0.16	0.81±0.55	0.50±0.21
	SE	0.44±0.21	0.49±0.11	0.64±0.26	0.46±0.20
	UE	0.43±0.17	0.48±0.16	0.52±0.17	0.49±0.19
A_1 (ml.min ⁻¹ .mmHg ⁻¹)	BS	1.92±1.09	2.75±0.64	1.93±1.17	2.46±2.08
	SE	2.49±0.65	2.14±1.36	3.05±1.25	3.69±2.90
	UE	2.48±0.79	3.09±1.19	3.19±1.62	2.31±1.55
TD ₁ (s)	BS	1.6±2.0	1.0±0.1	1.4±0.7	1.1±0.6
	SE	1.0±0.6	1.6±1.3	1.3±1.4	1.8±1.5
	UE	1.3±1.0	0.7±0.3	1.0±0.6	1.8±1.7
τ_1 (s)	BS	3.8±2.9	2.5±0.8	4.0±2.1	5.8±4.5
	SE	2.8±1.6	2.6±1.9	1.8±1.0	2.1±1.0
	UE	2.1±0.3	4.0±1.7	1.9±0.3	1.5±0.5
A_2 (ml.min ⁻¹ .mmHg ⁻¹)	BS	0.59±0.35	0.76±0.38	0.53±0.52	0.76±0.97
	SE	0.67±0.45	0.45±0.70	0.65±0.83	1.50±2.01
	UE	1.00±0.85	1.85±1.53	0.72±0.84	0.92±0.77
TD ₂ (s)	BS	10.3±6.0	8.4±7.0	11.2±6.1	12.6±9.7
	SE	10.6±8.2	12.8±19.2	12.6±12.2	5.4±3.8
	UE	8.5±3.8	5.2±1.9	7.9±3.6	6.5±3.0
τ_2 (s)	BS	6.1±2.7	6.2±1.2	11.2±12.2	6.0±3.4
	SE	10.0±18.7	3.1±3.2	5.3±5.9	8.1±2.9
	UE	3.6±2.8	8.4±2.4	7.1±4.0	7.9±6.9
A_3 (ml.min ⁻¹ .mmHg ⁻¹)	BS	0.75±0.39	1.26±0.61	1.10±0.80	0.76±0.81
	SE	0.94±0.66	0.96±0.32	1.80±0.71	0.98±0.95
	UE	1.02±0.66	1.56±0.86	1.39±0.66	1.14±0.63
TD ₃ (s)	BS	22.4±5.7	18.7±4.8	24.1±9.6	22.6±10.7
	SE	20.1±4.9	25.0±5.5	16.3±6.9	20.2±8.9
	UE	22.2±4.9	23.8±11.6	21.2±8.7	19.8±5.5
τ_3 (s)	BS	11.4±5.2 <i>b</i>	13.0±7.7	15.9±9.8	12.2±5.8 <i>b</i>
	SE	8.5±3.5 <i>b</i>	20.9±9.8	15.7±6.8	8.6±5.8 <i>b</i>
	UE	12.0±5.0 <i>b</i>	16.3±7.9	14.2±4.4	12.5±4.1 <i>b</i>
End A (ml.min ⁻¹ .mmHg ⁻¹)	BS	3.14±1.39	4.60±0.77	3.83±1.84	3.72±2.56
	SE	3.87±1.07	3.58±1.37	5.49±1.86	5.13±3.85
	UE	3.93±1.21	5.05±2.04	5.10±2.04	3.94±2.29

Significantly different from T2DM Con *bP*<0.05

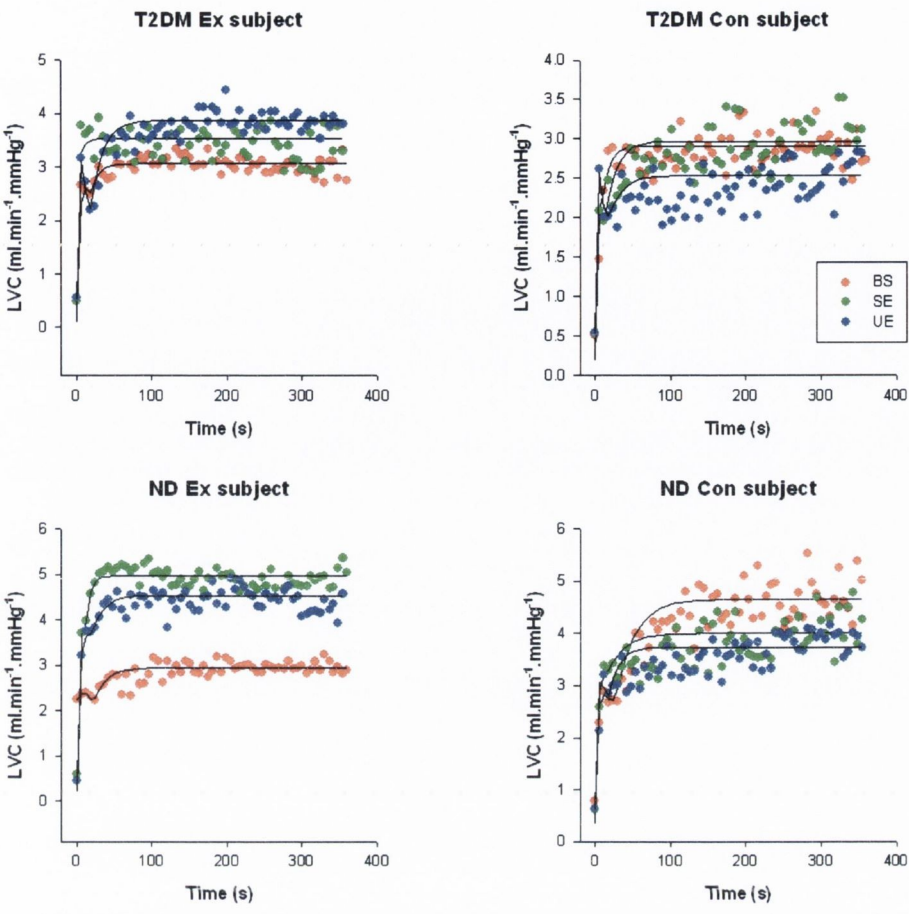


Fig. 5.10. LVC kinetic responses and predicted responses from the tri-exponential curve fits during plantar flexion exercise at 30% MVC from representative subjects from each group at BS, SE, and UE.

5.4.3.3 Heart rate and MAP responses during plantar flexion exercise at 30% MVC

The effect of exercise intervention on HR and MAP responses at rest and during plantar flexion exercise at 30% MVC for women with and without T2DM in exercise and control groups are presented in Fig. 5.11. Individual HR and MAP responses can be seen in Appendix 32.

HR responses both at rest and during exercise (30 s, 180 s, and 360 s) were not different either within or between groups. MAP responses were greater in women with T2DM than non-diabetic women both at rest ($P < 0.0001$) and during exercise (30 s, 180 s, and 360 s) ($P < 0.01$).

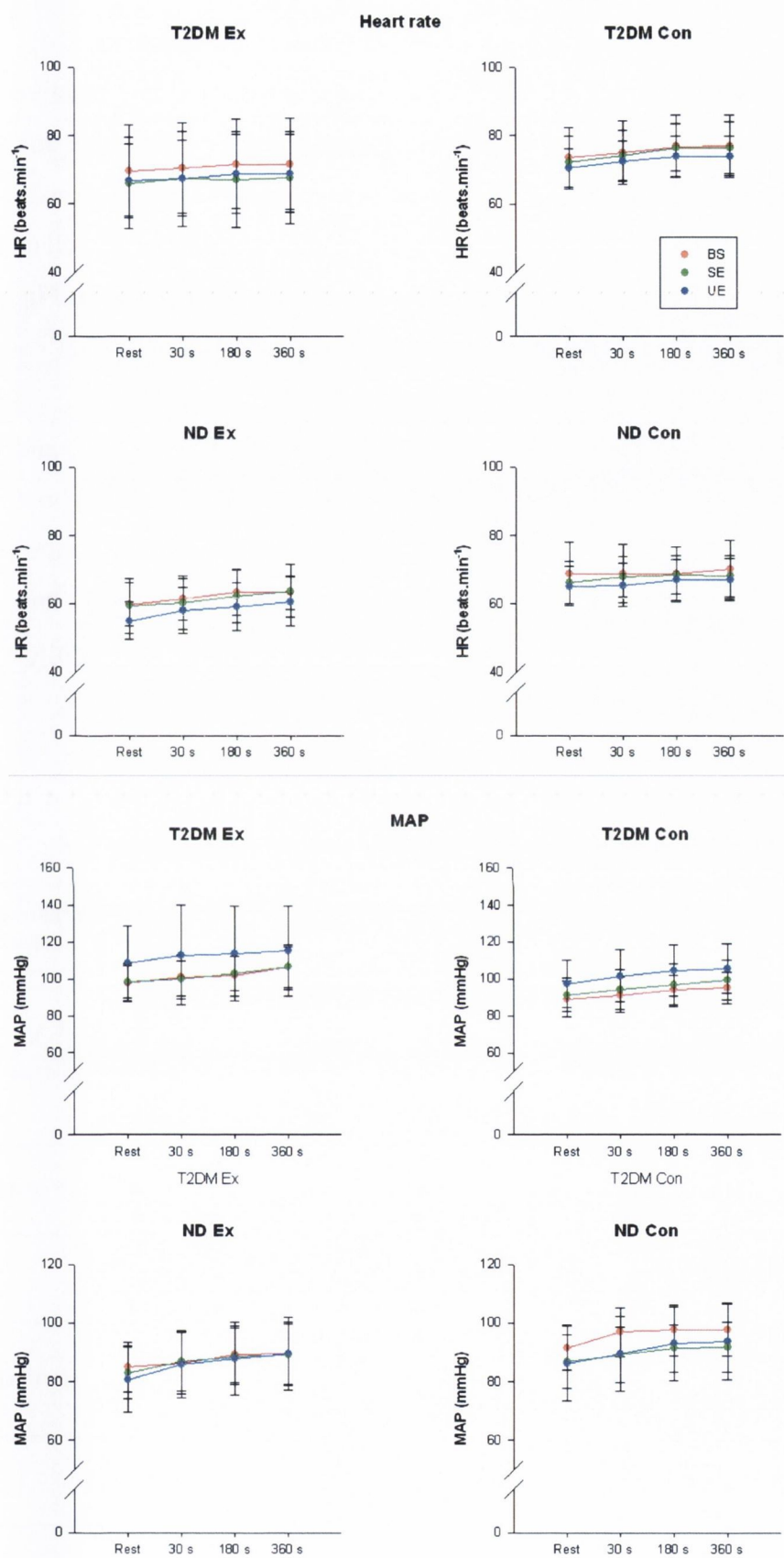


Fig. 5.11. Mean HR and MAP responses at rest and during plantar flexion exercise (30 s, 180 s, and 360 s) at 30% MVC at BS, SE, and UE for each group. There was no training effect for either HR or MAP responses. For between group comparisons see written text.

5.4.4 FVC kinetic responses during reactive hyperaemia of the forearm

The effect of exercise intervention on FVC kinetic responses during reactive hyperaemia of the forearm for women with and without T2DM in exercise and control groups is presented in Table 5.9. Representative FVC kinetic responses from women with and without T2DM in exercise and control conditions as well as the predicted responses from the mono-exponential curve fits are depicted in Fig. 5.12. Individual FVC kinetic responses can be seen in Appendix 33.

The resting FVC, predicted resting FVC (a), area under the curve (AUC), and decay constant (c) were not different either within or between groups throughout the intervention. Peak FVC (measured) was greater in exercise than control groups ($P<0.05$). There was a treatment effect for both the amplitude (A) and predicted peak FVC (a + A) with both being greater in exercise than control groups ($P<0.05$).

Table 5.9. Mean FVC kinetic responses during reactive hyperaemia of the forearm

		T2DM		Non-diabetic		Treatment effect
		Exercise (n=7)	Control (n=7)	Exercise (n=9)	Control (n=8)	
Resting FVC (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	BS	2.5±1.7	1.5±0.5	2.7±2.6	1.5±0.5	
	SE	2.7±1.9	2.3±1.3	3.3±2.2	1.7±0.5	
	UE	2.5±1.5	1.8±0.9	1.4±0.7	1.6±0.7	
a (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	BS	2.0±1.4	2.2±1.0	2.5±1.1	1.2±0.3	
	SE	1.9±1.2	2.0±1.6	2.7±1.9	1.7±0.5	
	UE	2.8±2.1	1.7±1.4	1.2±0.7	1.1±0.3	
A (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	BS	17.7±3.3	15.3±5.1	25.0±9.2	14.5±5.1	P<0.05
	SE	20.1±4.3	19.9±5.8	20.4±6.1	17.2±3.8	
	UE	16.5±4.6	18.9±7.0	23.9±4.4	16.3±3.8	
c	BS	0.06±0.02	0.04±0.03	0.06±0.05	0.05±0.02	
	SE	0.07±0.03	0.05±0.05	0.06±0.04	0.06±0.03	
	UE	0.10±0.09	0.06±0.06	0.07±0.02	0.06±0.02	
Predicted peak FVC (a + A) (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	BS	19.7±4.2	17.5±4.8	27.5±8.7	15.7±5.2	P<0.05
	SE	21.9±4.8	21.9±6.4	23.1±7.5	18.9±4.0	
	UE	19.3±5.6	20.6±7.2	25.1±4.7	17.3±3.9	
AUC (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	BS	993±512	1134±327	1423±435	659±189	
	SE	950±610	1134±774	1324±861	869±261	
	UE	1184±741	961±439	765±386	626±158	
Peak FVC (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)	BS	19.6±4.4	17.6±4.8	27.8±9.3	15.9±5.1	P<0.05
	SE	21.8±5.1	21.3±7.1	22.2±7.4	18.7±4.0	
	UE	19.2±5.6	21.9±7.1	24.9±4.7	17.2±3.9	

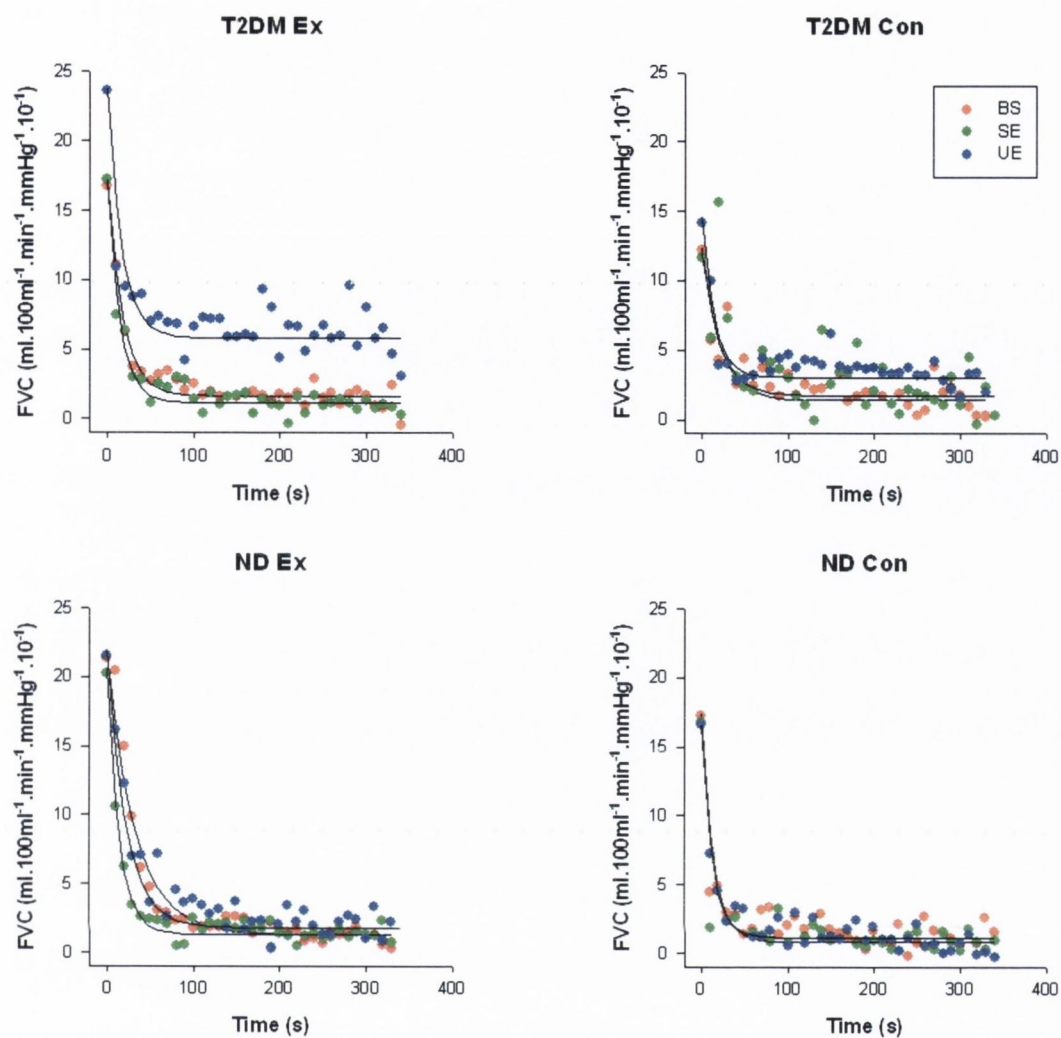


Fig. 5.12. FVC responses during reactive hyperaemia of the forearm from individual subjects from each group at BS, SE, and UE.

5.4.5 Vasodilatory and hyperaemic responses during incremental plantar flexion exercise

5.4.5.1 Resting LBF and LVC responses

The effect of exercise intervention on resting LBF (ml.min⁻¹) and resting LVC (ml.min⁻¹.mmHg⁻¹) for women with and without T2DM in exercise and control groups is presented in Table 5.10. Individual resting LBF and LVC responses are available in Appendix 34. There were no changes in resting LBF or LVC either within or between groups.

Table 5.10. Resting LBF and LVC

		T2DM		Non-diabetic	
		Exercise	Control	Exercise	Control
Resting LBF (ml.min ⁻¹)	BS	41.0±11.4	50.6±20.3	54.2±24.2	52.2±20.7
	SE	44.5±15.9	52.1±18.7	59.3±28.5	40.2±19.7
	UE	50.6±12.6	60.5±22.3	48.3±20.0	43.2±14.5
Resting LVC (ml.min ⁻¹ .mmHg ⁻¹)	BS	0.39±0.10	0.52±0.21	0.60±0.29	0.52±0.21
	SE	0.48±0.22	0.59±0.31	0.70±0.32	0.42±0.32
	UE	0.44±0.13	0.56±0.28	0.54±0.24	0.45±0.15

5.4.5.2 LBF and LVC responses during incremental plantar flexion exercise

The effect of exercise intervention on LBF (ml.min⁻¹) and LVC (ml.min⁻¹.mmHg⁻¹) responses during incremental plantar flexion exercise for women with and without T2DM in exercise and control groups can be seen in Fig. 5.13. Individual responses during incremental plantar flexion exercise can be seen in Appendix 34.

Training did not induce any effects on either peak LBF or peak LVC responses during the incremental test. In order to maintain the same n numbers throughout the intervention phases at each workload in each group, only data from workloads that were achieved in the three phases of the intervention (from each subject) are included.

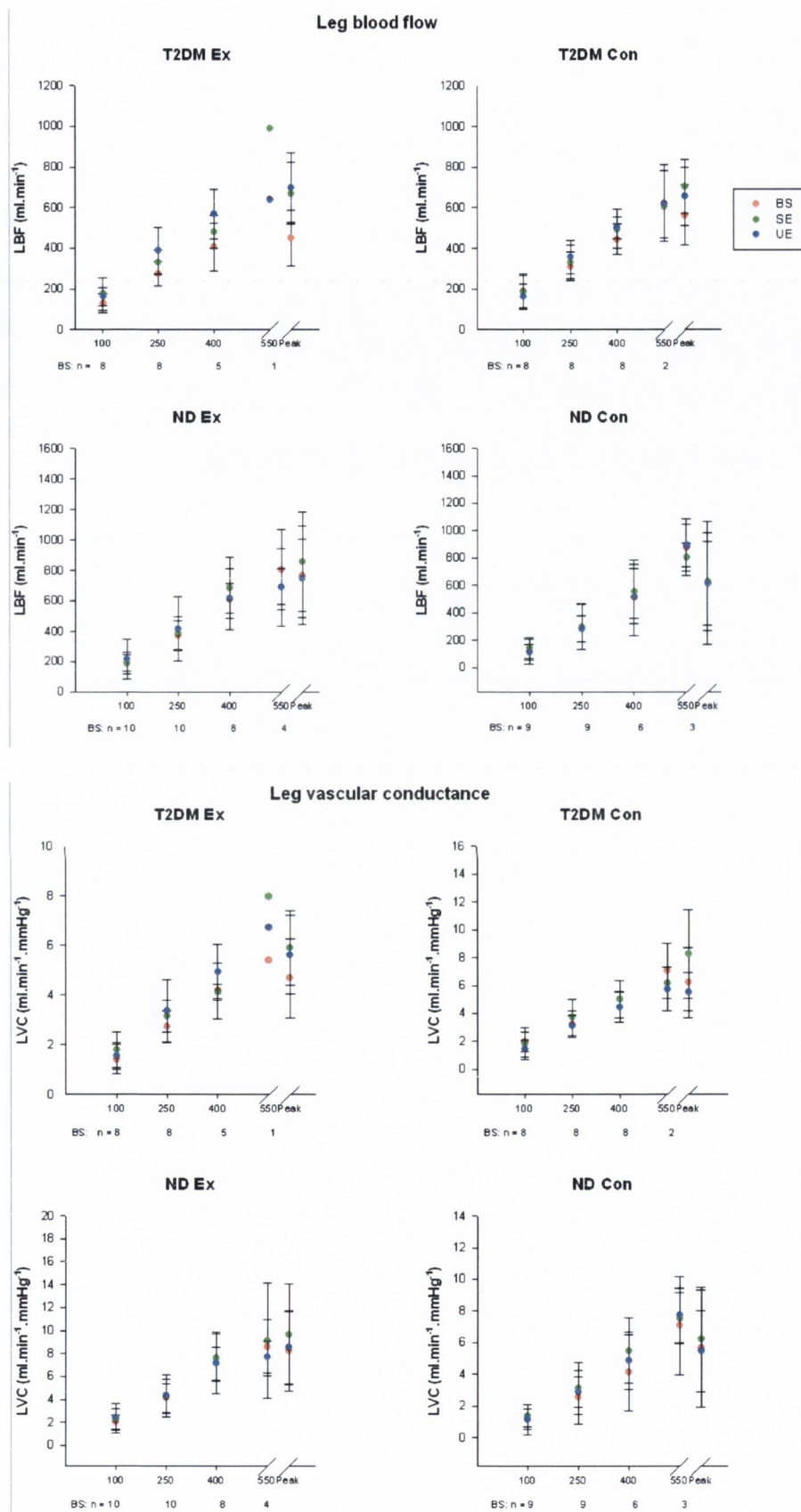


Fig. 5.13. Mean LBF and LVC responses during incremental plantar flexion exercise for each group.

5.4.5.3 *Hyperaemic and vasodilatory responses during incremental plantar flexion exercise*

The effect of exercise intervention on the hyperaemic (slope of LBF (ml.min⁻¹) vs. % of maximum force and slope of LBF (ml.min⁻¹) vs. force (N)) and vasodilatory (slope of LVC (ml.min⁻¹.mmHg⁻¹) vs. % of maximum force and slope of LVC (ml.min⁻¹.mmHg⁻¹) vs. force (N)) responses during incremental plantar flexion exercise for women with and without T2DM in exercise and control groups are presented in Table 5.11. Individual hyperaemic and vasodilatory responses can be seen in Appendix 34.

There were no differences either within or between groups with respect to hyperaemic or vasodilatory responses.

Table 5.11. Hyperaemic and vasodilatory responses during incremental plantar flexion exercise

		T2DM		Non-diabetic	
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=9)
Slope BF (ml.min ⁻¹) vs. % max force	BS	329±182	393±172	577±336	482±337
	SE	531±268	483±187	710±292	456±295
	UE	573±175	520±132	580±243	549±340
Slope VC (ml.min ⁻¹ .mmHg ⁻¹) vs. % max force	BS	3.3±1.7	4.1±2.2	6.2±3.4	3.9±3.0
	SE	4.2±1.6	5.9±2.9	7.8±4.5	4.7±2.4
	UE	4.8±2.0	4.5±1.2	6.1±2.8	4.9±2.4
Slope BF (ml.min ⁻¹) vs. force (N)	BS	0.9±0.3	0.9±0.3	1.3±0.6	1.6±1.7
	SE	1.0±0.5	1.0±0.2	1.2±0.5	1.0±0.5
	UE	1.0±0.3	1.1±0.1	1.2±0.7	1.2±0.3
Slope VC (ml.min ⁻¹ .mmHg ⁻¹) vs. force (N)	BS	0.01±0.003	0.01±0.010	0.01±0.01	0.01±0.010
	SE	0.01±0.003	0.01±0.004	0.01±0.01	0.01±0.003
	UE	0.01±0.004	0.01±0.002	0.01±0.01	0.01±0.003

5.5 Discussion

The main findings of the current experiment are as follows: Firstly $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) improved following 3 months of supervised aerobic plus resistance training in T2DM Ex and ND Ex groups, but these improvements were not maintained during unsupervised exercise. Secondly, exercise training did not enhance the rate of adjustment of the $\dot{V}O_2$ kinetic or CO responses to moderate intensity cycle exercise (80% VT) performed relative to the current VT at each phase (BS, SE, and UE). Thirdly, the speed of the vasodilatory response during low intensity (30% MVC) plantar flexion exercise relative to the phase-specific current MVC and both leg hemodynamic and vasodilatory responses during incremental plantar flexion were unchanged following exercise intervention. In addition, exercise training did not improve vasodilatory responses during reactive hyperaemia of the forearm.

Incremental cycle test

It has been well documented that regular aerobic exercise improves $\dot{V}O_{2\text{ peak}}$ in individuals with T2DM (Mourier *et al.*, 1997; Brandenburg *et al.*, 1999; Okada *et al.*, 2010). Consistent with previous research, we found improvements in both relative ($\text{ml.kg}^{-1}.\text{min}^{-1}$) and absolute (L.min^{-1}) $\dot{V}O_{2\text{ peak}}$ following 3 months of supervised aerobic plus resistance exercise in the two exercising groups. In addition to an increase in $\dot{V}O_{2\text{ peak}}$, peak workload increased in both exercise groups after 3 months of supervised exercise but was not improved any further following unsupervised exercise. The increase in relative $\dot{V}O_{2\text{ peak}}$ which was of the magnitude of 13% in T2DM Ex is somewhat similar to previous investigations reporting increases in relative $\dot{V}O_{2\text{ peak}}$ following 3 months of aerobic exercise (Brandenburg *et al.*, 1999), combined aerobic and resistance exercise (Okada *et al.*, 2010), and 12 months of combined aerobic and resistance exercise (Loimaala *et al.*, 2007). Although relative $\dot{V}O_{2\text{ peak}}$ was greater in both exercise groups at UE than at BS in the current investigation, the improvements acquired in $\dot{V}O_{2\text{ peak}}$ (expressed in $\text{ml.kg}^{-1}.\text{min}^{-1}$ or L.min^{-1}) were not maintained following the unsupervised exercise intervention. While the effect of home-based training in diabetic populations has not been well investigated, one former investigation has shown that home-based training following an initial period of supervised exercise

is not sufficient to maintain improvements in glycaemic control or body composition acquired during the initial period of supervised exercise in men and women with T2DM (Dunstan *et al.*, 2005).

In the current investigation, the reduction in training compliance is likely to have contributed to the reduction in $\dot{V}O_{2\text{ peak}}$ from SE to UE in both the T2DM Ex and ND Ex groups. Furthermore, as the intensity and frequency of training sessions were self reported it is possible that individuals 'overestimated' the volume of training completed. This is significant as research indicates that the majority of individuals with T2DM do not engage in sufficient regular exercise and that compliance with exercise prescription is low in diabetic individuals (Krug *et al.*, 1991; Morrato *et al.*, 2007). The failure to maintain or further improve $\dot{V}O_{2\text{ peak}}$ during unsupervised exercise has important implications in terms of exercise prescription for individuals with T2DM. It has been well documented that decreased cardiovascular fitness is associated with increased morbidity and mortality in diabetic populations (Wei *et al.*, 2000; Church *et al.*, 2004); therefore, it is important that individuals with T2DM adhere to exercise programmes.

In the current investigation, load at VT increased following SE in both exercise groups and was also accompanied by an increase in $\dot{V}O_2$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) at VT. VT represents the point at which ventilation increases disproportionately with $\dot{V}O_2$ during exercise due to the buffering of hydrogen ions (produced from the dissociation of lactic acid) by bicarbonate leading to excess $\dot{V}CO_2$. The increase in CO_2 stimulates the chemoreceptors to increase ventilation. The increase in load at VT following training intervention indicates that the point at which metabolic acidosis occurred during the graded exercise test was delayed and therefore is a reflection of training induced adaptations in lactate production and clearance. However, the training induced adaptations in load at VT and $\dot{V}O_2$ at VT were not maintained during the UE phase highlighting the importance of supervision during exercise intervention in both diabetic and non-diabetic populations.

$\dot{V}O_2$ kinetics

Training did not enhance the speed of the $\dot{V}O_2$ kinetic responses in either of the two exercising groups over the course of the 7-month intervention. This conflicts with at least two previous research findings where $\dot{V}O_2$ kinetic responses became faster (shorter time constant) after exercise training (Brandenburg *et al.*, 1999; Mac Ananey, 2010). First, Brandenburg *et al.*, (1999) observed faster $\dot{V}O_2$ kinetic responses during low (20 W) and moderate intensity (30 W) exercise but not high intensity (80 W) exercise following 3 months of supervised aerobic exercise in premenopausal women with T2DM. However, no improvements were found in $\dot{V}O_2$ kinetic responses in lean and obese non-diabetic control women with the exception of moderate intensity exercise in obese women (shorter time constant) (Brandenburg *et al.*, 1999). The authors suggested that the training programme (1 h of aerobic exercise at 70-85% of peak HR 3 d.wk⁻¹) was inadequate to enhance $\dot{V}O_2$ kinetic responses in non-diabetic individuals (Brandenburg *et al.*, 1999). Investigations conducted in non-diabetic populations indicate that higher intensity exercise programmes are required to impact on $\dot{V}O_2$ kinetic responses (Phillips *et al.*, 1995). This argument seems plausible given the fact that the $\dot{V}O_2$ kinetic responses were quite normal (reflecting adequate metabolic and cardiovascular responses to exercise) in non-diabetics at baseline in the study by Brandenburg *et al.*, (1999). Using exercise intensities calculated to each individual's VT and $\dot{V}O_{2\text{ peak}}$, a recent study from our laboratory has reported improvements in $\dot{V}O_2$ kinetic responses during low (50% VT), moderate (80% VT), and high intensity (50% Δ) cycle exercise in women with T2DM following 3 months of supervised aerobic and resistance exercise intervention (Mac Ananey, 2010). Furthermore, the improvements acquired during the initial 3-month period were maintained following a further 3 months of gym-based unsupervised exercise.

However, it is important to note that the exercise loads used post-training to determine $\dot{V}O_2$ kinetic responses in the two former studies (Brandenburg *et al.*, 1999; Mac Ananey, 2010) were the same as those used at baseline. In the investigation by Brandenburg *et al.*, (1999), subjects exercised at the same absolute intensities (20, 30, & 80 W) pre and post intervention, while in the study by Mac Ananey (2010) the exercise intensities were calculated relative to the VT

obtained at baseline. In contrast, we determined exercise intensities relative to each individual's current VT (i.e. phase-specific VT). While we did not find a speeding of the $\dot{V}O_2$ kinetic responses in either exercise group, the increase in load at VT meant that each exercise group was working at a greater absolute workload at SE than at BS. Thus, an increase in oxygen consumption accompanying an increase in workload following SE would affect the time taken to reach steady state conditions. Furthermore, despite a lower workload at VT in both the control groups compared to the exercise groups at SE, the $\dot{V}O_2$ kinetic response at SE was similar to that at BS in both control groups. Therefore, it seems plausible to suggest that some form of training-induced adaptations have taken place in both exercise groups with respect to the dynamics of $\dot{V}O_2$ at exercise onset.

Cardiac output

No training effect was observed for either CO at SE or UE in either exercise group in the current study. This finding is in agreement with that of Mac Ananey (2010) who failed to find improvements in CO during sub-maximal cycling exercise following supervised and unsupervised exercise intervention while using the same workloads established at baseline. The fact that we did not find an increase in CO at SE despite exercising at greater workloads following the initial supervised exercise intervention period suggests some form of metabolic or peripheral vascular adaptation to chronic exercise. The similar CO observed at SE and UE in comparison to BS in both exercising groups could reflect more effective distribution of blood flow and an increased capacity of the muscle to generate ATP at a lower P_{O_2} . The a- $\dot{V}O_2$ diff responses tended to be larger in the exercising groups after SE, and this could be linked to an enhanced oxygen extraction capacity, increased mitochondrial content (Phielix *et al.*, 2010), mitochondrial density (Toledo *et al.*, 2007), capillary density (Gavin *et al.*, 2007), and fibre size (Holten *et al.*, 2004).

Leg and arm hemodynamic responses

We did not observe any improvements in dynamic response of LVC kinetics during 30% MVC plantar flexion in either exercise group following supervised or unsupervised exercise intervention. However, the increase in MVC at SE and UE

in both T2DM Ex and ND Ex groups resulted in both groups exercising at a greater absolute workload at SE and UE compared to baseline. The resulting increase in workload would require a longer rate of adaptation in the vasodilatory response to an exercise load and this may have obscured any 'improvements' in LVC kinetic responses in the current study (i.e. similar speed of LVC kinetics post-supervised exercise and post-unsupervised exercise despite an increase in force). Our findings appear contradictory to a previous investigation where a faster rate of adjustment in LVC during plantar flexion at 70% MVC was reported following 3 months of supervised exercise; improvements which were maintained after a follow-up period of 3 months of unsupervised exercise (Mac Ananey, 2010). In the study by Mac Ananey (2010), the improvement in the temporal adjustment of the hyperaemic response was attributed to a speeding of the time constant of the second phase and occurred in the absence of any increase in the magnitude of the response (End A). However, it is important to note that in this former study (Mac Ananey, 2010) the workload at 70% MVC did not change over the course of the intervention (i.e. the force post-exercise training was the same as that used at baseline).

Although in the present study, both peak force and time to failure increased during incremental plantar flexion exercise in T2DM Ex and ND Ex at SE and UE from BS, the peak LVC vasodilatory responses were unaffected by exercise intervention. Furthermore, both the hyperaemic (slope of LBF vs. force) and vasodilatory (slope of LVC vs. force) responses were not different following exercise intervention. This finding is surprising considering the increase in peak force in both exercise groups at SE and UE, but could be reflective of training-induced adaptations in the extraction and/or utilisation of oxygen within the myocyte.

Similarly, forearm reactive hyperaemia responses were not affected by the training intervention in either exercise group. The aerobic and resistance training interventions focused predominantly on the lower limbs apart from 4 upper body resistance exercises. It is therefore possible that resistance exercise and/or cardiovascular exercise do not induce systemic adaptations that are noticeable in the upper limbs, at least at the intensities used in the current investigation. In

agreement with these results, a previous study conducted on non-diabetic men and women failed to find improvements in forearm endothelium-dependent vasodilation following a period of combined aerobic and resistance exercise despite improvements being found in $\dot{V}O_{2\text{ peak}}$ (Maiorana *et al.*, 2001b).

Mechanisms underpinning improvements

The faster rate of adaptation in $\dot{V}O_2$ kinetic responses in women with T2DM has been attributed to improvements in oxygen delivery and/or oxygen extraction (Brandenburg *et al.*, 1999). Due to the close temporal alignment of $\dot{V}O_2$ and blood flow responses (MacDonald *et al.*, 1998), it is possible that the improvement in LVC kinetic responses seen following supervised and unsupervised training in T2DM (Mac Ananey, 2010) might underpin an improvement in $\dot{V}O_2$ kinetic responses following exercise intervention. While we did not observe a speeding of either $\dot{V}O_2$ or LVC kinetic responses following training, the fact that the rate of adaptation in both $\dot{V}O_2$ and LVC was similar pre and post intervention at greater absolute workloads suggests some improvement in both parameters with chronic exercise. Studies conducted on non-diabetic men indicate that the faster $\dot{V}O_2$ kinetics observed following high intensity interval training or endurance training in the absence of any changes in muscle oxygenation kinetics could reflect adaptations in microvascular perfusion and oxygen delivery (McKay *et al.*, 2009; Murias *et al.*, 2010). High intensity interval training has been shown to increase oxidative enzymes, citrate synthase, and pyruvate dehydrogenase, in the initial 2 weeks of training in non-diabetic participants (Burgomaster *et al.*, 2005; Burgomaster *et al.*, 2006). A faster activation of oxidative enzymes in the initial period of exercise would lead to greater substrate provision for the tricarboxylic acid cycle and electron transport chain, which could subsequently lead to a more rapid achievement of steady state conditions through a speeding of the $\dot{V}O_2$ kinetic response.

The adaptation of heart rate during moderate intensity cycle exercise in the current investigation was used to reflect the adaptations in CO and bulk blood flow. While we did not find any change in heart rate kinetics following exercise intervention, it is possible that the redistribution of blood flow and subsequent rate

of adaptation in blood flow responses may be improved with training. In the study by Mac Ananey (2010), the speeding of the $\dot{V}O_2$ kinetic response (during low, moderate, and high intensity cycle exercise) following exercise intervention (both supervised and unsupervised) occurred in the absence of any increase in CO and was attributed to the faster rate of adaptation in LVC kinetic responses during high intensity plantar flexion exercise.

As the vascular endothelium is an important regulator in vascular tone and endothelial dysfunction is an early manifestation of the atherosclerotic process, the importance of exercise intervention in reversing this process cannot be underestimated. Studies that have reported improvements in conduit or resistance endothelial vessel function following exercise intervention have typically reported improvements in $\dot{V}O_{2\text{ peak}}$ (Maiorana *et al.*, 2001a; Colberg *et al.*, 2006; Okada *et al.*, 2010), indicating that an improvement in endothelial function may increase oxygen delivery during exercise.

The improvements observed in resistance and conduit vessel function accompanying exercise intervention in individuals with T2DM appear to be largely endothelial-dependent (Maiorana *et al.*, 2001a). It has been postulated that the exercise-induced training effects on vascular function could be mediated via an increased shear stress mechanism via increased NO production (leading to smooth muscle relaxation and subsequent vasodilation) and could be responsible for the improved endothelial function seen in individuals with T2DM (De Filippis *et al.*, 2006). Evidence supporting this theory can be found in a study on dogs where ACh mediated NO production increased in coronary arteries and microvessels following exercise training (Sessa *et al.*, 1994). Repetitive increases in blood flow and shear stress that accompany regular exercise induce an adaptive response that results in an increase in eNOS mRNA expression in skeletal muscle of individuals with T2DM (Sixt *et al.*, 2010). As nitric oxide synthase (NOS) mediates the production of NO, it therefore seems reasonable to suggest that increases in NOS should result in an increase in NO production and a subsequent improvement in vascular responsiveness.

Impaired mitochondrial function is an early event in the pathogenesis of T2DM. Numerous studies have reported improvements in morphological outcomes in response to exercise training. Increases in mitochondrial respiration and content have been reported with combined aerobic and endurance training (Phielix *et al.*, 2010). Muscle biopsies taken from the vastus lateralis muscle revealed an increase in oxidative capacity as determined from ex vivo respiratory measurements, which was accompanied by increased glucose disposal in men with T2DM (Phielix *et al.*, 2010). It would appear that modest increases in physical activity are sufficient to induce morphological changes in the mitochondria of diabetic individuals; improvements in mitochondrial density, mitochondrial size, and oxidative capacity have been reported following moderate intensity physical activity (30-40 min of aerobic exercise most days of the week) over a 5-month period (Toledo *et al.*, 2007). The increase in uncoupling protein (UCP) 3 (which is an initiator of mitochondrial biogenesis) following low intensity activity could enhance the oxidative capacity of skeletal muscle (Fritz *et al.*, 2006). It is possible that the improvements in $\dot{V}O_2$ peak in the current investigation are in part a reflection of increased mitochondrial function. Associations have been found between $\dot{V}O_2$ peak and mitochondrial density, mitochondrial size, and oxidative capacity (Toledo *et al.*, 2007).

In summary, supervised exercise intervention improves $\dot{V}O_2$ peak in previously sedentary women with and without T2DM. However, the training induced adaptations in peak exercise performance were not maintained during a subsequent unsupervised period of exercise. Exercise intervention did not enhance the rate of adaptation in either LVC or $\dot{V}O_2$ at the beginning of light and moderate exercise respectively at workloads specific to each training phase. However, as both exercise groups were exercising at greater absolute workloads following SE and the kinetic responses of both $\dot{V}O_2$ and LVC were unchanged, it is likely that training adaptations took place with respect to both of these parameters. Enhancing the rate of adaptation in $\dot{V}O_2$ at exercise onset has important implications for individuals with T2DM and may overcome a disincentive to engage in regular exercise.

Chapter 6: Inflammatory and endothelial markers

6.1 Aims:

- (a) To investigate the impact of T2DM on plasma levels of IL-6, IL-10, TNF- α , CRP, SAA, sICAM-1, and sVCAM-1 in postmenopausal women
- (b) To investigate the impact of T2DM on IL-6, TNF- α , visfatin, and resistin mRNA expression from whole blood in postmenopausal women
- (c) To examine the effect of a long-term exercise intervention on plasma levels of IL-6, IL-10, TNF- α , CRP, SAA, sICAM-1, and sVCAM-1
- (d) To examine the effect of a long-term exercise intervention on IL-6, TNF- α , visfatin, and resistin mRNA expression from whole blood

6.2 Subjects

A total of forty postmenopausal women were recruited to participate in the baseline study: 19 had T2DM and 21 were non-diabetic. From these forty women, a total of 34 women, 16 with T2DM and 18 non-diabetics, participated in an exercise intervention study (12-wk supervised exercise intervention followed by a 16-wk unsupervised intervention). For the intervention study, the women were divided into 4 groups: T2DM exercise n=8, T2DM control n=8, non-diabetic exercise n=10, and non-diabetic control n=8.

6.3 Results

6.3.1 Markers of inflammation and endothelial function (baseline study)

6.3.1.1 Subject characteristics

Physical characteristics and habitual physical activity levels in women with and without T2DM are presented in Table 6.1. Individual subject characteristics can be seen in Appendix 35.

In the group of postmenopausal women, significant differences were observed in BMI, body mass ($P<0.0001$), and WHR ($P<0.01$) between groups with and without T2DM.

In the subgroup of BMI matched postmenopausal women, there were no differences between women with and without T2DM with respect to age, physical activity levels, BMI, weight, height, WHR, or ABI.

Table 6.1. Physical characteristics and activity levels

	T2DM (n = 19)	Non-diabetic (n = 21)	T2DM (n = 11)	Non-diabetic (n = 9)
	Postmenopausal		BMI matched postmenopausal	
Age (yr)	57.0±4.0	56.1±5.7	57.7±4.7	56.5±3.5
BMI (kg.m ⁻²)	34.1±5.5***	26.4±4.0	30.7±3.0	29.7±1.9
Weight (kg)	85.7±14.2***	66.0±10.1	78.1±9.8	75.3±5.9
Height (m)	1.58±0.05	1.60±0.07	1.60±0.05	1.59±0.03
WHR	0.95±0.05**	0.90±0.08	0.93±0.05	0.91±0.08
ABI	1.06±0.11	1.09±0.13	1.04±0.11	1.10±0.10
LOPAR (MET h.wk ⁻¹)	104±47	112±49	93±45	124±61

Significantly different from non-diabetic ** $P<0.01$, *** $P<0.0001$.

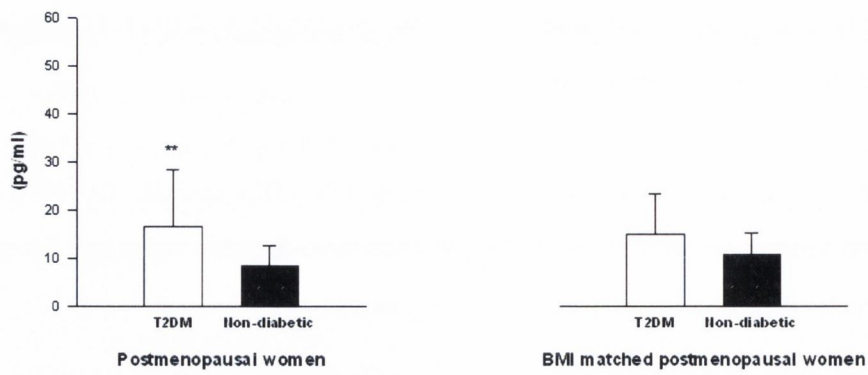
6.3.1.2 Plasma levels of IL-6, IL-10, and TNF- α

Plasma levels of IL-6, IL-10, and TNF- α are presented in Fig. 6.1. Individual results can be seen in Appendix 36.

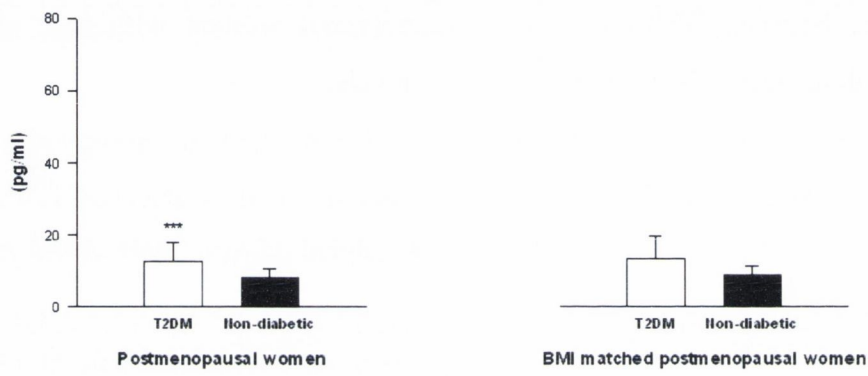
In the group of postmenopausal women, IL-6 ($P<0.01$) and IL-10 ($P<0.0001$) levels were greater in women with T2DM than non-diabetic women. However, TNF- α levels were similar between the two groups.

In the subgroup of BMI matched postmenopausal women, there were no differences between BMI-matched postmenopausal women with and without T2DM with respect to IL-6, IL-10 or TNF- α levels.

IL-6



IL-10



TNF- α

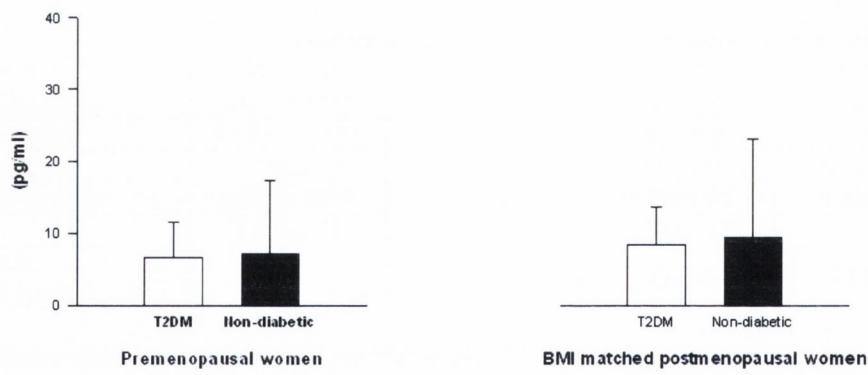


Fig. 6.1. Plasma levels of IL-6, IL-10, and TNF- α . Significantly different from non-diabetic ** $P<0.01$, *** $P<0.0001$.

6.3.1.3 *IL-6, TNF- α , visfatin, and resistin mRNA expression*

IL-6, TNF- α , visfatin, and resistin mRNA expression are presented in Fig. 6.2. Individual results can be seen in Appendix 36.

The expression of visfatin mRNA was greater in the group of postmenopausal women with T2DM than non-diabetic women ($P<0.01$). IL-6, TNF- α , and resistin mRNA expression were similar between the groups of postmenopausal women with and without T2DM.

Visfatin mRNA expression was numerically greater in the subgroup of BMI matched postmenopausal women with T2DM than non-diabetic women but the result did not reach statistical significance ($P=0.06$). There were no differences in IL-6, TNF- α , or resistin mRNA expression between the subgroups of BMI matched postmenopausal women with and without T2DM.

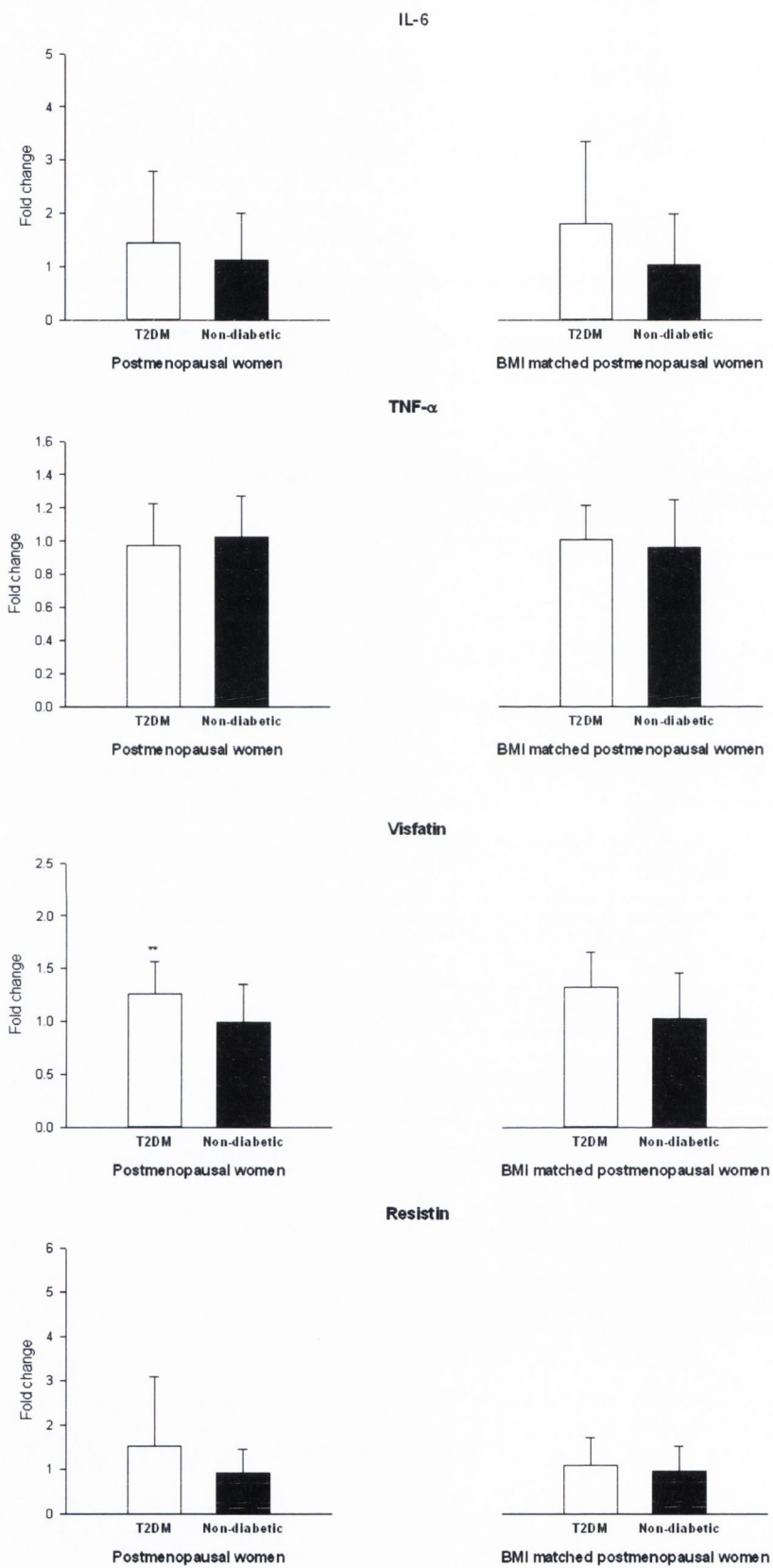


Fig. 6.2. IL-6, TNF- α , visfatin, and resistin mRNA expression. Significantly different from non-diabetic ** $P<0.01$.

6.3.1.4 Plasma levels of CRP, SAA, sICAM-1, and sVCAM-1

Plasma levels of CRP, SAA, sICAM-1, and sVCAM-1 are presented in Fig. 6.3. Individual results can be seen in Appendix 36.

Plasma levels of CRP were greater in postmenopausal women with T2DM than non-diabetic women ($P<0.01$). There were no differences between the subgroups of BMI matched postmenopausal women with and without T2DM in relation to CRP levels.

There were no differences between the group of postmenopausal women or subgroups of BMI matched postmenopausal women with and without T2DM in relation to plasma levels of SAA, sICAM-1, or sVCAM.

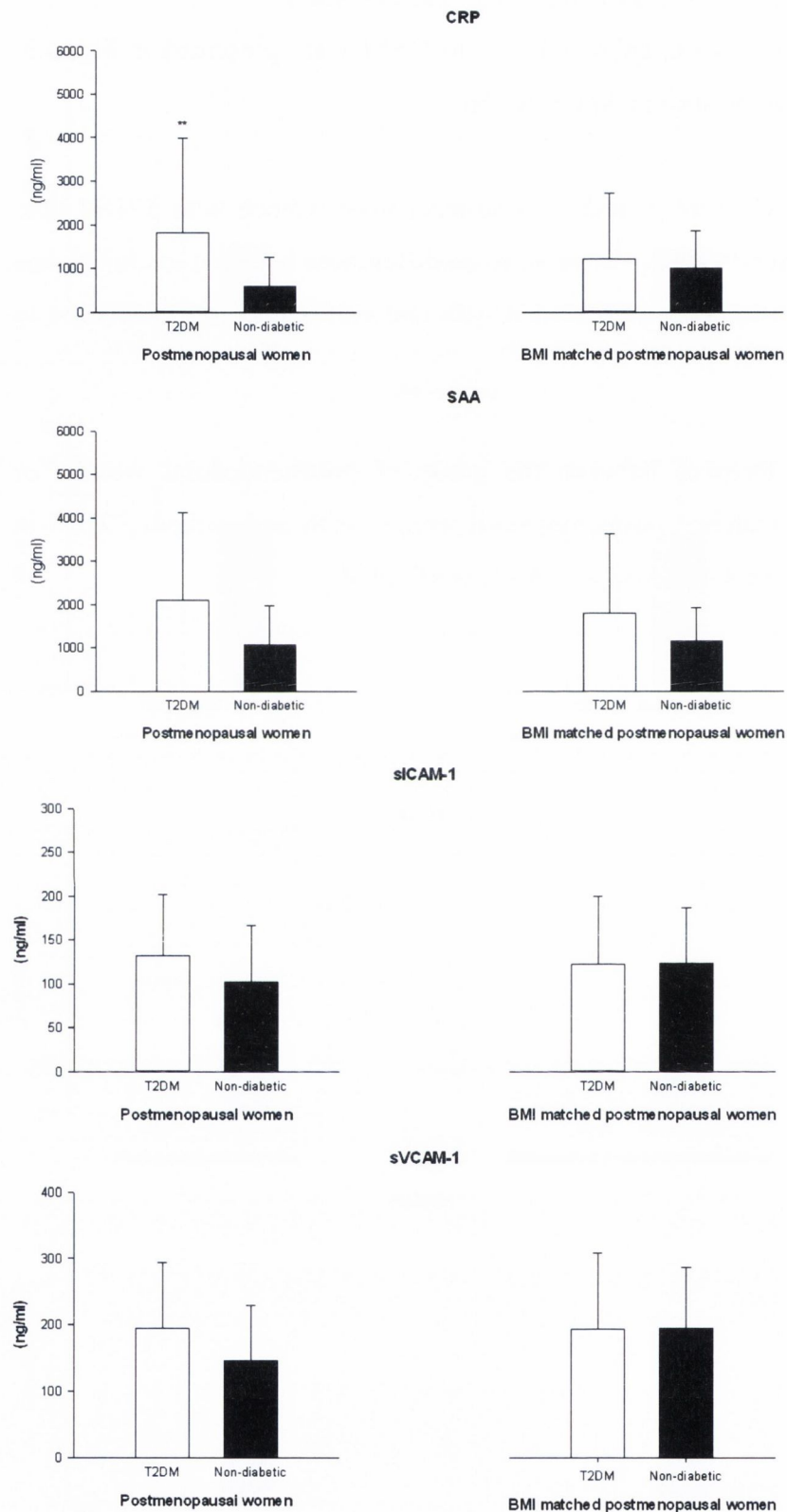


Fig. 6.3. Plasma levels of CRP, SAA, sICAM-1, and sVCAM-1. Significantly different from non-diabetic $**P<0.01$.

6.3.2 Effect of exercise intervention on markers of inflammation and endothelial function

6.3.2.1 Physical characteristics and activity levels

The effect of the exercise intervention on physical characteristics and activity levels for women with and without T2DM in the exercise and control groups at baseline (BS), supervised exercise (SE), and unsupervised exercise (UE) are presented in Table 6.2. Individual physical characteristics and activity levels can be seen in Appendix 37.

There was a main effect of diabetes status for body mass, BMI, and WHR at BS, SE, and UE ($P<0.01$) so that they were significantly greater in women with T2DM than non-diabetics.

Table 6.2. Physical characteristics and activity levels at BS, SE, and UE

		T2DM		Non-diabetic		Diabetes effect
		Exercise (n=8)	Control (n=8)	Exercise (n=10)	Control (n=8)	
Age (yr)		57.5±4.5	56.0±5.8	55.5±9.0	51.9±8.0	
Body mass (kg)	BS	78.1±10.7	86.7±14.2	65.3±10.4	67.4±10.9	$P<0.01$
	SE	76.5±9.7	85.9±14.2	64.3±9.8	67.4±11.2	
	UE	76.1±9.6	86.6±15.4	64.5±9.8	66.1±11.7	
BMI (kg.m ⁻²)	BS	31.3±4.2	34.0±5.6	26.2±3.4	25.4±3.8	$P<0.01$
	SE	30.6±3.9	33.7±5.5	25.7±2.8	25.4±3.9	
	UE	30.6±3.8	34.0±5.9	25.8±2.9	25.4±3.9	
WHR	BS	0.95±0.05	0.95±0.05	0.89±0.07	0.89±0.08	$P<0.01$
	SE	0.96±0.05	0.95±0.05	0.89±0.07	0.89±0.09	
	UE	0.95±0.05	0.95±0.05	0.88±0.08	0.89±0.09	
ABI	BS	1.08±0.08	1.11±0.10	1.07±0.12	1.09±0.16	
	SE	1.06±0.09	1.06±0.09	1.11±0.06	1.10±0.13	
	UE	1.13±0.09	1.03±0.05	1.05±0.08	1.07±0.07	
LOPAR (Met h.wk ⁻¹)	BS	98±43	125±65	101±56	121±27	
	SE	149±52	122±48	109±50	119±31	
	UE	126±49	166±84	119±50	120±55	

6.3.2.2 *Effect of exercise intervention on plasma levels of IL-6, IL-10, and TNF- α*

The effect of exercise intervention on plasma levels of IL-6, IL-10, and TNF- α in women with and without T2DM is shown in Fig. 6.4. Individual results can be seen in Appendix 38.

Plasma levels of IL-6, IL-10, and TNF- α were not different following exercise intervention in any of the groups.

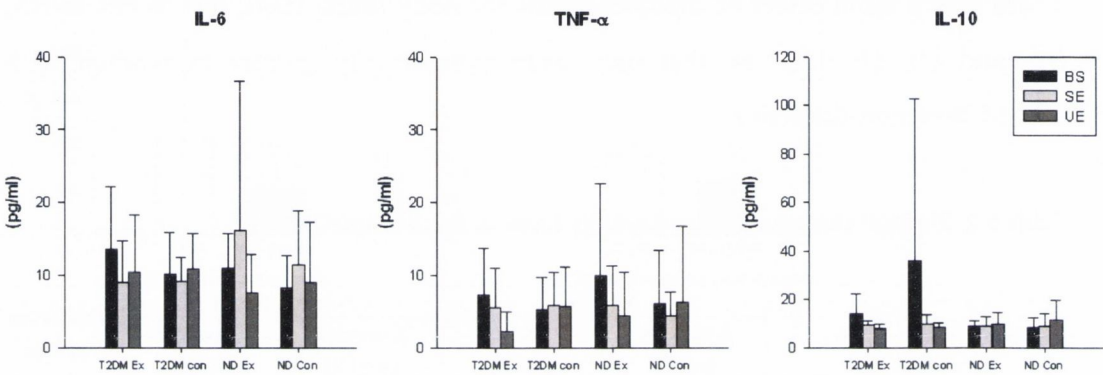


Fig. 6.4. Plasma levels of IL-6, IL-10, and TNF- α at BS, SE, and UE

6.3.2.3 Effect of exercise intervention on IL-6, TNF- α , visfatin, and resistin mRNA expression

The effect of exercise intervention on IL-6, TNF- α , visfatin, and resistin mRNA expression in women with and without T2DM is presented in Fig. 6.5. Individual results can be seen in Appendix 39.

There were no differences in IL-6, TNF- α , visfatin, and resistin mRNA expression following exercise intervention in any of the groups.

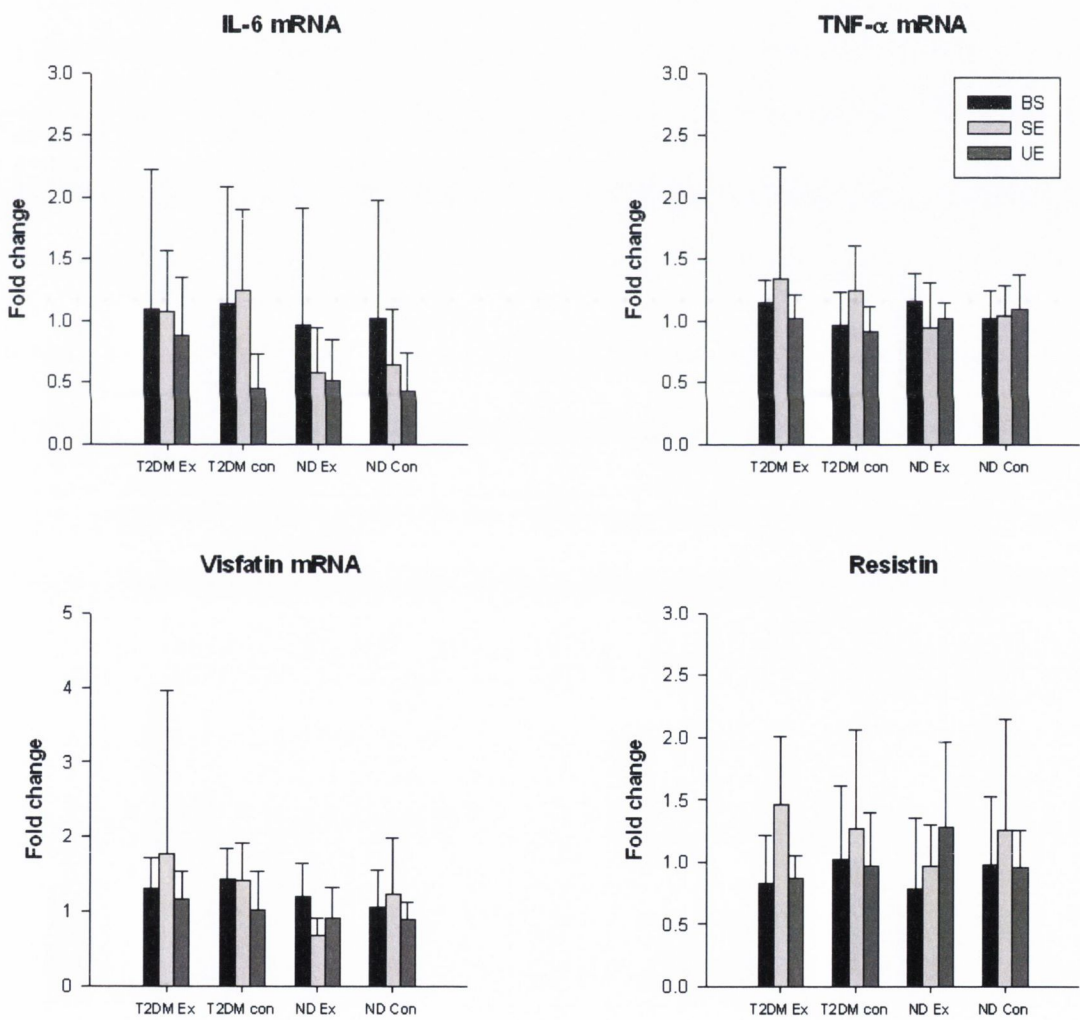


Fig. 6.5. IL-6, TNF- α , visfatin, and resistin mRNA expression at BS, SE, and UE.

6.3.2.4 Effect of exercise intervention on plasma levels of CRP, SAA, sICAM-1, and sVCAM-1

The effect of exercise intervention on plasma levels of CRP, SAA, sICAM-1 and sVCAM-1 is presented in Fig. 6.6. Individual results can be seen in Appendix 40.

Plasma levels of CRP, SAA, sICAM-1, and sVCAM-1 were not changed following exercise intervention for any of the groups.

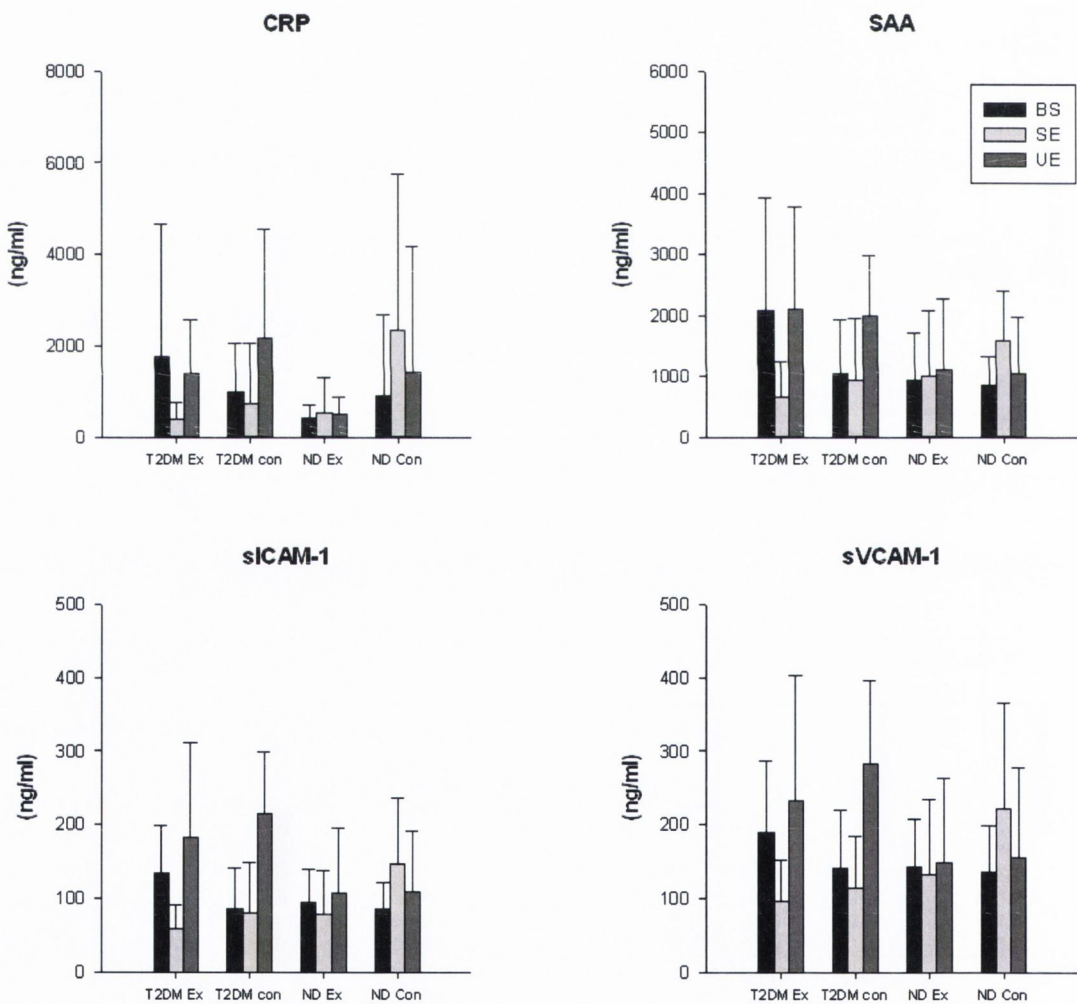


Fig. 6.6. Plasma levels of CRP, SAA, sICAM-1, and sVCAM-1 at BS, SE, and UE.

6.4 Discussion

The main findings of this study were as follows:

- (a) Plasma levels of IL-6, IL-10, and CRP are increased in postmenopausal women with T2DM compared with non-diabetic controls (not matched by BMI), but they are not different between BMI matched postmenopausal women with and without T2DM.
- (b) No differences were observed between plasma levels of TNF- α , sICAM, and sVCAM, or SAA between women with and without T2DM.
- (c) Visfatin mRNA expression was increased in postmenopausal women with T2DM in comparison to non-diabetic women (not matched by BMI).
- (d) IL-6, TNF- α , or resistin mRNA expression was not different between women with and without T2DM.
- (e) Exercise intervention, either supervised or unsupervised, did not affect plasma concentrations of IL-6, IL-10, TNF- α , CRP, SAA, sICAM or sVCAM in women with or without T2DM. In addition, IL-6, TNF- α , visfatin, or resistin mRNA expression was not altered in women with and without T2DM following exercise intervention.

Baseline responses

The research on pro-inflammatory cytokine levels in postmenopausal women with T2DM is limited. In a non-diabetic population, TNF- α expressed relative to total fat mass and plasma levels of leptin were lower in pre versus postmenopausal women (Hayase *et al.*, 2002). Furthermore, TNF- α was associated with visceral fat in premenopausal women only and increases in TNF- α levels following 10 wk of exercise intervention were associated with reduced amounts of visceral fat in premenopausal women (Hayase *et al.*, 2002). The authors suggested that TNF- α secretion after the menopause might be influenced by ageing and/or female hormones (Hayase *et al.*, 2002). Others have found that plasma levels of TNF- α , CRP, and IL-6 are increased in men and women with T2DM in comparison to BMI matched non-diabetic controls (Plomgaard *et al.*, 2007). Furthermore, positive associations were found between TNF- α and both insulin resistance and BMI in individuals with T2DM (Plomgaard *et al.*, 2007). It is possible that differences in visceral adipose tissue may account for the increased levels of

TNF- α seen in individuals with T2DM in previous investigations. Although we did not measure visceral adipose tissue in the current investigation, it is possible that the degree of visceral adiposity was not severe enough in women with T2DM included in the study to emphasise any differences in TNF- α levels between women with and without T2DM.

Previously, it has been suggested that adipose tissue may be an important source of IL-6 production due to the positive correlations found between IL-6 and BMI, waist circumference, and insulin resistance in individuals with T2DM (Leinonen *et al.*, 2003). In the current investigation, plasma levels of IL-6 were greater in the group of postmenopausal women with T2DM in comparison to non-diabetic women (not matched by BMI). In contrast, there were no differences between BMI matched postmenopausal women with and without T2DM in relation to IL-6 levels, indicating that BMI is an important indicator of IL-6 levels in individuals with T2DM. Confirming these findings, others have observed similarities in plasma concentrations of IL-6 and TNF- α between individuals with and without T2DM matched for age and BMI despite observing a 3-fold higher glucose infusion rate during a euglycaemic-hyperinsulinemic clamp in individuals with T2DM than controls (Carey *et al.*, 2004). The association found between IL-6 and BMI in individuals with T2DM indicate that IL-6 concentrations are related to fat mass rather than insulin responsiveness BMI (Carey *et al.*, 2004).

Muller *et al.*, (2002) reported elevated serum levels of IL-6, CRP, and SAA in individuals with T2DM and IGT while TNF- α levels were elevated in individuals with T2DM only. This implicates IL-6 but not TNF- α as a contributory factor in the inflammatory process prior to the onset of overt hyperglycaemia. As the range of HbA_{1C} in the former investigation (Muller *et al.*, 2002) was greater than that in the current investigation, it is probable a greater severity of hyperglycaemia may be required to increase TNF- α levels in individuals with T2DM. More recently it has been reported that plasma levels of both hs-CRP and IL-6 are increased in obese men with T2DM in comparison to non-obese men without T2DM (Hansen *et al.*, 2010). As in the study by Hansen *et al.*, (2010), no such differences were found between non-obese men with and without T2DM, the

results suggest that hs-CRP and IL-6 are more likely to be influenced by adiposity rather than hyperglycaemia (Hansen *et al.*, 2010). This is further supported by the fact that the association found between glycaemic control and both hs-CRP and IL-6 no longer existed when the data was adjusted for trunk adipose tissue mass (Hansen *et al.*, 2010). In agreement with this finding, we did not find any differences in plasma levels of CRP between BMI matched postmenopausal women with T2DM, while in contrast, CRP levels were greater in the group of postmenopausal women with T2DM in comparison to their non-diabetic counterparts (not matched by BMI).

Recent work conducted on cultured adipocytes indicates that visfatin synthesis is activated from adipose tissue in a hyperglycaemic environment, it increases NF- κ B transcriptional activity, and it leads to activation of pro-inflammatory cytokines (Kang *et al.*, 2010). Furthermore, plasma levels of visfatin were elevated in individuals with T2DM in comparison to non-diabetics matched by age but not BMI, a finding that persisted irrespective of the severity of microalbuminuria (Kang *et al.*, 2010). In agreement with these findings we found that visfatin mRNA expression was greater in the group of postmenopausal women with T2DM than their non-diabetic counterparts. In addition, there was a trend for greater visfatin mRNA expression in BMI matched postmenopausal women with T2DM.

Endothelial dysfunction is a common feature of T2DM and it is frequently associated with increased circulating levels of pro-inflammatory cytokines and soluble adhesion molecules as well as atherosclerosis. Recent evidence suggests that in normotensive individuals with T2DM, plasma levels of both ICAM-1 and VCAM-1 are associated with the severity of atherosclerosis (Rubio-Guerra *et al.*, 2009). An earlier investigation found positive associations between markers of endothelial activation (ICAM-1 and VCAM-1) and insulin resistance in men and women with T2DM (Leinonen *et al.*, 2003). The authors suggested that as the association between insulin resistance and inflammation is weaker after adjusting for BMI, other factors must contribute to the inflammatory milieu (Leinonen *et al.*, 2003). In the current investigation there were no differences between women

with and without T2DM with respect to plasma levels of SAA, sICAM or sVCAM. These findings are not altogether that surprising considering that total cholesterol levels were lower in women with T2DM than non-diabetic women and no differences were reported in ankle brachial index between women with and without T2DM (see chapter 3, sections 3.4.1.1 and 3.4.1.2). Furthermore, arm reactive hyperaemic responses were similar in women with and without T2DM (see chapter 3, section 4.4.5), suggesting similar systemic endothelial function (at least in the forearm) between women with uncomplicated T2DM and non-diabetic controls.

Effect of exercise intervention

The anti-inflammatory effect of exercise has received much debate but the results are somewhat conflicting. Data from a number of intervention studies have reported reductions in IL-6, CRP, and ICAM-1 concentrations in individuals with T2DM (Roberts *et al.*, 2006; Zoppini *et al.*, 2006; Dekker *et al.*, 2007), indicating that exercise may suppress systemic low-grade inflammation. In contrast, other exercise investigations have found no effects on levels of IL-6 or TNF- α (Jorge *et al.*, 2011). In the current investigation, exercise intervention over a 7-month period did not affect plasma levels of IL-6, IL-10, TNF- α , CRP, SAA, sICAM-1, or sVCAM-1 and similarly, no effects were found in IL-6, TNF- α , resistin, or visfatin whole blood mRNA expression in women with or without T2DM. While we did not measure adiposity, we did not find any improvement in either BMI or body mass in exercising women with and without T2DM, a fact that might explain the lack of improvement in the inflammatory and endothelial biomarkers.

Previous investigations in this area indicate that weight loss or loss of visceral adipose tissue is an important requirement for any improvements to take place in inflammatory markers. In older individuals with T2DM, a 6-month period of supervised aerobic exercise induced reductions in plasma concentrations of ICAM-1 but not hs-CRP or soluble TNF- α receptors (Zoppini *et al.*, 2006). It is possible that the lack of significant improvement in body composition in the individuals in the study by Zoppini *et al.*, (2006) may account for the lack of improvement in markers of inflammation and therefore, more intensive training

may have been required to affect change in concentrations of hs-CRP and soluble TNF- α receptors (Zoppini *et al.*, 2006). In support of these results, others have also failed to find any improvements in plasma concentrations of CRP and plasminogen activator inhibitor-1 (PAI-1) in men with T2DM and both lean and obese non-diabetic controls following 12-wk of combined dietary and exercise (60 min of aerobic exercise per day at 60% of $\dot{V}O_{2\text{ peak}}$) intervention (Dekker *et al.*, 2007). However, Dekker *et al.*, (2007) observed that IL-6 concentrations were lower in all groups, a factor that was attributed to the reduction in visceral adiposity and waist circumference in these men and thus, highlights the potential role that excess adiposity plays in contributing to the inflammatory process. The authors of the study suggested that CRP may be more closely aligned to insulin sensitivity, which was unchanged following intervention, rather than visceral adiposity and thus, improvements may be required in insulin sensitivity in order to reduce CRP levels (Dekker *et al.*, 2007).

In contrast, it has been shown that a combination of diet and exercise intervention over a 21-d period was effective in lowering serum levels of CRP along with sICAM-1 and sE-selectin in a group of overweight and obese men with T2DM (Roberts *et al.*, 2006). However, the more intensive intervention (45-60 min of exercise per day at 70-85% of peak HR) in the former investigation by Roberts *et al.*, (2006) resulted in a reduction in BMI (11.1%), which is likely to have accounted for the differences in results compared with previous investigations where body composition was unaffected (Zoppini *et al.*, 2006; Dekker *et al.*, 2007).

The research on plasma levels of visfatin following exercise intervention in individuals with T2DM is limited and somewhat conflicting. A recent investigation reported increased levels of plasma levels of visfatin in middle-aged individuals with T2DM following a 12-wk period of either aerobic, resistance, or combined (aerobic & resistance) exercise (Jorge *et al.*, 2011) while in contrast, others have reported reduced plasma levels of visfatin following 3-months of aerobic training in a young group with T2DM (Brema *et al.*, 2008). The mechanism underpinning the reduction in visfatin levels observed by Brema *et al.*,

(2008) is unclear as apart from a reduction in waist circumference, no improvements were observed in insulin sensitivity, percentage body fat, or lipid profile following intervention.

On the other hand, Katsuki *et al.*, (1998) investigated the effect of a dietary and exercise intervention programme of a 4-week duration on serum levels of TNF- α in both lean and obese men and women with T2DM. Visceral adiposity was reduced (similar magnitude) in both lean and obese groups following intervention (Katsuki *et al.*, 1998). However, serum levels of TNF- α were reduced in the obese group only and not in the lean group, a finding that might be explained by the greater level of visceral fat in the obese group at baseline (Katsuki *et al.*, 1998). In contrast, Giannopoulou *et al.*, (2005) reported no changes in plasma levels of TNF- α , adiponectin, resistin, and IL-6 in postmenopausal women with T2DM assigned to a diet and/or exercise interventions over a 14-wk period. It is likely that the increase in visceral fat in two of the groups (diet plus exercise and exercise) in the former investigation by Giannopoulou *et al.*, (2005) might account for the lack of effect in TNF- α levels. However, reductions were observed in plasma levels of CRP across all intervention groups, which might indicate that modest improvements in body mass rather than visceral fat are sufficient to affect changes in CRP levels at least in postmenopausal women with T2DM (Giannopoulou *et al.*, 2005).

Limitations

The majority of women with T2DM were receiving lipid-lowering therapy in the current investigation. This is likely to have accounted for the similarities between women with and without T2DM in relation to markers of endothelial dysfunction as lipid-lowering therapy has been known to lower circulating levels of adhesion molecules. Furthermore, the women in this investigation had well-controlled T2DM and it is possible that more advanced T2DM may be required to emphasise any differences in circulating levels of inflammatory cytokines and markers of endothelial dysfunction.

Conclusions

In summary, plasma levels of IL-6, IL-10, and CRP as well as visfatin mRNA expression were greater in the group of postmenopausal women with T2DM in comparison to non-diabetic women (not matched by BMI) at baseline. However, no differences were found in markers of inflammation or endothelial activation in the subgroups of BMI matched postmenopausal women with or without T2DM, implicating the potential negative impact that increasing levels of adiposity have on the inflammatory process. In addition, no improvements were found in markers of inflammation or endothelial activation in either exercising group following the supervised and unsupervised exercise interventions. It is possible that the lack of significant improvement in body composition following exercise training might explain the lack of differences in the levels of the biomarkers. Thus, the results indicate that a more intensive exercise intervention and/or a combination of exercise and diet might be required to affect change in body composition and pro-inflammatory cytokines and markers of endothelial function in women with T2DM.

Chapter 7: General discussion and conclusion

7.1 Discussion

Individuals with T2DM exhibit impaired peak exercise performance (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Baldi *et al.*, 2003; Mac Ananey *et al.*, 2011a; Wilkerson *et al.*, 2011), a finding that has been previously linked with increased mortality (Wei *et al.*, 2000). Despite the increased rates of morbidity and mortality among diabetic populations (Stamler *et al.*, 1993; Gu *et al.*, 1998; Haffner *et al.*, 1998), individuals with T2DM do not engage in sufficient regular exercise and adherence to exercise programmes is poor (Krug *et al.*, 1991; Morrato *et al.*, 2007). Numerous studies have confirmed the beneficial effects of regular exercise in diabetic populations promoting improvements in glycaemic control (Okada *et al.*, 2010), inflammatory markers (Roberts *et al.*, 2006), endothelial function (Maiorana *et al.*, 2001a), lipid profile (Sixt *et al.*, 2010), body composition (Cuff *et al.*, 2003), and blood pressure (Loimaala *et al.*, 2003). However, women with T2DM perceive exercise as being more difficult than their non-diabetic counterparts (Huebschmann *et al.*, 2009), which could be related to the exercise intolerance observed in individuals with T2DM during both maximal and sub-maximal exercise (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a).

In agreement with previous investigations (Regensteiner *et al.*, 1995; Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Baldi *et al.*, 2003), we found a reduction in $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) in postmenopausal women with T2DM in comparison to age and activity matched controls, the magnitude of which was approximately 20%. While $\dot{V}O_{2\text{ peak}}$ ($\text{ml.kg}^{-1}.\text{min}^{-1}$) was reduced in age and BMI matched postmenopausal women with T2DM, the magnitude of difference in $\dot{V}O_{2\text{ peak}}$ was somewhat smaller (~12%).

$\dot{V}O_2$ kinetics

Current research in this field indicates that the rate of adaptation in the $\dot{V}O_2$ response during the on-transient of exercise is slowed in individuals with T2DM during low and moderate intensity exercise premenopausal women, combined groups of pre and postmenopausal women, and combined groups of men and women with T2DM (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a) but not in older men with T2DM (Wilkerson *et al.*, 2011). The 'lack of significance' in $\dot{V}O_2$ kinetic responses between older men with and without T2DM has been attributed to an age-related defect in $\dot{V}O_2$ kinetic responses in non-diabetic men (Wilkerson *et al.*, 2011).

In the first experiment, we sought to establish if the slowed $\dot{V}O_2$ kinetic responses observed elsewhere in premenopausal women (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999) and combined groups of pre and postmenopausal women (Mac Ananey *et al.*, 2011a) are applicable to a population of postmenopausal women with T2DM. In addition, we wanted to establish if the findings persist in BMI matched postmenopausal women with and without T2DM.

For all group comparisons, a slowing in the rate of adaptation of the $\dot{V}O_2$ response was observed in women with T2DM in comparison to non-diabetic women, indicating that the impairment is a function of diabetes rather than BMI. This is the first study to report a slowing in $\dot{V}O_2$ kinetic responses in postmenopausal women with T2DM. The slowing of the $\dot{V}O_2$ response in women with T2DM, evident by the greater MRT, was attributed to the slower rate of adaptation in the time constant of phase 2. The rate of adjustment of phase 2 of the $\dot{V}O_2$ kinetic response closely reflects adjustment in oxidative metabolism at the level of the skeletal muscle. A slowing in the rate of adjustment in $\dot{V}O_2$ results in a greater oxygen deficit and subsequently increased reliance on substrate-level phosphorylation to provide ATP to enable exercise to be sustained. In contrast, the time constant of phase 1 of the $\dot{V}O_2$ kinetic response, where phase 1 represents the oxygen exchange associated with the initial increase in CO and pulmonary blood flow, was not different between women with and without T2DM. This

suggests that the underlying control mechanisms responsible for the increase in $\dot{V}O_2$ during phase 1 (rapid vasodilation, muscle pump effect, and neural control of heart rate) remain intact in T2DM.

As left ventricular diastolic dysfunction (LVDD) is prevalent in diabetic populations (Poirier *et al.*, 2001; Baldi *et al.*, 2006) and has previously been associated with reduced exercise performance (Poirier *et al.*, 2000; Ha *et al.*, 2008), it is possible that central factors may contribute to the slowed rate of adaptation in $\dot{V}O_2$ responses in individuals with T2DM seen in this experiment and previous investigations (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999; Bauer *et al.*, 2007; Mac Ananey *et al.*, 2011a).

To gain insight into the possible mechanistic basis for the impaired $\dot{V}O_2$ kinetic responses in women with T2DM, we measured CO responses during exercise (30 s and 240 s). Confirming reports of a prior investigation (Mac Ananey *et al.*, 2011a), we failed to find any differences in CO responses between women with or without T2DM, therefore suggesting that CO is not a limiting factor in the rate of adaptation of the $\dot{V}O_2$ response during exercise onset. However, due to the required inter-measurement timing-related limitations in the inert gas rebreathing technique, we were unable to continuously measure CO during exercise. The findings of impaired heart rate kinetic responses for all group comparisons in women with T2DM indicate a possible impairment in oxygen delivery. Furthermore, the similarities in oxygen extraction between women with and without T2DM in the current investigation together with the overshoot in [HHb] responses (reflective of tissue oxygen extraction) above that required in steady state exercise in individuals with T2DM at the onset of exercise observed by others (Bauer *et al.*, 2007), indicate that oxygen extraction might not be a limiting factor in $\dot{V}O_2$ kinetic response in individuals with uncomplicated T2DM. Thus, an increased reliance for oxygen extraction during the on-transient of exercise in disease states such as T2DM (Bauer *et al.*, 2007), is possibly reflective of a compensatory mechanism for impairments in microvascular blood flow.

LVC kinetics

In the absence of any cardiac dysfunction or limitations in oxygen extraction in the present experiment, it would appear that the slowed rate of adaptation in $\dot{V}O_2$ responses are associated with an impaired blood flow response or maldistribution of blood flow. Thus, in the second experiment, LVC kinetic responses were determined during 30% and 70% MVC plantar flexion exercise in the supine posture. The LVC data were resolved by fitting biphasic ‘growth only’ (70% MVC) and triphasic ‘growth and decay’ (30% MVC) models to the data on the basis that biphasic ‘growth only’ and triphasic ‘growth and decay’ responses were more prevalent in each respective case. Prior investigations conducted on blood flow responses during low intensity knee extension exercise (Shoemaker *et al.*, 1996; MacDonald *et al.*, 1998) indicate the presence of fast decay following the initial increase in blood flow during phase 1.

The rate of adjustment in LVC responses were similar between women with and without T2DM at 30% MVC irrespective of BMI. This suggests that low intensity exercise may be ineffective in emphasising any differences in LVC kinetic responses between women with and without T2DM. The elevated heart rate responses and similar MAP responses during exercise in women with T2DM might be a compensatory mechanism to maintain blood flow and hence LVC responses during exercise at 30% MVC. In keeping with the results of a prior investigation (Mac Ananey *et al.*, 2011b), women with T2DM displayed a slowing in the rate of adjustment in LVC responses during plantar flexion at 70% MVC and this occurred independent of BMI. As the second growth phase is a flow-mediated, endothelium-dependent phase (Tschakovsky *et al.*, 2006), the slowed adjustment in vasodilatory responses observed at 70% MVC in women with T2DM indicate a failure in endothelial-dependent vasodilatory mechanisms in adjusting the rate of adaptation in LVC during exercise onset to meet the exercise stimulus. In addition, the preserved MAP and heart rate responses during exercise at 70% MVC in women with T2DM add further weight to the argument that the impairment is not due to central cardiac function but suggests an abnormality in vascular function. Interestingly, the End A was not different

between women with and without T2DM indicating that the capacity to vasodilate during high intensity exercise is preserved in women with T2DM.

To investigate if endothelial dysfunction plays a role in contributing to the impaired LVC kinetic responses observed in women with T2DM during high intensity exercise, reactive hyperaemia of the forearm was performed. While we did not observe any differences in the vasodilatory responses between women with and without T2DM, it is possible that vascular function may be limb specific and that endothelial dysfunction might be more pronounced in the lower limbs of women with T2DM.

The presence of endothelial dysfunction is clinically significant as impaired endothelial-dependent vasodilatory responses are thought to precede atherosclerosis (Celermajer *et al.*, 1994). Extensive research indicates that individuals with T2DM have impaired vasodilatory responses to endothelium-dependent agonists such as ACh (McVeigh *et al.*, 1992) and metacholine (Williams *et al.*, 1996). Thus, it would appear that the pathogenesis of endothelial dysfunction in T2DM involves some defect in endothelial-derived NO. As ACh mediates vasodilation through the release of NO and vasodilator prostanoids via interaction with M₃ muscarinic receptors, it would appear that either the release of these substances or arterial smooth muscle reactivity to these substances is reduced in T2DM. Others have found an association between reduced leg blood flow observed during exercise in men with T2DM and fasting plasma glucose (Kingwell *et al.*, 2003). The mechanistic basis by which hyperglycaemia contributes to impaired endothelial-dependent vasodilation is multi-factorial and includes increased formation of oxygen derived free radicals, activation of protein kinase C, activation of the polyol pathway, and formation of AGEs (Matsubara & Ziff, 1986; Bucala *et al.*, 1991; Chung *et al.*, 2003; Kelly *et al.*, 2003).

In experiment 1, we reported a slowing in the rate of adaptation of the $\dot{V}O_2$ response during moderate intensity exercise in women with T2DM and it appears that CO does not contribute to the impaired $\dot{V}O_2$ kinetic responses. In the second experiment, we found an impaired rate of adaptation in LVC responses during

high intensity but not low intensity plantar flexion exercise in women with T2DM; this finding was again apparent after controlling for BMI. Due to the close temporal alignment of $\dot{V}O_2$ and blood flow, it is possible that the slowed rate of adaptation in LVC observed during high intensity plantar flexion exercise contributes to the slowed $\dot{V}O_2$ kinetic responses seen during cycle exercise in women with T2DM.

Exercise intervention

A slowing in the dynamic response of $\dot{V}O_2$ seen in the current investigation in women with T2DM at exercise onset has important implications in terms of exercise performance and adherence. If the capacity to exercise even at low intensities is reduced in T2DM, then individuals with T2DM may perceive exercise as more challenging than non-diabetics and may therefore, be discouraged from engaging in regular exercise. Therefore, strategies that reduce or alleviate the exercise intolerance in T2DM are of great importance as they may result in greater participation in physical activity. The third experiment, therefore, aimed to investigate the effect of supervised exercise (combined aerobic plus resistance) over 3 months on $\dot{V}O_2$ and LVC kinetic responses in women with T2DM. In addition, subjects completed a further period of unsupervised exercise (4 months) to investigate if the benefits acquired during the supervised phase could be maintained during the unsupervised exercise.

The initial 3-month period of supervised exercise was sufficient to induce changes in both relative ($\text{ml.kg}^{-1}.\text{min}^{-1}$) and absolute (L.min^{-1}) $\dot{V}O_{2 \text{ peak}}$ in exercising diabetics and non-diabetics. The magnitude of increase in $\dot{V}O_{2 \text{ peak}}$ (13%) in T2DM Ex observed in the present experiment was consistent with previous investigations reporting improvements in $\dot{V}O_{2 \text{ peak}}$ following an exercise training intervention (Brandenburg *et al.*, 1999; Loimaala *et al.*, 2007; Okada *et al.*, 2010). However, the benefits acquired in $\dot{V}O_{2 \text{ peak}}$ at SE were not maintained during UE. Prior research in this area is limited in T2DM, but recent evidence suggests that ‘unsupervised exercise’ training is sufficient to maintain improvements in $\dot{V}O_{2 \text{ peak}}$ when it is performed in the gymnasium (Mac Ananey, 2010). In the current investigation, most subjects carried out the unsupervised exercise intervention in

their homes or in the vicinities of their homes, which led to an important reduction in training compliance, and this most likely contributed to the reduction in $\dot{V}O_{2\text{ peak}}$ in both exercise groups between SE and UE. The reduction in maximal aerobic performance and decrease in training compliance in the current investigation has particular implications in terms of exercise prescription for individuals with T2DM. As shown by others (Dunstan *et al.*, 2005; Dunstan *et al.*, 2006), when unsupervised exercise is carried out exclusively in a gym-based setting, the subsequent benefits are greater than when the unsupervised exercise is performed 'home-based' or in a combination of both. Thus, from the exercise prescription point of view, gym-based unsupervised exercise programmes should be encouraged (which include some form of supervision) for individuals with T2DM.

In the current experiment, exercise intervention (either SE or UE) did not enhance the speed of the $\dot{V}O_2$ kinetic response at the beginning of moderate intensity cycle exercise in either exercise group. This finding appears contradictory to previous research where faster $\dot{V}O_2$ kinetics responses (shorter time constant) have been observed in men and women with T2DM during low and moderate intensity cycle exercise (Brandenburg *et al.*, 1999; Mac Ananey, 2010). The improvements in the rate of adaptation in $\dot{V}O_2$ in the former investigations (Brandenburg *et al.*, 1999) could reflect improvements in either oxygen delivery and/or oxygen utilisation. Consistent with the study by Mac Ananey (2010), we did not find any change in CO during exercise following the training intervention. However, there is an important point to take into consideration with the 'lack' of significant improvement in $\dot{V}O_2$ kinetic responses or CO following exercise intervention in the current investigation. The increase in workload at VT following SE resulted in both exercising groups exercising at greater absolute workloads than at BS and thus, the resulting increase in oxygen consumption during exercise would necessitate a longer rate of adaptation in $\dot{V}O_2$ before steady state was achieved. As the adjustment in $\dot{V}O_2$ kinetic responses were similar at BS and SE in both exercise groups, it is not unreasonable to suggest that some form of adaptation has taken place in the $\dot{V}O_2$ kinetic response following exercise intervention.

Mirroring the findings in $\dot{V}O_2$ kinetic responses, we failed to find any changes in LVC kinetic responses during low intensity plantar flexion exercise following either SE or UE. This conflicts with a prior investigation where faster LVC kinetic responses were observed in women with T2DM following SE exercise during high intensity plantar flexion exercise (Mac Ananey, 2010). The increase in MVC at SE and at UE from BS in both T2DM and ND Ex resulted in greater absolute workloads during constant load plantar flexion exercise and it could be argued that some improvement in endothelial-dependent vasodilation did take place.

The speeding of the $\dot{V}O_2$ kinetic responses during cycle exercise observed elsewhere (Mac Ananey, 2010) in men and women with T2DM following an exercise intervention have been attributed at least in part to the faster rate of adaptation in LVC kinetic responses during high intensity plantar flexion exercise. In the current investigation, heart rate kinetic responses were not enhanced by exercise intervention. However, the increase in absolute workload and similarities in heart rate kinetic responses between BS and SE could be indicative of improvements in the redistribution of blood flow and subsequent rate of adaptation in blood flow responses during exercise. The training induced adaptations in vascular function observed in individuals with T2DM (Maiorana *et al.*, 2001a) appear to be endothelial-dependent and could be mediated through an increase in shear stress mechanism via increased NO production. Alternatively, improvements in mitochondrial density, mitochondrial size, and oxidative capacity which have been observed in individuals with T2DM following exercise intervention (Toledo *et al.*, 2007) may contribute to an enhanced rate of adaptation in the $\dot{V}O_2$ response.

Markers of inflammation and endothelial function at baseline

It has been well established that concentrations of several pro-inflammatory cytokines such as IL-6, CRP, and TNF- α are increased in T2DM (Muller *et al.*, 2002; Bruun *et al.*, 2003; Verma *et al.*, 2003; Giannopoulou *et al.*, 2005; Wood *et al.*, 2005; Plomgaard *et al.*, 2007). However, some investigations have failed to find any differences in plasma concentrations of IL-6 and TNF- α between age and

BMI matched individuals with and without T2DM (Carey *et al.*, 2004). As positive associations have been found between IL-6 and BMI in individuals with T2DM in previous investigations (Leinonen *et al.*, 2003; Carey *et al.*, 2004), it would appear that obesity is an important contributor to the inflammatory process.

In the current investigation, plasma levels of IL-6, IL-10, and CRP as well as visfatin mRNA expression were increased in the group of postmenopausal women with T2DM but not BMI matched postmenopausal women with T2DM in comparison to non-diabetic controls. The 'lack' of a significant finding in markers of inflammation and endothelial activation between age and BMI matched postmenopausal women with and without T2DM in the current investigation, suggests that obesity may be more influential in contributing to the inflammatory milieu than hyperglycaemia *per se*.

Support for an obesity-related role in the inflammatory process can be found in a recent investigation by Hansen *et al.*, (2010), where plasma levels of both hs-CRP and IL-6 were increased in obese men with T2DM but not non-obese men with T2DM in comparison to respective BMI-matched non-diabetic men. In agreement with these findings, Plomgaard *et al.*, (2007) reported elevated plasma levels of TNF- α , CRP, and IL-6 in men and women with T2DM in comparison to non-diabetic age and BMI matched controls. Others have also reported elevated plasma levels of visfatin in individuals with T2DM in comparison to non-diabetic controls (Kang *et al.*, 2010). As in the current investigation where visfatin mRNA expression was greater in women with T2DM than non-diabetic women not matched by BMI, in the former investigation by Kang *et al.*, (2010), BMI was greater in individuals with T2DM, a finding that may account for the increased levels of visfatin in T2DM. Furthermore, in individuals with T2DM, positive associations have been found between TNF- α and BMI (Plomgaard *et al.*, 2007) and between IL-6 and BMI (Leinonen *et al.*, 2003), which implicates obesity as a possible contributor to the inflammatory process in individuals with T2DM.

Consistent with the findings of a previous investigation where similarities were reported in plasma levels of IL-6 and TNF- α between individuals with and

without T2DM matched for age and BMI (Carey *et al.*, 2004), we did not find any differences in IL-6, TNF- α , or resistin mRNA expression between women with and without T2DM, irrespective of BMI. However, it is possible that the method of determining cytokine mRNA expression in the current investigation (whole blood rather than peripheral mononuclear cells (PBMCs)), might explain the 'lack' of significance in cytokine expression between women with and without T2DM. A recent investigation compared expression profiles from PBMCs and from whole blood samples demonstrated that the quantity of present microRNA (miRNA), overall signal intensity, and intragroup correlation yielded similar results (Gaarz *et al.*, 2010). However, miRNA profiles obtained from whole blood exhibited a greater variance and results in miRNAs were differentially expressed in comparison to the PBMC-derived profile, a finding that might be attributed to the differences in cells types between whole blood and PBMCs (Gaarz *et al.*, 2010).

Furthermore, in the current investigation, plasma levels of TNF- α , SAA, sICAM, or sVCAM were not different between women with and without T2DM. However, these findings are not unusual considering the lower total cholesterol levels in women with T2DM and similar ankle brachial index between women with and without T2DM (see chapter 3, sections 3.4.1.1 and 3.4.1.2). In addition, arm reactive hyperaemic responses were not different between women with and without T2DM (see chapter 4, section 4.4.5), suggesting endothelial function is preserved (at least in the forearm) in women with uncomplicated T2DM.

Effect exercise intervention on markers of inflammation and endothelial function

In the current investigation, exercise intervention (supervised and unsupervised) did not affect plasma levels of IL-6, IL-10, TNF- α , CRP, SAA, sICAM-1, or sVCAM-1 and similarly, no changes were found in IL-6, TNF- α , resistin, or visfatin mRNA expression in women with and without T2DM. While visceral adiposity was not measured in the current investigation, no improvements were found in either BMI or body mass, a finding that might account for the 'lack' of improvement in markers of inflammation and endothelial activation.

Improvements in body composition, and in particular loss of visceral adipose tissue appears to be an important prerequisite for reducing subclinical, low-grade inflammation and markers of endothelial activation. In support of this theory, Dekker *et al.*, (2007) reported reductions in plasma concentrations of IL-6 in men with T2DM and in lean and obese non-diabetic controls following a 12-wk period of diet and exercise (60 min of aerobic exercise per day at 60% of $\dot{V}O_{2\text{ peak}}$). It is likely that the reduction in visceral adiposity observed in all groups in the former investigation (Dekker *et al.*, 2007) contributed to the reductions in IL-6 concentrations and therefore, highlights the potential negative role played by visceral adiposity in contributing to the inflammatory process. In contrast, a study conducted by Zoppini *et al.*, (2006), showed that 6 months of supervised aerobic exercise was effective in lowering plasma levels of ICAM-1 but not hs-CRP or soluble TNF- α receptors in older individuals with T2DM. As body composition was unchanged following exercise intervention in the study by Zoppini *et al.*, (2006), it is possible that a more intensive exercise intervention may have been required to reduce body mass and subsequently improve inflammation.

However, other investigations have reported reductions in plasma levels of CRP, along with sICAM-1 and sE-selectin in overweight and obese men with T2DM following 21 days of diet and exercise intervention (Roberts *et al.*, 2006). It would appear that the more intense exercise intervention (45-60 min of exercise per day at 70-85% of peak HR), which resulted in a subsequent reduction in BMI (11%) in the former study by Roberts *et al.*, (2006), might account for the disparities in results between the current and previous investigations (Zoppini *et al.*, 2006). The effectiveness of a diet and exercise intervention has also been confirmed by Katsuki *et al.*, (1998), who reported reduced serum levels of TNF- α in obese but not lean men with T2DM following a 4-wk intervention of diet and exercise, a finding that the authors attributed to the greater degree of visceral fat in the obese group at baseline as visceral fat was reduced to a similar degree in both groups following intervention.

As no improvements were found in markers of inflammation or endothelial activation following both supervised and unsupervised exercise intervention in the

current investigation, it would therefore appear that a more intensive exercise intervention and/or combination of diet and exercise is required to improve body composition and therefore, pro-inflammatory cytokines and markers of endothelial function.

Conclusion

In summary, the impaired $\dot{V}O_2$ kinetic responses observed in women with T2DM during moderate intensity cycle exercise (experiment 1) appear to reflect a diabetes-associated defect rather than being associated with. CO measured simultaneously during constant load cycle exercise revealed no differences in responses between women with and without T2DM and seem to suggest that CO does not contribute to a slowing in the adjustment of $\dot{V}O_2$ response from exercise onset to steady state and suggests a peripheral rather than central factor in the exercise abnormality probably together with a mitochondrial defect in T2DM. The slowed rate of adjustment in LVC kinetic responses in women with T2DM during high intensity plantar flexion exercise appears to confirm this notion. As the second growth phase of the LVC response is endothelial-dependent, this suggests a diabetes-related defect in endothelial function. In contrast, LVC kinetic responses were not different between women with and without T2DM during low intensity plantar flexion exercise, which possibly indicates that diabetes does not impair LVC responses during low intensity exercise. Supervised exercise intervention consisting of aerobic plus resistance exercise improved peak cycle exercise performance in both women with and without T2DM. However, the benefits acquired during supervised exercise were not maintained during a follow-up period of unsupervised exercise. The majority of individuals carried out the UE phase in their own homes or in the vicinity of their homes, which led to a reduction in training compliance in both exercise groups. This finding emphasises the importance of carrying out unsupervised exercise in a gym-based setting that also includes some form of supervision. While exercise intervention did not enhance the speed of either the $\dot{V}O_2$ or LVC kinetic response in either women with or without T2DM, the increased workloads during both constant load cycle exercise and plantar flexion following SE in both exercise groups may have obscured any improvements in either parameter. Thus, it is

possible that some adaptations have taken place in response to exercise intervention with respect to enhancing the rate of adaptation of $\dot{V}O_2$ and LVC responses.

In experiment 4, increased plasma levels of IL-6, IL-10, and CRP as well as visfatin mRNA expression were found in the group of postmenopausal women with T2DM. The increased expression of these cytokines appears to be a function of obesity rather than diabetes *per se*, as no differences were found in markers of inflammation or endothelial activation between BMI matched women with and without T2DM. Furthermore, no improvements were found in markers of inflammation or endothelial activation following supervised and unsupervised exercise intervention. As body composition was unchanged following the intervention, it is likely that a more intense exercise intervention and/or combination of diet and exercise may be required to improve subclinical low-grade inflammation and markers of endothelial activation, at least in women with uncomplicated T2DM.

6.2 Future work

In summary, the results of this investigation confirm that $\dot{V}O_2$ kinetic responses are slowed in postmenopausal women with T2DM. In the absence of any differences in CO responses, a slowed rate of adjustment in LVC kinetic responses at the onset of low intensity exercise appear to contribute to the slowed rate of adjustment in $\dot{V}O_2$ during the exercise transient. However, in the current investigation CO measurements were made at specific time points during the exercise protocol (30 and 240 s) and as such, steady-state measurements do not provide any indication as to the rate of adjustment in CO responses at exercise onset. Therefore, future studies should look at the adjustment in CO during the initial stages of exercise.

The individuals with T2DM in the current investigation were postmenopausal women with well-controlled T2DM. It would be interesting to investigate the impact of more advanced T2DM on $\dot{V}O_2$ kinetic responses and CO responses during exercise onset. In addition, the effects of chronic hyperglycaemia on vascular function have been well documented. Therefore, the mechanisms responsible for the slowed rate of increase in $\dot{V}O_2$ kinetic responses in this and prior investigations may be different in more advanced T2DM and may be related to central rather than peripheral factors. Thus, the potential mechanisms responsible for the slowed rate of increase in $\dot{V}O_2$ kinetic responses should be explored in individuals with more advanced T2DM.

To date, only one former study has examined the effect of supervised exercise training on $\dot{V}O_2$ kinetic responses over a 3-month period. As yet no study has looked at the effect of long-term (>7 months) exercise training (either supervised or unsupervised) on $\dot{V}O_2$ or LVC kinetic responses in individuals with T2DM. As regular exercise is a cornerstone in the treatment of T2DM, this is an area warrants further investigation in both individuals with well-controlled and advanced T2DM.

Appendix 1: Low level physical activity recall (LOPAR) questionnaire

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				

CARD A Selected List of Activities with MET Values (in parentheses)*
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)
Shoveling (7.0)	Machine tooling (4.0)	Teacher (2.5)	Computer work (1.5)
Heavy carpentry (7.0)	Forestry, chain saw (4.5)	Tailoring, machine (2.5)	Receptionist (1.5)

CARD B **HOUSEHOLD CHORES AND YARD WORK**

HEAVY	MODERATE	LIGHT	VERY LIGHT
Roofing (6.0)	Food shopping (3.5)	Preparing meals (2.5)	Sitting (1.5)
Digging (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)
Shoveling snow (6.0)	Weeding (4.5)	Fertilizing (2.5)	Sewing (1.5)
Manual lawn mowing (6.0)	Power lawn mowing (4.5)	Ironing (2.3)	

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 2: Participant information sheets

PARTICIPANT INFORMATION FORM FOR DIABETICS

1. Title of study: The effect of combined treatment with exercise and thiazolidinedione (TZD) class drug versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls.

2. Introduction: The current proposal **aims** to investigate the effect of combined treatment with exercise and thiazolidinediones versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) 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 120 volunteers recruited for this study and you will be between 30 and 75 years old. Out of 120, 60 will receive a thiazolidinedione therapy), and the other 60 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 (see Table 1). 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 (see **Table 1**)

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 40 watts every 3 minutes.

Visit 2: Submaximal cycling trials (session will last up to 180 min)

You will perform four 6-minute sub-maximal 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 force 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 Centre. 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 min 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 thiazolidinedione'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 or thigh cuff will be inflated at a pressure of 200 mmHg for 5 min. After this arterial occlusion, changes in girth of the forearm and calf 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.

Thiazolidinediones: One recent study has highlighted an increased risk of coronary heart failure with use of thiazolidinediones in some subjects. A qualified medical doctor will deem the subject suitable for prescription of thiazolidinediones 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 hemoglobin levels (HbA_{1c}) are <10% (adequate control on therapy), you are sedentary (≤ 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.

If you are a woman of childbearing age, you may participate in this study only if you are surgically sterilised or are using an effective contraceptive method. You must not be pregnant or lactating and you must have a negative pregnancy test before the study begins. The effects of exercise on the foetus or child may be harmful. The effects of thiazolidinediones on a foetus are unknown but may be harmful. If you should become pregnant, in spite of all the precautions, please notify your doctor immediately.

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, 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 any of the investigators (Mr Eamonn O'Connor (087-9631348/oconnoeg@tcd.ie), Ms. Catherine Kiely (087-9837055/kielyc@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.

PARTICIPANT INFORMATION FORM FOR NON-DIABETICS

1. Title of study: The effect of combined treatment with exercise and thiazolidinedione (TZD) class drug versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls.

2. Introduction: The current proposal **aims** to investigate the effect of combined treatment with exercise and thiazolidinediones versus exercise alone on the peripheral circulation, endothelial function and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) 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 non-diabetic participants recruited for this study and you will 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 $\dot{V}O_{2\text{ max}}$ (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 sub-maximal trials of constant-force 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 force 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 Centre. 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 or thigh cuff will be inflated at a pressure of 200 mmHg for 5 minutes. After this arterial occlusion, changes in girth of the forearm and calf 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.

$\dot{V}O_{2\text{ max}}$ and constant load cycle test: The study involves both a $\dot{V}O_{2\text{ max}}$ test and a constant load cycling test to determine $\dot{V}O_{2\text{ max}}$, $\dot{V}O_2$ 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 $\dot{V}O_{2\text{ max}}$ test there is always a very slight risk that participants may suffer a coronary attack (approximately one occurrence per 5,000 tests). All $\dot{V}O_{2\text{ max}}$ 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 (≤ 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.

If you are a woman of childbearing age, you may participate in this study only if you are surgically sterilised or are using an effective contraceptive method. You must not be pregnant or lactating and you must have a negative pregnancy test before the study begins. The effects of exercise on the foetus or child may be harmful. If you should become pregnant, in spite of all the precautions, please notify your doctor immediately.

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 any of the investigators (Mr Eamonn O'Connor (087-9631348/ oconnoeg@tcd.ie), Ms. Catherine Kiely (087-9837055/ kielyc@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

Appendix 3: Consent forms

INFORMED CONSENT FORM (EXERCISING DIABETICS)

PROJECT TITLE: The effect of combined treatment with exercise and Thiazolidinedione (TZD) class drug vs. exercise alone on the peripheral circulation, endothelial function, and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls.

PRINCIPAL INVESTIGATORS: Eamonn O'Connor (Student)
Catherine Kiely (Student)
Mikel Egaña (Supervisor)

SUMMARY: Having received clearance from the doctor, I will undergo two testing days at the human laboratory in the Department of Physiology (Trinity College Dublin) at least 72 hours apart, where I will perform the necessary calf ergometer and cycling testing. After this I will begin the 12-week training program at the Monkstown pool & fitness centre, attending for 1 hour at least 3 days a week, keeping a record of heart rate and blood pressure values. At the conclusion of the 12 weeks, I will return to Trinity College to repeat the initial two-day testing again, before beginning the 4-month home-based training program. I will continue to record heart rate and blood pressure readings from the 3 workouts per week, and at the conclusion of the 4 months I will return to Trinity to perform the last testing bout completing my participation in the study.

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:.....

INFORMED CONSENT FORM (NON EXERCISING DIABETICS)

PROJECT TITLE: The effect of combined treatment with exercise and Thiazolidinedione (TZD) class drug vs. exercise alone on the peripheral circulation, endothelial function, and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls.

PRINCIPAL INVESTIGATORS: Eamonn O'Connor (Student)
Catherine Kiely (Student)
Mikel Egaña (Supervisor)

SUMMARY: Having received clearance from the doctor, I will undergo two testing days at the human laboratory in the Department of Physiology (Trinity College Dublin) at least 72 hours apart, where I will perform the necessary calf ergometer and cycling testing. After this I will maintain my normal lifestyle, while recording my food intake 3 days a week for a twelve-week period. At the conclusion of the 12 weeks, I will return to Trinity College to repeat the initial two-day testing again. Again, I will maintain a normal lifestyle, recording food intake, and at the conclusion of the 4 months I will return to Trinity to perform the last testing bout completing my participation in the study.

DECLARATION:

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Date:.....

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INVESTIGATOR'S SIGNATURE:.....

Date:.....

INFORMED CONSENT FORM (EXERCISING NON-DIABETICS)

PROJECT TITLE: The effect of combined treatment with exercise and thiazolidinedione (TZD) class drug vs. exercise alone on the peripheral circulation, endothelial function, and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls.

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Date:.....

INFORMED CONSENT FORM (EXERCISING NON-DIABETICS)

PROJECT TITLE: The effect of combined treatment with exercise and thiazolidinedione (TZD) class drug vs. exercise alone on the peripheral circulation, endothelial function, and exercise tolerance in subjects with Type 2 Diabetes Mellitus (T2DM) and non-diabetic controls.

PRINCIPAL INVESTIGATORS: Eamonn O'Connor (Student)
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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 4: Medical form for non-diabetics

Pre-participation Medical Questionnaire & Examination Form

Date / /

Personal Details

Name:	Tel No. Home:
Age:	Mob:.....
Date of Birth:	Email:
Occupation:	G.P's name:
Address:	G.P's address:
.....	
.....	

For Type II Diabetic Subjects only

For Non-Diabetic Subjects only

(Please Circle)			(Please Circle)		
Diagnosis (yr)	< 5	> 5	Heart disease	Y	N
Treatment:			Stroke	Y	N
Diet only	Y	N	Diabetes	Y	N
Diet/ oral medication	Y	N	Asthma	Y	N
Insulin	Y	N	Epilepsy	Y	N
Heart disease	Y	N	Smoking	Y	N
Stroke	Y	N	Alcohol	Y	N
Asthma	Y	N	Tea	Y	N
Epilepsy	Y	N	Coffee	Y	N
Smoking	Y	N			
Alcohol	Y	N			
Tea	Y	N			
Coffee	Y	N			

Family History**State details and any other family illnesses**

	(Please Circle)		
Heart disease	Y	N	
Stroke	Y	N	
Diabetes	Y	N	
Asthma	Y	N	
Epilepsy	Y	N	

Drug History**State Details**

Medication	Y	N	
Over The Counter Meds	Y	N	
Allergies	Y	N	
Vitamins	Y	N	

Current medical problems in the last 3 months

Recent Illnesses:	Recent Injuries:

Last 7 days	Circle	At any time...	Circle	Details
Flu Symptoms	Y N	Hospital	Y N	
Sore Throat	Y N	admissions	Y N	
Cough	Y N	Operations	Y N	
Wheeze	Y N	Fractures	Y N	
Chest Pains	Y N	Sports Injuries	Y N	
Palpitations	Y N	Diabetes	Y N	
Nausea	Y N	Asthma	Y N	
Vomiting	Y N	Epilepsy	Y N	
Diarrhoea	Y N	Jaundice	Y N	
Headaches	Y N	Kidney problem	Y N	
Fits or Faints	Y N	Heart Murmur	Y N	
Others	Y N	Eye Problems	Y N	
		Other Illness		

General Examination (Doctors Use Only)

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

Medical Summary

Fit for Exercise Test to Exhaustion	Y	N	Signature:
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Appendix 5: Blood lactate levels prior to each bout of 6 min cycling

Non-diabetic	Pre 1	Pre 2	Pre 3	Pre 4
1	0.3	1.57	0.77	1.85
2	0.92	1.57	0.87	1.15
3	0.41	0.51	0.54	0.35
4	0.42	1.01	0.72	0.61
5	1.34	2.35	2.78	2.92
6	0.52	1.41	0.52	1.15
Mean	0.7	1.4	1.0	1.3
SD	0.4	0.6	0.9	0.9
T2DM	Pre 1	Pre 2	Pre 3	Pre 4
1	1.2	2.27	2.2	1.92
2	1.52	1.47	1.57	2.23
3	0.94	1.22	1.02	0.85
4	1.51	1.94	1.87	1.62
5	1.24	1.76	1.61	
6	1.11	1.59	2.01	1.55
7	0.73	1.83	2.02	1.92
8	3.02	2.42	2.24	2.2
9	0.69	2	1.74	1.89
10	1.16	2.2	1.92	2.04
11	1.19	1.53		1.87
Mean	1.3	1.8	1.8	1.8
SD	0.6	0.4	0.4	0.4

Appendix 6: Calf ergometer calibrations. Corresponding mV for 20 kg and 80kg are shown. Coefficient of variation (CV) is also shown.

Test day	20kg mV	80kg mV	Test day	20kg mV	80kg mV	Test day	20kg mV	80kg mV
1	735.551	2769.951	41	771.745	2931.745	81	770.6695	2897.67
2	723.001	2823.012	42	755.34	2915.34	82	789.0035	2988.004
3	720.632	2823.015	43	807.625	3087.625	83	810.561	3036.561
4	769.23	2809.221	44	729.69	264.969	84	813.603	3075.063
5	757.11	2857.103	45	773.21	2873.21	85	801.591	3024.591
6	741.725	2781.15	46	744.245	2814.245	86	804.6	3000.6
7	766.652	2896.665	47	654.34	2214.32	87	859.5825	3274.583
8	892.302	3172.31	48	667.223	2287.23	88	855.248	3246.248
9	765.624	2865.62	49	756.145	2925.145	89	794.405	3008.405
10	901.724	3421.72	50	695.57	2615.57	90		
11	897.117	3417.872	51	722.44	2702.44	91		
12	742.637	2752.054	52	734.65	2774.65	92		
13	701.602	2681.662	53	753.605	2853.605	93		
14	797.312	2807.341	54	731.96	2711.96	94		
15	674.745	2504.742	55	743.615	2753.615	95		
16	734.195	2624.195	56	760.445	2860.445	96		
17	763.815	2893.815	57	794.565	3059.505	97		
18	758.655	2918.655	58	825.213	3156.214	98		
19	727.545	2677.545	59	762.197	2937.194	99		
20	714.65	2694.65	60	762.807	2853.803	100		
21	715.835	2755.835	61	733.935	2749.935	101		
22	727.88	2779.788	62	767.551	2891.552	102		
23	735.79	2835.99	63	745.897	2785.898	103		
24	342.351	2714.235	64	790.531	2980.531	104		
25	755.257	2735.275	65	784.585	2953.585	105		
26	772.45	2992.45	66	786.8275	2973.828	106		
27	753.645	2883.645	67	764.983	2831.983	107		
28	701.97	2501.971	68	784.9775	3007.978	108		
29	753.29	2823.29	69	771.198	2865.198	109		
30	754.093	2854.095	70	789.8035	2958.804	110		
31	748.045	2908.453	71	789.7485	2982.749	111		
32	717.587	2697.582	72	750.5265	2784.527	112		
33	723.965	2733.965	73	779.0015	2912.002	113		
34	733.535	2893.535	74	819.1565	3120.157	114		
35	710.385	2750.385	75	752.9555	2864.956	115		
36	724.235	2750.385	76	800.563	3056.563	116		
37	737.115	2777.115	77	815.1755	3071.176	117		
38	734.33	2834.33	78	819.48	2976.48	118		
39	800.31	3020.31	79	750.032	2784.032	119		
40	741.39	2841.39	80	773.2165	2894.217	120		
						Mean	758.26	2841.20
						SD	63.39	335.15
						CV	8.36	11.80

Appendix 7: MAP responses during incremental plantar flexion exercise measured simultaneously using applanation tonometry (COLIN) and ‘volume clamp’ method (Finapres).

Subject	a		b		a-b	(a-b)2
	Colin		Finapres			
1 REST		83		79	5	21
1 Onset		83		82	1	1
1 Min 1		91		88	2	4
1 Min 2		92		89	3	7
1 Min 3		97		95	2	5
1 Min 4		79		81	-1	2
1 Min 5		68		69	-1	2
1 Min 6		90		85	5	26
1 Min 7		94		93	0	0
1 Min 8		88		89	-1	0
1 Min 9		98		101	-3	7
1 Min 10		101		102	-2	3
1 Min 11		97		91	5	30
1 Min 12		98		94	4	19
2 REST		74		77	-3	9
2 Onset		100		96	4	14
2 Min 1		95		91	4	17
2 Min 2		98		96	2	5
2 Min 3		101		97	4	16
2 Min 4		104		100	4	19
2 Min 5		95		93	1	2
2 Min 6		100		95	6	31
2 Min 7		100		96	4	18
2 Min 8		100		96	4	19
2 Min 9		100		96	4	13
2 Min 10		99		99	0	0
2 Min 11		100		99	1	1
2 Min 12		101		100	1	0
3 REST		83		85	-3	6
3 Onset		99		97	2	3
3 Min 1		109		96	13	181
3 Min 2		104		88	16	258
3 Min 3		112		104	8	70
3 Min 4		109		100	8	69
3 Min 5		113		99	13	180
3 Min 6		105		95	10	104
3 Min 7		110		104	6	41
3 Min 8		105		98	8	62
3 Min 9		107		103	4	20
3 Min 10		105		100	5	27
3 Min 11		105		111	-7	46
3 Min 12		106		110	-4	17
4 REST		86		83	3	9
4 Onset		90		92	-2	5
4 Min 1		89		91	-2	4
4 Min 2		92		91	0	0
4 Min 3		87		92	-6	31
4 Min 4		96		90	5	28
4 Min 5		98		98	0	0
4 Min 6		96		99	-3	9
4 Min 7		95		96	-1	1
4 Min 8		96		98	-2	3
4 Min 9		99		98	2	3
4 Min 10		107		105	2	5
4 Min 11		108		109	-1	0
4 Min 12		111		113	-2	3
5 REST		83		85	-3	6
5 Onset		99		97	2	3
5 Min 1		109		96	13	181
5 Min 2		104		88	16	258
5 Min 3		112		104	8	70
5 Min 4		101		100	1	0
5 Min 5		113		99	13	180
5 Min 6		105		104	1	2
5 Min 7		97		96	1	1
5 Min 8		105		102	3	11
5 Min 9		107		106	1	2
5 Min 10		105		100	5	27
5 Min 11		112		111	1	0
5 Min 12		106		110	-4	17
MEAN		99		96	3 SUM	2235.1
SD		10		8	5 SUM/2n	18.6
					10 Square root	4.3 TEM
						4.4 %TEM
ICC = 0.8						

Appendix 8: Physical characteristics and activity levels

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

T2DM	Age (yr)	Body mass (kg)	Height (m)	BMI (kg.m ⁻²)	WHR	ABI	LOPAR (Met h.wk ⁻¹)	Inactive (hr)	light (hr)	Moderate (hr)	Vigorous (hr)
1	54	108.1	1.56	44.4	1.01	1.00	65				
2	53	89.75	1.59	35.5	0.96	1.04	199.75	16.82	5.65	1.25	0.28
3	56	84.9	1.48	38.8	1.00	1.13	180.35	23.67	0.33	0	0
4	59	98.4	1.67	35.3	0.95	1.13	111.15	17.88	5.30	0.65	0.16
5	56	109.5	1.58	43.8	1.01	1.10	79.75	18.17	5.32	0.48	0.03
6	53	98.8	1.58	39.6	0.93	1.34	125.4	20.53	2.88	0.45	0.13
7	59	106.3	1.56	43.7	1.01	1.03	91.4				
8	57	72.5	1.51	31.8	0.98	1.02	103.5	21.41	2.59	0	0
9	56	60.25	1.53	25.7	0.96	0.95	109.6	16.23	6.61	1	0.16
10	55	82.35	1.57	33.4	0.86	1.00	68	23.69	0.31	0	0
11	60	69	1.56	28.4	0.99	1.19	97.3	18.43	5.15	0.41	0
12	67	78.3	1.63	29.5	0.97	1.03	85.15	17.77	5.71	0.52	0.003
13	52	84.1	1.61	32.4	1.01	1.05	106	18.22	5.26	0.49	0.02
14	56	94.3	1.66	34.2	0.95	1.14	28.94	16.49	5.53	1.55	0.42
15	64	68.7	1.58	27.5	0.99	1.03	48.5	23.87	0.13	0.00	0.00
16	57	81.1	1.66	29.4	0.93	1.03	57.35	23.60	0.39	0.00	0.00
17	57	81.6	1.54	34.4	0.90	1.11	166.45	16.26	6.24	1.13	0.36
18	58	72.2	1.58	28.9	0.89	1.06	171.4	22.99	1.00	0.00	0.00
19	49	88.9	1.66	32.3	0.87	1.10	78.97				
20	56	82.5	1.57	33.5		0.79		106.25			
Mean	56.7	88.1	1.58	34.1	0.96	1.06	112	24.8	3.7	0.5	0.1
SD	4.0	13.8	0.05	5.5	0.05	0.10	49	21.2	2.5	0.5	0.1
Non-diabetic	Age (yr)	Body mass (kg)	Height (m)	BMI	WHR	ABI	LOPAR (Met h.wk ⁻¹)	Inactive (hr)	light (hr)	Moderate (hr)	Vigorous (hr)
1	60	61.9	1.59	24.5	0.91	0.82	56.3	19.76	2.98	0.71	0.55
2	66	48.3	1.36	26.1	1.00	1.11	44.6	18.35	4.83	0.6	0.2
3	55	64.7	1.61	24.9	0.95	1.10	95.3	15.16	7.73	0.71	0.4
4	53	61.9	1.63	23.3	0.84	1.06	104.1	16.2	5.91	1.28	0.51
5	62	53.4	1.60	20.8	0.87	1.02	57.9	13.24	8.52	1.94	0.29
6	50	54.1	1.58	21.7	0.79	1.21	114.1	16.05	5.77	1.75	0.41
7	47	53.9	1.60	21.0	0.75	0.87	130.3	19.31	4.23	0.36	0.08
8	60	74.1	1.68	26.1	0.98	1.15	84.8	16.88	5.41	1.41	0.28
9	58	64.0	1.63	24.1	0.83	1.42	163.8	14.40	6.51	1.59	1.50
10	64	56.0	1.62	21.3	0.90	1.12	142.4	17.16	5.56	1.05	0.23
11	45	60.2	1.59	23.8	0.90	1.11	143.9				
12	50	64.6	1.62	24.6	0.98	1.03					
13	62	72.9	1.56	30.1	0.98	1.20	37.0	18.91	4.87	0.2	0.006
14	56	69.5	1.55	29.1	0.77	1.08	101.5	16.29	6.47	1.09	0.14
15	53	83.6	1.64	31.1	0.94	0.92	122.0	17.31	5.36	1.09	0.22
16	62	74.6	1.60	29.1	0.99	1.18	83.0	15.18	7.15	0.68	0.98
17	56	72.5	1.62	27.6	0.83	1.10	219.9				
18	54	69.4	1.59	27.4	0.88	1.22	129.3				
19	54	93.3	1.65	34.3	0.91	1.11	82.2				
20	54	74.6	1.58	29.9	0.90	1.04	96.8				
21	55	85.0	1.60	33.2	0.95	1.05	201.5				
Mean	56.0	67.2	1.60	26.4	0.90	1.09	111	16.7	5.8	1.0	0.4
SD	5.5	11.5	0.06	3.9	0.07	0.10	48	1.9	1.4	0.5	0.4

Appendix 9: Haematology.

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

T2DM	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Tri
	(mmol.L ⁻¹)	(%)	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)
1	6.3	6.1	43.2	5.1	3.5	1.3	1.4
2	6.1	6.4	46.4	5	3.3	1.7	1.4
3	7.6	7.9	62.8	4.8	3.5	1.2	1.0
4	5.1	5.2	33.3	3.4	2.3	0.8	2.4
5	9.9	7.3	56.3	4.7	3.8	1.1	1.7
6	5.2	5.5	36.6	4.8	3.3	1.5	1.2
7	6.5	6.8	50.8	3.1	2.0	1.0	1.9
8	13.4	9.4	79.0	3.4	1.4	1.3	1.7
9	7.2	6.4	46.4	3.4	2.2	1.2	1.2
10	8.4	6.5	47.5	4.9	3.0	1.5	2.8
11	7	6.8	50.8	4.4	2.6	1.9	1.4
12	6.7	6.7	50.0	5.3	3.2	1.5	1.3
13	7.2	6	42.0	4.1	2.1	1.5	1.3
14	7.7	6.6	48.6	3.8	2.5	1.4	1.0
15	8.1	6.2	44.3	4.8	3.4	1.4	1.1
16	13.1	5.3	34.4	4.6	3.2	1.4	2.3
17	9	7	53.0	5.1			2.1
18	8.3	7.5	58.0	4.9	3.3	1.2	1.0
19	10.3	7.2	55.2	6.3	4.4	1.6	1.4
20	12.9	8.1	65.0	4.5	3.0	1.2	2.5
Mean	8.3	6.7	50.2	4.5	2.9	1.3	1.6
SD	2.5	1.0	10.9	0.8	0.7	0.3	0.6
Non-diabetic	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Tri
	(mmol.L ⁻¹)	(%)	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)
1	3.8	5.3	34.4	5.7	3.4	2.5	0.7
2	4.5	5.1	32.2	5.3	3.2	2.0	1.7
3	4.5	5.7	38.8	8.6	6.4	2.3	1.1
4	4.9	5.6	37.7	8.5	5.7	2.6	1.2
5	5.7	5.4	35.5	5.6	3.9	1.9	0.9
6	4.4						
7	4.8	5.4	35.5	5.2	3.2	2.1	0.7
8	4	5.3	34.4	3.9	2.4	1.4	0.7
9	4.6	5.5	36.6	4.5	2.7	1.7	0.8
10	3.5			6.4	4.1	2.0	0.8
11	4.1	5.3	34.0	5.3	3.1	1.9	0.9
12	4.3			6.2	3.6	2.2	0.9
13				4.8	3.2	1.4	1.4
14	4	5.5	36.6	6.1	4.1	2.2	0.5
15	5	5.6	37.7	5.2	3.5	1.7	1.2
16	5.2	5.7	38.8	6.7	5.0	1.4	1.3
17	5.4	5.4	35.5	8.3	5.7	2.4	1.5
18	4.1	5.6	37.7	5.6	3.7	2.1	0.8
19							
20	4.5			5.6	2.9	2.33	0.82
21	4.8						
Mean	4.5	5.5	35.4	5.7	3.7	1.8	1.0
SD	0.6	0.2	1.9	1.3	1.1	0.4	0.3

Appendix 10: Peak cycling data.

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

T2DM											
	Peak HR	Peak HR	VO ₂	VO ₂	RER	V _E	Time	Peak workload	Workload at VT	VO ₂ at VT	VO ₂ at VT
	(beats.min ⁻¹)	%	(ml.kg ⁻¹ .min ⁻¹)	(L.min ⁻¹)		(L.min ⁻¹)	(min)	W	W	(ml.kg ⁻¹ .min ⁻¹)	(% VO _{2 peak})
1	123	74.1			1.02	40	9	80	80		
2	147	88.0	17.3	1.55	1.06	103.5	14	120	100	13.6	78.6
3	165	100.6	17.5	1.43	1.16	64.5	11.5	100	80	13.6	77.7
4	124	77.0	14.8	1.47	1.13	64.4	12	100	80	11.3	76.4
5	149	90.9	14.8	1.61	1.09	51.8	15	120	80	10.3	69.6
6	156	93.4	18.5	1.96	1.01	72.2	13	120	100	13.8	74.6
7	159	98.1	15.1	1.34	1.14	53.9	12	100	80	10.2	67.5
8	95	58.3	19.2	1.38	1.10	46.9	9	80	60	13.3	69.3
9	139	84.8	24	1.43	1.01	45.3	10	100	80	18.8	78.3
10	165	100.0	18.8	1.54	1.16	58.7	12	100	80	12.9	68.6
11	150	93.8	18.5	1.27	1.1	56.2	7	80	60	12.7	68.6
12	163	101.9	19.2	1.5		54.5	12.5	120	80	14.3	74.5
13	174	103.6	22.1	1.85		64.7	15	120	100	16.7	75.6
14	119	72.6	18.7	1.80	1.15	76.9	12	100	80	11.3	60.4
15	152	97.4	23.1	1.68	1.13	63.3	15	120	100	15.1	65.4
16	161	98.8	15.6	1.22		53	12	100	80	10.7	68.6
17	164	101.9	20.2	2.16	1.11	66.1	15	120	100	14.5	71.8
18	162	100.0	22.5	1.62	1.10	61.6	13	120	100	15.2	67.6
19	165	96.5	15.3	1.29	1.12	45.8	8	80	60	9.7	63.4
20	143	80.3	16.8	1.4		45.8	11	80	60	12.5	74.4
Mean	149	90.5	18.5	1.55	1.10	59.5	11.9	103.0	82.0	13.2	76.0
SD	20	12.3	2.9	0.25	0.05	14.2	2.4	16.3	14.4	2.3	20.7
Non-diabetic											
No	Peak HR	Peak HR	VO ₂	VO ₂	RER	V _E	Time	Peak workload	Workload at VT	VO ₂ at VT	VO ₂ at VT
	(beats.min ⁻¹)	%	(ml.kg ⁻¹ .min ⁻¹)	(L.min ⁻¹)		(L.min ⁻¹)	(min)	W	W	(ml.kg ⁻¹ .min ⁻¹)	(% VO _{2 peak})
1	164	102.5	29.3	1.82	1.11	76.9	13	120	100	21.6	73.7
2	174	113.0	17.7	0.87	1.20	42	9	80	80	13.7	77.4
3	173	104.8	23.3	1.52	1.07	58	14	120	100	17.9	76.8
4	150	89.8	35.7	2.23	1.08	61.9	19.5	160	140	28.3	79.3
5	148	93.7	22.1	1.22	1.14	60.8	10	100	80	16.7	75.6
6	155	91.2	33.8	1.92	1.09	64.4	18	140	120	19.8	58.6
7	170	98.3	23.8	1.27	1.13	43.9	10	100	80	18.7	78.6
8	164	102.5	21.6	1.62	1.12	60.2	12	100	100	16.5	76.4
9	163	100.6	24.7	1.56	1.01	55.7	11	100	80	19.4	78.5
10	166	106.4	19.1	1.05	1.10	48.3	10	100	60	13.5	70.7
11	180	108.4	22.5	1.35	1.12	51.6	10	100	60	14.5	64.4
12	156	91.8	23.2	1.48	1.06	64.1	12	100	100	19.9	85.8
13	148	93.7	18.01	1.27	1.04	41	9	80	60	12.1	67.2
14	152	92.7	22.8	1.55	1.08	49.9	13	120	100	14.8	64.9
15	148	88.6	22.5	1.9	1.11	31.1	15	120	120	18.8	83.6
16	138	87.3	24.7	1.86		70.3	18	140	120	18.5	74.9
17	155	94.5	22.2	1.63	1.06	68.8	18	140	140	12	54.1
18	152	91.6	26.5	1.82	1.19	78.8	16	140	120	19.9	75.1
19	143	85.6	21.3	1.84	1.01	55.7	16	140	120	16.8	78.9
20	147	88.6	23.8	1.42	1.14	48.9	14	120	80	11.7	49.2
21	133	80.6	18.3	1.52	1.05	64.8	12	100	100	14.2	77.6
Mean	156	95.5	23.7	1.56	1.10	59.7	13.3	115.2	98.1	17.1	73.2
SD	12	8.3	4.6	0.32	0.06	12.0	3.3	21.8	24.4	3.9	7.6

Appendix 11: $\dot{V}O_2$ kinetic responses.

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

T2DM	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	gain T2
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)
1	0.41	0.10	6.2	5.6	0.33	28.0	36.2	0.84	52.0	7.96
2	0.51	0.21	2.9	8.2	0.34	32.8	61.9	1.06	62.80	8.32
3	0.70	0.26	6.0	14.2	0.30	28.6	54.3	1.26	53.8	10.36
4	0.70	0.12	3.4	6.6	0.27	19.6	50.0	1.09	51.2	7.22
5	0.74	0.20	0.8	11.9	0.56	23.6	47.0	1.50	55.3	14.07
6	0.63	0.19	2.1	12.5	0.45	23.5	40.7	1.27	49.4	9.14
7	0.90	0.28	6.3	8.2	0.50	24.6	39.0	1.68	45.9	14.44
8	0.46	0.10	15.0	7.7	0.35	30.7	71.9	0.92	84.2	12.01
9	0.37	0.22	10.4	12.5	0.33	33.7	43.0	0.92	55.2	10.18
10	0.55	0.17	9.5	15.0	0.41	30.8	49.9	1.13	64.3	10.71
11	0.60	0.13	8.0	6.3	0.32	20.1	38.1	1.05	45.5	11.84
12	0.61	0.21	12.5	18.0	0.43	32.2	50.8	1.25	65.7	11.84
13	0.60	0.23	5.9	15.0	0.60	33.7	45.6	1.42	62.8	11.84
14	0.70	0.33	0.9	15.0	0.55	41.7	53.4	1.58	65.1	16.25
15	0.51	0.25	4.8	7.7	0.59	21.4	56.4	1.45	61.7	13.34
16	0.57	0.16	7.1	16.4	0.27	40.0	55.7	1.00	68.7	8.09
17	0.57	0.34	3.5	12.1	0.30	37.2	54.0	1.32	55.0	10.68
18	0.56	0.26	13.0	12.0	0.57	30.9	47.5	1.39	61.6	11.85
19	0.72	0.22	0.0	7.9	0.25	13.3	26.2	1.19	25.0	6.67
20	0.47	0.15	1.6	14.6	0.28	29.0	74.4	0.90	72.4	8.59
Mean	0.59	0.21	6.0	11.4	0.40	28.8	49.8	1.21	57.9	10.8
SD	0.13	0.07	4.3	3.8	0.12	7.2	11.5	0.24	12.2	2.6
Non-diabetic	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)
1	0.55	0.32	4.6	12.8	0.15	39.8	38.0	1.02	36.7	9.43
2	0.53	0.50	8.3	12.9	0.23	38.3	56.0	1.34	37.1	11.60
3	0.46	0.26	8.0	12.7	0.31	31.9	30.9	1.03	43.6	10.56
4	0.43	0.38	4.0	17.9	0.30	39.8	33.0	1.11	44.4	9.71
5	0.53	0.33	3.5	9.1	0.97	29.7	31.0	1.83	48.5	12.74
6	0.44	0.23	1.9	3.7	0.25	24.3	17.9	0.92	24.6	8.89
7	0.40	0.31	5.1	15.0	0.63	26.1	36.1	1.34	48.3	10.93
8	0.44	0.10	1.1	5.4	0.36	24.0	40.3	0.90	51.7	12.27
9	0.50	0.25	5.6	4.7	0.53	20.6	47.0	1.32	43.4	8.52
10	0.46	0.14	7.9	13.3	0.46	28.0	45.5	1.06	61.3	11.86
11	0.50	0.08	7.7	16.0	0.28	29.5	41.0	0.86	60.1	10.93
12	0.46	0.14	4.9	15.0	0.28	41.0	30.0	0.88	54.0	9.47
13	0.53	0.40	8.1	6.1	0.45	23.7	46.0	1.38	43.6	9.29
14	0.48	0.31	2.2	14.8	0.17	26.5	30.0	0.96	31.0	12.63
15	0.51	0.57	0.7	16.4	0.23	35.0	30.6	1.42	35.6	12.98
16	0.53	0.55	7.6	14.6	0.48	49.0	48.9	1.56	57.4	11.96
17	0.55	0.36	2.1	9.1	0.62	18.2	40.0	1.53	40.9	11.39
18	0.52	0.48	7.8	8.4	0.80	37.0	43.0	1.80	56.1	12.56
19	0.58	0.29	0.8	6.7	0.60	33.0	41.5	1.46	52.7	10.30
20	0.65	0.20	3.8	15.8	0.74	40.5	31.0	1.59	60.5	11.11
21	0.39	0.15	3.5	8.9	0.53	23.4	36.0	1.08	49.0	10.27
22	0.51	0.245	4.1	7.5	0.59	25.9	23.0	1.35	37.9	9.40
Mean	0.50	0.30	4.7	11.2	0.45	31.1	37.1	1.26	46.3	10.9
SD	0.06	0.14	2.6	4.3	0.22	8.0	8.9	0.30	10.0	1.6

Appendix 12: Heart rate kinetic responses.

T2DM	a	A1	TD1	Tau1	End A
	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)
1	98.5	21.4	3.8	57.0	119.9
2	93.0	21.4	1.5	68.4	114.3
3	119.5	21.5	12.1	85.1	140.6
4	83.0	9.1	4.1	51.4	92.1
5	116.8	22.2	3.0	71.2	138.8
6	108.5	29.0	0.5	57.8	137.4
7	76.0	13.0	2.1	50.0	88.9
8	114.0	23.0	6.0	85.6	136.6
9	94.4	34.5	0.1	143.7	126.1
10	114.8	24.5	7.7	79.8	138.9
11	105.6	33.1	0.5	69.7	138.5
12	114.0	28.9	0.1	76.3	142.6
13	108.1	34.0	0.1	98.4	141.2
14	134.9	12.9	2.0	68.0	147.8
15	86.0	41.9	0.1	66.0	127.7
Mean	104	25	2.9	75.2	129
SD	16	9	3.4	23.2	18

Non-diabetic	a	A1	TD1	Tau1	End A
	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)
1	103.1	47.5	7.6	44.8	150.6
2	78.1	48.2	9.8	55.9	126.2
3	80.5	38.8	8.6	60.0	119.2
4	84.2	43.5	2.0	49.3	127.6
5	97.0	39.7	15.7	76.0	136.2
6	99.0	47.2	6.4	56.0	146.1
7	81.0	32.1	2.4	68.1	113.0
8	96.5	42.5	6.9	72.0	138.6
9	83.0	28.5	3.2	76.2	111.2
10	88.9	40.9	1.2	51.0	129.8
11	83.0	33.3	4.0	59.2	116.2
12	91.3	39.6	2.9	61.6	130.7
13	83.5	27.9	2.6	48.0	111.3
Mean	88	39	5.6	59.8	127
SD	8	7	4.1	10.5	13

Appendix 13: Cardiovascular responses at rest and during cycle exercise at 80% VT.

CO, CI, SV, SVI, and HR responses at rest and during cycle exercise at 80% VT

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
 Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

T2DM	CO (L.min ⁻¹)			CI (L.min ⁻¹ .m ⁻²)			SV (ml)			SVI (ml.m ⁻²)			HR (beats.min ⁻¹)		
	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1	6.0	9.2	11.3	3.0	4.5	5.5	66	84	89	32.3	41.1	43.5	90	110	127
2	5.3	6.5	9.4	2.7	3.4	4.8	61	74	88	31.8	38.6	45.9	99	106	124
3	5.2	8.2	10.5	2.9	4.6	5.9	49	67	76	27.6	37.7	42.8	106	125	140
4	4.7	5.6	7.9	2.3	2.7	3.3	72	75	84	34.9	36.3	40.7	66	84	93
5	4.0	8.5	7.8	2.0	4.1	3.7	44	73	56	21.2	35.2	27.0	98	116	139
6	5.1	6.8	11.7	2.5	3.4	5.9	52	62	88	25.9	31.0	44.3	97	110	136
7	4.8	10.3	13.0	2.4	5.1	6.4	46	84	85	22.7	41.4	41.9	104	123	153
8	3.2	5.6	8.2	1.9	3.3	4.8	53	77	93	31.4	45.7	55.2	61	73	88
9	2.8	6.2	6.3	1.8	4.0	4.0	42	68	53	26.7	43.2	33.4	72	95	120
10	3.6	6.6	8.6	2.0	3.7	4.7	42	58	66	22.9	31.7	36.1	92	113	133
11	3.9	7.2	7.8	2.3	4.3	4.6	50	72	64	29.3	42.3	37.9	79	101	123
12	3.8	6.9	7.8	2.1	3.8	4.6	38	59	64	20.6	32.0	34.8	100	116	131
13	4.0	7.5	10.0	2.1	4.1	5.3	41	70	74	21.8	37.2	39.3	97	111	135
14	5.3	10.2	11.3	2.6	5.1	5.2	55	89	77	27.0	44.1	38.1	97	114	143
15	3.0	7.1	10.4		3.3	5.2		61	74		35.8	43.5	111	117	141
16	3.6	8.6	5.9	1.9	4.6	5.1	39	78	42	20.6	40.9	21.9	91	118	137
17	4.4	7.8	13.1	2.4	4.3	7.3	39	63	91	21.7	35.0	50.6	112	123	144
18	2.1	6.0	8.9	1.2	3.4	5.1	26	67	71	15.0	38.5	40.8	83	123	153
19	4.5	7.1	9.8	2.3	3.6	5.0	46	57	72	23.4	28.9	36.6	99	123	136
20	4.1	4.4	6.3	2.2	2.4	3.4	47	40	51	25.7	21.8	27.8	97	111	123
Mean	4.2	7.3	9.3	2.2	3.9	5.0	47.7	68.8	72.9	25.4	36.9	39.1	92.4	110.5	130.8
SD	1.0	1.5	2.1	0.4	0.7	1.0	10.7	11.3	14.7	5.0	5.8	7.8	13.8	13.5	16.6

Non-diabetic	CO (L.min ⁻¹)			CI (L.min ⁻¹ .m ⁻²)			SV (ml)			SVI (ml.m ⁻²)			HR (beats.min ⁻¹)		
	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1	3.8	7.8	10.1	2.3	4.8	6.2	50.0	74.0	75.5	30.6	45.2	46.2	76	105	135
2	2.8	5.9	6.9	1.6	4.5	5.3	31.0	45.5	41.5	23.6	34.6	31.6	111	128	164
3	5.2	8.7	10.8	3.1	5.2	6.4	60.0	79.0	80.0	35.7	47.0	47.6	77	116	136
4													69	98	123
5	4.2	5.5	8.2	2.8	3.6	4.5	51.0	59.5	69.0	33.1	38.6	44.7	82	93	120
6													72	94	126
7	4.5	7.1	8.3	1.0	4.5	5.2	66.0	64.5	63.5	42.6	41.6	41.0	85	109	131
8	4.1	6.3	9.0	2.2	3.4	4.9	50.0	57.0	60.0	27.2	31.0	32.6	84	111	150
9	4.4	6.5	7.9	2.6	3.9	4.7	61.0	66.5	73.5	36.1	39.4	43.5	74	91	109
10	3.8	5.3	7.0	2.4	3.4	4.4	40.0	47.0	59.0	25.2	29.6	37.1	95	114	120
11	3.9	6.7	7.5	2.4	4.2	4.7	52.0	62.0	56.0	32.2	38.4	34.6	76	108	135
12	4.7	7.4	10.1	2.8	4.4	6.0	72.8	80.3	67.0	43.1	47.5	39.7	72	98	124
13	3.0	5.9	6.8	1.7	3.4	4.0	38.0	60.0	64.0	22.0	34.7	37.0	78	92	117
14	3.7	8.1	9.1	2.6	4.8	5.5	57.0	67.0	78.0	33.9	39.8	46.3	76	111	124
15	3.6	8.2	10.7	1.3	4.4	5.6	61.0	71.5	84.0	32.1	37.6	44.2	76	102	127
16	3.9	6.5	8.8	3.7	4.9	3.9	52.0	69.0	77.5	29.2	38.8	43.6	73	94	113
17	4.5	7.7	9.9	4.7	4.3	5.5	60.0	71.0	71.0	33.8	40.0	40.0	75	108	138
18	3.6	6.9	11.3	2.4	5.2	7.0	53.0	84.0	92.0	30.9	48.9	53.6	87	106	130
19	4.7	9.0	10.0	2.4	4.5	5.0	64.0	92.0	80.0	32.0	46.0	40.0	74	98	125
20	4.5	6.0	8.3	2.6	3.2	4.7	59.0	60.0	73.5	33.5	34.0	41.7	72	103	114
21	3.9	6.8	8.7	2.1	3.6	4.7	50.0	67.0	67.0	26.6	35.6	35.6	77	103	130
Mean	4.0	6.9	8.9	2.5	4.2	5.2	54.1	67.2	70.1	31.7	39.4	41.1	78.8	103.6	127.9
SD	0.6	1.1	1.4	0.8	0.6	0.8	10.1	11.7	11.5	5.6	5.6	5.6	9.5	9.4	12.6

A-vO₂ diff, MAP, TPR responses at rest and during cycle exercise at 80% VT. CO, CI, and SV between rest and 30 s and between 30 s and 240 s.

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

T2DM										CO (L.min ⁻¹)		CI (L.min ⁻¹ .m ⁻²)		SV (ml)	
	A-VO ₂ DIFF (ml.100ml ⁻¹)			MAP (mmHg)			TPR (mmHg/L/min)			30s-0s	240s-30s	30s-0s	240s-30s	30s-0s	240s-30s
	0s	30s	240s	0s	30s	240s	0s	30s	240s	BS	BS	BS	BS	BS	BS
1	4.5	8.0	8.9	113.3	127.0	143.3	18.9	13.8	12.7	3.2	2.1	1.6	1.0	18.0	5.0
2	4.7	9.8	13.2	111.0	127.0	133.3	20.9	19.5	14.3	1.2	2.9	0.7	1.4	13.0	14.0
3	4.4	10.6	12.2	113.3	131.7	131.7	21.8	16.1	12.5	3.0	2.3	1.7	1.3	18.0	9.0
4	6.8	11.4	14.7	99.0	112.0	115.0	21.1	20.0	14.6	0.9	2.3	0.4	0.6	3.0	9.0
5	8.1	10.6	18.0	95.0	128.3	130.3	23.8	15.2	16.7	4.5	-0.6	2.1	-0.4	29.0	-17.0
6	6.3	11.2	11.4	117.3	139.0	148.7	23.2	20.4	12.7	1.8	4.9	0.9	2.5	10.0	26.5
7	6.4	10.1	13.4	99.5	138.7	141.3	20.9	13.5	10.9	5.6	2.7	2.8	1.3	38.0	1.0
8	6.9	10.3	12.1	99.3	102.3	106.7	31.0	18.3	13.0	2.4	2.6	1.4	1.5	24.0	16.0
9	7.7	9.7	16.9	89.7	110.7	115.7	32.0	17.8	18.5	3.4	0.0	2.2	0.0	26.0	-15.5
10	7.7	11.7	13.6	107.3	120.3	124.0	30.2	18.4	14.4	3.0	2.1	1.8	1.0	16.0	8.0
11	6.4	9.0	15.9	103.3	120.0	126.7	26.5	16.7	16.2	3.3	0.6	2.0	0.4	22.0	-7.5
12	6.7	11.3	15.9	98.7	106.7	110.0	26.0	15.5	14.1	3.1	0.9	1.7	0.8	21.0	5.0
13	7.7	10.9	13.0	105.3	112.7	130.0	26.3	15.0	13.0	3.5	2.5	2.0	1.2	29.0	4.0
14	5.4	9.5	14.2	117.3	121.8	147.7	22.3	11.9	13.1	5.0	1.1	2.5	0.1	34.5	-12.0
15	7.0	9.7	12.7	92.0	128.0	131.7	30.7	18.0	12.7	4.1	3.3	3.1	2.0	61.0	13.0
16	6.8	8.8	17.1	99.0	120.0	136.0	27.9	14.0	23.1	5.1	-2.7	2.8	0.5	38.5	-36.0
17	4.7	10.9	11.0	116.7	130.0	145.0	26.5	16.7	11.1	3.4	5.3	1.9	3.0	24.0	28.0
18	10.1	12.6	15.5	85.7	93.3	96.7	40.8	15.6	10.9	3.9	2.9	2.2	1.7	41.0	4.0
19	7.1	9.2	11.1	88.2	109.9	126.5	19.6	15.6	12.9	2.6	2.8	1.3	1.4	11.0	15.0
20	5.9	11.4	13.3	103.2	113.2	117.2	25.2	25.7	18.6	0.3	1.9	0.2	1.0	-7.0	11.0
Mean	6.6	10.3	13.7	102.7	119.6	127.9	25.8	16.9	14.3	3.2	2.0	1.7	1.1	23.5	4.0
SD	1.4	1.1	2.3	9.9	12.0	14.3	5.3	3.1	3.0	1.4	1.8	0.8	0.8	15.0	15.3

Non-diabetic										CO (L.min ⁻¹)		CI (L.min ⁻¹ .m ⁻²)		SV (ml)	
	A-VO ₂ DIFF (ml.100ml ⁻¹)			MAP (mmHg)			TPR (mmHg/L/min)			30s-0s	240s-30s	30s-0s	240s-30s	30s-0s	240s-30s
	0s	30s	240s	0s	30s	240s	0s	30s	240s	BS	BS	BS	BS	BS	BS
1	6.8	9.6	13.3	92.5	113.3	121.7	24.3	14.6	12.0	4.0	2.4	2.5	1.5	24.0	1.5
2	7.5	11.3	13.9	100.7	118.3	124.3	36.0	20.2	18.0	3.1	1.1	2.9	0.8	14.5	-4.0
3	5.8	8.8	12.4	91.0	120.0	118.3	17.5	13.8	11.0	3.5	2.1	2.1	1.2	19.0	1.0
4															
5	6.2	11.3	11.1	81.7	83.8	101.3	19.4	15.2	12.4	1.3	2.7	0.8	1.0	8.5	9.5
6				103.0	122.3	133.3									
7	5.8	9.9	11.6	100.2	115.3	127.0	22.5	16.4	15.3	2.6	1.3	3.5	0.8	-1.5	-1.0
8	6.5	11.3	15.0	96.7	116.0	117.7	23.6	18.4	13.1	2.2	2.7	1.2	1.5	7.0	3.0
9	5.9	10.2	13.5	90.3	102.7	113.3	20.5	15.8	14.3	2.1	1.4	1.3	0.8	5.5	7.0
10	6.6	11.6	12.8	98.7	116.7	124.7	26.0	22.0	17.8	1.5	1.7	1.0	1.0	7.0	12.0
11	5.8	9.5	12.9	90.0	99.3	102.7	23.1	14.8	13.7	2.8	0.8	1.8	0.5	10.0	-6.0
12	7.7	11.6	12.5	96.0	97.7	108.7	20.6	13.3	10.8	2.7	2.8	1.6	1.6	7.5	-13.3
13	8.2	11.9	15.0	96.7	108.5	111.7	32.2	18.5	16.4	2.9	1.0	1.7	0.6	22.0	4.0
14	7.1	10.6	14.8	96.7	110.0	116.7	26.1	13.7	12.8	4.4	1.1	2.2	0.7	10.0	11.0
15	7.3	10.2	14.3	84.3	111.7	113.3	23.4	13.7	10.6	4.6	2.5	3.1	1.2	10.5	12.5
16	6.4	13.7	17.0	90.8	111.2	112.0	23.3	17.2	12.8	2.6	2.3	1.3	-1.0	17.0	8.5
17	7.1	10.1	17.3	87.7	106.3	116.0	19.5	13.9	11.7	3.2	2.3	-0.4	1.2	11.0	0.0
18	7.5	9.6	12.0	106.3	120.0	133.3	29.5	17.4	11.8	3.3	4.4	2.8	1.8	31.0	8.0
19	4.8	11.3	15.6	95.7	117.3	123.3	20.4	13.0	12.3	4.3	1.0	2.1	0.5	28.0	-12.0
20	5.2	11.7	12.9	101.3	112.7	122.7	22.5	18.8	14.9	1.5	2.3	0.6	1.6	1.0	13.5
21		7.4	12.9	91.0	103.0	118.7	23.3	15.1	13.6	2.9	1.9	1.5	1.1	17.0	0.0
Mean	6.6	10.6	13.7	94.6	110.3	118.0	23.9	16.1	13.4	2.9	2.0	1.8	0.9	13.1	2.9
SD	0.9	1.4	1.7	6.3	9.4	8.7	4.6	2.6	2.2	1.0	0.9	1.0	0.6	8.8	7.9

SVI, HR, and a-vO₂ diff between rest and 30 s and between 30 s and 240 s during cycle exercise at 80% VT. Percentage steady state CO, CI, and a-vO₂ diff.

T2DM (postmenopausal 1-20, BMI matched postmenopausal 9-20)
Non-diabetic (postmenopausal 1-21, BMI matched postmenopausal 13-21)

	SVI (ml.m ⁻²)		HR (beats.min ⁻¹)		A-VO2 DIFF (ml.100ml ⁻¹ blood)		CO	CI	a-vO ₂ diff
	30s-0s	240s-30s	30s-0s	240s-30s	30s-0s	240s-30s	Steady state	Steady state	Steady state
	BS	BS	BS	BS	BS	BS	%	%	%
T2DM									
1	8.8	2.4	20.0	17.0	3.6	0.9	60.4	60.8	79.9
2	6.8	7.3	7.5	18.0	5.1	3.4	29.6	33.3	60.3
3	10.1	5.1	19.0	15.5	6.3	1.6	56.6	56.7	79.8
4	1.5	4.4	18.0	9.0	4.6	3.3	28.1	42.1	58.5
5	14.0	-8.2	17.5	23.5	2.5	7.4	117.1	120.0	25.5
6	5.0	13.3	13.0	26.0	5.0	0.2	26.3	26.5	96.3
7	18.7	0.5	19.5	30.0	3.7	3.2	67.3	67.9	53.4
8	14.2	9.5	12.0	15.0	3.4	1.9	48.0	48.3	64.5
9	16.5	-9.9	22.5	25.0	2.1	7.1	98.6	100.0	22.4
10	8.7	4.4	21.5	19.5	4.1	1.9	59.4	63.6	68.6
11	13.0	-4.4	22.0	22.0	2.7	6.9	84.6	84.8	27.9
12	11.4	2.7	16.0	15.0	4.6	4.6	77.5	68.0	50.2
13	15.4	2.1	14.0	24.0	3.2	2.1	58.3	62.5	60.7
14	17.1	-5.9	17.0	28.5	4.1	4.7	81.8	96.1	46.3
15	35.8	7.6	6.0	24.0	2.8	2.9	55.4	62.5	48.3
16	20.3	-19.0	27.5	18.5	2.0	8.3	214.9	84.6	19.6
17	13.3	15.6	11.0	21.0	6.2	0.2	39.1	38.8	97.2
18	23.6	2.3	40.0	30.0	2.5	2.9	57.4	56.4	46.3
19	5.6	7.6	24.0	13.0	2.1	1.9	48.1	48.1	52.6
20	-3.8	6.0	14.0	12.0	5.5	2.0	13.6	13.6	73.7
Mean	12.8	2.2	18.1	20.3	3.8	3.4	66.1	61.7	56.6
SD	8.5	8.2	7.5	6.1	1.3	2.4	43.2	26.0	22.4

	SVI (ml.m ⁻²)		HR (beats.min ⁻¹)		A-VO2 DIFF (ml.100ml ⁻¹ blood)		CO	CI	a-vO ₂ diff
	30s-0s	240s-30s	30s-0s	240s-30s	30s-0s	240s-30s	Steady state	Steady state	Steady state
	BS	BS	BS	BS	BS	BS	%	%	%
Non-diabetic									
1	14.7	0.9	29.0	30.0	2.7	3.7	62.7	62.8	42.3
2	11.0	-3.0	17.5	36.0	3.8	2.7	74.4	79.5	58.6
3	11.3	0.6	38.5	20.0	3.0	3.6	62.5	63.6	45.0
4			28.5	25.5					
5	5.5	6.2	10.5	27.0	5.1	-0.2	32.5	45.7	104.4
6			21.5	32.0					
7	-1.0	-0.6	24.0	22.0	4.1	1.7	67.5	82.1	70.0
8	3.8	1.6	27.5	38.5	4.8	3.7	45.4	45.3	56.3
9	3.3	4.1	17.0	18.0	4.3	3.3	60.0	61.9	56.9
10	4.4	7.6	19.0	6.0	5.0	1.2	46.9	50.0	80.3
11	6.2	-3.7	32.0	27.0	3.7	3.5	77.8	78.3	51.4
12	4.4	-7.8	26.5	25.5	3.8	0.9	49.5	49.5	80.8
13	12.7	2.3	14.0	25.0	3.7	3.1	75.0	75.6	54.8
14	5.9	6.5	35.0	13.0	3.4	4.2	80.6	75.9	44.8
15	5.5	6.6	26.5	25.0	2.9	4.1	64.5	71.8	41.8
16	9.6	4.8	21.0	19.0	7.3	3.3	52.6	500.0	69.1
17	6.2	0.0	33.0	30.5	3.0	7.2	58.3	-50.0	29.3
18	18.1	4.7	19.0	23.5	2.1	2.4	42.9	61.5	46.9
19	14.0	-6.0	24.0	27.0	6.5	4.3	81.1	80.8	60.3
20	0.6	7.7	30.5	11.5	6.5	1.1	40.0	26.2	85.2
21	9.0	0.0	26.5	26.5	7.4	5.6	60.4	58.8	57.0
Mean	7.6	1.7	24.8	24.2	4.4	3.1	59.7	80.0	59.7
SD	5.0	4.6	7.1	7.8	1.6	1.7	14.4	106.0	18.2

Appendix 14: Physical characteristics and activity levels.

T2DM (postmenopausal 1-22, BMI matched postmenopausal 11-22)
Non-diabetic (postmenopausal 1-22, BMI matched postmenopausal 13-22)

T2DM	Age	Body mass	Height	BMI	WHR	ABI	LOPAR
	(yr)	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	54	108.1	1.56	44.4	1.01	1.00	65
2	53	89.75	1.59	35.5	0.96	1.04	199.75
3	56	84.9	1.48	38.8	1.00	1.13	180.35
4	59	98.4	1.67	35.3	0.95	1.13	111.15
5	56	109.5	1.58	43.8	1.01	1.10	79.75
6	53	98.8	1.58	39.6	0.93	1.34	125.4
7	59	106.3	1.56	43.7	1.01	1.03	91.4
8	57	72.5	1.51	31.8	0.98	1.02	103.5
9	66	93.1	1.52	40.3	0.96	1.15	
10	56	104.0	1.63	39.1	1.01	1.23	
11	56	60.25	1.53	25.7	0.96	0.95	109.6
12	55	82.35	1.57	33.4	0.86	1.00	68
13	60	69	1.56	28.4	0.99	1.19	97.3
14	67	78.3	1.63	29.5	0.97	1.03	85.15
15	52	84.1	1.61	32.4	1.01	1.05	106
16	56	94.3	1.66	34.2	0.95	1.14	28.94
17	64	68.7	1.58	27.5	0.99	1.03	48.5
18	57	81.1	1.66	29.4	0.93	1.03	57.35
19	57	81.6	1.54	34.4	0.90	1.11	166.45
20	58	72.2	1.58	28.9	0.89	1.06	171.4
21	49	88.9	1.66	32.3	0.87	1.10	78.97
22	56	82.5	1.57	33.5		0.79	
Mean	57.1	86.7	1.58	34.6	0.96	1.07	104
SD	4.3	13.8	0.05	5.5	0.05	0.11	47
Non-diabetic	Age	Body mass	Height	BMI	WHR	ABI	LOPAR
	(yr)	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	60	61.9	1.59	24.5	0.91	0.82	56.3
2	66	48.3	1.36	26.1	1.00	1.11	44.6
3	55	64.7	1.61	24.9	0.95	1.10	95.3
4	53	61.9	1.63	23.3	0.84	1.06	104.1
5	62	53.4	1.60	20.8	0.87	1.02	57.9
6	50	54.1	1.58	21.7	0.79	1.21	114.1
7	47	53.9	1.60	21.0	0.75	0.87	130.3
8	60	74.1	1.68	26.1	0.98	1.15	84.8
9	58	64.0	1.63	24.1	0.83	1.42	163.8
10	64	56.0	1.62	21.3	0.90	1.12	142.4
11	45	60.2	1.59	23.8	0.90	1.11	143.9
12	50	64.6	1.62	24.6	0.98	1.03	
13	62	72.9	1.56	30.1	0.98	1.20	37.0
14	56	69.5	1.55	29.1	0.77	1.08	101.5
15	53	83.6	1.64	31.1	0.94	0.92	122.0
16	62	74.6	1.60	29.1	0.99	1.18	83.0
17	56	72.5	1.62	27.6	0.83	1.10	219.9
18	54	69.4	1.59	27.4	0.88	1.22	129.3
19	54	93.3	1.65	34.3	0.91	1.11	82.2
20	54	74.6	1.58	29.9	0.90	1.04	96.8
21	55	85.0	1.60	33.2	0.95	1.05	201.5
22	58	67.8	1.57	27.5	1.04	1.00	130.4
Mean	56.1	67.3	1.59	26.4	0.91	1.09	111
SD	5.4	11.2	0.06	3.9	0.08	0.13	47

Appendix 15: Haematology.

T2DM (postmenopausal 1-22, BMI matched postmenopausal 11-22)
Non-diabetic (postmenopausal 1-22, BMI matched postmenopausal 13-21)

T2DM	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Tri
	(mmol.L ⁻¹)	(%)	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)
1	6.3	6.1	43.2	5.1	3.5	1.3	1.4
2	6.1	6.4	46.4	5	3.3	1.7	1.4
3	7.6	7.9	62.8	4.8	3.5	1.2	1.0
4	5.1	5.2	33.3	3.4	2.3	0.8	2.4
5	9.9	7.3	56.3	4.7	3.8	1.1	1.7
6	5.2	5.5	36.6	4.8	3.3	1.5	1.2
7	6.5	6.8	50.8	3.1	2.0	1.0	1.9
8	13.4	9.4	79.0	3.4	1.4	1.3	1.7
9							
10							
11	7.2	6.4	46.4	3.4	2.2	1.2	1.2
12	8.4	6.5	47.5	4.9	3.0	1.5	2.8
13	7	6.8	50.8	4.4	2.6	1.9	1.4
14	6.7	6.7	50.0	5.3	3.2	1.5	1.3
15	7.2	6	42.0	4.1	2.1	1.5	1.3
16	7.7	6.6	48.6	3.8	2.5	1.4	1.0
17	8.1	6.2	44.3	4.8	3.4	1.4	1.1
18	13.1	5.3	34.4	4.6	3.2	1.4	2.3
19	9	7	53.0	5.1			2.1
20	8.3	7.5	58.0	4.9	3.3	1.2	1.0
21	10.3	7.2	55.2	6.3	4.4	1.6	1.4
22	12.9	8.1	65.0	4.5	3.0	1.2	2.5
Mean	8.3	6.7	50.2	4.5	2.9	1.3	1.6
SD	2.5	1.0	10.9	0.8	0.7	0.3	0.6
Non-diabetic	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Tri
	(mmol.L ⁻¹)	(%)	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)
1	3.8	5.3	34.4	5.7	3.4	2.5	0.7
2	4.5	5.1	32.2	5.3	3.2	2.0	1.7
3	4.5	5.7	38.8	8.6	6.4	2.3	1.1
4	4.9	5.6	37.7	8.5	5.7	2.6	1.2
5	5.7	5.4	35.5	5.6	3.9	1.9	0.9
6	4.4						
7	4.8	5.4	35.5	5.2	3.2	2.1	0.7
8	4	5.3	34.4	3.9	2.4	1.4	0.7
9	4.6	5.5	36.6	4.5	2.7	1.7	0.8
10	3.5			6.4	4.1	2.0	0.8
11	4.1	5.3	34.0	5.3	3.1	1.9	0.9
12	4.3			6.2	3.6	2.2	0.9
13				4.8	3.2	1.4	1.4
14	4	5.5	36.6	6.1	4.1	2.2	0.5
15	5	5.6	37.7	5.2	3.5	1.7	1.2
16	5.2	5.7	38.8	6.7	5.0	1.4	1.3
17	5.4	5.4	35.5	8.3	5.7	2.4	1.5
18	4.1	5.6	37.7	5.6	3.7	2.1	0.8
19							
20	4.5			5.6	2.9	2.33	0.82
21	4.8						
Mean	4.5	5.5	35.4	5.7	3.7	1.8	1.0
SD	0.6	0.2	1.9	1.3	1.1	0.4	0.3

Appendix 16: Peak calf performance.

T2DM (postmenopausal 1-22, BMI matched postmenopausal 11-22)
Non-diabetic (postmenopausal 1-22, BMI matched postmenopausal 13-21)

T2DM	MVC	Time	Peak force	Pk/MVC
	(N)	(s)	(N)	(%)
1	270	240	250	92.6
2	500	360	400	80.0
3	470	240	250	53.2
4	885	480	550	62.1
5	794	360	400	50.4
6	776	360	400	51.5
7	785	510	550	70.1
8	629	360	400	63.6
9	513	240	250	48.7
10	536	360	400	74.6
11	569	360	400	70.3
12	844	480	550	65.2
13	609	360	400	65.7
14	750	240	250	33.3
15	606	360	400	66.0
16	368	240	250	67.9
17	457	360	400	87.5
18	660	480	550	83.3
19	715	360	400	55.9
20	714	360	400	56.0
21	224	120	100	44.6
22	390	240	250	64.1
Mean	594	340	373	63.9
SD	183	98	119	14.4
Non-diabetic	MVC	Time	Peak force	Pk/MVC
	(N)	(s)	(N)	(%)
1	488	360	400	82.0
2	160	240	250	156.3
3	520	360	400	76.9
4	651	360	400	61.4
5	660	360	400	60.6
6	355	240	250	70.4
7	530	360	400	75.5
8	392	240	250	63.8
9	361	360	400	110.8
10	698	240	250	35.8
11	661	480	550	83.2
12	703	360	400	56.9
13	598	480	550	92.0
14	703	480	550	78.2
15	533	360	400	75.0
16	688	480	550	79.9
17	655	480	550	84.0
18	361	240	250	69.3
19	958	480	550	57.4
20	517	360	400	77.4
21	597	480	550	92.1
22	165	240	250	151.5
Mean	543	365	407	81.4
SD	188	94	118	28.0

Appendix 17: Incremental plantar flexion exercise.

T2DM (postmenopausal 1-22, BMI matched postmenopausal 11-22)
Non-diabetic (postmenopausal 1-22, BMI matched postmenopausal 13-21)

T2DM										Slope LVC		Slope LBF		Slope LVC		Slope LBF					
No.	Leg vol (ml)	LBF (ml.min ⁻¹)				550	LVC (ml.min ⁻¹ .mmHg ⁻¹)				550	(ml.min ⁻¹ .mmHg ⁻¹) vs force (N)	(ml.min ⁻¹) vs force (N)	(ml.min ⁻¹ .mmHg ⁻¹) vs % max	(ml.min ⁻¹) vs % max	rest LBF (ml.min ⁻¹ .mmHg ⁻¹)	rest LVC (ml.min ⁻¹)	Pk LBF (ml.min ⁻¹ .mmHg ⁻¹)	Pk LVC (ml.min ⁻¹)		
		100	250	400			100	250	400												
1	3535	64.9	196.6			0.635	1.66				0.006	0.88	1.71	220		68.8	0.52	208.9	1.74		
2	2797	186.9	343	537.3		1.313	2.671	4.367			0.01	1.17	4.07	467		47.3	0.35	655.4	5.23		
3	2810	232.4	371.9			2.152	2.98				0.005	0.92	1.38	233		25.4	0.21	446.5	3.61		
4	3307	182.2	225.2	407	758	1.904	2.065	3.503	8.466		0.014	1.27	7.75	700		55.5	0.62	864	11.95		
5	3325	185.1	289.8	603.8		2.139	3.175	6.159			0.013	1.40	5.36	558		89.4	0.92	699.7	6.57		
6	3272	354.5	400	481.9		3.216	3.693	4.176			0.003	0.42	1.28	170		69.7	0.72	540.8	5.32		
7	3395	162	513.7	593.2		1.609	4.86	5.229			0.0121	1.44	4.83	575		86.6	0.86	758.6	5.3		
8	2233	212.7	350.6	557.6		3.444	5.599	7.95			0.015	1.15	6.01	460		50.8	0.76	756.3	10.68		
9	2534	303.1	331.7			2.236	2.97				0.004	0.19	1.22	48		48.9	0.42	468	3.59		
10	3903	178.7	571.1	731.4		1.15	3.13	4.064			0.009	1.84	3.89	764		145.6	1.15	869.6	5.18		
11	1897	148	257.4	488.4		1.549	2.889	4.719			0.01	1.13	4.23	454		32.3	0.38	524.3	6.38		
12	2835	64.3	311.2	496.2	642.7	0.586	2.109	4.274	5.381		0.011	1.28	6.07	704		49.2	0.46	744.5	6.85		
13	2103	123.4	255	417.5		1.317	2.957	4.991			0.012	0.98	4.90	392		34.9	0.39	539.6	5.69		
14	2574	123.1	218.5			1.074	2.247				0.007	0.64	1.96	159		37.3	0.32	373.4	3.11		
15	3009	106.5	213	276.6		0.966	1.805	2.233			0.004	0.69	1.69	227		41.7	0.37	332.5	2.62		
16	2754	143.1	311.4			1.243	3.068				0.012	1.12	3.04	281		62.2	0.56	416.5	3.78		
17	2364	224.3	311.2	400		2.429	3.43	4.349			0.006	0.59	2.56	234		46.1	0.44	433.9	4.86		
18	2574	134.9	329.6	447.5	491	1.875	4.025	5.035	5.667		0.008	0.79	4.54	435		31.5	0.41	571.5	6.76		
19	2216	208.1	391.8	455.4		1.899	3.282	3.83			0.006	0.82	2.57	330		36.7	0.33	509.8	4.8		
20	2417	121.5	200.7	360.2		1.291	1.817	3.016			0.005	0.80	2.30	318		44.6	0.45	444.9	3.76		
21	2699	159.9				1.328										27	0.24	182	1.41		
22	2803	61.8	158.3			0.58	1.33				0.005	0.64	1.25	161		47.4	0.4	223.1	1.39		
Mean	2789	167.3	312.0	483.6	630.6	1.6	2.9	4.5	6.5		0.008	0.96	3.46	376		53.6	0.51	525.6	5.0		
SD	507	72.3	102.7	111.4	133.9	0.8	1.0	1.3	1.7		0.004	0.38	1.90	201		26.9	0.24	200.6	2.6		
Non-diabetics																					
No.	Leg vol (ml)	LBF (ml.min ⁻¹)				550	LVC (ml.min ⁻¹ .mmHg ⁻¹)				550	(ml.min ⁻¹ .mmHg ⁻¹) vs force (N)	(ml.min ⁻¹) vs force (N)	(ml.min ⁻¹ .mmHg ⁻¹) vs % max	(ml.min ⁻¹) vs % max	rest LBF (ml.min ⁻¹ .mmHg ⁻¹)	rest LVC (ml.min ⁻¹)	Pk LBF (ml.min ⁻¹ .mmHg ⁻¹)	Pk LVC (ml.min ⁻¹)		
		100	250	400			100	250	400												
1	2308	73.6	208.3	354.4		0.9	2.3	4.2			0.011	0.94	4.45	374		31.3	0.28	501.7	4.83		
2	1465	283.7	295.0			2.5	3.0				0.003	0.25	0.92	19		48.7	0.52	346.5	3.26		
3	3150	225.5	485.4	938.0		2.3	4.9	9.3			0.023	2.38	9.40	950		55.4	0.55	958.4	9.64		
4	2135	172.4	460.4	771.7		1.8	4.8	8.2			0.021	2.00	8.59	799		42.9	0.49	846.8	9.08		
5	2511	224.1	381.4	774.9		2.8	5.0	10.2			0.024	1.84	9.76	734		51.8	0.68	990.8	12.9		
6	1981	84.6	155.6			0.9	1.5				0.004	0.47	1.03	118		39.9	0.43	169.1	1.73		
7	2272	43.8	122.1	175.2		0.4	1.3	1.7			0.004	0.44	1.77	175		23.7	0.22	246	2.47		
8	2593	79.8	326.5			0.9	3.1				0.01	1.14	3.74	411		66.1	0.69	395.3	4.46		
9	2170	138.5	233.4	405.7		1.4	2.5	4.3			0.009	0.89	3.87	356		56.4	0.6	478.3	5.2		
10	2257	101.5	203.5			0.7	1.3				0.004	0.68	1.15	170		49.5	0.47	298.7	2.94		
11	2649	75.6	229.6	595.7	664.1	0.8	2.1	5.8	5.9		0.012	1.42	6.95	782		39.3	0.4	729.2	6.15		
12	2257	209.7	326.3	499.1		2.6	5.1	8.2			0.018	0.96	7.44	386		59.1	0.91	685.3	9.35		
13	2494	205.6	485.4	694.2	1109.3	2.0	4.9	6.1	9.6		0.016	1.95	8.86	1071		60.7	0.59	1252.3	11.9		
14	2389	222.2	285.3	316.9	519.5	2.5	3.0	3.3	5.4		0.006	0.62	3.31	339		27.1	0.31	551.4	5.95		
15	2974	205.6	444.2	750.3		1.6	3.6	5.7			0.012	1.72	5.46	727		70	0.64	1069	8.09		
16	2720	117.2	149.6	335.9	480.2	1.0	1.3	2.9	3.3		0.005	0.85	3.13	468		77.9	0.64	523	3.88		
17	2624	154.0	484.4	765.9	918.3	2.0	6.5	10.8	11.2		0.021	1.72	11.66	944		112.7	1.34	1040.2	12.07		
18	2550	163.7	290.1			1.6	3.5				0.031	1.40	3.26	211		53.7	0.51	332.9	4.45		
19	3018	99.5	366.5	734.2	776.3	0.6	1.9	3.6	3.8		0.007	1.60	4.10	879		79.8	0.81	988.2	8.44		
20	2287	87.0	186.0	303.5		0.6	1.0	1.7			0.003	0.72	1.49	289		44.9	0.37	322.5	2.59		
21	3218	83.2	237.6	573.7		1.0	2.8	6.8			0.019	1.64	7.73	654		53.2	0.54	670.9	6.92		
22	1611	133.4	446.2			2.4	5.1				0.017	5.87	4.45	521		27.6	0.34	509.3	5.42		
Mean	2438	144.7	309.2	561.8	744.6	1.5	3.2	5.8	6.5		0.013	1.43	5.11	517		53.3	0.56	632.1	6.4		
SD	441	65.8	119.7	224.4	241.3	0.8	1.6	2.9	3.2		0.008	1.15	3.20	306		20.3	0.24	311.3	3.3		

Appendix 18: LVC kinetic responses at 30% MVC.

T2DM (postmenopausal 1-22, BMI matched postmenopausal 11-22)
Non-diabetic (postmenopausal 1-22, BMI matched postmenopausal 13-21)

T2DM	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A
	*	*	(s)	(s)	*	(s)	(s)	*	(s)	(s)	*
1	0.75	0.97	4.35	9.6	0.38	4.8	7.0	0.30	35.2	14.7	2.02
2	0.71	1.94	1.04	4.2	0.30	9.0	7.9	0.35	27.2	14.0	3.00
3	0.40	1.19	5.60	4.9	0.94	7.4	9.3	0.50	24.1	15.0	2.09
4	0.66	2.79	1.07	3.2	0.11	2.3	9.0	0.49	19.0	4.9	3.95
5	0.71	2.68	1.00	2.0	1.01	11.0	6.0	1.82	18.0	17.1	5.21
6	0.91	2.69	1.08	2.0	0.57	11.0	5.9	1.84	17.0	12.2	5.44
7	1.02	2.60	1.46	4.0	0.95	7.0	7.1	0.90	23.3	25.0	4.52
8	0.47	5.29	0.00	2.0	4.01	5.2	8.5	2.89	12.8	10.4	8.66
9	0.45	3.10	0.94	2.6	0.55	5.3	38.9	0.25	14.3	16.0	3.80
10	1.20	2.17	1.14	3.1	1.80	7.4	7.1	2.71	22.7	44.0	6.08
11	0.50	1.13	0.00	3.2	0.37	14.9	6.2	0.73	19.0	14.2	2.36
12	0.49	2.27	1.08	1.5	0.37	6.2	5.9	0.67	17.2	9.0	3.43
13	0.41	1.99	3.59	10.5	0.41	22.1	6.2	0.25	34.3	8.8	2.65
14	0.44	2.20	0.00	2.7	0.99	10.1	6.0	1.39	24.2	7.5	4.03
15	0.30	2.25	0.99	2.0	1.02	7.0	6.0	1.15	24.1	16.2	3.70
16	0.45	0.31	0.93	3.2	0.10	3.3	8.8	0.40	18.8	18.0	1.16
17	0.50	1.66	1.01	2.0	0.97	6.0	5.9	1.93	12.1	9.2	4.09
18	0.56	3.85	0.88	2.0	0.90	8.0	5.9	1.10	17.1	8.6	5.51
19	0.41	2.70	1.03	4.0	0.80	0.9	4.9	1.60	17.4	29.6	4.71
20	0.46	3.25	0.99	2.0	1.32	5.0	6.0	0.81	20.0	14.2	4.52
21	0.30	0.86	0.99	3.0	0.55	5.0	9.5	0.50	15.0	16.0	1.65
22	0.37	0.39	0.00	1.9	0.56	5.4	8.1	0.29	17.3	12.2	1.06
Mean	0.57	2.19	1.3	3.4	0.86	7.7	8.5	1.04	20.5	15.3	3.80
SD	0.24	1.10	1.0	2.3	0.81	5.5	6.6	0.74	6.1	9.4	1.68
(ml.min ⁻¹ .mmHg ⁻¹)											
Non-diabetics	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A
	*	*	(s)	(s)	*	(s)	(s)	*	(s)	(s)	*
1	0.29	1.16	0.52	4.8	0.07	7.3	3.5	0.24	21.6	8.0	1.69
2	0.61	1.56	1.47	3.8	0.65	15.4	14.8	1.40	23.8	12.0	3.57
3	0.60	2.97	0.73	12.8	0.20	1.9	9.5	0.83	30.5	7.0	4.40
4	0.46	2.01	0.50	5.0	0.19	2.3	5.1	0.65	16.1	7.0	3.12
5	0.73	4.28	0.95	3.7	1.22	3.1	8.2	3.01	19.4	21.1	8.02
6	0.41	3.30	0.67	4.8	3.10	1.0	8.1	0.52	24.1	13.0	4.22
7	0.23	1.00	1.77	8.5	0.75	8.4	8.5	0.50	20.0	8.1	1.72
8	0.49	0.47	1.55	7.1	0.42	36.0	11.0	0.80	41.0	23.5	1.76
9	0.62	1.17	1.68	6.6	0.02	6.4	0.4	0.49	12.8	15.2	2.28
10	0.75	2.48	1.22	4.3	0.40	3.6	8.0	0.28	6.2	11.9	3.50
11	0.37	0.80	1.31	1.9	0.43	7.8	4.5	1.57	14.0	15.0	2.74
12	0.69	1.25	2.79	2.8	0.41	8.0	4.8	1.94	13.3	16.0	3.87
13	0.46	1.44	1.97	1.3	0.20	16.8	7.4	1.00	19.8	24.1	2.90
14	2.24	0.14	1.37	3.8	0.23	14.4	4.4	0.80	17.3	25.0	3.19
15	1.00	2.60	1.64	8.5	1.55	14.3	44.3	1.64	41.0	35.4	5.24
16	0.43	0.76	1.53	6.4	0.18	20.9	10.5	0.51	28.0	9.5	1.70
17	0.98	1.95	3.04	1.4	0.07	10.0	3.6	1.15	14.3	9.4	4.08
18	0.55	1.05	1.50	3.0	0.30	21.0	11.0	0.29	41.0	9.3	1.89
19	0.66	4.53	0.82	2.2	1.00	10.8	1.5	0.87	18.1	6.3	6.06
20	0.20	0.45	1.27	1.2	0.19	14.1	4.6	0.38	18.0	8.6	1.03
21	0.70	1.78	4.00	12.2	0.16	30.9	0.8	0.15	31.2	2.3	2.63
22	0.39	0.48	1.50	7.0	0.12	27.0	11.0	0.26	41.0	18.3	1.13
Mean	0.64	1.92	1.5	5.1	0.55	12.1	8.7	0.88	23.6	13.0	3.40
SD	0.37	1.21	0.9	3.5	0.61	9.3	8.1	0.66	9.5	8.0	1.92
(ml.min ⁻¹ .mmHg ⁻¹)											

Appendix 19: HR and MAP responses during plantar flexion at 30% MVC.

T2DM (postmenopausal 1-22, BMI matched postmenopausal 11-22)
Non-diabetic (postmenopausal 1-22, BMI matched postmenopausal 13-21)

30% MVC		HR (beats.min ⁻¹)				MAP (mmHg)			
T2DM		Rest	30s	180s	360s	Rest	30s	180s	360s
	1	62.0	62.0	66.0	64.5	116.6	115.8	118.0	118.0
	2	67.0	73.0	75.0	77.5	99.8	116.9	120.0	120.0
	3	79.0	80.0	81.0	80.0	109.4	116.0	116.0	116.0
	4	66.3	69.0	68.3	70.5	90.6	93.0	95.0	96.0
	5	72.0	73.0	75.6	76.3	95.1	93.5	94.8	93.5
	6	68.0	69.0	70.0	71.0	87.8	89.0	89.8	88.0
	7	57.0	61.0	62.3	65.0	99.7	90.0	104.3	104.0
	8	48.0	49.0	51.5	51.5	59.7	62.3	62.0	68.0
	9	89.0	90.0	90.0	91.0	103.0	106.0	108.0	107.0
	10	55.0	57.3	67.7	67.0	126.6	133.3	130.2	141.7
	11	48.0	49.0	48.0	48.0	80.1	81.8	83.9	85.4
	12	89.0	90.0	90.0	91.0	97.5	98.9	101.4	107.5
	13	67.0	67.0	69.0	67.0	89.9	91.5	91.0	90.0
	14	81.0	80.0	82.0	83.0	90.4	92.2	94.0	95.0
	15	73.0	74.0	74.0	75.0	104.0	108.0	107.0	108.0
	16	63.0	63.0	64.7	64.3	105.2	111.2	115.9	112.0
	17	72.0	74.0	77.0	75.0	93.1	99.6	102.5	101.0
	18	75.0	76.0	76.0	77.6	69.9	76.8	79.6	80.0
	19	73.6	74.0	81.0	77.3	104.4	106.0	105.0	107.0
	20	68.0	69.0	70.0	71.0	87.4	92.0	94.0	95.6
	21	78.0	78.0	82.0	80.0	102.0	102.7	106.9	108.5
	22	73.0	76.3	74.3	74.3	113.5	118.2	121.4	121.6
Mean		69.3	70.6	72.5	72.6	96.6	99.8	101.8	102.9
SD		11.0	10.8	10.5	10.5	14.9	15.9	15.7	16.1
30% MVC		HR (beats.min ⁻¹)				MAP (mmHg)			
Non-diabetic		Rest	30s	180s	360s	Rest	30s	180s	360s
	1	63.0	66.0	66.3	67.7	82.8	104.0	106.0	105.0
	2	70.0	72.0	76.3	61.7	95.4	83.0	87.5	88.0
	3	54.6	55.0	57.5	77.4	92.1	96.5	98.0	99.6
	4	51.3	52.0	54.0	55.7	84.8	83.6	84.6	83.0
	5	56.6	57.0	62.7	66.6	75.0	76.9	81.0	81.0
	6	59.0	60.0	61.0	60.0	85.5	91.1	95.0	92.0
	7	71.0	71.0	72.3	75.0	95.2	100.0	101.3	101.5
	8	53.0	55.0	57.3	59.0	90.9	100.6	100.0	101.5
	9	61.0	61.0	63.0	65.5	84.4	92.5	93.3	93.6
	10	78.5	80.0	80.0	79.0	81.0	88.3	87.0	88.0
	11	65.0	67.0	67.0	73.0	98.2	109.0	110.7	110.7
	12	56.0	56.5	59.0	59.5	81.5	81.5	81.7	82.0
	13	64.6	65.0	67.0	72.3	90.0	94.0	98.3	103.0
	14	63.0	64.0	67.0	65.0	92.7	92.3	93.9	93.0
	15	50.0	56.0	56.0	60.3	82.4	82.0	82.0	81.3
	16	58.0	63.0	65.0	64.0	93.0	110.9	116.0	116.0
	17	55.0	55.9	56.7	58.6	79.2	77.0	81.0	81.0
	18	63.7	63.3	65.1	65.6	95.7	99.4	101.0	103.0
	19	56.0	57.0	59.0	58.0	94.0	95.8	95.0	95.6
	20					103.7	109.7	109.7	109.3
	21	56.0	56.5	59.0	61.0	82.6	84.5	86.6	87.0
	22	68.0	68.0	67.0	69.0	97.0	100.0	99.0	102.0
Mean		60.6	62.0	63.7	65.4	89.0	93.3	94.9	95.3
SD		7.2	7.0	6.7	6.7	7.4	10.3	10.2	10.6

Appendix 20: LVC kinetic parameters at 70% MVC.

T2DM	a	A1	TD1	r1	A2	TD2	r2	End A	MRT
	*	*	(s)	(s)	*	(s)	(s)	*	(s)
1	0.47	0.87	0.10	3.03	1.91	16.22	31.76	3.25	33.96
2	0.42	3.56	1.21	2.04	4.20	11.10	40.00	8.17	29.16
3	0.96	3.20	1.90	7.26	2.84	26.00	42.62	6.99	37.16
4	0.66	2.20	0.94	5.95	2.65	19.73	71.00	5.48	52.70
5	0.30	2.00	0.10	3.15	1.25	16.21	22.51	3.54	16.88
6	0.83	2.59	1.24	3.53	2.09	15.82	42.00	5.51	28.45
7	0.57	3.57	1.15	3.50	2.17	16.71	16.00	6.31	15.26
Mean	0.60	2.57	0.9	4.1	2.44	17.4	38.0	5.61	30.5
SD	0.23	0.98	0.7	1.8	0.93	4.6	17.8	1.77	12.7
Non-diabetic	a	A1	TD1	r1	A2	TD2	r2	End A	MRT
	*	*	(s)	(s)	*	(s)	(s)	*	(s)
1	0.63	1.80	0.10	1.69	1.10	10.92	12.68	3.52	10.06
2	0.64	2.36	0.05	4.37	2.74	18.98	27.87	5.74	27.22
3	0.64	7.38	0.10	1.46	0.88	9.11	4.60	8.90	2.85
4	0.27	2.02	1.31	4.15	3.93	16.00	20.00	6.21	25.64
5	0.28	0.93	1.57	3.80	1.05	17.12	22.99	2.26	23.79
Mean	0.49	2.90	0.6	3.1	1.94	14.4	17.6	5.33	17.9
SD	0.20	2.56	0.7	1.4	1.34	4.2	9.1	2.57	10.8
(ml.min ⁻¹ .mmHg ⁻¹)									

Appendix 21: HR and MAP responses at 70% MVC.

T2DM	HR (beats.min ⁻¹)				MAP (mmHg)			
	Rest	30s	180s	360s	Rest	30s	180s	360s
1	2.0	80.0	83.0	86.0	144.0	152.0	156.4	156.6
2	62.0	67.0	73.0	73.0	102.0	104.0	106.0	106.0
3	77.5	80.0	84.5	84.5	83.0	88.0	93.0	94.0
4	75.0	75.0	80.0	80.0	101.0	111.2	121.0	121.0
5	67.0	71.0	75.0	81.0	114.0	118.0	125.0	132.0
6	75.0	75.0	80.0	80.0	71.0	95.0	96.3	95.5
7	49.0	51.5	54.0	55.0	66.0	68.0	72.0	72.0
Mean	58.2	71.4	75.6	77.1	97.3	113.8	110.0	111.0
SD	26.7	9.9	10.4	10.6	27.0	27.2	27.2	28.0
Non-diabetic	HR (beats.min ⁻¹)				MAP (mmHg)			
	Rest	30s	180s	360s	Rest	30s	180s	360s
1	75.0	76.0	76.0	76.5	96.0	103.0	115.0	118.0
2	56.0	57.0	57.0	60.0	81.0	84.9	86.0	87.0
3	65.0	65.0	77.0	77.0	85.0	102.3	120.0	121.0
4	67.0	74.0	81.5	81.5	103.4	113.0	115.5	115.6
5					115.2	117.0	117.0	118.0
Mean	65.8	68.0	72.9	73.8	87.3	96.7	107.0	108.7
SD	7.8	8.8	10.9	9.4	7.8	10.3	18.4	18.8

Appendix 22: Reactive hyperaemic responses.

T2DM (1-19 postmenopausal, 9-19 BMI matched postmenopausal)
Non-diabetic (1-18 postmenopausal, 12-18 BMI matched postmenopausal)

T2DM	resting vc	a	A	c	a+A	AUC	Peak VC
	*	*	*		*		*
1	1.5	2.0	15.3	0.07	17.4	905	17.7
2	6.1	4.6	20.4	0.06	25.0	1923	25.6
3	1.7	3.0	20.3	0.02	23.4	1643	23.2
4	1.7	3.9	12.0	0.10	15.9	1379	15.8
5		2.0	12.7	0.06	14.7	864	14.6
6	6.1	3.6	18.5	0.02	22.1	1933	22.1
7	1.9	0.8	9.4	0.01	10.2	916	8.2
8	2.1	1.9	8.0	0.05	9.9	749	9.8
9	1.2	0.5	15.5	0.09	16.0	326	15.7
10	1.6	2.7	14.8	0.03	17.5	1240	17.4
11	2.1	1.1	17.4	0.06	18.5	663	18.5
12	2.7	1.3	15.8	0.03	17.1	881	17.0
13	1.9	1.5	15.8	0.05	17.2	760	16.8
14	1.6	2.0	10.0	0.07	12.0	810	12.2
15	1.2	1.0	20.6	0.05	21.6	694	21.3
16	2.1	2.1	11.1	0.01	13.3	1255	13.3
17	0.7	1.4	21.0	0.03	22.4	1047	22.9
18	2.5	1.3	17.0	0.04	18.2	904	18.0
19	1.3	2.1	25.9	0.09	26.0	1009	27.7
Mean	2.2	2.0	15.9	0.05	17.8	1047	17.8
SD	1.5	1.1	4.6	0.03	4.7	424	5.1
* (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)							
Non-diabetic	resting vc	a	A	c	a+A	AUC	Peak VC
	*	*	*		*		*
1	9.4	4.4	11.8	0.01	16.2	2181	13.3
2	2.2	2.2	24.1	0.02	26.3	1965	26.4
3	2.1	0.1	10.9	0.06	11.1	250	11.1
4	2.7	1.3	43.6	0.03	44.9	1556	44.3
5	1.9	0.9	8.5	0.03	9.4	431	9.3
6	2.0	2.0	23.8	0.08	25.8	961	25.6
7	1.8	1.3	13.9	0.03	15.2	876	15.5
8	1.3	1.1	20.3	0.07	21.5	668	21.8
9	0.9	1.1	13.4	0.08	14.5	543	14.8
10	2.2	1.9	13.5	0.04	15.4	925	15.4
11	2.5	1.7	23.9	0.04	25.6	1153	27.1
12	1.9	4.0	20.5	0.07	24.4	1366	24.8
13	2.0	3.0	34.8	0.08	37.8	1500	38.4
14	2.2	2.1	21.0	0.19	23.1	878	23.1
15	1.0	1.6	23.0	0.04	24.6	858	24.4
16	1.3	2.0	25.4	0.04	27.4	1263	32.6
17	1.3	1.0	11.1	0.05	12.2	508	12.3
18	0.7	1.6	19.6	0.03	21.2	1184	19.7
Mean	2.2	1.8	20.2	0.05	22.0	1059	22.2
SD	1.9	1.1	8.9	0.04	9.1	519	9.5
* (ml.100ml ⁻¹ .min ⁻¹ .mmHg ⁻¹ .10 ⁻¹)							

Appendix 23: Physical characteristics at BS, SE, and UE.

ND Ex				Baseline						SE						UE					
Code	Age	Height	Leg vol	Body mass	Height	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR		
		(m)	ml	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)		
1	60	1.59	2308	61.9	1.59	24.5	0.91	0.82	56	62.3	24.6	0.91	1.15	83	61.9	24.5	0.91	0.99	85		
2	33	1.64	2616	59.7	1.64	22.2	0.82	1.14	141	60.5	22.5	0.83	1.15	132	61.5	22.8	0.79	1.08	204		
3	62	1.56	2494	72.9	1.56	30.1	0.98	1.20	37	70.8	28.7	0.99	1.08	56	70.8	28.7	0.99	0.90	79		
4	56	1.55	2389	69.5	1.55	29.1	0.77	1.08	101	66.3	27.6	0.78	1.08	95	68.4	28.5	0.77	1.06	105		
5	53	1.64	2974	83.6	1.64	31.1	0.94	0.92	122	82.2	30.2	0.94	1.14	121	81.4	30.2	0.94	1.18	145		
6	56	1.36	1465	48.3	1.36	26.1	1.00	1.11	45	46.5	25.1	1.00	1.01	65	46.9	25.7	1.00	1.01	52		
7	56	1.62	2624	72.5	1.62	27.6	0.83	1.10	220	71.1	27.1	0.84	1.08	220	71.8	27.4	0.84	1.09	188		
8	53	1.63	2135	61.9	1.63	23.3	0.84	1.06	104	61.4	23.1	0.84	1.19	142	61.7	23.2	0.86	1.09	121		
9	54	1.59	2550	69.4	1.59	27.4	0.88	1.22	129	67.5	26.7	0.87	1.15	118	67.4	26.3	0.86	1.09	141		
10	62	1.60	2511	53.4	1.60	20.8	0.87	1.02	58	54.2	21.4	0.88	1.04	59	53.4	21.1	0.87	1.06	70		
Mean	55.5	1.58	2407	65.3	1.58	26.2	0.89	1.07	101	64.3	25.7	0.89	1.11	109	64.5	25.8	0.88	1.05	119		
SD	9.0	0.08	397	10.4	0.08	3.4	0.07	0.12	56	9.8	2.8	0.07	0.06	50	9.8	2.9	0.08	0.08	50		

ND Con				Baseline						SE						UE					
Code	Age	Height	Leg vol	Body mass	Height	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR		
		(m)	ml	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)		
1	58	1.57	1981	67.8	1.57	27.5	1.04	1.00	130	66.8	27.1	1.06	0.91	140	69.4	28.5	1.06	1.00	120		
2	50	1.58	1611	54.1	1.58	21.7	0.79	1.21	114	53.0	21.2	0.78	1.39	100	52.8	21.2	0.79	1.15	162		
3	47	1.60	2272	53.9	1.60	21.0	0.75	0.87	130	53.5	20.9	0.76	1.12	124	53.8	21.0	0.76	1.03	104		
4	58	1.63	2170	64.0	1.63	24.1	0.83	1.42	164	63.8	23.7	0.83	1.02	185	65.3	24.6	0.84	1.19	189		
5	54	1.65	3018	93.3	1.65	34.3	0.91	1.11	82	92.6	34.0	0.91	1.07	206	91.5	33.6	0.92	0.95	155		
6	64	1.62	2257	56.0	1.62	21.3	0.90	1.12	142	57.0	21.7	0.92	1.27	150	56.3	21.5	0.89	1.01	167		
7	42	1.65	3215	72.9	1.65	26.8	0.90	1.05	118	71.4	26.2	0.93	0.99	113	71.1	26.1	0.93	1.09	118		
8	54	1.58	2287	74.6	1.58	29.9	0.90	1.04	97	75.3	30.1	0.87	1.01	117	74.6	29.9	0.88	1.00	117		
9	42	1.66	3462	90.6	1.66	32.9	0.90	1.08	139	91.0	33.0	0.95	1.03	105	89.4	32.4	0.95	1.00	101		
Mean	52.1	1.62	2475	69.7	1.62	26.6	0.88	1.10	126	69.3	26.4	0.89	1.09	137	69.3	26.5	0.89	1.05	123		
SD	7.5	0.03	615	14.8	0.03	5.0	0.08	0.15	24	14.8	5.0	0.09	0.15	35	14.2	4.9	0.09	0.08	53		

T2DM Ex				Baseline						SE						UE					
Code	Age	Height	Leg vol	Body mass	Height	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR		
		(m)	ml	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)		
1	56	1.53	1897	60.25	1.53	25.7	0.96	0.95	110	58.45	25.0	0.96	0.93	105	58.0	24.78	0.95	1.05	107		
2	56	1.6	2626	71.8	1.6	28.0	0.89	1.12	111	71.4	27.9	0.93	1.17	141	70.3	27.81	0.91	1.21	83		
3	56	1.48	2810	84.9	1.48	38.8	1.00	1.13	180	82.95	37.9	1.02	1.01	194	81.2	37.07	0.98	1.11	240		
4	55	1.57	2835	82.35	1.57	33.4	0.86	1.00	68	80.25	32.6	0.85	1.04	145	82.5	33.90	0.84	1.20	119		
5	60	1.56	2103	69	1.56	28.4	0.99	1.19	97	69.95	28.7	0.97	1.03	155	69.5	28.56	0.97	1.26	132		
6	67	1.63	2574	78.3	1.63	29.5	0.97	1.03	85	78.05	29.0	0.97	1.00	237	78.2	29.07	0.98	0.99	111		
7	52	1.61	3009	84.1	1.61	32.4	1.01	1.05	106	81.1	31.3	1.02	1.05	149	81.3	31.36	1.01	1.12	130		
8	56	1.66	2754	94.3	1.66	34.2	0.95	1.14	29	90.2	32.7	0.95	1.22	65	87.8	31.86	0.96	1.14	91		
Mean	57.3	1.58	2576	78.1	1.58	31.3	0.95	1.08	98	76.5	30.6	0.96	1.06	149	76.1	30.6	0.95	1.13	126		
SD	4.5	0.06	383	10.7	0.06	4.2	0.05	0.08	43	9.7	3.9	0.05	0.09	52	9.6	3.8	0.05	0.09	49		

T2DM Con				Baseline						SE						UE					
Code	Age	Height	Leg vol	Body mass	Height	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR		
		(m)	ml	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)		
1	64	1.58	2364	68.7	1.58	27.5	0.99	1.03	49	67.4	27.0	1.01	1.12	127	69.0	28.0	1.01	1.04	210		
2	59	1.67	3307	98.4	1.67	35.3	0.95	1.13	111	96.1	34.4	0.95	1.04	95	98.0	35.2	0.94	1.01	222		
3	56	1.58	3325	109.5	1.58	43.8	1.01	1.10	80	108.7	43.0	1.01	1.14	64	109.9	43.5	1.00	1.01	43		
4	44	1.59	2298	83.5	1.59	33.0	1.00	1.06	238	83.5	33.9	1.01	1.17	183	81.4	33.0	0.98	1.00	137		
5	57	1.66	2574	81.1	1.66	29.4	0.93	1.03	57	80.8	29.3	0.94	1.00	68	80.6	29.2	0.94	1.02	99		
6	53	1.58	3272	98.8	1.58	39.6	0.93	1.34	125	99.4	39.8	0.92	0.96	103	104.1	41.7	0.92	1.13	119		
7	57	1.54	2216	81.6	1.54	34.4	0.90	1.11	166	79.5	33.5	0.90	0.91	188	79.3	33.4	0.91	0.98	309		
8	58	1.58	2417	72.2	1.58	28.9	0.89	1.06	171	71.7	28.7	0.89	1.10	146	70.5	27.9	0.88	1.04	192		
Mean	56.0	1.60	2722	86.7	1.60	34.0	0.95	1.11	125	85.9	33.7	0.95	1.06	122	86.6	34.0	0.95	1.03	166		
SD	5.8	0.04	491	14.2	0.04	5.6	0.05	0.10	65	14.2	5.5	0.05	0.09	48	15.4	5.9	0.05	0.05	84		

Appendix 24: Haematology at BS, SE, and UE.

Baseline							Supervised Exercise					Unsupervised Exercise						
ND Ex	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	
	(mmol.L ⁻¹)	%	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	
1	3.8	5.3	34.4		5.7	3.4	2.5	0.7	5.1	5.3	3.0	2.4	0.9	4.1	5.3	3.0	2.4	0.8
2	4.4				3.4	1.8	1.9	0.3	4.3	3.7	2.0	1.9	0.4	4.9	3.6	2.0	1.8	0.4
3					4.8	3.2	1.4	1.4	5.3	5.4	3.6	1.8	1.1	4.7	4.8	3.2	1.5	1.3
4	4.0	5.5	36.6		6.1	4.1	2.2	0.5	4.8	5.7	4.0	1.7	0.5	4.7	5.9	4.2	1.8	0.8
5	5.0	5.6	37.7		5.2	3.5	1.7	1.2	5.2	6.5	4.7	1.5	1.3	5.6	4.7	3.1	1.2	2.8
6	4.5	5.1	32.2		5.3	3.2	2.0	1.7	4.3	5.9	3.4	2.4	1.1	4.5	5.0	2.9	2.1	1.0
7	5.4	5.4	35.5		8.3	5.7	2.4	1.5	5.2	6.4	4.5	1.8	0.9					
8	4.9	5.6	37.7		8.5	5.7	2.6	1.2	4.8	6.5	4.4	2.1	0.8	5.1	7.4	5.2	2.3	0.8
9	4.1	5.6	37.7		5.6	3.7	2.1	0.8	4.5	5.2	3.4	1.8	0.8	5.1	5.8	3.7	1.8	0.7
10	5.7	5.4	35.5		5.6	3.9	1.9	0.9	4.0	5.3	3.5	1.9	0.9	5.3	6.0	3.8	1.8	0.9
Mean	4.5	5.4	35.9		5.9	3.8	2.1	1.0	4.8	5.6	3.7	2.0	0.9	4.9	5.4	3.5	1.9	1.1
SD	0.7	0.2	1.9		1.5	0.4	0.4	0.5	0.5	0.8	0.8	0.3	0.3	0.4	1.1	0.9	0.4	0.7

Baseline							Supervised Exercise					Unsupervised Exercise						
ND Con	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	
	(mmol.L ⁻¹)	%	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	
1	4.4	5.9	41.0		4.0	2.9	1.0	1.7		4.8	3.4	1.2	2.1	5.2				
2	4.4								5.5					4.6	7.1	4.2	2.4	1.0
3	4.8	5.4	35.5		5.2	3.2	2.1	0.7	4.8	4.8	2.9	2.1	0.6	5.1	4.7	2.7	2.0	1.0
4	4.6	5.5	36.6		4.5	2.7	1.7	0.8						4.7	4.1	2.2	1.5	0.8
5	3.5				6.4	4.1	2.0	0.8	4.6	6.1	3.8	1.5	1.6	4.9	6.1	3.5	2.0	1.3
6	5.3				6.4	4.2	1.6	1.2	4.6	6.1	4.1	1.7	0.7	4.0	6.4	4.3	1.8	0.8
7	4.5				5.6	2.9	2.3	0.8	5.2	5.2	2.4	2.1	1.5	5.0	5.0	2.6	2.0	1.1
8	5.1	4.7	28.0		4.7	3.3	1.2	1.0						4.5	4.9	3.4	1.3	0.6
Mean	4.6	5.4	35.3		5.3	3.3	1.7	1.0	4.9	5.4	3.3	1.7	1.3	4.8	5.5	3.3	1.9	0.9
SD	0.5	0.5	5.4		0.9	0.6	0.5	0.4	0.4	0.7	0.7	0.4	0.6	0.4	1.1	0.8	0.3	0.2

Baseline							Supervised Exercise					Unsupervised Exercise						
TZDM Ex	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	
	(mmol.L ⁻¹)	%	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	
1	7.2	6.4	46.4		3.4	2.2	1.2	1.2	5.8	3.3	1.9	1.1	1.4	6.6	3.4	2.2	1.1	1.1
2	8.0	6.6	48.6		4.5	2.6	1.7	1.4	7.5	4.1	2.4	1.6	1.4	8.1	3.8	2.3	1.5	0.9
3	7.6	7.9	62.8		4.8	3.5	1.2	1.0	9.4									
4	8.4	6.5	47.5		4.9	3.0	1.5	2.8	7.9	3.5	2.2	1.2	2.1	8.3	3.4	2.0	1.2	1.7
5	7.0	6.8	50.8		4.4	2.6	1.9	1.4	6.7	4.3	2.7	1.7	1.5		3.9	2.2	1.8	1.2
6	6.7	6.7	50.0		5.3	3.2	1.5	1.3	7.4	5.1	2.7	1.6	1.8	8.0	4.7	2.3	1.7	1.4
7	7.2	6.0	42.0		4.1	2.1	1.5	1.3	7.0	4.1	1.9	1.5	1.5	7.9	4.6	2.1	1.8	1.6
8	7.7	6.6	48.6		3.8	2.5	1.4	1.0						6.6	4.9	3.1	1.5	1.7
Mean	7.5	6.7	49.6		4.4	2.7	1.5	1.4	7.4	4.1	2.3	1.5	1.6	7.6	4.1	2.3	1.5	1.4
SD	0.6	0.5	6.0		0.6	0.5	0.2	0.6	1.1	0.6	0.3	0.2	0.3	0.8	0.6	0.4	0.3	0.3

Baseline							Supervised Exercise					Unsupervised Exercise						
TZDM Con	FPG	HbA1c	HbA1c	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	FPG	TCL	LDL	HDL	Triglycerides	
	(mmol.L ⁻¹)	%	(mmol.mol ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	(mmol.L ⁻¹)	
1	8.1	6.2	44.3		4.8	3.4	1.4	1.1	8.9	4.4	2.6	1.2	1.5	10.3	3.4	1.8	1.2	0.8
2	5.1	5.2	33.3		3.4	2.3	0.8	2.4	5.3	3.8	2.6	0.9	2.3	5.7	4.0			1.8
3	9.9	7.3	56.3		4.7	3.8	1.1	1.7										
4	8.4	6.3	45.4						10.2	4.3	3.1	1.1	1.5	7.8	5.0	3.4	1.2	2.7
5	13.1	5.3	34.4		4.6	3.2	1.4	2.3						6.5	3.8	2.2	1.3	1.4
6	5.2	5.5	36.6		4.8	3.3	1.5	1.2	5.3	5.7	4.1	1.5	1.4	5.7	5.7	4.1	1.5	1.1
7	9.0	7.0	53.0		5.1		2.1											
8	8.3	7.5	58.0		4.9	3.3	1.2	1.0	6.8					7.3				
Mean	8.4	6.3	45.2		4.6	3.2	1.2	1.7	7.3	4.6	3.1	1.2	1.7	7.2	4.4	2.9	1.3	1.5
SD	2.6	0.9	9.9		0.6	0.5	0.2	0.6	2.2	0.8	0.7	0.2	0.4	1.7	0.9	1.0	0.2	0.7

Appendix 25: Peak cycling performance at BS, SE, and UE.

ND Ex	Baseline											
No	Pk HR (beats.min ⁻¹)	Pk HR %	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO ₂ pk)	
1	164	103	29.3	1.82	1.11	76.9	13	120	100	21.6	73.7	
2	169	90	30.5	1.81		66.4	19	160	120	20.9	68.5	
3	148	94	18.01	1.27	1.04	41	9	80	60	12.1	67.2	
4	152	93	22.8	1.55	1.08	49.9	13	120	100	14.8	64.9	
5	148	89	22.5	1.9	1.11	31.1	15	120	120	18.8	83.6	
6	174	113	17.7	0.87	1.20	42	9	80	80	13.7	77.4	
7	155	95	22.2	1.63	1.06	68.8	18	140	140	12	54.1	
8	150	90	35.7	2.23	1.08	61.9	19.5	160	140	28.3	79.3	
9	152	92	26.5	1.82	1.19	78.8	16	140	120	19.9	75.1	
10	148	94	22.1	1.22	1.14	60.8	10	100	80	16.7	75.6	
Mean	156	95	24.7	1.61	1.11	57.8	14.2	122.0	106.0	17.9	71.9	
SD	10	7	5.7	0.40	0.06	16.1	4.0	29.0	26.7	5.1	8.5	
ND Con	Pk HR (beats.min ⁻¹)	Pk HR %	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO ₂ pk)	
No	(beats.min ⁻¹)	%	(ml.kg ⁻¹ .min ⁻¹)	(L.min ⁻¹)		(L.min ⁻¹)	(min)	(W)	(W)	(ml.kg ⁻¹ .min ⁻¹)	(% VO ₂ pk)	
1	155	91	33.8	1.92	1.09	64.4	18	140	120	19.8	58.6	
2	170	98	23.8	1.27	1.13	43.9	10	100	80	18.7	78.6	
3	163	101	24.72	1.56	1.01	55.7	11	100	80	19.4	78.5	
4	143	86	21.3	1.84	1.01	55.7	16	140	120	16.8	78.9	
5	166	106	19.1	1.05	1.10	48.3	10	100	60	13.5	70.7	
6	178	100	25.8	1.86	1.11	74.1	16	140	100	19.5	75.6	
7	147	89	23.8	1.42	1.14	48.9	14	120	80	11.7	49.2	
8	162	91	14.3	1.28	1.00	54.5	17	140	100	10.8	75.5	
Mean	161	95	23.3	1.53	1.07	55.7	14.0	122.5	92.5	16.3	70.7	
SD	12	7	5.6	0.32	0.06	9.7	3.3	19.8	21.2	3.7	11.0	
T2DM Ex	Pk HR (beats.min ⁻¹)	Pk HR %	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO ₂ pk)	
No	(beats.min ⁻¹)	%	(ml.kg ⁻¹ .min ⁻¹)	(L.min ⁻¹)		(L.min ⁻¹)	(min)	(W)	(W)	(ml.kg ⁻¹ .min ⁻¹)	(% VO ₂ pk)	
1	139	85	24	1.43	1.01	45.3	10	100	80	18.8	78.3	
2	140	85	31.2	2.23	1.06	65.9	19	160	140	25.6	82.1	
3	165	101	17.5	1.43	1.16	64.5	11.5	100	80	13.6	77.7	
4	165	100	18.8	1.54	1.16	58.7	12	100	80	12.9	68.6	
5	150	94	18.5	1.27	1.1	56.2	7	80	60	12.7	68.6	
6	163	102	19.2	1.5		54.5	12.5	120	80	14.3	74.5	
7	174	104	22.1	1.85		64.7	15	120	100	16.7	75.6	
8	119	73	18.7	1.8	1.15	76.9	12	100	80	11.3	60.4	
Mean	152	93	21.3	1.63	1.11	60.8	12.4	110.0	87.5	15.7	73.2	
SD	18	11	4.6	0.31	0.06	9.4	3.5	23.9	23.8	4.6	6.9	
T2DM Con	Pk HR (beats.min ⁻¹)	Pk HR %	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO ₂ pk)	
No	(beats.min ⁻¹)	%	(ml.kg ⁻¹ .min ⁻¹)	(L.min ⁻¹)		(L.min ⁻¹)	(min)	(W)	(W)	(ml.kg ⁻¹ .min ⁻¹)	(% VO ₂ pk)	
1	152	97	23.1	1.68	1.13	63.3	15	120	100	15.1	65.4	
2	124	77	14.8	1.47	1.13	64.4	12	100	80	11.3	76.4	
3	149	91	14.8	1.61	1.09	51.8	15	120	80	10.3	69.6	
4	160	91	18.1	1.5		42.2	9	80	60	12.4	68.5	
5	161	99	15.6	1.22		53	12	100	80	10.7	68.6	
6	156	93	18.5	1.96	1.01	72.2	13	120	100	13.8	74.6	
7	159	98	15.1	1.34	1.14	53.9	12	100	80	10.2	67.5	
8	162	100	22.5	1.62	1.10	61.6	13	120	100	15.2	67.6	
Mean	153	93	17.8	1.55	1.10	57.8	12.6	107.5	85.0	12.4	69.8	
SD	13	8	3.4	0.23	0.05	9.4	1.9	14.9	14.1	2.1	3.8	

Peak cycling performance

ND Ex	Supervised Exercise											
No	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})	
1	164	103	31.6	1.99	1.11	68.6	17	140	100	24.1	76.3	
2	171	91	27	1.71	1.08	61.5	23.33	180	160	20.5	75.9	
3	153	97	27.7	2.1	1.14	85.6	13	120	100	20.2	72.9	
4	174	106	38.8	2.56	1.09	99.2	18	160	120	25.1	64.7	
5	147	88	26.1	2.2	1.00	64.4	18	140	120	20.7	79.3	
6	176	114	28.3	1.32	1.08	39	12	100	80	19.4	68.6	
7	170	104	33.3	2.38	1.13	79.8	21	160	140	23.6	70.9	
8	157	94	44.4	2.76	1.12	72.2	21.5	180	160	36.6	82.4	
9	152	92	33.3	2.29	1.16	89.9	21.5	180	140	26.2	78.7	
10	153	97	28.2	1.48	1.08	60.2	12	100	80	19.7	69.9	
Mean	162	99	31.9	2.1	1.10	72.0	17.7	146.0	120.0	23.6	72.2	
SD	11	8	5.9	0.5	0.05	17.4	4.2	31.3	29.8	5.2	8.4	
ND Con	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO2 (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO2 at VT (ml.kg ⁻¹ .min ⁻¹)	VO2 at VT (% VO2 pk)	
No												
1	153	90	34.3	1.85		55.6	15.5	140	120	23.1	67.3	
2	169	98	22.3	1.21	1.04	39.8	9.5	100	80	17.4	78.0	
3	162	100	25.1	1.89	1.07	72.9	12	100	80	18.3	72.9	
4	143	86	21.5	1.97		47.3	16	140	120	17	79.1	
5	153	98	21.6	1.12		44.2	10	100	60	13.7	63.4	
6	183	103	27.4	1.94	1.10	91.6	17	140	100	19.8	72.3	
7	153	92	19.1	1.78	1.07	86.8	15	120	100	15.2	79.6	
8	160	90	20.8	1.87	1.07	79.9	18	140	100	12.3	59.1	
Mean	160	95	24.0	1.70	1.07	64.8	14.1	122.5	95.0	17.1	71.5	
SD	12	6	4.9	0.34	0.02	20.5	3.2	19.8	20.7	3.4	7.6	
T2DM Ex	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})	
No												
1	134	82	24.5	1.46	1.01	51.2	12	100	80	18.7	76.3	
2	146	89	34.5	2.44	1.06	72.5	22	180	140	25.6	74.2	
3	159	97	21	1.74		57	15	120	100	16.6	79.0	
4	164	99	21.6	1.72	1.06	64.3	15	120	100	15.3	70.8	
5	150	94	22.1	1.52	1.11	58.5	11	100	80	14.6	66.1	
6	166	104	20.9	1.6	1.05	60	15	120	100	15.1	72.2	
7	171	102	26	2.25	1.10	69.5	18	140	120	16.9	65.0	
8	115	70	20.7	1.94	1.21	71.8	15	120	100	14	67.6	
Mean	151	92	23.9	1.83	1.09	63.1	15.4	125.0	102.5	17.1	71.4	
SD	19	11	4.7	0.35	0.06	7.7	3.4	25.6	19.8	3.7	5.0	
T2DM Con	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})	
No												
1	152	97	22.1	1.5		54	12	120	80	14.6	66.1	
2	129	80	13.2	1.31	1.12	51	10	100	80	9.8	74.2	
3	152	93	14.3	1.55	1.04	48.8	15.5	140	80	7.5	52.4	
4	184	105	20.2	1.67	1.02	58.4	13	120	80	13.5	66.8	
5	160	98	19.9	1.61	1.12	66.4	12	100	80	14.1	70.9	
6	162	97	21.8	2.13	1.13	75.6	14.5	120	100	15.6	71.6	
7	166	102	15.9	1.25	1.06	65	10	100	80	10.4	65.4	
8	159	98	22.7	1.61	1.10	51.9	13	120	80	15.3	67.4	
Mean	158	96	18.8	1.58	1.08	58.9	12.5	115.0	82.5	12.6	66.9	
SD	16	8	3.7	0.27	0.04	9.3	1.9	14.1	7.1	3.0	6.6	

Peak cycling performance

ND Ex		Unsupervised Exercise											
No	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})		
1	164	103	22.7	1.41	1.14	58.5	18	140	120	16.4	72.2		
2	174	93	32.1	1.95	1.07	90.8	19.5	160	120	22.4	69.8		
3	148	94	20.1	1.46	1.06	47.6	12	100	80	12.9	64.2		
4	170	104	29.1	1.91	1.03	65.9	18	140	120	21.2	72.9		
5	150	90	26.2	2.16	1.01	74.4	18	140	120	21.8	83.2		
6	168	109	26.1	1.19	1.10	45.5	9	80	60	15.9	60.9		
7	170	104	27.7	1.96	1.10	76.8	16	140	120	20	72.2		
8	155	93	37.5	2.44	1.06	65	23	180	160	31.9	85.1		
9	155	93	29.4	1.96	1.12	80.1	16	140	100	22.8	77.6		
10	155	98	27.1	1.43	1.11	62	12	100	80	18.1	66.8		
Mean	161	98	27.8	1.79	1.08	66.7	16.2	132.0	108.0	20.3	72.5		
SD	9	6	4.8	0.39	0.04	14.3	4.1	30.1	28.6	5.2	7.8		
ND Con													
No	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})		
1	155	91	34.3	1.85		60.1	16	140	120?	24.4	71.1		
2	169	98	26.9	1.42	1.02	49.2	9.5	100	80	20.2	75.1		
3	162	100	24.9	1.56	1.11	63.3	12	100	80	18.4	73.9		
4	142	85	21	1.77	1.00	47.3	16	140	120	16.9	80.5		
5	153	98	19.6	1.11	1.10	44.1	10.5	100	60	12.1	61.7		
6	178	100	28.5	2.02	1.04	76.4	17	140	100	21	73.7		
7	147	89	22.6	1.69	1.13	78.4	14	120	100	16	70.8		
8	172	97	21.5	1.93	1.04	84	17	140	100	16.8	78.1		
Mean	160	95	24.9	1.67	1.06	62.9	14.0	122.5	91.4	18.2	73.1		
SD	13	6	4.9	0.30	0.05	15.4	3.0	19.8	19.5	3.7	5.6		
T2DM Ex													
No	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})		
1	144	88	27.1	1.62		52.8	10.5	100	80	20.6	76.0		
2	137	84	30.5	2.13	1.11	67.6	20	160	140	22.7	74.4		
3	157	96	17.6	1.39	1.18	55.7	12	100	80	12.4	70.5		
4	162	98	19.2	1.53	1.10	63.4	13	120	80	14.6	76.0		
5	157	98	23.5	1.61	1.11	52.4	12	100	80	14	59.6		
6	159	99	21.2	1.65	1.04	40.2	13	120	80	15.8	74.5		
7	165	98	22.8	1.91	1.10	55	13	120	80	13	57.0		
8	121	74	22.3	1.94	1.07	74.1	15	120	100	16.2	72.6		
Mean	150	92	23.0	1.72	1.10	57.7	13.6	117.5	90.0	16.2	70.1		
SD	15	9	4.2	0.25	0.04	10.5	2.9	19.8	21.4	3.7	7.5		
T2DM Con													
No	Pk HR (beats.min ⁻¹)	Pk HR (%)	VO ₂ (ml.kg ⁻¹ .min ⁻¹)	VO2 (L.min ⁻¹)	RER	VE (L.min ⁻¹)	Time (min)	Pk workload (W)	Load VT (W)	VO ₂ at VT (ml.kg ⁻¹ .min ⁻¹)	VO ₂ at VT (% VO _{2 pk})		
1	150	96	20.3	1.61	1.09	61.5	10	100	60	13.1	64.5		
2	122	76	15.6	1.52	1.06	63.1	10	100	80	9.6	61.5		
3	153	93	13.9	1.52	1.02	62.6	15	120	80	9.9	71.2		
4	174	99	22.4	1.81	1.04	67.4	12	100	80	16.2	72.3		
5	150	92	21.5	1.73	1.14	70.4	11.5	100	80	15.7	73.0		
6	158	95	19	1.97	1.03	61.5	15	120	100	14	73.7		
7	162	100	15.6	1.23	1.04	52.1	9	80	60	11.8	75.6		
8	157	97	21.1	1.48	1.12	54.3	14	120	100	16.1	76.3		
Mean	153	93	18.7	1.61	1.07	61.6	12.1	105.0	80.0	13.3	71.0		
SD	15	8	3.2	0.23	0.04	6.1	2.4	14.1	15.1	2.7	5.3		

Appendix 26: $\dot{V}O_2$ kinetics responses at BS, SE, and UE.

Baseline											
ND Ex	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.53	0.50	8.29	12.91	0.23	38.25	56.00	1.34	37.14	11.60	
2	0.53	0.64	2.08	14.61	0.26	37.99	39.53	1.43	34.26	11.84	
3	0.48	0.31	2.22	14.80	0.17	26.50	29.99	0.96	31.00	12.63	
4	0.51	0.57	0.67	16.40	0.23	35.00	30.58	1.42	35.61	12.98	
5	0.53	0.55	7.55	14.64	0.48	48.98	48.92	1.56	57.43	11.85	
6	0.46	0.26	7.99	12.70	0.31	31.87	30.92	1.03	43.59	10.56	
7	0.52	0.48	7.75	8.39	0.80	36.99	43.00	1.80	56.08	11.86	
8	0.53	0.33	3.50	9.13	0.97	29.68	30.99	1.83	48.48	12.74	
9	0.58	0.29	0.83	6.72	0.60	33.00	41.46	1.46	52.71	9.98	
10	0.44	0.23	1.86	3.66	0.25	24.32	17.91	0.92	24.64	8.89	
MEAN	0.51	0.42	4.3	11.4	0.43	34.3	36.9	1.38	42.1	11.5	
SD	0.04	0.15	3.2	4.2	0.28	7.0	11.0	0.32	11.3	1.3	
ND Con	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.40	0.31	5.05	14.99	0.63	26.12	36.06	1.34	48.28	12.63	
2	0.44	0.10	1.06	5.40	0.36	23.99	40.33	0.90	51.74	10.93	
3	0.46	0.14	7.92	13.28	0.46	27.99	45.53	1.06	61.31	8.52	
4	0.65	0.20	3.80	15.82	0.74	40.52	30.99	1.59	60.47	9.37	
5	0.50	0.08	7.66	15.99	0.28	29.48	40.98	0.86	60.06	11.70	
6	0.68	0.28	3.65	15.82	0.44	35.76	30.00	1.40	47.79	9.47	
7	0.39	0.15	3.47	8.88	0.53	23.39	36.00	1.08	48.95	10.27	
8	0.46	0.22	5.39	9.64	0.44	36.50	43.00	1.12	58.04	12.20	
MEAN	0.50	0.19	4.7	12.5	0.49	30.5	37.9	1.17	54.6	9.40	
SD	0.11	0.08	2.3	4.0	0.15	6.4	5.6	0.25	5.9	1.5	
T2DM Ex	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.37	0.22	10.44	12.50	0.33	33.72	42.99	0.92	55.20	10.18	
2	0.52	0.12	3.63	4.14	1.07	16.47	42.50	1.71	53.81	8.32	
3	0.70	0.26	5.99	14.21	0.30	28.56	54.29	1.26	53.76	10.36	
4	0.55	0.17	9.55	15.00	0.41	30.79	49.85	1.13	64.26	10.36	
5	0.60	0.13	7.99	6.33	0.32	20.07	38.07	1.05	45.48	11.84	
6	0.61	0.21	12.52	17.99	0.43	32.18	50.75	1.25	65.73	11.84	
7	0.60	0.23	5.85	14.99	0.60	33.73	45.57	1.42	62.80	10.14	
8	0.70	0.33	0.90	15.00	0.55	41.74	53.40	1.58	65.12	16.44	
MEAN	0.58	0.21	7.1	12.5	0.50	29.7	47.2	1.29	58.3	11.2	
SD	0.11	0.07	3.8	4.8	0.25	8.1	5.8	0.27	7.3	2.4	
T2DM Con	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.51	0.25	4.78	7.67	0.59	21.38	56.39	1.45	61.74	13.34	
2	0.70	0.12	3.42	6.57	0.27	19.62	49.96	1.09	51.24	7.22	
3	0.74	0.20	0.76	11.91	0.56	23.57	47.00	1.50	55.33	14.07	
4	0.41	0.27	3.96	17.64	0.16	32.03	53.00	0.84	45.20	11.57	
5	0.57	0.16	7.12	16.35	0.27	40.00	55.73	1.00	68.67	8.26	
6	0.63	0.19	2.10	12.48	0.45	23.49	40.68	1.27	49.45	9.14	
7	0.57	0.34	3.53	12.12	0.30	37.18	54.00	1.32	55.03	10.68	
8	0.56	0.26	12.99	12.00	0.57	30.87	47.49	1.39	61.64	11.85	
MEAN	0.59	0.22	4.8	12.1	0.40	28.5	50.5	1.23	56.0	10.8	
SD	0.10	0.07	3.8	3.8	0.17	7.6	5.3	0.23	7.6	2.4	

$\dot{V}O_2$ kinetic responses

SE										
ND Ex	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)
1	0.53	0.27	7.17	15.95	0.49	24.36	40.99	2.17	76.27	23.43
2	0.48	0.55	2.64	18.49	0.52	22.99	24.65	1.55	34.01	9.07
3	0.70	0.41	3.66	13.97	1.07	27.75	70.99	2.17	76.27	21.00
4	0.55	0.46	2.35	14.74	0.61	23.08	46.99	1.62	47.29	12.44
5	0.37	0.34	4.19	17.39	0.53	32.20	38.99	1.24	51.80	10.11
6	0.42	0.30	7.01	8.05	0.28	27.23	33.99	1.00	37.34	10.74
7	0.48	0.64	7.99	11.21	0.49	39.61	46.99	1.61	48.43	11.07
8	0.59	0.44	8.55	14.69	0.92	27.40	31.30	1.95	47.23	11.53
9	0.52	0.48	7.85	16.35	0.65	28.47	29.99	1.65	43.91	11.08
10	0.53	0.27	5.03	13.14	0.37	28.53	33.99	1.17	43.81	11.85
MEAN	0.52	0.42	5.6	14.4	0.59	28.2	39.9	1.61	50.6	13.2
SD	0.09	0.12	2.3	3.1	0.24	4.9	13.1	0.40	14.5	4.9
ND Con	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)
1	0.43	0.34	2.32	17.44	0.71	29.98	32.99	1.48	48.98	12.63
2	0.43	0.12	1.78	16.95	0.35	24.99	42.66	0.90	55.16	12.21
3	0.45	0.12	12.33	6.15	0.45	28.84	49.99	1.02	66.12	8.70
4	0.56	0.21	6.86	10.90	0.72	31.18	37.99	1.49	57.56	8.90
5	0.35	0.11	2.35	12.87	0.25	39.99	42.12	0.71	61.75	10.81
6	0.62	0.23	10.61	14.49	0.52	30.63	43.00	1.37	58.77	9.42
7	0.52	0.24	4.64	20.00	0.56	33.83	50.86	1.31	66.76	10.73
8	0.64	0.26	3.68	13.14	0.46	34.14	36.00	1.35	50.95	11.37
MEAN	0.50	0.20	5.6	14.0	0.50	31.7	42.0	1.20	58.3	10.2
SD	0.10	0.08	4.0	4.3	0.16	4.4	6.3	0.29	6.5	1.5
T2DM Ex	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)
1	0.51	0.12	14.98	3.29	0.52	24.73	32.70	1.15	50.09	11.85
2	0.57	0.51	8.69	14.99	0.66	33.32	42.15	1.74	52.89	11.47
3	0.69	0.30	10.47	14.99	0.40	35.65	57.78	1.39	64.30	9.98
4	0.66	0.34	8.07	13.69	0.46	27.78	51.99	1.46	55.12	11.42
5	0.58	0.19	8.08	13.81	0.40	35.26	40.99	1.17	58.74	10.92
6	0.51	0.39	7.80	18.30	0.43	33.38	40.99	1.33	51.41	11.71
7	0.57	0.21	2.35	8.57	0.80	20.09	39.56	1.58	49.52	11.74
8	0.67	0.20	11.91	13.74	0.55	27.31	40.99	1.42	56.93	10.71
MEAN	0.60	0.28	9.0	12.7	0.53	29.7	43.4	1.40	54.9	11.2
SD	0.07	0.13	3.7	4.6	0.14	5.6	7.8	0.20	5.0	0.6
T2DM Con	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)
1	0.56	0.10	0.28	11.99	0.56	34.07	57.00	1.22	79.13	12.19
2	0.65	0.38	0.47	17.99	0.32	24.39	57.99	1.35	47.68	12.94
3	0.90	0.08	0.40	7.16	0.78	15.99	47.87	1.76	58.62	15.91
4	0.47	0.14	0.33	11.42	0.26	16.96	32.00	0.87	35.94	7.41
5	0.61	0.08	1.05	8.75	0.41	11.40	43.00	1.10	47.12	9.07
6	0.67	0.19	0.78	11.99	0.51	27.90	62.79	1.37	69.54	9.96
7	0.59	0.23	6.25	9.91	0.45	26.60	62.00	1.27	64.10	12.55
8	0.55	0.15	13.29	7.49	0.39	24.99	44.87	1.09	56.23	10.00
MEAN	0.63	0.17	2.9	10.8	0.46	22.8	50.9	1.25	57.3	11.3
SD	0.13	0.10	4.7	3.5	0.16	7.4	10.8	0.26	13.8	2.7

VO₂ kinetic responses

UE											
ND Ex	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.53	0.25	3.84	15.35	0.68	31.99	32.47	1.46	52.29	11.81	
2	0.60	0.31	3.82	14.13	0.91	29.45	39.99	1.82	56.36	11.18	
3	0.51	0.10	6.10	9.69	0.49	25.99	37.36	1.10	55.29	11.92	
4	0.61	0.26	12.83	9.17	0.48	38.99	32.08	1.35	53.83	8.60	
5	0.60	0.21	6.14	9.24	0.75	29.99	41.74	1.56	59.40	11.16	
6	0.60	0.12	1.34	4.83	0.35	20.99	22.71	1.07	34.12	12.37	
7	0.48	0.24	3.70	8.00	0.87	23.99	44.35	1.59	56.09	12.90	
8	0.61	0.41	9.15	6.94	1.36	28.92	43.99	2.38	59.75	11.99	
9	0.45	0.26	6.88	9.43	0.54	25.96	43.99	1.25	52.52	11.42	
10	0.51	0.19	11.25	17.71	0.32	36.03	31.99	1.02	53.47	9.44	
MEAN	0.55	0.24	6.5	10.4	0.68	29.2	37.1	1.46	53.3	11.7	
SD	0.06	0.09	3.8	4.0	0.31	5.4	7.1	0.41	7.2	2.0	
ND Con	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.57	0.34	9.30	19.96	0.62	28.85	33.06	1.53	50.35	11.26	
2	0.54	0.13	6.99	17.71	0.40	31.02	38.00	1.07	58.15	11.16	
3	0.50	0.23	8.18	14.72	0.39	35.99	45.84	1.12	59.97	9.81	
4	0.58	0.17	4.20	15.03	0.78	29.56	33.99	1.53	55.62	9.68	
5	0.39	0.15	2.70	18.07	0.27	39.99	41.93	0.81	60.16	11.05	
6	0.62	0.14	11.00	12.30	0.59	29.53	39.37	1.34	60.36	11.94	
7	0.55	0.19	2.40	17.51	0.39	33.65	41.44	1.13	57.05	11.30	
8	0.64	0.15	3.51	9.99	0.48	22.68	39.99	1.27	51.00	8.21	
MEAN	0.55	0.19	6.0	15.7	0.49	31.4	39.2	1.22	56.6	8.97	
SD	0.08	0.07	3.3	3.3	0.16	5.2	4.2	0.25	4.0	1.0	
T2DM Ex	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.48	0.28	7.52	18.25	0.33	29.99	33.42	1.09	46.13	11.30	
2	0.56	0.29	4.82	13.00	0.79	22.14	31.99	1.64	44.38	11.59	
3	0.71	0.13	4.90	11.05	0.42	27.56	45.99	1.26	59.94	11.18	
4	0.68	0.32	8.66	19.99	0.26	42.66	33.00	1.25	49.72	11.70	
5	0.57	0.22	1.99	12.84	0.54	37.59	60.99	1.33	74.34	11.02	
6	0.58	0.20	4.90	19.13	0.37	35.09	37.32	1.15	55.23	11.59	
7	0.58	0.26	12.08	17.10	0.26	46.05	32.09	1.09	53.66	9.59	
8	0.62	0.27	10.61	12.66	0.36	32.55	30.00	1.25	45.74	9.01	
MEAN	0.60	0.25	6.9	15.5	0.42	34.2	38.1	1.26	53.6	10.7	
SD	0.07	0.06	3.4	3.5	0.18	7.9	10.5	0.18	9.9	1.5	
T2DM Con	a	A1	TD1	r1	A2	TD2	r2	End A	MRT	VO2 gain	
	(L.min ⁻¹)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(s)	(L.min ⁻¹)	(s)	(ml.min ⁻¹ .W ⁻¹)	
1	0.50	0.15	14.39	16.46	0.22	35.23	44.00	0.87	59.62	11.27	
2	0.71	0.08	10.65	13.95	0.53	28.76	47.98	1.32	69.90	11.29	
3	0.80	0.26	4.85	18.61	0.53	31.99	50.03	1.59	62.75	11.62	
4	0.69	0.22	16.31	14.99	0.34	35.13	58.10	1.25	68.90	11.35	
5	0.60	0.10	7.86	9.36	0.45	24.99	58.96	1.15	71.82	11.16	
6	0.63	0.15	6.34	5.72	0.78	21.27	54.99	1.56	65.91	11.19	
7	0.55	0.21	12.64	14.99	0.48	35.02	70.22	1.24	81.62	11.69	
8	0.58	0.24	9.70	13.60	0.43	31.45	50.00	1.25	60.62	9.56	
MEAN	0.63	0.18	10.3	13.5	0.47	30.5	54.3	1.28	67.6	12.0	
SD	0.10	0.07	4.0	4.1	0.16	5.2	8.2	0.23	7.2	2.7	

Appendix 27: Heart rate kinetic responses at BS, SE, and UE.

	Baseline					Supervised exercise					Unsupervised exercise				
	a	A1	TD	tau	End A	a	A1	TD	tau	End A	a	A1	TD	tau	End A
ND Ex	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)
1						92.0	43.3	0.6	53.6	135.3	107.5	28.7	1.6	56.7	136.1
2	83.0	26.7	9.2	53.0	109.7	89.9	44.2	0.1	64.5	133.9	89.31	49.3	0.1	73.3	138.2
3	83.0	28.5	3.2	76.2	111.2	89.3	47.8	0.5	68.1	136.9	83.5	35.1	2.1	64.5	118.4
4															
5	88.9	40.9	1.2	51.0	129.8	81.0	42.4	13.0	55.6	123.3	82.62	42.5	7.9	58.6	125.0
6						108.9	22.4	9.0	66.3	131.2	114.21	19.4	0.1	56.8	133.6
7						94.6	69.8	6.3	70.0	163.9	82.25	57.6	1.8	63.4	139.6
8	78.1	48.2	9.8	55.9	126.2	77.8	52.4	8.1	40.5	130.2	77.62	60.3	3.0	55.3	137.8
9	91.3	39.6	2.9	61.6	130.7	83.0	40.1	3.0	36.7	123.2	74.68	37.1	18.4	54.5	111.7
10	80.5	38.8	8.6	60.0	119.2	80.7	30.9	1.7	47.6	111.5					
MEAN	84	37	5.8	59.6	121	89	44	4.7	55.9	132	89	41	4.4	60.4	130
SD	5	8	3.8	9.1	9	10	13	4.6	12.3	14	14	14	6.2	6.4	10
ND Con	a	A1	TD	tau	End A	a	A1	TD	tau	End A	a	A1	TD	tau	End A
ID	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)
1	84.2	43.5	2.0	49.3	127.6	87.2	40.3	13.4	28.0	127.4	81.3	50.0	9.4	34.2	131.2
2	97.0	39.7	15.7	76.0	136.2	95.4	47.0	9.0	91.0	141.4	115.5	31.4	3.0	86.7	146.3
3	81.0	32.1	2.4	68.1	113.0	82.0	30.0	9.0	60.6	111.9	92.8	32.7	3.5	63.2	125.4
4						86.0	41.5	5.5	32.0	127.5	84.8	36.7	4.0	51.1	121.5
5															
6	97.0	51.1	8.6	68.0	147.7	78.3	66.5	5.1	61.0	144.6	99.3	26.3	5.3	37.0	125.5
7	83.5	27.9	2.6	48.0	111.3	83.9	60.9	6.4	61.0	144.6	94.0	15.0	35.8	81.0	108.7
8	98.0	28.6	0.8	67.0	126.4	107.6	27.4	5.3	61.0	134.9	84.4	64.5	4.3	50.0	148.8
MEAN	90	37	5.3	62.7	127	89	45	7.7	56.4	133	93	37	9.3	57.6	130
SD	8	9	5.8	11.4	14	10	15	3.0	21.0	12	12	16	12.0	20.4	14
TZDM Ex	a	A1	TD	tau	End A	a	A1	TD	tau	End A	a	A1	TD	tau	End A
ID	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)
1						88.5	30.24	0.4	58.3	118.7	83.0	38.2	3.1	42.6	121.2
2	79.7	38.6	2.3	27.7	118.2	90.3	29.9	0.4	35.3	120.1	78.9	34.9	0.5	29.9	113.8
3	119.5	21.5	12.1	85.1	140.6	116.0	10.0	9.0	101.5	125.6	117.0	22.0	5.2	65.0	138.9
4	114.0	23.0	6.0	85.6	136.6	112.7	31.0	0.1	67.1	143.5	120.1	22.5	21.9	47.0	142.6
5	94.4	34.5	0.1	143.7	126.1						110.4	39.0	11.2	85.0	148.7
6	114.8	24.5	7.7	79.8	138.9	103.0	19.2	9.0	112.5	121.3	103.9	33.4	10.4	62.0	137.2
7	105.6	33.1	0.5	69.7	138.5	95.5	47.4	7.0	62.0	142.8	87.9	24.9	6.8	39.0	112.9
8						80.0	19.0	13.7	43.0	98.9	73.3	29.6	0.0	13.1	102.9
MEAN	105	29	4.8	81.9	133	98	27	5.6	68.5	124	97	31	7.4	47.9	127
SD	15	7	4.7	37.2	9	13	12	5.4	28.7	15	18	7	7.2	22.4	17
TZDM Con	a	A1	TD	tau	End A	a	A1	TD	tau	End A	a	A1	TD	tau	End A
ID	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)	(beats.min ⁻¹)	(beats.min ⁻¹)	(s)	(s)	(beats.min ⁻¹)
1	114.0	28.9	0.1	76.3	142.6	126.3	9.8	2.9	82.4	135.9	100.7	9.5	20.0	190.1	108.5
2	83.0	9.1	4.1	51.4	92.1	89.5	20.0	0.1	138.3	108.0					
3	116.8	22.2	3.0	71.2	138.8						96.4	31.4	3.0	84.2	127.4
4	135.9	17.0	3.0	80.3	152.7	126.2	12.1	0.1	57.3	138.3	118.3	26.3	0.4	90.0	144.1
5	108.1	34.0	0.1	98.4	141.2	123.2	19.3	0.1	164.1	140.3	110.9	21.6	0.5	79.3	132.3
6	108.5	29.0	0.5	57.8	137.4	114.4	19.1	0.1	94.4	133.0					
7	134.9	12.9	2.0	68.0	147.8	129.8	16.2	0.1	91.5	145.7	112.6	33.1	7.2	100.0	144.7
8	86.0	41.9	0.1	66.0	127.7	82.3	27.5	8.8	40.6	109.8					
MEAN	111	24	1.6	71.2	135	113	17	1.7	95.5	130	108	24	6.2	108.7	131
SD	20	11	1.6	14.4	19	19	6	3.3	43.2	15	9	9	8.2	46.2	15

Appendix 28: Cardiovascular responses during cycle exercise at 80% VT at BS, SE, and UE.

ND Ex		CO (L.min ⁻¹)									CI (L.min ⁻¹ .m ⁻²)								
		BS			SE			UE			BS			SE			UE		
No		0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1		3.8	7.8	10.1	4.0	6.7	8.9	4.1	7.6	9.2	2.3	4.8	6.2	2.5	4.1	5.2	2.5	4.7	5.6
2		4.7	8.5	9.1	4.5	8.1	10.2	4.9	7.7	9.3	2.8	5.2	6.0	2.7	3.4	4.8	2.9	4.6	5.6
3		3.0	5.9	6.8	3.3	5.7	8.5	3.1	6.4	7.6	1.7	3.4	4.0	1.6	3.4	4.9	1.8	3.7	4.4
4		3.7	8.1	9.1	4.7	5.7	10.2	1.8	7.1	8.0	2.6	4.8	5.5	2.9	3.4	6.2	1.1	4.3	4.9
5		3.6	8.2	10.7	4.8	8.7	10.6	5.2	8.8	10.7	1.3	4.4	5.6	2.5	4.2	5.4	2.8	4.8	5.7
6		2.8	5.9	6.9	2.0	4.0	6.2	3.9	5.4	5.6	1.6	4.5	5.3	1.5	3.1	4.8	3.0	4.2	4.3
7		4.5	7.7	9.9	5.0	8.4	11.1	4.4	8.0	10.5	4.7	4.3	5.5	2.4	4.7	5.8	2.5	4.5	5.9
8					5.2	9.9		4.0	9.8	11.7				3.2	5.9		2.3	5.7	6.9
9		3.6	6.9	11.3	4.5	8.4	11.2	3.9	7.4	10.2	2.4	5.2	7.0	2.2	4.9	6.6	2.0	4.0	5.7
10		4.2	5.5	8.2	2.9	6.5	7.0	4.9	6.6		2.8	3.6	4.5	1.9	4.2	4.6	3.2	4.3	
MEAN		3.8	7.1	9.1	4.1	7.2	9.3	4.0	7.5	9.2	2.5	4.4	5.5	2.3	4.1	5.3	2.4	4.5	5.4
SD		0.6	1.1	1.6	1.0	1.8	1.8	1.0	1.3	1.9	1.0	0.6	0.9	0.5	0.9	0.7	0.6	0.5	0.8

ND Con		CO (L.min ⁻¹)									CI (L.min ⁻¹ .m ⁻²)								
		BS			SE			UE			BS			SE			UE		
No		0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1																			
2		4.5	7.1	8.3	4.9	7.0	8.5	3.8	6.6	7.7	1.0	4.5	5.2	3.1	4.4	5.4	2.1	4.2	4.9
3		4.4	6.5	7.9	3.3	5.0	5.2	3.2	7.0	8.0	2.6	3.9	4.7	1.9	3.0	2.9	2.1	4.1	4.7
4		4.7	9.0	10.0	5.2	8.8	10.4	4.9	9.9	12.1	2.4	4.5	5.0	2.6	4.4	5.2	2.1	5.0	6.1
5		3.8	5.3	7.0	4.8	5.1	6.7	4.6	5.2	6.5	2.4	3.4	4.4	3.0	2.2	4.2	2.9	3.3	4.2
6		5.9	7.9	10.9	5.5	8.5	9.5	5.1	5.3	10.0	3.3	4.4	6.0	3.1	4.8	5.3	2.9	3.0	5.6
7		4.5	6.0	8.3	3.8	6.1	9.7	4.1	7.0	8.9	2.6	3.2	4.7	2.2	3.5	5.5	2.3	4.0	5.0
8		5.7	7.1	8.4	5.7	7.9	8.5	4.2		9.8	2.9	3.6	4.2	2.9	4.0	4.3	2.1		4.9
MEAN		4.8	7.0	8.7	4.7	6.9	8.4	4.3	6.8	9.0	2.5	3.9	4.9	2.7	3.8	4.7	2.4	3.9	5.1
SD		0.8	1.2	1.3	0.9	1.6	1.8	0.7	1.7	1.8	0.7	0.5	0.6	0.5	0.9	1.0	0.4	0.7	0.6

T2DM Ex		CO (L.min ⁻¹)									CI (L.min ⁻¹ .m ⁻²)								
		BS			SE			UE			BS			SE			UE		
No		0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1		2.8	6.2	6.3	3.3	9.1	7.5	2.7	5.6	6.1	1.8	4.0	4.0	2.1	5.8	4.8	1.7	3.6	3.5
2		3.6	8.8	10.6	4.3	9.8	11.7	3.3	9.1	12.1	2.1	5.1	6.1	2.4	5.6	6.7	1.9	5.2	7.0
3		5.2	8.2	10.5	5.6	9.0	13.5	4.8	9.3	10.3	2.9	4.6	5.9	3.2	5.1	7.1	2.8	5.4	5.9
4		3.6	6.6	8.6	3.7	6.5	9.4	5.2		9.1	2.0	3.7	4.7	2.0	3.6	5.2	2.9		5.0
5		3.9	7.2	7.8	5.9	7.0	8.0	4.7	5.7	8.0	2.3	4.3	4.6	3.5	4.1	4.7	2.8	3.3	4.7
6		3.8	6.9	7.8	3.9	7.3	9.6	3.3	6.2	7.5	2.1	3.8	4.6	2.1	4.0	5.2	1.8	3.4	4.1
7		4.0	7.5	10.0	3.9	7.8	6.1	4.2	6.9	8.6	2.1	4.1	5.3	2.1	4.2	3.3	2.2	3.7	4.6
8		5.3	10.2	11.3	5.1	7.6	8.7	6.5	7.9	10.2	2.6	5.1	5.2	2.5	4.5	4.3	3.3	4.0	5.2
MEAN		4.0	7.7	9.1	4.5	8.0	9.3	4.3	7.2	9.0	2.2	4.3	5.0	2.5	4.6	5.2	2.4	4.1	5.0
SD		0.8	1.3	1.8	0.9	1.1	2.4	1.2	1.5	1.9	0.4	0.5	0.7	0.6	0.8	1.2	0.6	0.9	1.1

T2DM Con		CO (L.min ⁻¹)									CI (L.min ⁻¹ .m ⁻²)								
		BS			SE			UE			BS			SE			UE		
No		0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1		3.0	7.1	10.4				3.0	5.3	6.1			3.3	5.2			1.7	3.1	3.6
2		4.7	5.6	7.9	5.0	8.0	9.7	4.5	8.6	9.9	2.3	2.7	3.3	2.4	3.9	4.7	2.2	4.2	4.8
3		4.0	8.5	7.8	4.6	8.9	10.4	4.5	9.0	10.6	2.0	4.1	3.7	2.2	4.5	5.0	2.1	4.3	5.1
4		3.9	6.7	8.5	4.8	8.6	9.3	4.2	8.1	8.0	2.1	3.6	4.6	2.6	4.6	5.6	2.3	4.4	5.4
5		3.6	8.6	5.9	3.8	4.0	8.3	3.6	7.5	6.6	1.9	4.6	5.1	2.0	2.1	4.4	1.9	4.0	4.1
6		5.1	6.8	11.7	5.7	9.0	10.5	5.7	8.6	9.4	2.5	3.4	5.9	3.0	4.5	5.4	1.0	4.2	4.6
7		4.4	7.8	13.1	3.5	7.1	9.0	2.6	5.6	5.3	2.4	4.3	7.3	1.9	4.0	5.1	1.5	3.1	3.0
8		2.1	6.0	8.9	2.0	7.0	8.0	2.6	7.0	9.1	1.2	3.4	5.1		4.1	4.6	1.5	4.1	5.3
MEAN		3.8	7.1	9.3	4.2	7.5	9.3	3.8	7.5	8.1	2.0	3.7	5.0	2.3	4.0	5.0	1.8	3.9	4.5
SD		1.0	1.1	2.3	1.2	1.7	1.0	1.1	1.4	1.9	0.4	0.6	1.3	0.4	0.9	0.4	0.4	0.5	0.9

Cardiovascular responses during cycle exercise at 80% VT

ND Ex	SV (ml)									SVI (ml.m ⁻²)									
	BS			SE			UE			BS			SE			UE			
	No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
	1	50.0	74.0	75.5	50.5	62.5	65.5	50.0	72.0	67.5	30.6	45.2	46.2	30.8	38.1	39.9	30.6	44.0	41.3
	2	61.0	68.0	75.0	60.0	80.5	81.0	68.0	95.0	71.0	37.0	41.3	45.5	36.2	48.6	48.9	40.8	56.9	42.6
	3	38.0	60.0	64.0	39.0	57.5	63.5	37.0	67.5	63.0	22.0	34.7	37.0	22.7	33.5	37.0	21.6	39.3	36.7
	4	57.0	67.0	78.0	59.0	51.0	85.0	24.0	66.0	71.5	33.9	39.8	46.3	35.7	30.8	51.4	14.3	39.4	42.7
	5	61.0	71.5	84.0	60.5	72.0	83.5	68.0	87.5	84.5	32.1	37.6	44.2	31.9	38.0	44.0	36.2	46.6	45.0
	6	31.0	45.5	41.5	20.0	30.0	41.0	47.0	46.5	39.0	23.6	34.6	31.6	15.5	23.2	31.7	36.4	36.0	30.2
	7	60.0	71.0	71.0	57.0	64.5	68.0	62.0	85.0	73.0	33.8	40.0	40.0	32.4	36.7	38.7	35.1	48.1	41.3
	8				78.0	96.0		55.0	90.0	86.0				47.0	57.8		33.1	54.1	51.7
	9	53.0	84.0	92.0	51.0	97.5	94.5	48.0	82.0	91.0	30.9	48.9	53.6	30.0	57.4	55.7	28.2	48.1	53.4
	10	51.0	59.5	69.0	60.0	70.5	63.0	59.0	73.0		33.1	38.6	44.7	38.8	45.6	40.8	38.4	47.5	
	MEAN	51.3	66.7	72.2	53.5	68.2	71.7	51.8	76.5	71.8	30.8	40.1	43.2	32.1	41.0	43.1	31.5	46.0	42.8
	SD	10.5	10.9	14.2	15.4	20.3	16.0	13.8	14.4	15.4	4.9	4.7	6.3	8.6	11.3	7.6	8.2	6.6	7.0

ND Con	SV (ml)									SVI (ml.m ⁻²)									
	BS			SE			UE			BS			SE			UE			
	No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
	1																		
	2	66.0	64.5	63.5	55.0	65.0	65.0	39.5	59.0	55.5	42.6	41.6	41.0	35.6	42.1	42.1	25.5	38.1	35.8
	3	61.0	66.5	73.5	41.5	58.0	48.0	37.0	74.0	70.0	36.1	39.4	43.5	24.5	34.2	28.3	21.7	43.4	41.1
	4	64.0	92.0	80.0	69.0	94.0	87.0	69.0	105.0	103.0	32.0	46.0	40.0	34.6	47.1	43.6	34.8	52.9	51.9
	5	40.0	47.0	59.0	64.0	38.0	46.0	84.0	109.0	118.0	25.2	29.6	37.1	40.0	23.8	28.8	52.7	68.4	74.1
	6	69.0	67.0	72.0	66.0	72.0	63.0	61.5	49.0	69.0	38.3	37.2	40.0	37.0	40.3	35.3	34.5	27.5	38.7
	7	59.0	60.0	73.5	45.0	48.0	74.0	60.0	80.0	80.0	33.5	34.0	41.7	25.4	27.1	41.8	34.0	45.4	45.4
	8	62.0	65.0	61.0	52.0	61.0	61.0	47.0		80.0	31.2	32.8	30.7	26.2	30.7	30.7	23.8	0.0	40.5
	MEAN	60.1	66.0	68.9	56.1	62.3	63.4	56.9	79.3	82.2	34.1	37.2	39.1	31.9	35.0	35.8	32.4	39.4	46.8
	SD	9.5	13.4	7.8	10.7	17.9	14.2	16.9	24.1	21.4	5.6	5.6	4.2	6.4	8.5	6.7	10.5	21.5	13.1

T2DM Ex	SV (ml)									SVI (ml.m ⁻²)									
	BS			SE			UE			BS			SE			UE			
	No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
	1	42.0	68.0	52.5	50.0	99.0	62.0	40.0	58.0	54.5	26.7	43.2	33.4	32.2	63.8	39.9	25.8	37.5	35.2
	2	53.5	84.5	87.5	63.5	104.5	100.5	50.0	93.0	107.0	30.6	48.3	50.0	36.4	59.8	57.5	28.9	53.8	62.0
	3	49.0	67.0	76.0	64.0	75.0	93.0	46.0	76.0	73.0	27.6	37.7	42.8	36.4	42.6	52.9	26.4	43.6	41.9
	4	42.0	58.0	66.0	37.0	56.0	67.0	52.0		66.0	22.9	31.7	36.1	19.4	29.4	35.2	28.5		36.2
	5	49.5	71.5	64.0	83.0	76.0	64.0	48.0	49.0	56.0	29.3	42.3	37.9	48.8	44.7	37.6	28.3	28.9	33.0
	6	38.0	59.0	64.0	39.0	61.0	71.0	35.0	57.0	55.0	20.6	32.0	34.8	21.1	33.0	38.4	18.9	30.8	29.8
	7	41.0	70.0	74.0	40.0	68.5	42.0	50.0	68.0	76.0	21.8	37.2	39.3	21.6	37.0	22.7	27.0	36.7	41.0
	8	54.5	89.0	77.0	73.0	86.5	86.0	101.0	93.0	99.0	27.0	44.1	38.1	36.8	43.7	43.4	51.6	47.5	50.5
	MEAN	46.2	70.9	70.1	56.2	78.3	73.2	52.8	70.6	73.3	25.8	39.6	39.0	31.6	44.3	41.0	29.4	39.8	41.2
	SD	6.2	11.0	10.7	17.2	17.3	19.0	20.3	17.6	20.2	3.6	5.9	5.3	10.2	12.1	10.7	9.5	9.0	10.5

T2DM Con	SV (ml)									SVI (ml.m ⁻²)									
	BS			SE			UE			BS			SE			UE			
	No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
	1		61.0	74.0				36.0	48.0	53.0		35.8	43.5				21.2	28.3	31.2
	2	72.0	75.0	84.0	77.0	94.0	88.5	54.0	87.0	82.0	34.9	36.3	40.7	37.7	46.0	43.3	26.2	42.2	39.8
	3	44.0	73.0	56.0	51.5	81.0	78.0	55.0	82.0	82.0	21.2	35.2	27.0	24.8	39.0	37.5	26.3	39.3	39.3
	4	32.0	46.0	53.0	49.5	64.0	73.5	44.0	64.5	52.0	17.2	24.8	28.5	26.9	34.8	39.9	24.2	35.4	28.5
	5	39.0	77.5	41.5	40.0	34.0	62.0	40.0	67.0	62.0	20.6	40.9	21.9	21.2	18.0	32.8	21.2	35.5	32.8
	6	51.5	61.5	88.0	60.0	87.0	87.0	26.0	90.0	75.0	25.9	31.0	44.3	30.1	43.7	43.7	12.8	44.3	36.9
	7	39.0	63.0	91.0	36.0	64.0	60.5	28.0	50.0	37.0	21.7	35.0	50.6	20.3	36.0	34.0	15.8	28.1	20.8
	8	26.0	67.0	71.0		77.0	74.0	33.0	75.0	85.0	15.0	38.5	40.8		44.4	42.7	19.1	43.4	49.2
	MEAN	43.4	65.5	69.8	52.3	71.6	74.8	39.5	70.4	66.0	22.4	34.7	37.2	26.8	37.4	39.1	20.8	37.1	34.8
	SD	15.0	10.1	18.0	14.8	19.9	10.9	11.0	15.9	17.6	6.5	4.9	10.1	6.5	9.6	4.5	4.8	6.4	8.5

Cardiovascular responses during cycle exercise at 80% VT

ND Ex	HR (beats.min ⁻¹)									A-VO ₂ DIFF (ml.100ml ⁻¹ blood)								
	BS			SE			UE			BS			SE			UE		
	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
No																		
1	75.5	104.5	134.5	78.5	106.5	129.0	82.0	105.5	135.0	6.8	9.6	13.3	7.3	11.0	14.0	7.1	10.6	15.2
2	78.0	109.0	129.5	78.0	101.0	131.0	72.0	101.0	132.0	7.1	10.4	14.6	6.0	9.9	16.2	6.3	11.3	20.7
3	77.5	91.5	116.5	84.5	100.0	130.5	79.5	94.0	123.0	8.2	11.9	15.0	8.3	17.5	25.5	7.1	11.0	14.9
4	75.5	110.5	123.5	80.5	108.0	121.0	78.0	107.0	111.5	7.1	10.6	14.8	6.8	14.2	15.9	17.2	11.6	17.7
5	75.5	102.0	127.0	79.0	111.0	120.5	73.0	102.5	125.5	7.3	10.2	14.3	4.6	7.5	11.7	5.7	10.4	14.8
6	110.5	128.0	164.0	96.5	134.5	152.5	81.5	114.5	140.0	7.5	11.3	13.9	10.7	18.0	20.5	5.5	14.9	19.4
7	74.5	107.5	138.0	85.5	129.0	165.5	71.0	94.0	144.0	7.1	10.1	17.3	4.2	8.6	13.1	6.8	10.0	16.0
8	69.0	97.5	123.0	66.5	103.5	133.0	72.0	109.0	138.0				6.4	10.4		7.4	9.8	20.2
9	87.0	106.0	129.5	75.0	86.0	119.0	70.5	91.5	113.0	7.5	9.6	12.0	6.6	13.6	15.0	6.7	8.7	12.3
10	82.0	92.5	119.5	74.5	92.0	114.0	83.0	93.0	128.0	6.2	11.3	11.1	7.4	11.8	18.0	5.9	9.8	
MEAN	80.5	104.9	130.5	79.9	107.2	131.6	76.3	101.2	129.0	7.2	10.5	14.0	6.8	12.3	16.7	7.6	10.8	16.8
SD	11.6	10.5	13.5	7.9	15.0	16.0	5.0	7.9	11.0	0.5	0.8	1.8	1.8	3.6	4.2	3.4	1.6	2.9

ND Con	HR (beats.min ⁻¹)									A-VO ₂ DIFF (ml.100ml ⁻¹ blood)								
	BS			SE			UE			BS			SE			UE		
	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
No																		
1	72.0	93.5	125.5	71.5	107.5	130.0	74.0	99.0	130.0									
2	84.5	108.5	130.5	89.0	108.5	130.5	93.5	112.0	139.0	5.8	9.9	11.6	5.8	8.7	10.6	6.1	9.8	14.0
3	73.5	90.5	108.5	79.0	86.0	108.5	87.0	94.0	114.0	5.9	10.2	13.5	6.6	12.4	19.1	15.5	10.5	14.7
4	74.0	98.0	125.0	75.0	94.0	87.0	72.0	94.0	118.0	4.8	11.3	15.6	5.2	8.8	14.7	4.9	9.2	13.1
5	95.0	114.0	120.0	74.0	135.0	145.0	84.0	109.0	118.0	6.6	11.6	12.8	5.0	10.4	11.0	5.1	10.2	12.7
6	85.0	118.0	152.0	84.0	118.0	151.0	81.0	108.0	144.0	5.2	10.5	12.5	5.5	10.0	14.4	5.7	13.1	13.8
7	72.0	102.5	114.0	84.0	125.0	131.0	67.0	88.0	111.0	5.2	11.7	12.9	6.3	10.6	13.4	6.9	10.0	12.8
8	92.0	104.0	138.0	110.0	130.0	138.0	89.0	108.0	122.0	3.9	8.8	11.1	5.1	10.3	15.8	6.9		13.2
MEAN	81.0	103.6	126.7	83.3	113.0	127.6	80.9	101.5	124.5	5.3	10.6	12.9	5.7	10.2	14.1	7.3	10.5	13.5
SD	9.4	9.6	13.8	12.3	17.2	20.7	9.2	8.9	12.0	0.9	1.1	1.4	0.6	1.3	2.9	3.7	1.4	0.7

T2DM Ex	HR (beats.min ⁻¹)									A-VO ₂ DIFF (ml.100ml ⁻¹ blood)								
	BS			SE			UE			BS			SE			UE		
	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
No																		
1	72.0	94.5	119.5	66.0	92.0	122.0	72.5	97.5	112.0	7.7	9.7	16.9	7.9	8.6	16.0	8.8	11.1	17.1
2	67.5	104.0	121.5	66.5	93.0	116.5	65.0	97.0	113.0	6.4	12.0	16.2	6.6	10.0	15.4	6.6	9.8	14.3
3	105.5	124.5	140.0	102.0	120.5	146.5	106.0	123.0	140.0	4.4	10.6	12.2	6.7	9.7	10.9	5.4	9.8	11.9
4	91.5	113.0	132.5	100.0	117.0	140.0	90.0	100.0	138.0	7.7	11.7	13.6	5.6	13.3	15.7	4.4		13.2
5	79.0	101.0	123.0	71.0	92.0	125.0	78.0	116.0	144.0	6.4	9.0	15.9	4.1	11.4	16.5	5.6	11.5	16.9
6	100.0	116.0	131.0	100.0	119.0	136.0	95.0	116.0	138.0	6.7	11.3	15.9	5.9	11.7	14.2	6.3	10.7	15.0
7	97.0	111.0	135.0	97.0	114.0	145.5	85.0	101.0	114.0	7.7	10.9	13.0	7.5	11.7	28.0	6.4	10.3	13.7
8	97.0	114.0	142.5	69.5	88.5	101.0	64.0	85.0	103.0	5.4	9.5	14.2	4.6	9.4	16.9	5.1	9.8	13.1
MEAN	88.7	109.8	130.6	84.0	104.5	129.1	81.9	104.4	125.3	6.5	10.6	14.7	6.1	10.7	16.7	6.1	10.4	14.4
SD	14.0	9.5	8.6	17.0	14.2	15.8	14.8	12.7	16.2	1.2	1.1	1.7	1.3	1.5	5.0	1.3	0.7	1.9

T2DM Con	HR (beats.min ⁻¹)									A-VO ₂ DIFF (ml.100ml ⁻¹ blood)								
	BS			SE			UE			BS			SE			UE		
	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
No																		
1	110.5	116.5	140.5	95.0	118.0	136.0	83.0	111.0	114.0	7.0	9.7	12.7				6.7	11.3	14.3
2	66.0	84.0	93.0	63.5	85.0	109.0	83.0	99.0	121.0	6.8	11.4	14.7	4.6	8.4	14.6	6.4	9.5	13.7
3	98.0	115.5	139.0	87.5	110.5	135.0	81.0	109.0	129.0	8.1	10.6	18.0	6.1	11.8	16.8	5.6	10.6	14.9
4	116.5	145.5	157.5	97.0	132.5	141.0	96.0	124.5	152.5	6.1	8.7	10.9	6.3	8.2	11.4	7.1	10.9	15.9
5	90.5	118.0	136.5	93.0	116.5	134.0	89.5	111.5	130.5	6.8	8.8	17.1	6.6	17.6	13.7	6.7	10.1	16.3
6	97.0	110.0	136.0	83.0	104.5	125.5	79.0	96.0	126.0	6.3	11.2	11.4	8.8	9.3	13.0	5.5	12.0	16.8
7	112.0	123.0	144.0	95.5	111.5	148.5	93.0	111.0	144.0	4.7	10.9	11.0	6.7	9.7	15.0	8.4	12.2	22.3
8	83.0	123.0	153.0	72.5	91.0	108.0	81.0	93.0	107.0	10.1	12.6	15.5	12.6	10.4	14.0	9.0	10.5	13.2
MEAN	96.7	116.9	137.4	85.9	108.7	129.6	85.7	106.9	128.0	7.0	10.5	13.9	7.4	10.8	14.1	6.9	10.9	15.9
SD	16.8	17.0	19.6	12.2	15.2	14.6	6.3	10.3	14.9	1.6	1.4	2.8	2.6	3.2	1.7	1.3	0.9	2.9

Cardiovascular responses during cycle exercise at 80% VT

ND Ex	MAP (mmHg)									TPR (mmHg.L ⁻¹ .min ⁻¹)								
	BS			SE			UE			BS			SE			UE		
No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1	92.5	113.3	121.7	91.3	116.7	129.3	96.7	123.3	133.3	24.3	14.6	12.0	23.1	17.5	14.5	23.6	16.3	14.6
2	76.2	86.3	87.0	76.8	80.0	83.7	83.3	90.0	91.7	16.2	10.2	9.6	17.1	9.9	8.2	17.0	11.7	9.9
3	96.7	108.5	111.7	108.7	115.0	126.7	99.3	126.7	140.0	32.2	18.5	16.4	32.9	20.2	14.9	32.0	19.9	18.4
4	96.7	110.0	116.7	98.7	117.0	119.8	78.7	90.0	100.0	26.1	13.7	12.8	21.0	20.5	11.7	43.7	12.8	12.5
5	84.3	111.7	113.3	85.8	108.3	121.3	86.0	101.7	115.0	23.4	13.7	10.6	18.1	12.5	11.4	16.5	11.6	10.9
6	100.7	118.3	124.3	107.0	131.0	133.7	81.7	111.7	116.7	36.0	20.2	18.0	53.5	32.8	21.6	20.9	20.9	20.8
7	87.7	106.3	116.0	86.3	99.0	110.3	86.7	100.0	106.7	19.5	13.9	11.7	17.3	11.9	9.9	19.7	12.5	10.2
8				79.3	103.3	120.0	87.3	106.7	113.3				15.3	10.5		21.8	10.9	9.7
9	106.3	120.0	133.3	76.7	125.7	129.7	90.7	120.0	122.7	29.5	17.4	11.8	17.0	15.0	11.6	23.2	16.2	12.0
10	81.7	83.8	101.3	88.8	95.0	95.0	74.7	97.3	102.7	19.4	15.2	12.4	30.6	14.6	13.6	15.2	14.7	
MEAN	91.4	106.5	113.9	90.0	109.1	117.0	86.5	106.7	114.2	25.2	15.3	12.8	24.6	16.5	13.1	23.4	14.8	13.2
SD	9.7	12.9	13.4	11.6	15.2	16.2	7.6	13.3	14.9	6.5	3.0	2.7	11.8	6.8	3.8	8.6	3.5	4.0

ND Con	MAP (mmHg)									TPR (mmHg.L ⁻¹ .min ⁻¹)								
	BS			SE			UE			BS			SE			UE		
No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1	103.0	122.3	133.3	93.7	117.0	126.7	93.3	113.3	126.7									
2	100.2	115.3	127.0	92.3	112.7	124.3	94.3	114.2	122.3	22.5	16.4	15.3	18.8	16.1	14.6	25.2	17.4	15.9
3	90.3	102.7	113.3	90.2	109.0	116.3	82.0	94.3	103.3	20.5	15.8	14.3	27.7	21.8	22.4	25.6	13.5	12.9
4	95.7	117.3	123.3	84.0	108.0	111.3	81.3	97.3	108.0	20.4	13.0	12.3	16.2	12.3	10.7	16.6	9.8	8.9
5	98.7	116.7	124.7	92.7	116.7	121.3	95.3	112.7	120.0	26.0	22.0	17.8	19.3	22.9	18.1	20.7	21.7	18.5
6	91.3	112.0	111.3	93.0	106.3	112.0	82.3	96.0	100.0	15.5	14.2	10.2	16.9	12.5	11.8	16.1	18.1	10.0
7	101.3	112.7	122.7	96.7	100.0	119.3	100.3	110.0	114.7	22.5	18.8	14.9	25.4	16.4	12.3	24.5	15.7	12.9
8	88.0	93.3	103.3	92.3	94.7	94.0	84.0	90.0	91.3	15.4	13.1	12.3	16.2	12.0	11.1	20.0		9.3
MEAN	96.1	111.5	119.9	91.9	108.0	115.7	89.1	103.5	110.8	20.4	16.2	13.9	20.1	16.3	14.4	21.2	16.0	12.6
SD	5.6	9.3	9.7	3.7	7.8	10.3	7.5	10.0	12.2	3.8	3.3	2.5	4.7	4.5	4.3	4.0	4.1	3.6

T2DM Ex	MAP (mmHg)									TPR (mmHg.L ⁻¹ .min ⁻¹)								
	BS			SE			UE			BS			SE			UE		
No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1	89.7	110.7	115.7	98.0	103.3	110.0	82.0	94.0	102.0	32.0	17.8	18.5	29.7	11.4	14.7	30.9	16.8	16.7
2	90.0	122.3	123.7	86.7	113.3	116.7	99.3	116.7	120.0	25.0	13.9	11.7	20.4	11.6	10.0	30.1	12.8	9.9
3	113.3	131.7	131.7	120.0	130.0	133.3	103.3	130.0	133.3	21.8	16.1	12.5	21.6	14.4	9.9	21.5	14.0	12.9
4	107.3	120.3	124.0	104.3	118.0	136.7	99.3	126.7	130.0	30.2	18.4	14.4	28.2	18.2	14.5	19.1		14.3
5	103.3	120.0	126.7	101.3	113.3	123.3	97.3	109.3	116.7	26.5	16.7	16.2	17.2	16.2	15.4	20.7	19.2	14.6
6	98.7	106.7	110.0	92.7	106.7	112.0	91.3	102.7	109.3	26.0	15.5	14.1	23.8	14.6	11.7	27.7	16.6	14.6
7	105.3	112.7	130.0	113.3	130.0	136.7	107.3	119.3	126.7	26.3	15.0	13.0	29.1	16.7	22.4	25.6	17.3	14.7
8	117.3	121.8	147.7	109.3	116.7	120.0	100.0	108.7	115.3	22.3	11.9	13.1	21.4	15.4	13.8	15.4	13.8	11.3
MEAN	103.1	118.3	126.2	103.2	116.4	123.6	97.5	113.4	119.2	26.3	15.7	14.2	23.9	14.8	14.0	23.9	15.8	13.6
SD	10.0	7.9	11.3	10.9	9.7	10.8	7.8	12.1	10.6	3.5	2.1	2.2	4.6	2.4	4.0	5.6	2.3	2.2

T2DM Con	MAP (mmHg)									TPR (mmHg.L ⁻¹ .min ⁻¹)								
	BS			SE			UE			BS			SE			UE		
No	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s	0s	30s	240s
1	92.0	128.0	131.7	95.3	116.7	120.0	96.7	112.7	115.3	30.7	18.0	12.7				32.2	21.3	18.9
2	99.0	112.0	115.0	98.2	106.7	118.3	96.0	109.3	113.3	21.1	20.0	14.6	19.6	13.3	12.2	21.3	12.7	11.4
3	95.0	128.3	130.3	90.0	126.7	126.7	92.3	98.3	109.0	23.8	15.2	16.7	19.8	14.2	12.2	20.5	10.9	10.3
4	109.7	118.2	128.0	118.0	135.0	136.7	98.0	114.0	116.0	28.1	17.6	15.1	24.6	15.8	14.7	23.3	14.2	14.6
5	99.0	120.0	136.0	87.3	106.7	106.7	95.0	113.3	116.7	27.9	14.0	23.1	23.3	26.7	12.9	26.4	15.2	17.7
6	117.3	139.0	148.7	106.7	126.7	133.3	96.7	126.7	137.0	23.2	20.4	12.7	18.7	14.1	12.7	17.0	14.7	14.6
7	116.7	130.0	145.0	96.3	116.7	133.3	91.0	112.0	115.0	26.5	16.7	11.1	27.9	16.4	14.8	35.0	20.0	21.7
8	85.7	93.3	96.7	90.3	103.3	111.7	80.7	98.0	100.0	40.8	15.6	10.9	45.2	14.9	14.0	31.0	14.0	11.0
MEAN	101.8	121.1	128.9	97.8	117.3	123.3	93.3	110.5	115.3	27.8	17.2	14.6	25.6	16.5	13.3	25.8	15.4	15.0
SD	11.6	14.0	16.7	10.2	11.4	10.9	5.6	9.2	10.4	6.1	2.3	4.0	9.2	4.6	1.1	6.4	3.5	4.1

Appendix 29: Percentage steady state CO, CI, and a-vO₂ diff at BS, SE, and UE

ND Ex									
No	% steady state CO			% steady state CI			% steady state a-vO ₂ diff		
	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	62.7	54.5	68.3	62.8	61.1	69.4	42.3	55.0	43.1
2	86.4	63.2	63.6	73.4	33.3	63.0	43.7	38.8	34.8
3	75.0	46.2	72.2	75.6	53.0	73.1	54.8	53.8	49.6
4	80.6	18.2	84.7	75.9	15.2	85.3	44.8	81.8	
5	64.5	67.5	66.1	71.8	59.6	68.4	41.8	41.0	51.0
6	74.4	47.6	85.3	79.5	48.5	88.5	58.6	74.7	67.4
7	58.3	54.9	59.0	-50.0	69.1	58.8	29.3	49.0	35.1
8			75.3			73.9			18.8
9	42.9	58.2	55.6	61.5	62.1	54.8	46.9	83.8	35.5
10	32.5	87.8		45.7	85.2		104.4	41.7	
MEAN	64.1	55.3	70.0	55.1	54.1	70.6	51.9	57.7	41.9
SD	17.6	18.7	10.4	40.8	20.4	11.2	21.4	17.8	14.5
ND Con									
No	% steady state CO			% steady state CI			% steady state a-vO ₂ diff		
	BS	SE	UE	BS	SE	UE	BS	SE	UE
1									
2	67.5	58.3	70.9	82.1	57.8	74.5	70.0	60.7	46.8
3	60.0	89.7	79.2	61.9	110.5	76.9	56.9	46.5	
4	81.1	69.2	69.4	80.8	69.2	72.5	60.3	37.6	53.1
5	46.9	15.8	31.6	50.0	-66.7	30.8	80.3	91.0	66.9
6	40.0	75.0	4.1	40.7	77.3	5.5	71.7	50.4	91.8
7	40.0	39.0	60.4	26.2	39.4	63.0	85.2	61.6	52.6
8	51.9	78.6		53.8	78.6		68.3	48.1	
MEAN	55.3	60.8	52.6	56.5	52.3	53.9	70.4	56.6	62.2
SD	15.2	25.6	28.9	20.4	56.8	29.2	10.1	17.3	18.1
T2DM Ex									
No	% steady state CO			% steady state CI			% steady state a-vO ₂ diff		
	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	98.6	138.1	85.5	100.0	137.0	105.6	22.4	9.4	28.1
2	74.3	73.8	65.9	75.0	73.3	64.7	56.7	38.7	40.7
3	56.6	43.4	81.8	56.7	49.4	83.9	79.8	71.3	68.0
4	59.4	49.1		63.6	50.0		68.6	76.0	
5	84.6	52.4	30.3	84.8	50.0	26.3	27.9	58.7	52.2
6	77.5	59.6	69.0	68.0	61.3	69.6	50.2	69.5	51.0
7	58.3	177.3	61.4	62.5	179.2	62.5	60.7	20.1	53.8
8	81.8	69.4	37.8	96.1	111.1	36.8	46.3	39.2	58.1
MEAN	73.9	82.9	61.7	75.8	88.9	64.2	51.6	47.9	50.3
SD	14.9	48.4	20.8	16.2	48.6	26.8	19.4	24.9	12.8
T2DM Con									
No	% steady state CO			% steady state CI			% steady state a-vO ₂ diff		
	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	55.4		74.2	62.5		73.7	48.3		60.1
2	28.1	63.8	75.9	42.1	65.2	76.9	58.5	37.9	43.2
3	117.1	74.4	73.8	120.0	82.5	73.3	25.5	53.7	53.9
4	60.9	83.3	102.7	60.0	67.8	67.7	52.5	38.5	42.9
5	214.9	5.5	128.3	84.6	4.2	93.2	19.6	154.3	36.1
6	26.3	68.8	78.4	26.5	62.5	88.9	96.3	11.6	57.4
7	39.1	65.8	111.1	38.8	66.7	106.7	97.2	36.0	27.7
8	57.4	82.5	67.7	56.4	88.0	68.4	46.3		35.2
MEAN	74.9	63.4	89.0	61.4	62.4	81.1	55.5	55.3	44.6
SD	63.4	26.7	22.1	29.5	27.4	13.8	28.6	50.4	11.6

Appendix 30: Peak calf performance at BS, SE, and UE.

BS					SE				UE			
ND Ex	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)
1	488	360	400	82.0	951	600	700	73.6	422	240	250	59.2
2	1015	480	550	54.2	1108	600	700	63.2	1242	600	700	56.4
3	598	480	550	92.0	686	480	550	80.2	970	600	700	72.2
4	703	480	550	78.2	825	480	550	66.7	422	480	550	130.3
5	533	360	400	75.0	781	540	700	89.6	967	600	700	72.4
6	160	240	250	156.3	425	360	400	94.1	472	360	400	84.7
7	655	480	550	84.0	1040	600	700	67.3	870	480	550	63.2
8	651	360	400	61.4	1146	480	550	48.0	848	480	550	64.9
9	361	240	250	69.3	620	360	400	64.5	570	360	400	70.2
10	660	360	400	60.6	710	480	550	77.5	655	196	400	61.1
Mean	582	384	430	81.3	829	498	580	72.5	744	440	520	73.5
SD	225	95	118	28.8	231	90	118	13.6	278	147	155	21.6
BS					SE				UE			
ND Con	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)
1	165	240	250	151.5	474	240	250	52.7	416	240	250	60.1
2	355	240	250	70.4	534	240	250	46.8	372	240	250	67.2
3	530	360	400	75.5	358	240	250	69.8	480	360	400	83.3
4	361	360	400	110.8	649	480	550	84.7	620	360	400	64.5
5	958	480	550	57.4	1079	522	550	51.0	1041	600	700	67.2
6	698	240	250	35.8	706	360	400	56.7	700	210	400	57.1
7	977	480	550	56.3	892	480	550	61.7	965	600	700	72.5
8	517	360	400	77.4	465	360	400	86.0	534	360	400	74.9
9	1090	480	550	50.5	990	480	550	55.6	860	480	550	64.0
Mean	511	320	350	83.6	633	347	375	60.3	586	360	400	68.5
SD	283	98	122	41.4	251	129	147	14.3	271	147	184	8.8
BS					SE				UE			
T2DM Ex	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)
1	569	360	400	70.3	682	480	550	80.6	534	360	400	74.9
2	725	360	400	55.2	770	480	550	71.4	733	480	550	75.0
3	470	240	250	53.2	633	360	400	63.2	770	360	400	51.9
4	844	480	550	65.2	1050	480	550	52.4	794	480	550	69.3
5	609	360	400	65.7	772	480	550	71.2	736	440	550	74.7
6	750	240	250	33.3	880	240	250	28.4	716	360	400	55.9
7	606	360	400	66.0	850	480	550	64.7	737	480	550	74.6
8	368	240	250	67.9	886	480	550	62.1	885	480	550	62.1
Mean	618	330	363	59.6	815	435	494	61.8	738	430	494	67.3
SD	154	85	106	12.2	131	89	112	15.8	98	60	78	9.4
BS					SE				UE			
T2DM Con	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)	MVC (N)	Time (s)	Pk force (N)	Pk/MVC (%)
1	457	360	400	87.5	730	402	550	75.3	702	360	400	57.0
2	885	480	550	62.1	902	480	550	61.0	1233	600	700	56.8
3	794	360	400	50.4	850	480	550	64.7	740	360	400	54.1
4	643	360	400	62.2	455	360	400	87.9	750	360	400	53.3
5	660	480	550	83.3	357	240	250	70.0	704	480	550	78.1
6	776	360	400	51.5	758	480	550	72.6	676	480	550	81.4
7	715	360	400	55.9	837	360	400	47.8	715	360	400	55.9
8	714	360	400	56.0	682	360	400	58.7	835	480	550	65.9
Mean	706	390	438	63.6	696	395	456	67.2	794	435	494	62.8
SD	127	56	69	14.2	194	84	112	12.1	184	89	112	11.2

Appendix 31: LVC kinetic responses at BS, SE, and UE.

Baseline											
ND Ex	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*
1	0.29	1.16	0.52	4.83	0.07	7.28	3.50	0.24	21.57	8.00	1.69
2	0.73	3.15	0.73	5.02	0.87	7.92	9.90	0.83	26.87	8.20	4.70
3	0.46	1.44	1.97	1.29	0.20	16.75	7.36	1.00	19.78	24.07	2.90
4	2.24	0.14	1.37	3.82	0.23	14.35	4.36	0.80	17.30	24.99	3.19
5	1.00	2.60	1.64	8.52	1.55	14.31	44.35	1.64	40.99	35.40	5.24
6	0.61	1.56	1.47	3.78	0.65	15.41	14.80	1.40	23.78	11.99	3.57
7	0.98	1.95	3.04	1.44	0.07	10.01	3.65	1.15	14.33	9.36	4.08
8	0.46	2.01	0.50	5.03	0.19	2.26	5.08	0.65	16.06	7.00	3.12
9	0.55	1.05	1.50	3.00	0.30	21.00	11.00	0.29	40.99	9.32	1.89
10	0.73	4.28	0.95	3.66	1.22	3.08	8.24	3.01	19.38	21.10	8.02
MEAN	0.81	1.93	1.4	4.0	0.53	11.2	11.2	1.10	24.1	15.9	3.84
SD	0.55	1.17	0.7	2.1	0.52	6.1	12.2	0.80	9.6	9.8	1.84
ND Con	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*
1	0.39	0.48	1.50	7.00	0.12	27.00	11.00	0.26	40.99	18.32	1.13
2	0.41	3.30	0.67	4.76	3.10	1.05	8.06	0.52	24.11	13.00	4.22
3	0.23	1.00	1.77	8.50	0.75	8.36	8.52	0.50	19.96	8.05	1.72
4	0.62	1.17	1.68	6.58	0.02	6.37	0.35	0.49	12.77	15.17	2.28
5	0.66	4.53	0.82	2.20	1.00	10.82	1.52	0.87	18.05	6.29	6.06
6	0.75	2.48	1.22	4.26	0.40	3.61	7.98	0.28	6.18	11.93	3.50
7	0.50	6.67	0.00	15.68	1.23	28.88	6.25	1.62	35.30	5.39	8.79
8	0.20	0.45	1.27	1.19	0.19	14.06	4.57	0.38	18.00	8.62	1.03
9	0.77	2.06	1.06	2.02	0.17	12.89	5.82	1.95	28.29	22.88	4.78
MEAN	0.50	2.46	1.1	5.8	0.78	12.6	6.0	0.76	22.6	12.2	3.72
SD	0.21	2.08	0.6	4.5	0.97	9.7	3.4	0.81	10.7	5.8	2.56
T2DM Ex	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*
1	0.50	1.13	0.00	3.21	0.37	14.89	6.18	0.73	19.03	14.16	2.36
2	0.75	3.98	0.28	1.95	0.54	11.57	0.39	0.94	17.61	2.78	5.67
3	0.40	1.20	5.61	4.94	0.94	7.39	9.34	0.50	24.07	15.00	2.09
4	0.49	2.27	1.08	1.52	0.37	6.18	5.87	0.67	17.19	8.99	3.43
5	0.41	1.99	3.59	10.51	0.41	22.15	6.23	0.25	34.30	8.81	2.65
6	0.44	2.20	0.00	2.74	0.99	10.12	5.97	1.39	24.21	7.48	4.03
7	0.30	2.25	0.99	1.99	1.02	7.02	6.02	1.15	24.08	16.19	3.70
8	0.45	0.31	0.93	3.16	0.10	3.31	8.77	0.40	18.81	18.00	1.16
MEAN	0.47	1.92	1.6	3.8	0.59	10.3	6.1	0.75	22.4	11.4	3.14
SD	0.13	1.09	2.0	2.9	0.35	6.0	2.7	0.39	5.7	5.2	1.39
T2DM Con	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*
1	0.50	1.66	1.01	2.00	0.97	5.99	5.95	1.93	12.05	9.19	4.09
2	0.66	2.79	1.07	3.23	0.11	2.33	9.00	0.49	18.95	4.94	3.95
3	0.71	2.68	1.00	2.00	1.01	11.00	6.00	1.82	18.00	17.10	5.21
4	0.51	2.34	1.29	3.07	0.41	23.01	5.97	0.51	29.01	8.19	3.36
5	0.56	3.85	0.88	1.95	0.90	8.02	5.92	1.10	17.08	8.57	5.51
6	0.91	2.69	1.08	2.03	0.57	11.00	5.90	1.84	17.03	12.19	5.44
7	0.41	2.70	1.03	4.02	0.80	0.90	4.93	1.60	17.39	29.55	4.71
8	0.46	3.25	0.99	1.99	1.32	5.00	6.00	0.81	20.04	14.19	4.52
MEAN	0.59	2.75	1.0	2.5	0.76	8.4	6.2	1.26	18.7	13.0	4.60
SD	0.16	0.64	0.1	0.8	0.38	7.0	1.2	0.61	4.8	7.7	0.77
* (ml.min ⁻¹ .mmHg ⁻¹)											

LVC responses

Supervised exercise												
ND Ex	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	1.20	3.02	1E+00	1.59	0.01	24.20	0.41	1.65	9.96	16.44	5.86	
2	0.45	3.87	1E+00	1.60	0.00	30.31	6.00	2.45	6.97	11.96	6.76	
3	0.37	2.29	1E-03	1.31	2.56	7.98	1.84	2.29	9.23	15.62	4.96	
4	0.60	3.35	6E-01	2.00	0.06	2.00	1.81	1.00	10.53	6.85	4.95	
5	0.85	3.45	1E-03	0.90	0.84	5.03	3.01	1.51	11.73	7.16	5.80	
6	0.44	0.74	1E-01	0.91	0.32	5.05	6.00	1.26	16.73	18.93	2.45	
7	0.90	3.95	5E-01	0.98	0.51	4.65	19.17	2.77	18.00	28.49	7.61	
8	0.49	4.00	3E+00	2.01	0.68	3.72	5.51	2.18	23.40	15.50	6.67	
9	0.56	1.24	3E+00	4.17	0.02	34.68	0.04	0.57	28.38	12.15	2.37	
10	0.58	4.57	4E+00	2.34	1.57	8.00	8.90	2.31	17.61	23.85	7.46	
MEAN	0.64	3.05	1.3	1.8	0.65	12.6	5.3	1.80	16.3	15.7	5.49	
SD	0.26	1.25	1.4	1.0	0.83	12.2	5.9	0.71	6.9	6.8	1.86	
ND Con	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.24	3.59	2.55	1.67	2.46	2.87	7.99	1.48	12.20	13.00	5.30	
2	0.29	1.51	0.99	1.69	0.01	4.10	5.95	0.22	12.67	13.00	2.02	
3	0.32	2.91	3.88	3.08	1.99	4.28	7.08	0.41	24.04	2.08	3.65	
4	0.43	4.10	1.87	3.71	0.83	4.50	10.00	0.55	16.94	6.37	5.08	
5	0.79	11.00	3.85	1.87	6.38	6.01	5.98	2.88	17.62	10.25	14.67	
6	0.71	2.88	2.13	2.21	0.71	13.95	13.00	0.49	40.88	5.90	4.08	
7	0.43	3.34	0.00	0.70	0.52	6.31	3.63	2.06	13.56	5.53	5.83	
8	0.32	1.08	0.01	0.68	0.52	6.88	11.38	0.13	22.38	1.91	1.52	
9	0.61	2.77	1.05	2.83	0.05	0.00	7.49	0.62	21.49	19.34	3.99	
MEAN	0.46	3.69	1.8	2.1	1.50	5.4	8.1	0.98	20.2	8.6	5.13	
SD	0.20	2.90	1.5	1.0	2.01	3.8	2.9	0.95	8.9	5.8	3.85	
T2DM Ex	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.28	1.62	0.78	2.10	0.63	3.21	6.87	0.49	17.22	8.00	2.39	
2	0.52	2.33	1.61	5.13	0.96	18.01	3.41	2.04	23.44	5.66	4.89	
3	0.25	2.10	0.50	5.26	0.59	18.34	5.85	1.06	23.49	7.87	3.41	
4	0.48	2.96	1.30	1.00	0.01	0.10	56.00	0.18	12.00	7.40	3.62	
5	0.43	1.84	0.01	2.84	0.10	23.05	2.51	0.46	26.22	4.36	2.73	
6	0.31	3.25	1.58	1.90	1.33	9.67	2.06	1.78	18.18	10.00	5.33	
7	0.35	2.41	1.28	1.78	1.00	7.61	1.52	0.90	24.00	15.90	3.66	
8	0.90	3.39	0.94	1.98	0.71	5.04	2.06	0.62	16.03	9.00	4.90	
MEAN	0.44	2.49	1.0	2.8	0.67	10.6	10.0	0.94	20.1	8.5	3.87	
SD	0.21	0.65	0.6	1.6	0.45	8.2	18.7	0.66	4.9	3.5	1.07	
T2DM Con	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.35	1.56	4.15	1.27	0.30	5.27	2.24	0.37	28.52	10.75	2.28	
2	0.57	1.35	2.29	5.39	0.30	2.00	2.05	0.98	27.07	20.44	2.90	
3	0.66	1.66	2.29	1.40	0.01	2.45	2.15	1.46	19.25	25.00	3.77	
4	0.53	1.59	1.36	1.39	0.16	7.08	2.11	1.02	19.12	25.00	3.13	
5	0.43	2.15	1.74	1.51	0.19	11.83	2.13	1.18	27.31	38.00	3.76	
6	0.37	1.58	0.00	3.43	0.03	59.51	0.72	0.90	31.55	26.35	2.84	
7	0.47	1.78	0.33	0.88	0.50	9.23	2.16	1.01	17.31	12.00	3.24	
8	0.53	5.46	0.80	5.58	2.13	4.85	10.99	0.75	29.55	9.46	6.74	
MEAN	0.49	2.14	1.6	2.6	0.45	12.8	3.1	0.96	25.0	20.9	3.58	
SD	0.11	1.36	1.3	1.9	0.70	19.2	3.2	0.32	5.5	9.8	1.37	
* (ml.min ⁻¹ .mmHg ⁻¹)												

LVC responses

Unsupervised exercise												
ND Ex	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.51	2.45	1.00	1.80	0.10	9.60	9.20	1.14	23.00	12.40		4.10
2	0.41	6.08	1.02	2.42	0.31	14.21	10.00	0.71	24.36	5.69		7.20
3	0.29	2.30	0.99	1.80	0.01	9.16	0.15	1.50	13.23	9.94		4.09
4	0.44	3.47	2.34	2.26	0.63	11.36	3.58	1.15	14.30	12.27		5.06
5	0.70	4.30	0.96	1.79	0.79	7.13	9.98	1.78	19.76	17.00		6.78
6	0.50	1.05	1.12	1.84	0.10	2.77	9.40	0.41	40.00	17.61		1.95
7	0.84	3.39	0.00	1.50	1.63	4.29	13.82	1.96	30.92	20.14		6.19
8	0.45	3.90	1.01	1.80	0.60	9.81	4.98	2.28	17.06	19.00		6.62
9	0.40	0.70	0.92	1.77	0.32	5.21	4.64	0.78	13.58	14.00		1.87
10	0.64	4.29	0.66	1.67	2.70	5.10	5.61	2.23	15.39	14.00		7.16
MEAN	0.52	3.19	1.0	1.9	0.72	7.9	7.1	1.39	21.2	14.2		5.10
SD	0.17	1.62	0.6	0.3	0.84	3.6	4.0	0.66	8.7	4.4		2.04
ND Con	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.36	2.15	3.25	1.74	0.65	8.86	9.43	0.95	30.40	22.00		3.45
2	0.47	1.82	0.02	0.59	1.48	3.32	8.30	0.81	23.05	7.22		3.09
3	0.19	0.60	4.04	0.92	0.23	9.65	24.79	0.44	22.56	11.00		1.23
4	0.70	1.41	3.00	1.60	0.01	1.00	1.00	1.36	16.00	11.00		3.47
5	0.66	4.99	0.59	1.46	2.21	7.06	7.84	2.23	21.30	11.00		7.87
6	0.35	0.81	0.07	2.02	0.11	8.05	5.25	0.49	14.75	14.21		1.65
7	0.70	4.37	0.40	2.19	1.68	9.83	5.52	2.04	21.31	10.21		7.11
8	0.31	1.50	0.95	1.58	0.63	5.01	3.02	0.80	12.08	13.00		2.60
9	0.64	3.20	3.53	1.62	1.26	6.12	6.00	1.12	17.04	13.00		4.95
MEAN	0.49	2.31	1.8	1.5	0.92	6.5	7.9	1.14	19.8	12.5		3.94
SD	0.19	1.55	1.7	0.5	0.77	3.0	6.9	0.63	5.5	4.1		2.29
T2DM Ex	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.19	3.90	1.93	1.57	3.00	7.89	9.92	1.76	21.05	13.30		5.85
2	0.41	1.71	3.07	1.75	0.24	4.73	2.62	0.33	30.00	5.94		2.45
3	0.24	2.24	2.09	2.54	0.26	4.76	1.71	0.31	18.84	10.00		2.79
4	0.56	2.74	0.06	2.14	1.12	10.79	1.94	1.64	23.00	15.66		4.94
5	0.54	1.95	1.24	2.06	0.56	11.18	4.67	1.27	18.41	8.47		3.76
6	0.39	3.38	0.97	1.93	0.77	15.67	4.04	0.68	29.28	6.41		4.45
7	0.70	2.01	0.00	2.10	1.19	5.79	1.75	1.78	17.60	18.99		4.49
8	0.39	1.90	0.99	2.50	0.85	7.01	2.00	0.44	19.01	17.00		2.72
MEAN	0.43	2.48	1.3	2.1	1.00	8.5	3.6	1.02	22.2	12.0		3.93
SD	0.17	0.79	1.0	0.3	0.85	3.8	2.8	0.66	4.9	5.0		1.21
T2DM Con	a	A1	TD1	Tau1	A2	TD2	tau2	A3	TD3	tau3	End A	
	*	*	(s)	*	*	(s)	(s)	*	(s)	(s)	*	*
1	0.29	2.13	0.50	5.48	1.01	5.04	9.73	1.63	15.63	11.99		4.05
2	0.44	3.5	1	2.5	0.85	7	6	2.44	30.8	31.9		8.30
3	0.75	4.85	0.71	5.50	3.90	5.00	11.10	2.70	17.40	15.08		5.67
4	0.54	3.79	0.73	5.54	3.20	4.96	10.95	1.34	17.49	11.99		5.67
5	0.28	1.99	0.10	2.24	0.41	1.56	8.55	0.55	44.88	13.37		2.82
6	0.62	4.39	0.69	5.50	3.90	4.99	9.87	2.34	12.77	11.99		7.35
7	0.44	2.30	0.98	2.49	0.90	8.09	6.07	0.48	34.81	9.00		3.21
8	0.51	1.80	0.78	2.42	0.65	5.05	5.21	1.00	16.41	25.00		3.30
MEAN	0.48	3.09	0.7	4.0	1.85	5.2	8.4	1.56	23.8	16.3		5.05
SD	0.16	1.19	0.3	1.7	1.53	1.9	2.4	0.86	11.6	7.9		2.04
* (ml.min ⁻¹ .mmHg ⁻¹)												

Appendix 32: HR and MAP responses during plantar flexion exercise at 30% MVC at BS, SE, and UE.

Baseline					Supervised exercise																Unsupervised exercise															
ND Ex	Heart rate (beats.min ⁻¹)				MAP (mmHg)				Heart rate (beats.min ⁻¹)				MAP (mmHg)				Heart rate (beats.min ⁻¹)				MAP (mmHg)															
	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s												
1	63.0	66.0	66.3	67.7	82.8	104.0	106.0	105.0	69.7	70.1	74.7	76.0	89.1	92.3	93.3	96.6	54.3	55.3	57.6	60.0	90.0	94.6	103.3	105.6												
2	62.0	63.0	62.3	60.7	71.4	71.0	78.0	76.0	51.1	52.6	52.3	52.2	66.5	72.9	73.0	71.3	45.3	46.3	50.0	51.0	62.0	68.6	67.3	69.6												
3	64.6	65.0	67.0	72.3	90.0	94.0	98.3	103.0	63.8	65.6	65.6	70.6	90.0	103.6	105.0	105.0	63.0	64.6	69.0	66.3	98.0	104.6	105.0	106.0												
4	63.0	64.0	67.0	65.0	92.7	92.3	93.9	93.0	71.4	72.2	73.4	73.5	88.9	91.9	91.3	92.0	59.0	60.6	59.6	63.0	70.0	78.0	87.3	87.6												
5	50.0	56.0	56.0	60.3	82.4	82.0	82.0	81.3	59.2	60.4	61.3	62.6	79.0	83.1	85.3	84.0	55.0	60.6	58.0	63.0	75.0	77.6	78.0	80.0												
6	70.0	72.0	76.3	61.7	95.4	83.0	87.5	88.0	63.6	63.6	66.0	64.4	98.2	101.0	102.0	103.0	57.0	69.5	73.0	76.5	90.0	92.0	95.0	95.0												
7	55.0	55.9	56.7	58.6	79.2	77.0	81.0	81.0	50.7	51.6	57.3	58.0	80.0	82.7	84.9	84.0	55.0	55.6	57.6	57.3	77.0	81.0	80.0	81.6												
8	51.3	52.0	54.0	55.7	84.8	83.6	84.6	83.0	48.6	49.1	51.8	56.8	78.3	79.0	80.3	81.0	46.6	50.0	51.0	53.5	75.0	79.3	80.0	80.6												
9	63.7	63.3	65.1	65.6	95.7	99.4	101.0	103.0	55.2	56.1	57.7	59.1	79.6	83.0	85.0	89.6	56.0	58.0	57.0	57.0	91.8	101.0	99.0	104.0												
10	56.6	57.0	62.7	66.6	75.0	76.9	81.0	81.0	61.2	62.2	64.0	64.8	78.5	78.0	86.0	86.6	60.0	60.0	60.0	60.0	79.0	83.0	84.0	85.0												
Mean	59.9	61.4	63.3	63.4	85.0	86.3	89.3	89.4	59.5	60.4	62.4	63.8	82.8	86.8	88.6	89.3	55.1	58.1	59.3	60.8	80.8	86.0	87.9	89.5												
SD	6.4	6.0	6.6	4.9	8.4	10.7	9.8	10.8	8.0	7.9	7.9	7.7	8.8	10.1	9.6	10.3	5.5	6.7	7.1	7.2	11.3	11.5	12.3	12.6												

ND Con	Heart rate (beats.min ⁻¹)				MAP (mmHg)				Heart rate (beats.min ⁻¹)				MAP (mmHg)				Heart rate (beats.min ⁻¹)				MAP (mmHg)			
	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s
1	68.0	68.0	67.0	69.0	97.0	100.0	99.0	102.0	57.4	58.0	60.7	59.0	81.1	82.0	83.0	83.0	67.0	68.3	68.6	65.3	77.0	87.0	85.9	86.0
2	59.0	60.0	61.0	60.0	85.5	91.1	95.0	92.0	66.8	68.1	67.7	67.2	101.6	106.0	104.0	105.0	63.0	65.0	66.0	67.0	66.0	68.0	76.0	75.0
3	71.0	71.0	72.3	75.0	95.2	100.0	101.3	101.5	70.4	70.9	69.9	70.4	83.3	84.3	85.0	86.0	68.6	69.3	71.0	71.6	95.0	99.0	100.0	101.0
4	61.0	61.0	63.0	65.5	84.4	92.5	93.3	93.6	59.5	64.0	64.7	63.9	78.5	80.4	86.0	85.0	56.0	56.0	58.0	58.0	70.0	72.0	74.0	74.0
5	56.0	57.0	59.0	58.0	94.0	95.8	95.0	95.6	63.0	63.0	63.0	63.0	80.0	84.0	88.0	89.0	61.0	62.0	61.0	61.0	93.0	95.0	99.0	102.0
6	78.5	80.0	80.0	79.0	81.0	88.3	87.0	88.0	74.3	75.0	75.0	75.0	83.6	83.2	88.7	90.5	69.0	69.7	71.7	71.0	101.6	105.0	110.3	112.1
7	76.0	76.0	79.0	79.0	93.0	102.0	111.3	110.1	65.7	67.0	69.7	71.7	84.8	88.0	91.5	93.4	63.3	67.6	74.3	73.0	87.2	90.0	94.2	97.5
8					103.7	109.7	109.7	109.3					99.3	100.0	102.7	102.8					93.0	96.7	102.2	101.7
9	80.0	80.0	80.0	81.5	85.5	86.6	86.9	86.2	73.0	75.0	76.7	76.0	94.8	98.5	98.5	100.3	74.3	75.0	76.3	77.3	97.0	98.8	101.4	100.4
Mean	65.6	66.2	67.1	67.8	89.5	94.6	95.1	95.5	65.2	65.3	65.8	65.1	84.7	86.7	89.1	89.7	63.1	64.1	64.9	64.6	80.2	84.2	87.0	87.6
SD	8.5	8.6	7.9	8.3	6.7	4.8	5.0	5.5	6.5	5.6	4.0	4.9	8.5	9.6	7.6	7.9	5.0	5.4	5.4	5.3	13.2	13.7	12.3	13.5

T2DM Ex	Heart rate (beats.min ⁻¹)				MAP				Heart Rate				MAP				Heart Rate				MAP			
	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s
1	48.0	49.0	48.0	48.0	80.1	81.8	83.9	85.4	44.3	45.0	45.0	48.3	102.0	103.5	105.0	103.0	57.0	58.0	61.0	62.0	81.3	85.0	86.0	88.3
2	58.0	61.0	65.0	65.5	106.6	108.1	108.0	112.0	61.3	62.3	59.6	58.6	104.0	102.0	102.0	103.0	56.0	56.0	56.0	56.0	126.0	140.0	141.0	141.0
3	79.0	80.0	81.0	80.0	109.4	116.0	116.0	116.0	84.0	87.0	87.0	88.0	102.0	106.5	111.0	110.0	71.0	71.6	73.0	73.0	111.3	115.0	116.0	117.0
4	89.0	90.0	90.0	91.0	97.5	98.9	101.4	107.5	73.0	74.0	75.0	75.0	99.2	104.0	108.0	106.0	73.0	74.3	76.7	77.0	103.4	106.4	111.6	115.6
5	67.0	67.0	69.0	67.0	89.9	91.5	91.0	90.0	61.0	61.0	62.0	62.0	85.0	85.0	88.0	88.0	62.0	63.0	63.6	63.3	96.4	98.0	99.3	98.5
6	81.0	80.0	82.0	83.0	90.4	92.2	94.0	95.0					93.0	96.0	100.0	101.0					115.3			
7	73.0	74.0	74.0	75.0	104.0	108.0	107.0	108.0	78.3	79.0	78.3	78.0	111.2	114.3	116.0	123.0	86.0	87.5	89.0	89.0	89.1	89.2	88.6	90.2
8	63.0	63.0	64.7	64.3	105.2	111.2	115.9	112.0	61.0	63.0	63.0	64.0	88.3	89.0	93.3	94.0	62.0	62.0	62.0	62.3	144.1	157.1	154.1	157.8
Mean	69.8	70.5	71.7	71.7	97.9	101.0	102.2	103.2	66.1	67.3	67.1	67.7	98.1	100.0	102.9	103.5	66.7	67.5	68.8	68.9	108.4	113.0	113.8	115.5
SD	13.4	13.1	13.1	13.4	10.2	11.8	11.7	11.5	13.3	13.8	14.0	13.4	8.7	9.6	9.2	10.5	10.7	11.1	11.4	11.4	20.4	26.8	25.8	26.2

T2DM Con	Heart rate (beats.min ⁻¹)				MAP				Heart Rate				MAP				Heart Rate				MAP			
	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s	Rest	30s	180s	360s
1	72.0	74.0	77.0	75.0	93.1	99.6	102.5	101.0	81.3	83.0	85.0	85.0	92.0	94.6	99.0	99.0	78.5	80.0	80.0	80.0	119.4	125.0	131.0	131.0
2	66.3	69.0	68.3	70.5	90.6	93.0	95.0	96.0	62.0	65.0	66.3	65.6	86.0	88.0	90.0	91.6	62.0	64.0	64.0	64.0	91.0	94.0	95.0	96.5
3	72.0	73.0	75.6	76.3	95.1	93.5	94.8	93.5	66.3	67.6	70.0	70.0	100.0	103.0	105.0	106.0	72.0	78.0	78.5	77.0	83.0	85.0	92.0	97.0
4	93.6	97.0	96.9	97.6	84.4	80.3	91.3	90.0	84.0	86.0	87.0	89.0	89.0	91.3	97.3	98.0	75.5	76.5	81.0	81.0	81.0	83.0	88.0	88.0
5	75.0	76.0	76.0	77.6	69.9	76.8	79.6	80.0	71.3	73.0	77.0	77.5	92.0	92.3	92.0	93.0	67.0	67.0	71.0	70.0	94.0	101.0	102.5	102.0
6	68.0	69.0	70.0	71.0	87.8	89.0	89.8	88.0	66.3	70.0	75.5	72.0	100.0	103.0	104.0	105.0	73.0	75.0	76.0	76.0	103.0	103.0	103.0	105.0
7	73.6	74.0	81.0	77.3	104.4	106.0	105.0	107.0	70.3	72.0	76.6	75.0	99.0	107.2	112.0	112.6	72.0	72.0	72.0	74.0	106.0	113.0	114.0	117.0
8	68.0	69.0	70.0	71.0	87.4	92.0	94.0	95.6	76.0	76.0	75.0	77.5	73.0	73.0	74.0	77.5	64.3	67.0	69.0	68.0	101.5	108.5	111.5	109.0
Mean	73.6	75.1	76.9	77.0	89.1	91.3	94.0	93.9	72.2	74.1	76.6	76.5	91.4	94.1	96.7	97.8	70.5	72.4	73.9	73.8	97.4	101.6	104.6	105.5
SD	8.6	9.2	9.2	8.8	9.9	9.5	7.8	8.2	7.7	7.3	6.9	7.7	9.1	10.8	11.6	10.8	5.6	5.9	5.9	6.0	12.7	14.2	14.0	13.5

Appendix 33: Arm reactive hyperaemic responses at BS, SE, and UE.

Baseline	resting vc	a	A	c	a+A	AUC	Peak VC
ND Ex	*	*	*		*		*
1	9.4	4.4	11.8	0.01	16.2	2181	13.3
2	0.8	1.5	20.2	0.03	21.6	1138	21.4
3	1.9	4.0	20.5	0.07	24.4	1366	24.8
4	2.0	3.0	34.8	0.08	37.8	1500	38.4
5	2.2	2.1	21.0	0.19	23.1	878	23.1
6	2.2	2.2	24.1	0.02	26.3	1965	26.4
7	2.7	1.3	43.6	0.03	44.9	1556	44.3
8	1.3	2.0	25.4	0.04	27.4	1263	32.6
9	2.0	2.0	23.8	0.08	25.8	961	25.6
MEAN	2.7	2.5	25.0	0.06	27.5	1423	27.8
SD	2.6	1.1	9.2	0.05	8.7	435	9.3
	resting vc	a	A	c	a+A	AUC	Peak VC
ND Con	*	*	*		*		*
1	1.9	0.9	8.5	0.03	9.4	431	9.3
2	1.8	1.3	13.9	0.03	15.2	876	15.5
3	0.9	1.1	13.4	0.08	14.5	543	14.8
4	1.3	1.0	11.1	0.05	12.2	508	12.3
5	2.2	1.9	13.5	0.04	15.4	925	15.4
6	1.2	1.3	24.7	0.06	26.0	726	25.6
7							
8	1.4	1.2	16.2	0.07	17.4	603	18.4
MEAN	1.5	1.2	14.5	0.05	15.7	659	15.9
SD	0.5	0.3	5.1	0.02	5.2	189	5.1
	resting vc	a	A	c	a+A	AUC	Peak VC
T2DM Ex	*	*	*		*		*
1	1.2	0.5	15.5	0.09	16.0	326	15.7
2	1.7	2.5	24.0	0.07	26.5	1156	26.3
3	6.1	4.6	20.4	0.06	25.0	1923	25.6
4	1.6	2.7	14.8	0.03	17.5	1240	17.4
5	2.1	1.1	17.4	0.06	18.5	663	18.5
6	2.7	1.3	15.8	0.03	17.1	881	17.0
7	1.9	1.5	15.8	0.05	17.2	760	16.8
MEAN	2.5	2.0	17.7	0.06	19.7	993	19.6
SD	1.7	1.4	3.3	0.02	4.2	512	4.4
	resting vc	a	A	c	a+A	AUC	Peak VC
ND Con	*	*	*		*		*
1	1.6	2.0	10.0	0.07	12.0	810	12.2
2	1.7	3.0	20.3	0.02	23.4	1643	23.2
3	1.7	3.9	12.0	0.10	15.9	1379	15.8
4	1.8	2.3	11.9	0.02	14.2	1113	14.2
5	1.2	1.0	20.6	0.05	21.6	694	21.3
6	2.1	2.1	11.1	0.01	13.3	1255	13.3
7	0.7	1.4	21.0	0.03	22.4	1047	22.9
MEAN	1.5	2.2	15.3	0.04	17.5	1134	17.6
SD	0.5	1.0	5.1	0.03	4.8	327	4.8

Arm reactive hyperaemic responses

SE	resting vc	a	A	c	a+A	AUC	Peak VC
ND Ex	*	*	*		*		*
1	8.1	7.3	26.7	0.02	34.1	3296	33.0
2	2.1	1.4	19.0	0.06	20.4	734	20.3
3	1.9	2.0	19.5	0.08	21.5	887	21.1
4	2.6	2.6	21.7	0.04	24.3	1408	23.3
5	3.0	2.6	24.1	0.03	26.7	1353	26.5
6	1.2	0.6	8.3	0.16	8.8	236	8.9
7	3.2	3.4	28.6	0.03	32.0	1775	31.3
8	5.6	2.1	19.2	0.06	21.3	1025	17.3
9	1.6	2.4	16.0	0.04	18.4	1200	18.6
MEAN	3.3	2.7	20.4	0.06	23.1	1324	22.2
SD	2.2	1.9	6.1	0.04	7.5	861	7.4
	resting vc	a	A	c	a+A	AUC	Peak VC
ND Con	*	*	*		*		*
1	1.4	2.1	18.0	0.03	20.1	1098	19.8
2	1.1	1.8	19.0	0.06	20.8	917	20.7
3	2.4	1.9	12.3	0.04	14.1	893	14.0
4	1.5	1.0	13.9	0.12	14.8	448	14.6
5	1.5	1.8	23.8	0.07	25.6	975	25.5
6	2.4	2.4	17.4	0.05	19.8	1165	19.4
7							
8	1.4	1.1	15.9	0.07	17.0	591	16.8
MEAN	1.7	1.7	17.2	0.06	18.9	869	18.7
SD	0.5	0.5	3.8	0.03	4.0	261	4.0
	resting vc	a	A	c	a+A	AUC	Peak VC
T2DM Ex	*	*	*		*		*
1	6.0	1.4	19.0	0.09	20.4	669	20.4
2	1.7	0.9	26.0	0.06	26.9	722	26.9
3	1.5	2.9	17.6	0.04	20.4	1349	19.3
4							
5	0.6	1.0	16.9	0.11	17.9	330	17.9
6	3.1	3.9	25.0	0.03	28.9	1992	29.4
7	3.0	1.2	16.0	0.06	17.2	635	17.3
MEAN	2.7	1.9	20.1	0.07	21.9	950	21.8
SD	1.9	1.2	4.3	0.03	4.8	610	5.1
	resting vc	a	A	c	a+A	AUC	Peak VC
ND Con	*	*	*		*		*
1	1.3	1.9	13.0	0.03	14.9	930	11.7
2	2.1	1.6	13.6	0.02	15.2	988	14.8
3	2.6	0.1	25.6	0.13	25.7	304	25.8
4	2.8	2.1	23.1	0.02	25.2	1452	25.1
5	0.4	0.3	19.3	0.12	19.6	277	19.7
6	2.3	3.1	17.1	0.03	20.2	1480	19.7
7	4.5	4.9	27.9	0.02	32.7	2509	32.6
MEAN	2.3	2.0	19.9	0.05	21.9	1134	21.3
SD	1.3	1.6	5.8	0.05	6.4	774	7.1

Arm reactive hyperaemic responses

UE		resting vc	a	A	c	a+A	AUC	Peak VC
ND Ex		*	*	*		*		*
	1	1.4	1.1	26.7	0.08	27.8	694	28.3
	2	2.7	2.1	20.2	0.04	22.2	1104	21.6
	3	0.4	0.3	18.0	0.11	18.3	274	18.4
	4	1.5	0.6	25.2	0.06	25.8	589	25.4
	5	1.4	1.0	21.3	0.06	22.2	553	22.2
	6	0.9	0.7	20.9	0.08	21.6	475	21.3
	7	1.8	1.8	32.8	0.04	34.6	1408	34.2
	8	1.6	0.9	24.9	0.08	25.8	559	25.8
	9	0.8	2.3	24.8	0.06	27.2	1230	27.4
MEAN		1.4	1.2	23.9	0.07	25.1	765	24.9
SD		0.7	0.7	4.4	0.02	4.7	386	4.7
		resting vc	a	A	c	a+A	AUC	Peak VC
ND Con		*	*	*		*		*
	1	2.0	0.8	13.3	0.03	14.1	679	13.6
	2	0.7	0.8	17.3		18.1	448	18.4
	3	2.1	1.6	14.9	0.05	16.4	801	16.6
	4	2.0	1.2	12.2	0.07	13.4	585	13.1
	5	0.6	0.7	16.4	0.06	17.1	505	16.8
	6	2.3	1.3	24.0	0.06	25.3	858	24.9
	7							
	8	1.5	0.9	15.9	0.08	16.8	507	16.7
MEAN		1.6	1.1	16.3	0.06	17.3	626	17.2
SD		0.7	0.3	3.8	0.02	3.9	158	3.9
		resting vc	a	A	c	a+A	AUC	Peak VC
T2DM Ex		*	*	*		*		*
	1	3.3	5.4	17.6	0.30	23.0	1874	23.0
	2	3.5	3.5	18.3	0.04	21.8	1644	21.5
	3	0.8	1.4	24.7	0.07	26.2	832	25.9
	4	1.5	1.5	11.2	0.07	12.7	649	12.5
	5	1.1	1.0	12.6	0.09	13.6	465	13.5
	6	2.1	0.9	13.3	0.06	14.2	523	14.0
	7	5.0	5.9	17.9	0.06	23.8	2298	23.6
MEAN		2.5	2.8	16.5	0.10	19.3	1184	19.2
SD		1.5	2.1	4.6	0.09	5.6	741	5.6
		resting vc	a	A	c	a+A	AUC	Peak VC
ND Con		*	*	*		*		*
	1	1.8	3.4	11.1	0.08	14.6	1293	14.1
	2	1.3	1.8	17.6	0.01	19.4	1544	22.9
	3	3.6	3.5	29.4	0.18	32.9	1387	33.0
	4	1.5	0.3	26.1	0.06	26.4	472	25.8
	5	1.1	0.3	15.6	0.04	15.9	510	15.5
	6	1.1	1.0	11.7	0.02	12.6	744	13.5
	7	1.9	1.3	20.9	0.06	22.2	778	21.9
MEAN		1.8	1.7	18.9	0.06	20.6	961	20.9
SD		0.9	1.4	7.0	0.06	7.2	439	7.1

Appendix 34: LBF and LVC at rest and during incremental plantar flexion exercise at BS, SE, and UE.

Baseline													Slope LVC		Slope LBF		Slope LVC		Slope LBF	
ND Ex	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg (ml.min ⁻¹)	(ml.min ⁻¹)				
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100	250	400	550	100	250	400	550	vs force (N)	vs force (N)	vs % max	vs % max				
1	31.3	0.28		501.7	4.8	73.6	208.3	354.4		0.9	2.3	4.2		0.011	0.9	4.5	374			
2	43	0.59		746.4	9.9	206.8	355.1	448.2	676.8	2.8	4.7	6.2	8.1	0.001	1.0	6.3	551			
3	60.7	0.59		1252.3	11.9	205.6	485.4	694.2	1109.3	2.0	4.9	6.1	9.6	0.016	1.9	8.9	1071			
4	27.1	0.31		551.4	6.0	222.2	285.3	316.9	519.5	2.5	3.0	3.3	5.4	0.006	0.6	3.3	339			
5	70	0.64		1069	8.1	205.6	444.2	750.3		1.6	3.6	5.7		0.012	1.7	5.5	727			
6	48.7	0.52		346.5	3.3	283.7	295.0			2.5	3.0			0.003	0.2	0.9	19			
7	112.7	1.34		1040.2	12.1	154.0	484.4	765.9	918.3	2.0	6.5	10.8	11.2	0.021	1.7	11.7	944			
8	42.9	0.49		846.8	9.1	172.4	460.4	771.7		1.8	4.8	8.2		0.021	2.0	8.6	799			
9	53.7	0.51		332.9	4.5	163.7	290.1			1.6	3.5			0.031	1.4	3.3	211			
10	51.8	0.68		990.8	12.9	224.1	381.4	774.9		2.8	5.0	10.2		0.024	1.8	9.8	734			
Mean	54.2	0.60		767.8	8.2	191.2	369.0	609.6	806.0	2.0	4.1	6.8	8.6	0.01	1.3	6.3	577			
SD	24.2	0.29		323.2	3.5	55.4	97.6	200.7	260.4	0.6	1.3	2.7	2.5	0.01	0.6	3.4	336			

													Slope LVC		Slope LBF		Slope LVC		Slope LBF	
ND Con	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg (ml.min ⁻¹)	(ml.min ⁻¹)				
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100.0	250.0	400.0	550.0	100.0	250.0	400.0	550.0	vs force (N)	vs force (N)	vs % max	vs % max				
1	39.9	0.43		169.1	1.7	84.6	155.6			0.9	1.5			0.004	0.5	1.0	118			
2	27.6	0.34		509.3	5.4	133.4	446.2			2.4	5.1			0.017	5.9	4.5	521			
3	23.7	0.22		246	2.5	43.8	122.1	175.2		0.4	1.3	1.7		0.004	0.4	1.8	175			
4	56.4	0.6		478.3	5.2	138.5	233.4	405.7		1.4	2.5	4.3		0.009	0.9	3.9	356			
5	79.8	0.81		988.2	8.4	99.5	366.5	734.2	776.3	0.6	1.9	3.6	3.8	0.007	1.6	4.1	879			
6	49.5	0.47		298.7	2.9	101.5	203.5			0.7	1.3			0.004	0.7	1.2	170			
7	68.9	0.67		1466.6	11.7	342.7	636.2	914.9	1117.7	3.1	5.6	8.1	9.9	0.015	1.7	8.5	955			
8	44.9	0.37		322.5	2.6	87.0	186.0	303.5		0.6	1.0	1.7		0.003	0.7	1.5	289			
9	79.5	0.81		1055.6	10.3	23.6	301.0	527.8	739.8	0.2	2.9	5.3	7.6	0.016	1.6	8.9	871			
Mean	52.2	0.52		614.9	5.6	117.2	294.5	510.2	877.9	1.1	2.6	4.1	7.1	0.01	1.6	3.9	482			
SD	20.7	0.21		448.5	3.7	92.4	165.0	275.7	208.4	1.0	1.7	2.4	3.1	0.01	1.7	3.0	337			

													Slope LVC		Slope LBF		Slope LVC		Slope LBF	
T2DM Ex	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg (ml.min ⁻¹)	(ml.min ⁻¹)				
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100.0	250.0	400.0	550.0	100.0	250.0	400.0	550.0	vs force (N)	vs force (N)	vs % max	vs % max				
1	32.3	0.38		524.3	6.4	148.0	257.4	488.4		1.5	2.9	4.7		0.01	1.1	4.2	454			
2	45.3	0.43		488.9	5.4	244.2	360.2	382.0		2.3	3.8	4.6		0.007	0.5	3.1	184			
3	25.4	0.21		446.5	3.6	232.4	371.9			2.2	3.0			0.005	0.9	1.4	233			
4	49.2	0.46		744.5	6.9	64.3	311.2	496.2	642.7	0.6	2.1	4.3	5.4	0.011	1.3	6.1	704			
5	34.9	0.39		539.6	5.7	123.4	255.0	417.5		1.3	3.0	5.0		0.012	1.0	4.9	392			
6	37.3	0.32		373.4	3.1	123.1	218.5			1.1	2.2			0.007	0.6	2.0	159			
7	41.7	0.37		332.5	2.6	106.5	213.0	276.6		1.0	1.8	2.2		0.004	0.7	1.7	227			
8	62.2	0.56		416.5	3.8	143.1	311.4			1.2	3.1			0.012	1.1	3.0	281			
Mean	41.0	0.39		483.3	4.7	148.1	287.3	412.1	642.7	1.4	2.7	4.2	5.4	0.01	0.9	3.3	329			
SD	11.4	0.10		127.5	1.6	61.4	60.7	89.7	#DIV/0!	0.6	0.6	1.1		0.003	0.3	1.7	182			

													Slope LVC		Slope LBF		Slope LVC		Slope LBF	
T2DM Con	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg (ml.min ⁻¹)	(ml.min ⁻¹)				
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100.0	250.0	400.0	550.0	100.0	250.0	400.0	550.0	vs force (N)	vs force (N)	vs % max	vs % max				
1	46.1	0.44		433.9	4.9	224.3	311.2	400.0		2.4	3.4	4.3		0.006	0.6	2.6	234			
2	55.5	0.62		864	12.0	182.2	225.2	407.0	758.0	1.9	2.1	3.5	8.5	0.014	1.3	7.7	700			
3	89.4	0.92		699.7	6.6	185.1	289.8	603.8		2.1	3.2	6.2		0.013	1.4	5.4	558			
4	30.9	0.29		455	5.6	104.9	334.9	401.8		1.0	4.7	5.6		0.015	1.0	6.1	396			
5	31.5	0.41		571.5	6.8	134.9	329.6	447.5	491.0	1.9	4.0	5.0	5.7	0.008	0.8	4.5	435			
6	69.7	0.72		540.8	5.3	354.5	400.0	481.9		3.2	3.7	4.2		0.003	0.4	1.3	170			
7	36.7	0.33		509.8	4.8	208.1	391.8	455.4		1.9	3.3	3.8		0.006	0.8	2.6	330			
8	44.6	0.45		444.9	3.8	121.5	200.7	360.2		1.3	1.8	3.0		0.005	0.8	2.3	318			
Mean	50.6	0.52		565.0	6.2	189.4	310.4	444.7	624.5	2.0	3.3	4.5	7.1	0.01	0.9	4.1	393			
SD	20.3	0.21		148.8	2.5	78.9	71.1	74.9	188.8	0.7	0.9	1.1	2.0	0.01	0.3	2.2	172			

LBF and LVC at rest and during incremental plantar flexion exercise

Supervised exercise										Slope LVC		Slope LBF		Slope LVC		Slope LBF	
ND Ex	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100	250	400	550	100	250	400	550	vs force (N)	vs force (N)	vs % max	vs % max	
1	102.7	1.09	1070.2	11.0	242.2	513.2	681.4		2.3	4.9	7.1		0.013	1.2	9.1	859	
2	18.6	0.32	721.6	8.9	258.6	400	504.1	553.3	4.0	6.2	7.7	7.4	0.006	0.6	4.2	450	
3	56.6	0.56	482.2	4.8	143.1	225.9	403.5	432.5	1.4	2.1	3.6	4.2	0.006	0.7	3.6	383	
4	52	0.58	1151.8	18.5	256.7	511.5	878.9	1014.5	2.2	5.0	9.4	16.1	0.03	1.8	16.8	968	
5	113.4	1.4	998.1	10.2	187.7	466.4	845.5		2.1	5.1	9.0		0.012	1.3	8.5	937	
6	46.1	0.6	380.5	3.6	166	275			2.5	2.6			0.003	0.6	1.2	231	
7	63.8	0.74	1066	10.1	128.3	327.6	596	759.9	1.7	3.3	6.3	8.9	0.016	1.4	10.2	950	
8	52.1	0.76	830.7	12.1	115.7	461.7	589.8		2.0	6.2	8.4		0.019	1.3	10.6	734	
9	38.7	0.38	522.8	5.2	92.5	261.3			1.0	2.6			0.007	1.0	4.2	517	
10	48.5	0.61	1369.2	12.4	303.9	458.3	986		3.6	4.9	9.2	10.5	0.016	1.9	9.2	1067	
Mean	59.3	0.70	859.3	9.7	189.5	390.1	685.7	690.1	2.3	4.3	7.6	9.4	0.01	1.2	7.8	710	
SD	28.5	0.32	326.0	4.4	71.9	108.7	201.0	255.1	0.9	1.5	1.9	4.4	0.01	0.5	4.5	292	

ND Con	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				Slope LVC		Slope LBF		Slope LVC		Slope LBF	
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100	250	400	550	100.0	250.0	400.0	550.0	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)
													vs force (N)	vs force (N)	vs % max	vs % max				
1	15.0	0.14	305.5	3.8	225.4	243			1.6	3.5			0.013	0.1	3.3	29				
2	31.0	0.32	447.9	4.5	128.4	386.1			1.2	3.3			0.011	1.1	3.4	430				
3	23.7	0.28	159.9	1.8	68.7	145.7			0.8	1.6			0.005	0.5	1.3	128				
4	23.5	0.25	864	9.9	44.3	141	488.6		0.7	2.0	5.7		0.013	1.2	7.4	661				
5	76.3	0.76	1139.7	10.3	254	635.9	870.9	897.1	2.5	6.5	8.7	8.8	0.014	1.4	7.7	794				
6	55.9	0.66	406.9	4.3	174.3	266.3			1.7	2.7			0.007	0.7	3.0	265				
7	35.0	0.39	1095.4	10.5	227.9	476.7	537.3	821.1	2.3	4.7	5.1	8.0	0.011	1.2	6.5	675				
8	46.2	0.44	417	3.7	107.5	210.6	329		1.0	1.9	3.0		0.006	0.8	2.7	295				
9	55.1	0.51	815.7	7.1	70.5	177.8	570.5	695.5	0.6	1.8	5.0	5.7	0.012	1.5	6.7	831				
Mean	40.2	0.42	628.00	6.2	144.6	298.1	559.3	804.6	1.4	3.1	5.5	7.5	0.010	1.0	4.7	456				
SD	19.7	0.32	357.11	3.3	78.5	168.8	197.3	101.8	0.7	1.6	2.1	1.6	0.003	0.5	2.4	295				

T2DM Ex	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				Slope LVC		Slope LBF		Slope LVC		Slope LBF	
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100	250	400	550	100.0	250.0	400.0	550.0	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)
													vs force (N)	vs force (N)	vs % max	vs % max				
1	29.1	0.33	446.8	4.8	116.9	274.1	400.4		1.2	2.7	4.1		0.007	0.7	4.0	396				
2	58.0	0.56	727.6	5.9	158.5	340.8	470.7		1.6	3.1	4.1		0.008	1.1	4.9	595				
3	29.4	0.27	668	5.1	129.7	416			1.3	3.6			0.012	1.5	5.0	607				
4	48.7	0.66	863.9	8.0	156.7	368.7	612.4	989.4	2.2	3.7	4.6	8.0	0.012	1.8	6.6	1005				
5	52.3	0.61	722	5.3	141.8	234.9	425.5		1.4	2.4	4.0		0.007	1.0	3.9	561				
6	33.0	0.34	444.9	4.6	344	388.1			2.9	3.7			0.005	0.3	1.3	74				
7	33.0	0.25	691.8	5.1	118.5	302.9	496.8		0.9	2.3	3.8		0.008	1.2	4.8	643				
8	72.3	0.85	808.5	8.5	231.9	331.9			2.7	3.9			0.005	0.7	3.2	368				
Mean	44.5	0.48	671.7	5.9	174.8	332.2	481.2	989.4	1.8	3.1	4.1		0.01	1.0	4.2	531				
SD	15.9	0.22	152.9	1.5	77.6	60.1	82.5		0.7	0.6	0.3		0.003	0.5	1.6	268				

T2DM Con	rest LBF	rest LVC	Pk LBF	Pk LVC	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				Slope LVC		Slope LBF		Slope LVC		Slope LBF	
No.	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	100	250	400	550	100.0	250.0	400.0	550.0	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)	(ml.min ⁻¹ .mmHg ⁻¹)	(ml.min ⁻¹)
													vs force (N)	vs force (N)	vs % max	vs % max				
1	30.9	0.26	846.2	15.2	168.5	355	658.6		1.8	4.6	7.3		0.021	1.2	11.7	682				
2	56.4	0.6	659.1	7.2	87	202.5	467.8	607.3	0.9	2.3	4.4	6.2	0.011	1.2	6.5	670				
3	81.8	1.13	839.7	6.4	247.4	346.5	542.4		2.0	3.1	4.3		0.007	1.0	3.9	572				
4	37.2	0.39	716.2	8.5	173.6	283.6	517.3		1.4	3.3	6.0		0.015	1.1	6.1	458				
5	42.5	0.46	610.6	7.3	361.3	477			4.3	5.9			0.01	0.8	2.7	193				
6	40.1	0.4	806.3	9.5	92.3	264.1	409.4		0.7	2.1	3.3		0.013	1.1	7.5	603				
7	50.5	0.49	448.7	4.5	184.8	334.4	361.2		1.3	3.7	4.3		0.01	0.6	4.1	235				
8	77.2	0.98	716.2	7.5	176.1	389.8	514.1		2.3	4.8	5.5		0.01	1.1	4.3	451				
Mean	52.1	0.59	705.4	8.3	186.4	331.6	495.8	607.3	1.8	3.7	5.0	6.2	0.01	1.0	5.9	483				
SD	18.7	0.31	133.8	3.2	87.5	83.5	96.5	#DIV/0!	1.1	1.3	1.3		0.004	0.2	2.9	187				

LBF and LVC at rest and during incremental plantar flexion exercise

Unsupervised exercise													Slope LVC		Slope LBF		Slope LVC		Slope LBF	
ND Ex No.	rest LBF (ml.min ⁻¹ .mmHg)	rest LVC (ml.min ⁻¹)	Pk LBF (ml.min ⁻¹ .mmHg)	Pk LVC (ml.min ⁻¹)	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs % max)	(ml.min ⁻¹ .mmHg vs % max)				
1	59.3	0.57	1103	10.3	512.5	933.8			4.8	7.7			0.019	2.8	4.9	702				
2	16.9	0.25	877.9	12.4	180.6	336.8	501.2	574.0	2.9	4.8	6.9	8.7	0.013	0.9	9.4	625				
3	29.2	0.28	913	8.6	76.0	370.0	563.5	652.3	0.8	3.6	4.9	5.6	0.012	1.3	6.9	858				
4	40.5	0.45	936.6	9.1	279.0	416.5	710.1	848.1	3.1	4.5	7.4	8.4	0.009	1.2	6.9	734				
5	60.3	0.76	822.3	10.1	197.9	438.7	654.7		2.4	5.1	8.4		0.016	0.9	7.3	608				
6	68.9	0.7	246.8	2.8	178.3	186.9			1.5	1.7			0.002	0.1	0.8	34				
7	68.2	0.76	756.2	9.6	99.8	361.6	527.2	702.6	1.1	4.0	6.4	8.1	0.015	1.3	8.6	724				
8	31.6	0.4	533.2	7.9	323.4	488.6	602.2		3.5	5.4	6.7		0.009	0.7	5.0	383				
9	34.1	0.33	467.1	3.4	106.7	172.0			1.0	1.5			0.006	1.0	2.6	397				
10	73.6	0.95	846.8	10.9	221.7	461.8	769.6		2.9	5.1	9.4		0.021	1.8	8.7	731				
Mean	48.3	0.55	750.3	8.5	217.6	416.7	618.4	694.3	2.4	4.3	7.1	7.7	0.01	1.2	6.1	580				
SD	20.0	0.24	257.6	3.1	129.7	210.7	98.2	115.4	1.3	1.8	1.4	1.4	0.01	0.7	2.8	243				

ND Con No.	rest LBF (ml.min ⁻¹ .mmHg)	rest LVC (ml.min ⁻¹)	Pk LBF (ml.min ⁻¹ .mmHg)	Pk LVC (ml.min ⁻¹)	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs % max)	(ml.min ⁻¹ .mmHg vs % max)
1	28.4	0.27	335.9	3.5	117.8	286.4			1.2	3.0			0.011	1.1	2.9	281
2	32.9	0.43	407.4	4.1	123.0	298.7			1.6	3.5			0.012	1.2	3.2	293
3	20.2	0.2	267.2	2.5	45.9	183.7	227.3		0.4	1.7	2.1		0.005	0.6	2.3	242
4	50.8	0.6	533.8	5.4	100.4	272.2	384.2		1.1	3.0	4.6		0.011	0.9	4.7	378
5	56.5	0.56	1047.7	7.6	137.6	395.9	732.2	986.0	1.3	3.7	5.9	7.6	0.009	1.5	6.7	1029
6	34.1	0.29	481.1	3.6	84.2	257.0			0.7	2.2			0.009	0.8	3.9	310
7	48.5	0.54	1119	10.8	247.2	456.7	717.3	977.7	2.6	4.6	7.5	9.5	0.013	1.5	9.5	1057
8	56.9	0.57	557.2	5.2	103.7	232.8	453.1		1.0	2.8	4.0		0.01	1.2	4.0	466
9	60.2	0.56	797.9	6.8	57.8	167.9	614.3	710.4	0.6	1.5	5.1	6.1	0.013	1.6	7.3	882
Mean	43.2	0.45	616.4	5.5	113.1	283.5	521.4	891.4	1.2	2.9	4.9	7.7	0.010	1.2	4.9	549
SD	14.5	0.15	305.1	2.6	58.5	93.3	200.7	156.8	0.6	1.0	1.8	1.7	0.003	0.3	2.4	340

T2DM Ex No.	rest LBF (ml.min ⁻¹ .mmHg)	rest LVC (ml.min ⁻¹)	Pk LBF (ml.min ⁻¹ .mmHg)	Pk LVC (ml.min ⁻¹)	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs % max)	(ml.min ⁻¹ .mmHg vs % max)
1	41.4	0.46	570.4	5.5	217.6	385.4	491.9		2.5	3.3	4.9		0.007	0.9	3.2	366
2	38.9	0.23	679.7	4.0	133.8	349.8	504.6		1.0	2.5	3.2		0.005	0.9	2.8	489
3	31.8	0.26	466.5	3.4	155.2	341.5			1.3	2.5			0.006	0.9	2.7	375
4	62.7	0.52	1024.2	7.3	210.6	456.5	784.6	866.1	1.7	3.9	5.9	6.7	0.011	1.5	6.2	841
5	45.6	0.45	603	6.1	177.6	338.3	563.0		1.8	3.4	5.8		0.012	1.1	7.0	589
6	61.1	0.53	786.4	6.6	187.6	627.1			1.7	5.9			0.014	0.7	5.9	664
7	59.6	0.62	806.5	7.8	82.4	359.7	503.2		0.9	3.7	5.0		0.013	1.4	7.1	767
8	63.7	0.44	675.5	4.4	129.8	252.5			1.2	1.7			0.005	0.9	3.2	491
Mean	50.6	0.44	701.5	5.6	161.8	388.8	569.5	866.1	1.5	3.4	5.0		0.01	1.0	4.8	573
SD	12.6	0.13	171.4	1.6	45.6	111.6	123.4		0.5	1.3	1.1	#DIV/0!	0.004	0.3	2.0	175

T2DM Con No.	rest LBF (ml.min ⁻¹ .mmHg)	rest LVC (ml.min ⁻¹)	Pk LBF (ml.min ⁻¹ .mmHg)	Pk LVC (ml.min ⁻¹)	LBF (ml.min ⁻¹)				LVC (ml.min ⁻¹ .mmHg ⁻¹)				(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs force (N))	(ml.min ⁻¹ .mmHg vs % max)	(ml.min ⁻¹ .mmHg vs % max)
1	54.7	0.34	566.1	3.5	129.3	449.4	522.1		0.9	2.6	3.3		0.007	1.3	3.1	524
2	69.2	0.66	904	7.6	133.8	485.9	578.9	734.4	1.3	4.4	5.5	6.9	0.009	1.1	6.6	786
3	98.9	1.13	682	6.8	266.8	384.8	562.5		2.6	4.0	6.5		0.013	1.0	5.2	394
4	46.5	0.5	554.9	6.3	124.1	256.9	457.1		1.4	2.9	5.0		0.011	1.1	4.8	444
5	43.4	0.41	573.8	5.2	124.7	270.0	460.9	499.9	1.2	2.5	4.1	4.6	0.008	0.9	4.4	483
6	87.4	0.77	786.6	5.7	255.0	380.8	489.6		2.2	3.4	3.9		0.006	0.9	3.3	519
7	40.4	0.34	464.8	4.0	136.7	331.1	426.0		1.1	2.6	3.4		0.007	1.0	3.1	386
8	43.8	0.36	720.9	5.4	132.1	311.9	515.3		1.1	2.7	4.1		0.009	1.1	5.0	625
Mean	60.5	0.56	656.6	5.6	162.8	358.9	501.6	617.2	1.5	3.1	4.5	5.7	0.01	1.1	4.5	520
SD	22.3	0.28	144.0	1.4	60.8	81.7	53.2	165.8	0.6	0.7	1.1	1.6	0.002	0.1	1.2	132

Appendix 35: Physical characteristics and activity levels

T2DM (1-19 postmenopausal, 9-19 BMI matched postmenopausal)
Non-diabetic (1-21 premenopausal, 12- 21 BMI matched postmenopausal)

T2DM	Age	Weight (kg)	Height (m)	BMI	WHR	ABI	LOPAR
	(yr)	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	54	108.1	1.56	44.4	1.01	1.00	65
2	53	89.75	1.59	35.5	0.96	1.04	199.75
3	56	84.9	1.48	38.8	1.00	1.13	180.35
4	59	98.4	1.67	35.3	0.95	1.13	111.15
5	56	109.5	1.58	43.8	1.01	1.10	79.75
6	53	98.8	1.58	39.6	0.93	1.34	125.4
7	59	106.3	1.56	43.7	1.01	1.03	91.4
8	57	72.5	1.51	31.8	0.98	1.02	103.5
9	56	60.25	1.53	25.7	0.96	0.95	109.6
10	55	82.35	1.57	33.4	0.86	1.00	68
11	60	69	1.56	28.4	0.99	1.19	97.3
12	67	78.3	1.63	29.5	0.97	1.03	85.15
13	56	94.3	1.66	34.2	0.95	1.14	28.94
14	64	68.7	1.58	27.5	0.99	1.03	48.5
15	57	81.1	1.66	29.4	0.93	1.03	57.35
16	57	81.6	1.54	34.4	0.90	1.11	166.45
17	58	72.2	1.58	28.9	0.89	1.06	171.4
18	49	88.9	1.66	32.3	0.87	1.10	78.97
19	56	82.5	1.57	33.5		0.79	
Mean	56.9	85.6	1.58	34.7	0.95	1.06	104
SD	4.0	14.2	0.05	5.6	0.05	0.1	50.0
Non-diabetic	Age	Body mass	Height	BMI	WHR	ABI	LOPAR
	(yr)	(kg)	(m)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	60	61.9	1.59	24.5	0.91	0.82	56.3
2	66	48.3	1.36	26.1	1.00	1.11	44.6
3	55	64.7	1.61	24.9	0.95	1.10	95.3
4	53	61.9	1.63	23.3	0.84	1.06	104.1
5	62	53.4	1.60	20.8	0.87	1.02	57.9
6	50	54.1	1.58	21.7	0.79	1.21	114.1
7	47	53.9	1.60	21.0	0.75	0.87	130.3
8	60	74.1	1.68	26.1	0.98	1.15	84.8
9	58	64.0	1.63	24.1	0.83	1.42	163.8
10	64	56.0	1.62	21.3	0.90	1.12	142.4
11	45	60.2	1.59	23.8	0.90	1.11	143.9
12	50	64.6	1.62	24.6	0.98	1.03	
13	62	72.9	1.56	30.1	0.98	1.20	37.0
14	56	69.5	1.55	29.1	0.77	1.08	101.5
15	53	83.6	1.64	31.1	0.94	0.92	122.0
16	62	74.6	1.60	29.1	0.99	1.18	83.0
17	56	72.5	1.62	27.6	0.83	1.10	219.9
18	54	69.4	1.59	27.4	0.88	1.22	129.3
19	54	74.6	1.58	29.9	0.90	1.04	96.8
20	55	85.0	1.60	33.2	0.95	1.05	201.5
21	58	67.8	1.57	27.5	1.04	1.00	130.4
Mean	51.0	66.0	1.6	26.1	0.90	1.09	113
SD	5.5	9.8	0.06	3.5	0.08	0.1	48

Appendix 37: Physical characteristics and activity levels at BS, SE, and UE.

ND Ex		Baseline					SE					UE				
Code	Age	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR
		(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	60	61.9	24.5	0.91	0.82	56	62.3	24.6	0.91	1.15	83	61.9	24.5	0.91	0.99	85
2	33	59.7	22.2	0.82	1.14	141	60.5	22.5	0.83	1.15	132	61.5	22.8	0.79	1.08	204
3	62	72.9	30.1	0.98	1.20	37	70.8	28.7	0.99	1.08	56	70.8	28.7	0.99	0.90	79
4	56	69.5	29.1	0.77	1.08	101	66.3	27.6	0.78	1.08	95	68.4	28.5	0.77	1.06	105
5	53	83.6	31.1	0.94	0.92	122	82.2	30.2	0.94	1.14	121	81.4	30.2	0.94	1.18	145
6	66	48.3	26.1	1.00	1.11	45	46.5	25.1	1.00	1.01	65	46.9	25.7	1.00	1.01	52
7	56	72.5	27.6	0.83	1.10	220	71.1	27.1	0.84	1.08	220	71.8	27.4	0.84	1.09	188
8	53	61.9	23.3	0.84	1.06	104	61.4	23.1	0.84	1.19	142	61.7	23.2	0.86	1.09	121
9	54	69.4	27.4	0.88	1.22	129	67.5	26.7	0.87	1.15	118	67.4	26.3	0.86	1.09	141
10	62	53.4	20.8	0.87	1.02	58	54.2	21.4	0.88	1.04	59	53.4	21.1	0.87	1.06	70
Mean	55.5	65.3	26.2	0.89	1.07	101	64.3	25.7	0.89	1.11	109	64.5	25.8	0.88	1.05	119
SD	9.0	10.4	3.4	0.07	0.12	56	9.8	2.8	0.07	0.06	50	9.8	2.9	0.08	0.08	50

ND Con		Baseline					SE					UE				
Code	Age	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR
		(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	58	67.8	27.5	1.04	1.00	130	66.8	27.1	1.06	0.91	140	69.4	28.5	1.06	1.00	120
2	50	54.1	21.7	0.79	1.21	114	53.0	21.2	0.78	1.39	100	52.8	21.2	0.79	1.15	162
3	47	53.9	21.0	0.75	0.87	130	53.5	20.9	0.76	1.12	124	53.8	21.0	0.76	1.03	104
4	58	64.0	24.1	0.83	1.42	164	63.8	23.7	0.83	1.02	185	65.3	24.6	0.84	1.19	189
5	64	56.0	21.3	0.90	1.12	142	57.0	21.7	0.92	1.27	150	56.3	21.5	0.89	1.01	167
6	42	72.9	26.8	0.90	1.05	118	71.4	26.2	0.93	0.99	113	71.1	26.1	0.93	1.09	118
7	54	74.6	29.9	0.90	1.04	97	75.3	30.1	0.87	1.01	117	74.6	29.9	0.88	1.00	117
8	42	90.6	32.9	0.90	1.08	139	91.0	33.0	0.95	1.03	105	89.4	32.4	0.95	1.00	101
Mean	51.9	67.4	25.4	0.88	1.09	121	67.4	25.4	0.89	1.10	119	66.6	25.4	0.89	1.07	120
SD	8.0	10.9	3.8	0.08	0.16	27	11.2	3.9	0.09	0.13	31	11.7	3.9	0.09	0.07	55

T2DM Ex		Baseline					SE					UE				
Code	Age	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR
		(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	56	60.25	25.7	0.96	0.95	110	58.45	25.0	0.96	0.93	105	58.0	24.78	0.95	1.05	107
2	56	71.8	28.0	0.89	1.12	111	71.4	27.9	0.93	1.17	141	70.3	27.81	0.91	1.21	83
3	56	84.9	38.8	1.00	1.13	180	82.95	37.9	1.02	1.01	194	81.2	37.07	0.98	1.11	240
4	55	82.35	33.4	0.86	1.00	68	80.25	32.6	0.85	1.04	145	82.5	33.90	0.84	1.20	119
5	60	69	28.4	0.99	1.19	97	69.95	28.7	0.97	1.03	155	69.5	28.56	0.97	1.26	132
6	67	78.3	29.5	0.97	1.03	85	78.05	29.0	0.97	1.00	237	78.2	29.07	0.98	0.99	111
7	52	84.1	32.4	1.01	1.05	106	81.1	31.3	1.02	1.05	149	81.3	31.36	1.01	1.12	130
8	56	94.3	34.2	0.95	1.14	29	90.2	32.7	0.95	1.22	65	87.8	31.86	0.96	1.14	91
Mean	57.3	78.1	31.3	0.95	1.08	98	76.5	30.6	0.96	1.06	149	76.1	30.6	0.95	1.13	126
SD	4.5	10.7	4.2	0.05	0.08	43	9.7	3.9	0.05	0.09	52	9.6	3.8	0.05	0.09	49

T2DM Con		Baseline					SE					UE				
Code	Age	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR	Body mass	BMI	WHR	ABI	LOPAR
		(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)	(kg)	(kg.m ⁻²)			(Met h.wk ⁻¹)
1	64	68.7	27.5	0.99	1.03	49	67.4	27.0	1.01	1.12	127	69.0	28.0	1.01	1.04	210
2	59	98.4	35.3	0.95	1.13	111	96.1	34.4	0.95	1.04	95	98.0	35.2	0.94	1.01	222
3	56	109.5	43.8	1.01	1.10	80	108.7	43.0	1.01	1.14	64	109.9	43.5	1.00	1.01	43
4	44	83.5	33.0	1.00	1.06	238	83.5	33.9	1.01	1.17	183	81.4	33.0	0.98	1.00	137
5	57	81.1	29.4	0.93	1.03	57	80.8	29.3	0.94	1.00	68	80.6	29.2	0.94	1.02	99
6	53	98.8	39.6	0.93	1.34	125	99.4	39.8	0.92	0.96	103	104.1	41.7	0.92	1.13	119
7	57	81.6	34.4	0.90	1.11	166	79.5	33.5	0.90	0.91	188	79.3	33.4	0.91	0.98	309
8	58	72.2	28.9	0.89	1.06	171	71.7	28.7	0.89	1.10	146	70.5	27.9	0.88	1.04	192
Mean	56.0	86.7	34.0	0.95	1.11	125	85.9	33.7	0.95	1.06	122	86.6	34.0	0.95	1.03	166
SD	5.8	14.2	5.6	0.05	0.10	65	14.2	5.5	0.05	0.09	48	15.4	5.9	0.05	0.05	84

Appendix 38: Plasma levels of IL-6, IL-10, and TNF-α at BS, SE, and UE.

ND Ex	BS			SE			UE		
	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)
1	1.8	6.2	0.0	1.8	8.3	0.0	11.9	10.4	0.7
2	15.2	10.3	7.7	11.5	9.6	7.3	0.4	4.5	0.0
3	14.4	7.7	4.7				10.4	10.6	5.1
4	13.3	11.8	42.3	14.8	14.7	12.0	8.9	6.5	2.6
5	14.0	12.1	19.0	22.9	15.1	15.3	14.1	10.2	12.8
6	6.3	8.3	1.5	2.6	9.9	2.6	13.4	20.3	16.1
7	17.0	10.3	9.1	17.8	8.0	9.5			
8	6.7	5.9	4.0	0.7	4.1	2.6	0.7	11.7	0.0
9	8.9	7.5	1.8	66.9	5.8	3.3	5.2	6.7	2.6
10	12.2	7.7	10.2	5.9	5.0	0.0	3.3	7.8	0.0
Mean	11.0	8.8	10.0	16.1	8.9	5.8	7.6	9.9	4.4
SD	4.8	2.2	12.6	20.5	3.9	5.5	5.3	4.6	6.0
ND Con	BS			SE			UE		
	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)
1	8.5	7.4	4.4				11.5	7.6	6.6
2	6.7	6.4	2.2	2.2	3.9	0.0	5.6	8.8	2.6
3	12.6	12.5	24.1	9.3	6.1	5.1	0.7	5.2	0.0
4	4.4	6.1	0.7				8.9	7.8	32.9
5	5.2	8.5	3.6	20.4	13.0	8.8	28.2	28.3	0.0
6	18.1	17.1	8.8	7.8	5.0	2.6	5.6	9.1	2.6
7	4.8	4.0	1.5	20.8	17.7	7.3	13.0	21.6	8.1
8	6.7	7.9	3.3				2.2	3.4	0.0
Mean	8.4	8.7	6.1	12.1	9.2	4.8	9.5	11.5	6.6
SD	4.7	4.2	7.7	8.2	5.9	3.5	8.7	8.7	11.1
T2DM Ex	BS			SE			UE		
	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)
1	4.1	23.2	0.0	1.1	9.6	0.0	25.6	10.6	2.9
2	2.2	4.6	1.8	5.2	10.1	1.8	6.7	5.2	0.0
3	10.3	11.9	1.5	5.5	9.0	0.7	3.7	6.3	2.2
4	11.8	8.8	17.1	7.0	7.5	15.0	4.8	9.7	1.1
5	22.2	10.1	9.8	15.2	11.0	9.5	5.9	8.6	0.0
6	20.3	12.1	8.8	14.4	10.8	8.4	16.7	9.3	7.3
7				6.7	6.4	0.7	15.2	8.2	5.1
8	23.7	27.4	12.4	17.0	10.1	8.4	4.8	5.2	0.0
Mean	13.5	14.0	7.3	9.0	9.3	5.6	10.5	7.9	2.3
SD	8.7	8.2	6.4	5.7	1.6	5.5	7.9	2.1	2.7
T2DM Con	BS			SE			UE		
	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-alpha (pm/ml)
1	17.7	16.2	12.0	15.4	14.6	13.3	16.4	9.3	14.3
2	8.1	14.0	5.1	9.2	14.2	5.5	14.1	11.2	8.4
3									
4	18.1	186.9	2.2	5.6	4.9	2.0	4.1	5.8	1.1
5	6.3	9.4	10.6	8.9	9.3	9.2	14.9	8.9	8.1
6	10.7	8.6	2.9						
7	5.5	9.2	0.0	8.7	9.0	2.1	8.5	8.2	2.2
8	4.8	7.9	3.6	6.8	7.9	2.6	7.1	8.4	0.0
Mean	10.2	36.0	5.2	9.1	10.0	5.8	10.8	8.6	5.7
SD	5.6	66.6	4.5	3.4	3.8	4.6	4.9	1.8	5.5

Appendix 39: IL-6, TNF- α , visfatin, and resistin mRNA expression at BS, SE, and UE.

ND Ex	IL-6 fold change			TNF-alpha fold change			Reistin fold change			Visfatin fold change		
	BS	SE	UE	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	1.59		0.45	1.40	1.86	1.05	0.40	0.22	0.86	1.65	0.82	1.57
2	0.29	0.30	0.26	1.17	0.94	1.06	0.63	0.84	0.48	2.01	0.64	1.45
3	3.24	1.11	1.24	1.36	0.83	1.16	0.53	1.04	0.74	0.83	0.46	0.80
4	0.35	0.22	0.29	1.00	0.70	0.90	0.57	1.02	1.23	1.63	0.86	0.70
5	0.59	0.43	0.28	0.74	0.58	0.98	0.36	1.15	2.51	0.85	0.47	1.20
6	0.69	0.52	0.36	1.11	1.00	1.24	1.81	0.60	0.70	1.05	0.49	0.40
7	0.28	0.16		0.92	0.80		0.09	0.67		0.76	0.53	
8	0.60		0.30	1.26		0.85	0.88		1.15	1.15		0.49
9	1.02	0.94	0.74	1.42	0.97	0.88	0.69	1.00	1.10	0.86	0.64	0.56
10		0.93	0.72		0.83	1.04		0.80	1.00		1.15	0.97
Mean	0.96	0.58	0.52	1.15	0.94	1.02	0.66	0.82	0.59	1.20	0.67	0.90
SD	0.95	0.37	0.33	0.23	0.37	0.13	0.48	0.29	0.59	0.45	0.23	0.42

ND Con	IL-6 fold change			TNF-alpha fold change			Reistin fold change			Visfatin fold change		
	BS	SE	UE	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	1.14	0.59		1.13	1.21		1.05	1.09		2.17	1.41	
2	0.88	1.06	0.88	0.94	0.75	0.94	0.80	0.61	0.77	0.92	0.91	0.63
3	0.98	0.25	0.23	1.38	1.12	1.60	0.41	0.62	0.67	1.01	0.41	0.82
4	2.91	0.90		1.12	1.41		0.09	2.76		1.07	2.78	
5	0.13	0.30	0.14	0.82	0.76	1.05	0.75	0.60	0.52	0.49	0.46	0.66
6	0.36	0.03	0.35	0.89	1.03	1.06	1.41	0.90	0.88	0.79	1.16	1.14
7	0.71	1.14	0.34	0.73	0.87	0.89	1.43	1.45	1.21	0.97	1.30	1.09
8	0.37	0.41		1.26	1.31		0.73	0.52		0.78	1.25	
Mean	0.93	0.59	0.39	1.03	1.06	1.11	0.83	1.07	0.81	1.03	1.21	0.87
SD	0.87	0.41	0.29	0.22	0.25	0.28	0.46	0.75	0.26	0.50	0.74	0.24

T2DM EX	IL-6 fold change			TNF-alpha fold change			Reistin fold change			Visfatin fold change		
	BS	SE	UE	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	1.13	1.80	0.56	1.31	1.15	0.69	0.86	1.11	0.68	1.09	0.80	1.00
2	0.46	1.04	1.30	1.19	1.43	1.24	0.87	1.85	1.00	0.74	1.08	0.50
3	0.77	0.54	0.85	1.20	0.96	1.11	0.29	1.96	1.00	1.38	1.49	1.34
4	1.09	1.41	0.96	1.22	3.51	1.27	1.63	2.37	0.99	1.17	7.14	1.23
5	0.73	1.05	0.77	1.37	1.17	1.00	0.62	0.72	0.75	1.15	0.73	0.87
6	0.26	0.28	0.29	1.10	0.83	0.83	0.87	1.44	0.64	1.14	0.80	1.21
7	0.53	0.96	0.53	0.97	1.01	1.03	0.89	1.12	1.05	2.11	1.08	1.49
8	3.80	1.49	1.77	0.84	0.71	0.96	0.58	1.11		1.66	1.03	1.72
Mean	1.10	1.07	0.88	1.15	1.35	1.02	0.82	1.46	0.87	1.31	1.77	1.17
SD	1.13	0.50	0.47	0.18	0.90	0.19	0.39	0.55	0.18	0.42	2.18	0.38

T2DM Con	IL-6 fold change			TNF-alpha fold change			Reistin fold change			Visfatin fold change		
	BS	SE	UE	BS	SE	UE	BS	SE	UE	BS	SE	UE
1	0.56	1.28	0.87	1.45	1.85	1.33	0.55	2.13	1.55	1.90	1.22	0.97
2	1.02	0.16	0.22	0.71	1.17	0.95	0.95	0.84	0.88	1.72	1.67	0.69
3	0.33	1.36	0.54	1.06	1.34	0.77	0.54	0.91	0.49	1.02	0.72	2.09
4			0.24		1.33	0.90		0.72	0.61		1.91	1.19
5	0.45	1.62	0.51	0.82	0.95	0.91	1.39	2.42	1.49	0.98	1.00	0.70
6	2.77		0.11	0.87		0.69	2.02		1.08	1.74		0.59
7	1.71	1.83	0.69	0.89	0.84	0.82	0.67	0.57	0.63	1.24	1.91	0.91
Mean	1.14	1.25	0.45	0.97	1.25	0.91	1.02	1.27	0.96	1.43	1.41	1.02
SD	0.94	0.65	0.28	0.26	0.36	0.21	0.59	0.80	0.43	0.40	0.50	0.51

Appendix 40: CRP, SAA, sICAM-1, and sVCAM-1 at BS, SE, and UE.

ND Ex	BS				SE				UE			
	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM
	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
1	450.8	2873.1	171.4	223.7	260.2	1631.3	104.0	158.6	198.0	1226.0	63.0	86.0
2	631.0	1595.8	129.4	255.0	1164.2	3431.9	126.6	245.0	211.3	427.3	28.8	48.5
3	943.9	572.0	101.4	132.2	453.9	345.3	55.3	97.0	512.6	352.2	62.5	77.6
4	581.1	873.0	116.6	180.2	261.9	546.4	46.5	86.7	845.4	971.7	145.5	185.7
5	520.4	863.4	57.9	110.3	2566.4	2101.1	215.0	356.6	539.7	578.6	36.0	53.7
6	120.1	445.8	29.2	50.9	136.7	436.2	35.8	62.5	522.7	1439.1	108.2	171.2
7	576.5	759.4	104.0	127.6	124.2	230.9	36.0	43.3				
8	99.0	458.6	46.2	73.1	225.1	1014.6	106.2	168.3	1319.6	4002.9	299.1	394.3
9	338.5	811.9	127.0	172.0	47.5	97.4	24.7	43.7	40.5	173.4	53.2	72.1
10	94.4	255.5	62.1	97.4	98.5	199.9	32.7	62.5	330.2	865.7	171.7	244.1
Mean	435.6	950.9	94.5	142.2	533.9	1003.5	78.3	132.4	502.2	1115.2	107.6	148.1
SD	275.6	767.8	44.5	65.1	783.9	1081.4	60.1	102.1	388.5	1160.2	87.0	114.7

ND Con	BS				SE				UE			
	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM
	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
1	549.2	331.4	39.2	63.3					8528.6	3189.6	310.9	468.9
2	371.6	364.5	97.4	126.5	430.1	345.5	110.0	121.5	118.7	403.7	119.2	148.5
3	207.3	543.1	61.7	83.5	236.9	812.2	44.6	73.0	266.9	1261.8	90.0	114.1
4	270.9	1251.8	141.9	213.2					258.3	1061.8	93.8	138.3
5	192.4	1560.3	109.7	200.1	429.6	1726.1	109.1	188.3	105.2	520.6	74.8	123.6
6	5645.7	1494.7	130.0	225.2	8940.9	2274.9	287.5	426.9	1340.5	454.2	79.4	122.7
7	503.1	844.1	51.3	66.6	3231.3	2265.2	111.2	146.6	1986.7	1704.3	136.5	154.9
8	368.9	708.0	76.8	105.6					114.0	396.1	35.5	53.7
Mean	1013.6	887.2	88.5	135.5	2653.8	1484.8	132.5	191.3	1589.9	1124.0	117.5	165.6
SD	1876.0	491.3	37.4	67.5	3728.1	872.1	91.2	138.2	2889.3	962.7	83.7	126.4

T2DM Ex	BS				SE				UE			
	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM
	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
1	145.5	387.0	32.6	58.4	89.4	259.8	45.1	73.1	1401.6	2345.4	100.7	117.1
2	522.8	1346.3	110.1	169.8	800.8	1753.0	126.2	211.0	162.5	361.5	51.1	83.6
3	8287.8	6097.8	228.3	308.8	1052.5	1255.0	43.1	71.5	2094.4	2415.2	93.2	125.9
4	1103.5	1783.1	118.5	124.2	434.9	607.4	53.3	77.9	3867.4	5307.5	299.5	307.7
5	767.6	2059.1	189.3	234.6	46.3	115.5	10.3	18.0	1058.6	2707.3	233.6	286.6
6	678.8	1119.7	99.8	128.0	228.5	582.7	58.3	91.1	253.8	347.2	49.7	52.8
7	830.5	1747.6	162.8	305.0	222.9	557.1	70.9	112.6	1434.9	2768.8	410.2	555.5
Mean	1762.3	2077.2	134.5	189.8	410.8	732.9	58.2	93.6	1467.6	2321.8	176.9	218.5
SD	2892.5	1856.0	64.6	95.9	380.6	575.5	35.4	59.2	1258.4	1683.9	139.9	178.3

T2DM Con	BS				SE				UE			
	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM	CRP	SAA	ICAM	VCAM
	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
1	3275.3	2428.3	191.0	276.0	141.4	162.5	19.4	38.5	1731.2	1282.6	195.1	219.0
2	820.5	650.2	113.5	201.5	131.4	244.8	32.2	60.0				
3	1228.7	313.2	49.7	77.5	3380.7	873.0	165.2	199.3	6492.4	1439.1	299.6	345.8
4	562.4	2044.0	83.4	152.2	309.2	1549.5	61.7	140.9	908.2	2647.6	100.8	185.4
5	618.4	1209.9	88.3	151.1	471.2	2672.8	165.9	186.1	378.1	1907.0	142.1	175.9
6	410.5	436.2	32.0	60.8					3185.6	3639.8	307.0	465.6
7	85.3	261.9	41.2	68.2	48.0	195.6	34.4	65.8	303.4	1069.3	243.0	307.4
Mean	1000.2	1049.1	85.6	141.1	747.0	949.7	79.8	115.1	2166.5	1997.6	214.6	283.2
SD	1063.2	876.9	54.8	79.5	1299.1	1002.0	67.8	69.5	2374.6	981.0	83.8	112.2

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