Pulmonary oxygen uptake and muscle deoxygenation responses during ramp incremental exercise and moderate- and heavy-intensity exercise subsequent to priming exercise in type 2 diabetes.

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

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I. DECLARATION

I declare that this thesis is entirely my own work and has not been previously submitted as an exercise for a degree in this or any other university. Trinity College Dublin may lend or copy this thesis on request.

Signature: _____ Date: _____

II. ACKNOWLEDGEMENTS

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III. ABSTRACT

Middle-aged and young individuals with uncomplicated type 2 diabetes mellitus (T2DM) consistently demonstrate impairments in submaximal and maximal exercise performance, which are independent of obesity, and present in the absence of clinically apparent cardiovascular disease. Such limitations have the potential to contribute to the tenacious excess cardiovascular and all-cause mortality observed in T2DM. Whilst the precise pathophysiological mechanisms responsible for this exercise intolerance remain to be elucidated, with both central and peripheral factors likely implicated, the primary aim of this thesis was to further investigate those mechanisms with an emphasis on the contribution of peripheral factors. As such, cardiorespiratory and estimated microvascular responses were simultaneously investigated during graded ramp incremental cycle exercise and during submaximal cycling exercise at moderate- and heavy-intensities subsequent to a prior heavy-intensity "priming" exercise.

Given that muscle oxygen supply may limit maximal exercise capacity in T2DM, *Experiment 1*, examined the influence of T2DM on the profile of muscle fractional oxygen (O_2) extraction (estimated using deoxygenated haemoglobin and myoglobin [HHb+Mb]) during ramp incremental cycle exercise in 17 middle-aged individuals with T2DM (48 ± 7 yr; 31.9 ± 4.8 $kg.m^{-2}$; 12 males/5 females) and 17 individuals without T2DM (ND/controls) (44 ± 8 yr; 30.8 ± 3.5 $kg.m^{-2}$; 12 males/5 females). Maximum oxygen uptake ($\dot{V}O_{2max}$) was significantly reduced in individuals with T2DM compared with controls (22.5 ± 3.7 *vs.* 28.6 ± 5.5 $mL.kg.min^{-1}$), representing a reduction of 21% in peak exercise capacity. This impairment was accompanied by the demonstration of a steeper primary slope of the bi-linear regression of relative [HHb+Mb] (% [HHb+Mb]) as a function of relative power output (%PO) in individuals with T2DM (1.48 ± 0.46 *vs.* 1.14 ± 0.21), thus, indicative of a greater rate of fractional O_2 extraction for a given increase in oxygen uptake ($\dot{V}O_2$). This suggests a reduced O_2 delivery is a likely underlying cause of exercise intolerance during a maximum graded test in T2DM.

The subsequent three experiments were designed to investigate the influence of heavy-intensity (50% delta) priming exercise on the $\dot{V}O_2$ and [HHb+Mb] kinetics responses during subsequent moderate-, heavy- and heavy-intensity work-to-work (initiated from an elevated baseline) exercise bouts in T2DM. *Experiment 2* examined the influence of priming exercise and T2DM on the $\dot{V}O_2$ and [HHb+Mb] dynamic response during moderate-intensity (80% ventilatory threshold (VT)) cycle exercise. Twelve middle-aged individuals with T2DM (48 ± 8 yr; 32.1 ± 5.6 $kg.m^{-2}$; 7 males/5 females) and 12 controls (44 ± 9 yr; 30.4 ± 4.1 $kg.m^{-2}$; 7 males/5 females)

were tested. Individuals with T2DM demonstrated an accelerated rate of adjustment of the primary phase of the $\dot{V}O_2$ kinetics ($\tau\dot{V}O_{2p}$) response (43 ± 41 *vs.* 34 ± 11 s), whilst Δ[HHb+Mb] kinetics remained unchanged (29 ± 6 *vs.*28 ± 6, s) in a subsequent bout of primed moderate-intensity (80%VT) cycling exercise. This was accompanied by the amelioration of an 'overshoot' relative to steady-state in the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio (1.18 ± 0.17 *vs.* 1.05 ± 0.15) in the primed on-transient exercise response, attributed to an enhanced matching of microvascular O_2 delivery to utilisation consequent to priming exercise.

Experiment 3 examined the influence of priming exercise on $\dot{V}O_2$ and Δ[HHb+Mb] kinetics during heavy-intensity cycle exercise in T2DM. Twelve middle-aged individuals with T2DM (46 ± 8 yr; 31.4 ± 4.8 $kg.m^{-2;}$ 8 males/4 females) and 12 controls (43 ± 10 yr; 30.6 ± 3.8 $kg.m^{-2}$; 8 males/4 females) were tested. Priming exercise significantly accelerated the overall $\dot{V}O_2$ kinetics profile or mean response time (MRT) in the subsequently primed heavy-intensity exercise bout (75 ± 10 *vs.* 55 ± 14 s). This was facilitated via a significant acceleration of $\tau\dot{V}O_{2p}$ (37 ± 10 *vs.* 31 ± 9 s) combined with a substantial reduction (*P*=0.1) in the $\dot{V}O_2$ amplitude of the slow component (0.26 ± 0.15 *vs.* 0.15 ± 0.07 $L.min^{-1}$). Given that this acceleration of the overall $\dot{V}O_2$ kinetics occurred in the presence of an unaffected dynamic Δ[HHb+Mb] response (33 ± 27 *vs.* 16 ± 6) is thus further supportive of a superior O_2 delivery relative to utilisation associated with heavy-intensity priming exercise.

Healthy individuals display a constrained $\dot{V}O_2$ kinetics response when constant-load exercise is initiated from an elevated baseline (work-to-work). Thus, when combined with the notion of an already constrained muscle O_2 supply in T2DM, the inclusion of a priming exercise intervention with the work-to-work model should provide superior insight into potential mechanisms implicated in the impaired $\dot{V}O_2$ kinetics response consistently demonstrated in T2DM. Thus, in *Experiment 4* the influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity, work-to-work (w-to-w) cycle exercise in T2DM was investigated. Seven middle-aged individuals with T2DM (46 ± 8 yr; 30 ± 6 $kg.m^{2;}$ 3 males/4 females) and 7 controls (41 ± 10 yr; 31 ± 5.0 $kg.m^{-2}$; 3 males/4 females) were tested. The MRT of the $\dot{V}O_2$ kinetics during w-to-w cycling transitions was significantly accelerated subsequent to the prior bout of heavy-intensity priming exercise (72 ± 10 *vs.* 53 ± 19 s). This was a consequence of a significant reduction (~40%) in the amplitude of the $\dot{V}O_2$ slow component (0.13 ± 0.15 *vs.* 0.08 ± 0.10 $L.min^{-1}$), a substantial reduction of ~22% in primary phase of the $\dot{V}O_2$ kinetics response (54 ± 14 *vs*. 42 ± 17 s), with a tendency for the overall dynamic responses of Δ[HHb+Mb] (MRT) to be accelerated (52 ± 32 *vs.* 37 ± 24 s; *P*<0.10). Thus, the speeding of

the $\dot{V}O_2$ MRT following priming exercise was attributed to a combination of an increased O_2 delivery and the potential enhancement of motor unit recruitment.

Thus, the accumulated data in this thesis offer a further insight into potential contributory mechanisms for the evidenced exercise intolerance in individuals with T2DM. The demonstration of a greater reliance on O_2 extraction for a given increase in power output (PO) suggests that a reduced O_2 delivery is in fact an important fundamental cause of exercise intolerance during maximal graded efforts in T2DM. This is further corroborated by the demonstration of improvements in oxidative metabolism with a concomitant improvement in the matching of O_2 delivery to utilisation at a microcirculatory level consequent to an acute bout of heavy-intensity priming exercise, prior to both moderate- and heavy-intensity exercise (with and without an elevated baseline). Collectively, these findings suggest that factors beyond the heart substantially contribute to the diminished exercise tolerance consistently evidenced in T2DM.

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V. ABBREVIATIONS AND SYMBOLS

A	**absorption of light**
A	**asymptotic amplitude of a mathematically modelled response**
~	**approximately**
β	**beta**
[ ]	**concentration**
ºC	**degrees Celsius**
Δ	**delta; a difference or a change in value**
ε	**extinction coefficient**
≥	**greater than or equal to**
≤	**less than or equal to**
λ	**wavelength**
ACE	**angiotensin-converting enzyme**
ADA	**American Diabetes Association**
ADP	**adenosine diphosphate**
ARBs	**angiotensin II receptor blockers**
ATP	**adenosine triphosphate**
ATPase	**enzyme that catalyses the decomposition of ATP into ADP**
a.u.	**arbitrary units**
a-vO_2 diff	**arteriovenous oxygen difference**
BbB	**breath-by-breath**
beats.min^{-1}	**beats per minute**
BMI	**body mass index**
BP	**blood pressure**
BP	**break point**
BSL	**baseline**
CAD	**coronary artery disease**
CAN	**cardiac autonomic neuropathy**
Ca^{2+}	**calcium**
CaO_2	**arterial oxygen concentration**
CK	**creatine kinase**
cm	**centimetre**
CO	**cardiac output**

CODEIRE	**Cost of Diabetes in Éire study**
CO_2	**carbon dioxide**
CVD	**cardiovascular disease**
CvO_2	**venous oxygen concentration**
CW	**continuous wave**
CWR	**constant work rate**
DBP	**diastolic blood pressure**
deoxy[Hb+Mb]	**concentration of deoxygenated haemoglobin and myoglobin**
DPP-4	**Dipeptidyl peptidase 4**
ECG	**electrocardiogram**
EDTA	**ethylenediaminetetraacetate**
EMG	**electromyogram**
EQN	**equation**
ESC	**European Society of Cardiology**
ESH	**European Society of Hypertension**
F_{peak}	**peak force**
FPG	**fasting plasma glucose**
G	**change in VO_2per unit change in external work (i.e. $\Delta VO_2/\Delta PO$)**
GET	**gas exchange threshold**
GLP-1	**Glucagon-like peptide-1**
(ΔG_{ATP})	**negative Gibbs free energy of ATP hydrolysis**
H^+	**hydrogen ion**
Hb	**haemoglobin**
HbA_{1c}	**glycated haemoglobin**
HbO_2	**oxyhaemoglobin**
$h.day^{-1}$	**hours per day**
HDL-C	**high-density lipoprotein cholesterol**
HHb	**deoxyhaemoglobin**
Δ[HHb+Mb]	**changes in the deoxy haemoglobin and myoglobin concentration**
[HHb+Mb]-*BP*	**Deoxyhaemoglobin and myoglobin break point**
$\Delta[HHb+Mb]/\Delta\dot{V}O_2$	**haemoglobin and myoglobin deoxygenation to oxygen uptake ratio**
HR	**heart rate**
HR_{max}	**maximum heart rate**
HR_{peak}	**peak heart rate**

HRT	**hormone replacement therapy**
H_z	**hertz**
IDF	**International Diabetes Federation**
iEMG	**integrated electromyogram**
IFG	**impaired fasting glucose**
IGT	**impaired glucose tolerance**
K^+	**potassium**
kg	**kilogram**
$Kg.m^{-2}$	**kilogram per metre squared**
LBF	**leg blood flow**
LDL-C	**low-density lipoprotein cholesterol**
$L.min^{-1}$	**litre per minute**
LOPAR	**Low Level Physical Activity Recall questionnaire**
LT	**lactate threshold**
LVC	**leg vascular conductance**
m	**metre**
MAP	**mean arterial pressure**
max	**maximum**
Mb	**myoglobin**
MET	**metabolic equivalent of task**
$Mg.dL^{-1}$	**milligrams per decilitre per minute**
min	**minute**
mL	**millilitre**
$mL.kg^{-1}.min^{-1}$	**millilitre per kilogram per minute**
mm	**millimetre**
mM	**millimolar**
mmHg	**millimetre of mercury**
$mmol.L^{-1}$	**millimoles per litre**
Mod	**moderate-intensity**
MRT	**mean response time**
ms^{-1}	**millisecond**
mV	**milliVolt**
MVC	**maximum voluntary contraction**
n	**number of participants**

Na^+	**sodium**
ND	**without type 2 diabetes**
NO	**nitric oxide**
NOS	**nitric oxide synthase**
NIRS	**near infra-red spectroscopy**
nm	**nanometre**
N_2O	**nitrous oxide**
O_2	**oxygen**
P	**probability (statistical)**
PAD	**peripheral arterial disease**
PCr	**phosphocreatine**
PDH	**pyruvate dehydrogenase**
PE	**priming exercise**
pH	**a logarithmic scale used to express the acidity or alkalinity of a solution**
Phase I	**cardiodynamic phase of the $\dot{V}O_2$ kinetics response**
Phase II	**primary phase of the $\dot{V}O_2$ kinetics response**
Phase III	**attainment of steady state of the $\dot{V}O_2$ kinetics response**
Pi	**inorganic phosphate**
PIL	**participant information leaflet**
$Pmvo_2$	**microvascular O_2 partial pressure**
PO	**power output**
PO_2	**partial pressure of oxygen**
PWV	**pulse wave velocity**
Qm	**muscle blood flow**
Q_{10}	**temperature coefficient**
r	**correlation coefficient**
RAAS	**renin-angiotensin-aldosterone system**
RCP	**respiratory compensation point**
RER	**respiratory exchange ratio**
RPE	**rating of perceived exertion**
RPM	**revolutions per minute**
s	**second**
SBP	**systolic blood pressure**
SD	**standard deviation**

SF_6	**sulphur hexaflouride**
SLAN	**Survey of Lifestyle, Attitudes and Nutrition in Ireland**
SRS	**spatially resolved spectroscopy**
SV	**stroke volume**
SVUH	**St. Vincent's University Hospital**
S_1	**slope of $\dot{V}O_2$/PO below VT**
S_2	**slope of $\dot{V}O_2$/PO above VT**
t	**time**
τ	**time constant**
τ'	**effective time constant**
TCD	**Trinity College Dublin**
TCL	**total cholesterol**
TD	**time delay**
TTF	**time to failure**
T2DM	**type 2 diabetes mellitus**
$\tau\ \dot{V}O_{2p}$	**rate of adaptation of the primary phase of the $\dot{V}O_2$ kinetics response**
UKPDS	**UK Prospective Diabetes Study**
$\dot{V}CO_2$	**carbon dioxide output**
$\dot{V}_E$	**expired minute ventilation**
$\dot{V}_E/\dot{V}CO_2$	**ventilatory equivalent for CO_2**
$\dot{V}_E/\dot{V}O_2$	**ventilatory equivalent for O_2**
VL	***vastus lateralis***
$\dot{V}O_2$	**pulmonary oxygen uptake**
$\dot{V}O_{2max}$	**maximal oxygen uptake**
$\dot{V}O_{2peak}$	**peak oxygen uptake**
$\dot{V}O_{2s}/\dot{V}O_{2sc}$	**oxygen uptake 'slow-component'**
vs	**versus**
VT	**ventilatory threshold**
W	**watts**
WHO	**World Health Organisation**
WHR	**waist to hip ratio**
w-to-w	**work-to-work**
yr	**year**

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Chapter 1: General introduction

1.1 *Overview*

Diabetes mellitus, long considered a disease of minor significance to world health, is now one of the main threats to human health in the 21st century. Type 2 diabetes mellitus (T2DM) is currently classified as one of the most common chronic diseases in nearly all countries (Whiting *et al.* 2011), having reached epidemic proportions in the last two decades. Of particular importance is the consistent finding that individuals with T2DM demonstrate limitations in physical fitness, which have been shown to correlate strongly with cardiovascular and all-cause mortality.

Being such a complex disease, it is likely that multiple factors contribute to the impaired exercise capacity. However, a well-established key determinant of exercise tolerance is the rate at which pulmonary oxygen uptake adjusts ($\dot{V}O_2$ kinetics) at the onset of exercise. During the transition from rest to exercise, $\dot{V}O_2$ does not meet metabolic demands and thus an oxygen deficit is incurred. However, this dynamic response has been demonstrated to be constrained in individuals with T2DM resulting in a greater O_2 deficit which ultimately contributes to premature muscular fatigue. However, the precise mechanisms responsible for this abnormal exercise response in T2DM require further investigation. Understanding the physiological mechanisms influencing exercise capacity in this clinical population may help to provide specific therapeutic interventions aimed at optimising exercise tolerance and subsequently reduce mortality risk.

Although the overall body of knowledge is beyond the scope of a single review, this thesis will critically appraise the current literature pertaining to the impaired exercise tolerance evidenced by individuals with uncomplicated T2DM. The introduction will initially address the contributing factors to T2DM, the associated health consequences, and the current modes of intervention used to treat and manage this disease. It will then address the aetiology of the exercise intolerance evidenced in this clinical population, the physiological mechanisms responsible for the same, before providing an insight into an acute intervention aimed at increasing exercise tolerance; priming exercise.

1.2 Introduction

The term diabetes mellitus describes a group of metabolic disorders characterised by chronic hyperglycaemia resulting, either, from a deficiency of insulin, a decreased ability to transduce the insulin signal, or both (Hussain *et al.* 2007). There are two main forms:

- Type 1 diabetes mellitus is due primarily to autoimmune-mediated destruction of pancreatic β-cell islets, resulting in absolute insulin deficiency. Individuals with type 1 diabetes must take exogenous insulin for survival to prevent the development of ketoacidosis. Its frequency is low relative to type 2 diabetes, which accounts for over 90% of cases globally (Zimmet *et al.* 2001; Adeghate *et al.* 2006).
- Type 2 diabetes mellitus is a heterogeneous cluster of conditions rather than a uniform entity (Savage *et al.* 2007). It is characterised by two interrelated metabolic defects: insulin resistance coupled with impaired insulin secretion by β-cells in the pancreas, either of which may predominate (Ahmad & Crandall, 2010). Individuals with T2DM are not dependent on exogenous insulin, but may require it for control of blood glucose levels if this is not achieved with diet alone and/or with hypoglycaemic agents (Zimmet *et al.* 2001).

Currently, the American Diabetes Association (ADA) recommends the use of any of the following four criteria for diagnosing diabetes (Colberg *et al.* 2010);

i. glycated haemoglobin (HbA_{1c}) value of 6.5% or higher,

ii. fasting plasma glucose $\geq$126 $mg.dL^{-1}$ (7.0 $mmol.L^{-1}$),

iii. 2-h plasma glucose $\geq$200 $mg.dL^{-1}$ (11.1 $mmol.L^{-1}$) during an oral glucose tolerance test using 75 g of glucose,

iv. classic symptoms of hyperglycaemia (e.g., polyuria, polydipsia, and unexplained weight loss) or hyperglycaemic crisis with a random plasma glucose of 200 $mg.dL^{-1}$ (11.1 $mmol.L^{-1}$) or higher.

The current diabetes epidemic pertains specifically to T2DM. Numerous reports and reviews have been written concerning the rise in the prevalence of diabetes worldwide especially within the past two decades. The World Health Organisation (WHO) had estimated the global prevalence for all age groups would rise from 2.8% in 2000 to 4.4% in 2030 (Wild *et al.* 2004), more than doubling the number of persons affected; from 171 million to 366 million. However, that latter number was in fact reached by 2011 (Whiting *et al.* 2011). The International Diabetes Federation (IDF) produced similar estimates with predicted numbers among adults (20+ years)

increasing from 194 million in 2003 (Sicree & Shaw, 2007) to 366 million in 2011 (Whiting *et al.* 2011). As displayed in Figure 1.1, the IDF released more recent worldwide figures in 2015 and revealed that there are now ~415 million individuals living with T2DM, and it is estimated that the number of cases will increase to 642 million by 2040. It is also estimated, that ~1.6 million new cases of T2DM are diagnosed each year in the United States (Ahmad & Crandall, 2010). Boyle *et al.* (2010), project that the prevalence of both diagnosed and undiagnosed diabetes in the US will increase from its current level of 1 in 10 adults to between 1 in 5 and 1 in 3 adults by 2050.

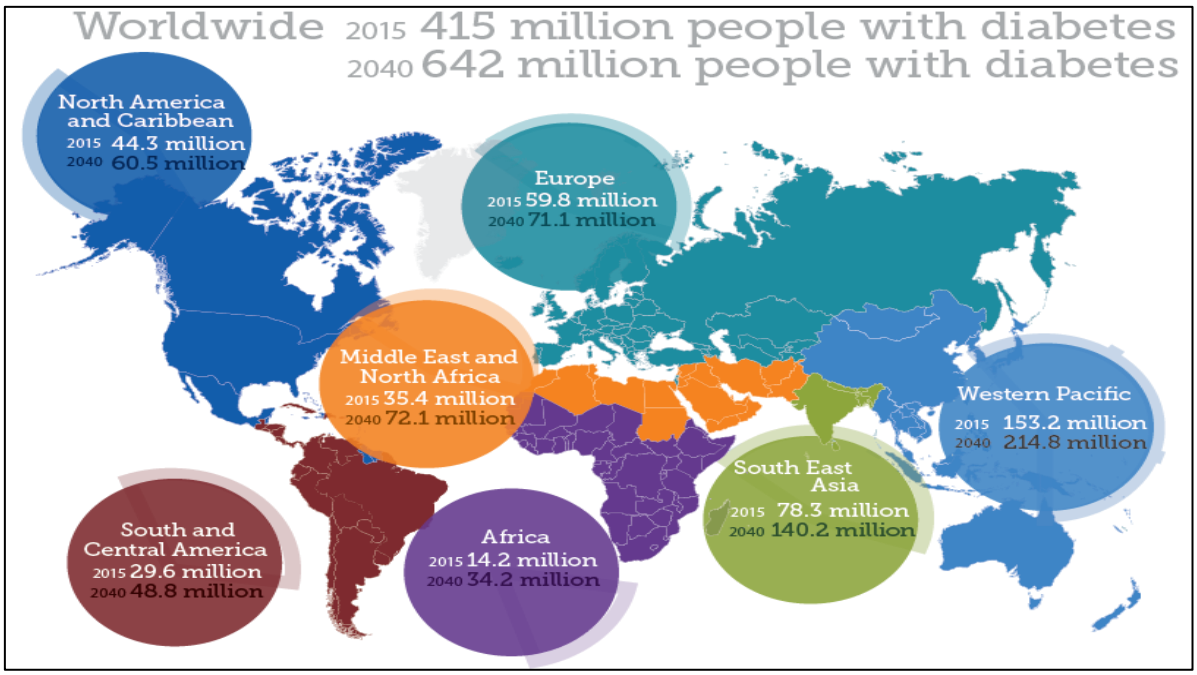

Figure 1.1. ***Worldwide current and predicted cases of diagnosed T2DM (IDF, 2015).***

At a more local level, a comprehensive analysis of the prescription of anti-diabetic therapies in Ireland from 2003-2012 was carried out by Zaharan *et al.* (2013). Findings estimated the prevalence of diabetes (both type 1 and type 2) in the Irish adult population to be in the region of 4.7% in 2007 and expected to increase to 5.9% by 2020. Females presented with a higher prevalence of diabetes (5.1%) compared to males (3.9%) which increased with age to 13.2% in females 60+ years (Balanda *et al.* 2010). In contrast to these findings, the 'Diabetes Briefing Irish Public Health Document' estimated that in 2010 more than 106,000 (6.9%) adults aged 18+ years in the Republic of Ireland had clinically diagnosed diabetes, and there were more than 41,000 (2.7%) adults aged 45+ years with undiagnosed diabetes.

1.3 Health consequences of diabetes

Diabetes mellitus is a major cause of illness and premature death in most countries (Sluik *et al.* 2012). It is well known that T2DM is an important and independent risk factor for cardiovascular mortality and morbidity. Individuals with T2DM are at increased risk of developing diverse microvascular, macrovascular and neuropathic complications that seriously erode quality of life (Boyle *et al.* 2010), having a two- to four-fold increased risk of developing cardiovascular disease (CVD), peripheral vascular disease, and stroke (Berry *et al.* 2007; Ahmad & Crandall, 2010). Meta-analysis data suggest a similar cardiovascular mortality risk in otherwise healthy individuals with T2DM as compared to individuals with a history of established coronary artery disease (CAD) (Carnethon *et al.* 2010). In addition to the aforementioned higher overall mortality associated with the high risk of cardiovascular complications, there is also a higher mortality due to cancers in these same individuals (Vigneri *et al.* 2009).

1.4 Economic cost of diabetes.

The economic burden associated with diabetes is substantial. It represents 10-15% of the total health costs in developed countries (Williams *et al.* 2002). The healthcare costs of an individual with diagnosed T2DM have been estimated to approximate 2.3 times that of an individual without (Sieverdes *et al.* 2009; Boyle *et al.* 2010). Once again, at a more local level, the ingredient cost of diabetes medications borne by the Irish government through reimbursement schemes was approximately €14 million in 2005, and this cost had increased to over €45 million in 2009 (Zaharan *et al.* 2013). Of significant importance is that within this report, these costs were related to diabetes specific pharmacotherapy and did not include additional medications which individuals with diabetes often require for other complications. Thus, it is expected that the medication costs would in fact be markedly greater as reported in the 'Cost of Diabetes in ÉIRE (CODÉIRE)' study (Nolan *et al.* 2006). Using data from 1999 to 2000, the authors estimated that the total cost for treating diagnosed T2DM patients in Ireland was ~ €377.2 million. Moreover, since then, newer and more expensive antidiabetic agents such as insulin analogues, thiazolidinediones, meglitinides, dipeptidyl peptidase 4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) agonists have been marketed (Figure 1.2) and subsequently will have most likely further increased the costs of caring for patients with diabetes (Zaharan *et al.* 2013). Additionally, it can be expected that the indirect costs associated with diabetes are also high, and include those incurred through the loss of productivity due to absenteeism,

disability and premature mortality. Although more difficult to measure further research is required to ascertain the total spectrum of these costs.

1.5 Risk factors for diabetes

The causes of diabetes are incompletely understood although it is widely accepted that they are multifactorial, resulting from the interaction between a genetic predisposition and behavioural and environmental risk factors (Tuomilehto *et al.* 2001; Adeghate *et al.* 2006). Although strongly implicated, the exact genetic basis of T2DM is complex and not clearly defined (Colberg *et al.* 2010) however, it is postulated that genetic susceptibility plays a crucial role in the aetiology and manifestation of the disease with concordance in monozygotic twins approaching 100% (Adeghate *et al.* 2006). Positive family history confers a 2.4-fold increased risk for T2DM with 15-25% of first-degree relatives of patients with T2DM developing impaired glucose tolerance or T2DM (Stumvoll *et al.* 2005).

There is strong evidence, however, to suggest that modifiable risk factors such as obesity and physical inactivity are the main non-genetic determinants of the disease (Manson *et al*, 1992; Wannamethee *et al.* 2000; Boule *et al.* 2003; Hu *et al.* 2004) with approximately half of the risk of T2DM being attributed to such environmental exposure and the other half to genetics (Hussain *et al.* 2007). This epidemic is taking place both in developed and developing nations and continues to increase in numbers and significance as economic development and urbanisation lead to changing lifestyles characterised by reduced physical activity and increased obesity (Whiting *et al.* 2011).

The worldwide prevalence of obesity has also reached epidemic proportions in recent years, with the WHO (2009) predicting that by 2015, 2.3 billion adults worldwide would be overweight and 700 million would be obese. Unfortunately, a similar trend is evident in Ireland. Representative data for Ireland revealed that ~37% of the Irish adult population is overweight and a further 24% are obese, with predictions that this prevalence of being overweight and obesity will reach 89% and 85% in adult males and females respectively (Keaver *et al.* 2013). A large body of evidence now exists demonstrating a firm relationship between the two medical entities of obesity and diabetes, with direct associations apparent. Indeed, it has been reported that for every 1 kg in weight gain there is a relative 4.5-9% increase in diabetes prevalence (Russell-Jones, 2008).

Over the last number of decades, environment and lifestyles have been engineered to favour increased sedentary time. The epidemiological data that is available regarding the change in physical activity patterns over time revealed that more than 60% of adults do not engage in

sufficient levels of physical activity worldwide. That is, they do not meet the basic American College of Sports Medicine (ACSM) guidelines for health, which is 30 minutes (min) of moderate physical activity at least 5 times per week, or vigorous activity for 20 min at least 3 times per week. Similar findings for Irish adults were revealed in the "Survey of Lifestyle, Attitudes and Nutrition in Ireland" (SLAN) report (2007), whereby only 41% of Irish adults took part in moderate or vigorous activity for at least 20 min on 3 or more days per week.

Mounting evidence has suggested that physical activity or cardiorespiratory fitness has protective influences on hyperglycaemia and incident diabetes (Wei *et al.* 2000; Boule *et al.* 2003). Many large epidemiological studies have examined cardiorespiratory fitness and diabetes risk, and the findings indicate that both cardiorespiratory fitness and physical activity are inversely associated with the risks of developing diabetes (Lynch *et al.* 1996; Wei *et al.* 1999; Sawada *et al.* 2003; Sui *et al*. 2008; Sieverdes *et al.* 2009), with physical inactivity estimated to account for 27% of diagnosed cases of diabetes globally (WHO, 2010).

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), both categories of abnormal glucose metabolism, are now collectively referred to as prediabetes (Ford *et al.* 2010). Prediabetes occurs when glucose values intermediate between normal and diabetes, with IGT indicated by a blood glucose value of 140-200 $mg.dL^{-1}$ after a 2-h oral glucose test, and IFG defined by a blood glucose concentration of 100-125 $mg.dL^{-1}$ after an overnight fast (Ford *et al.* 2010). Prediabetes is estimated to affect at least 200 million individuals worldwide (Zimmet *et al.* 2001); which includes 41 million adult Americans aged 40-74 years. This condition dramatically increases the risk for progression to clinical diabetes, with approximately 40% of these individuals progressing to diabetes over a 5-10 year period (Zimmet *et al.* 2001). Furthermore, these individuals also have a heightened risk of macrovascular disease. The emerging epidemic of diabetes is thus contributing to a worldwide cardiovascular public health crisis.

1.6 Treatment and management of diabetes

A considerable amount of resources have been devoted to the development of therapies for the treatment and management of diabetes. Exercise prescription is a well-established, although relatively underutilised strategy that in addition to dietary modification and medication is central in the treatment and management of T2DM (Zanuso *et al.* 2010). Management of cardiovascular risk factors is paramount among individuals with T2DM considering CVD is the leading cause of morbidity and mortality within this group. The aim of treatment for T2DM is to prevent or to slow the progression of the disease and the development of the micro- and

macro-vascular complications associated with this condition to which these patients are more susceptible (O'Hagan *et al.* 2013).

The first mode of treatment is usually diet and exercise intervention, as weight control and management are imperative in reducing the development and/or worsening of impaired glucose tolerance. Failing that, pharmacotherapy can be used in a bid to prevent the disease from progressing further with the concomitant development of other metabolic and physiological complications (Russell-Jones, 2008). Most commonly used anti-diabetic treatments include insulin, insulin sensitisers, insulin secretagogues and modulators of hepatic glucose production, of which some are outlined below (Haslam, 2010). These drugs collectively serve to improve insulin sensitivity of adipose, liver and muscle tissue (Colagiuri, 2010). Figure 1.2 displays the pharmacological treatment of hyperglycaemia according to site of action.

- ***Thiazolidinediones*** are drugs which primarily serve to enhance insulin sensitivity. In addition, they enhance vascular function, via serving to decrease macrophage and smooth muscle cell activation, proliferation, and mitigation, and reduce plague formation thereby, enhancing the dyslipidaemia and inflammatory situation which accompanies T2DM (Reusch *et al.* 2003).
- ***Metformin*** is a highly effective anti-hyperglycaemic drug which works independently of the pancreas and serves to inhibit gluconeogenesis whilst increasing tissue sensitivity to insulin mediated glucose transport (Haslam, 2012).
- ***Sulfonylurea derivatives*** are drugs which act by closing pancreatic cell potassium channels, thus, leading to enhanced insulin secretion. Sulfonylureas are frequently used in conjunction with metformin to increase pancreatic β-cell insulin secretion in a glucose-independent manner (Niswender, 2010).

However, weight gain can be a major resultant downfall of these drugs; specifically insulin, sulphonylurea derivatives and thiazolidinediones, which can be especially problematic in some individuals as it simply further aggravates the condition by increasing insulin resistance (Colagiuri, 2010). There are however, new drugs emerging that are weight neutral such as GLP-1 analogues and DPP-4. These serve to improve β-cell function and in addition, control glycaemia by stimulating glucose dependent insulin secretion, which in turn decreases glucagon secretion and inhibits gastric emptying (Haslam, 2010).

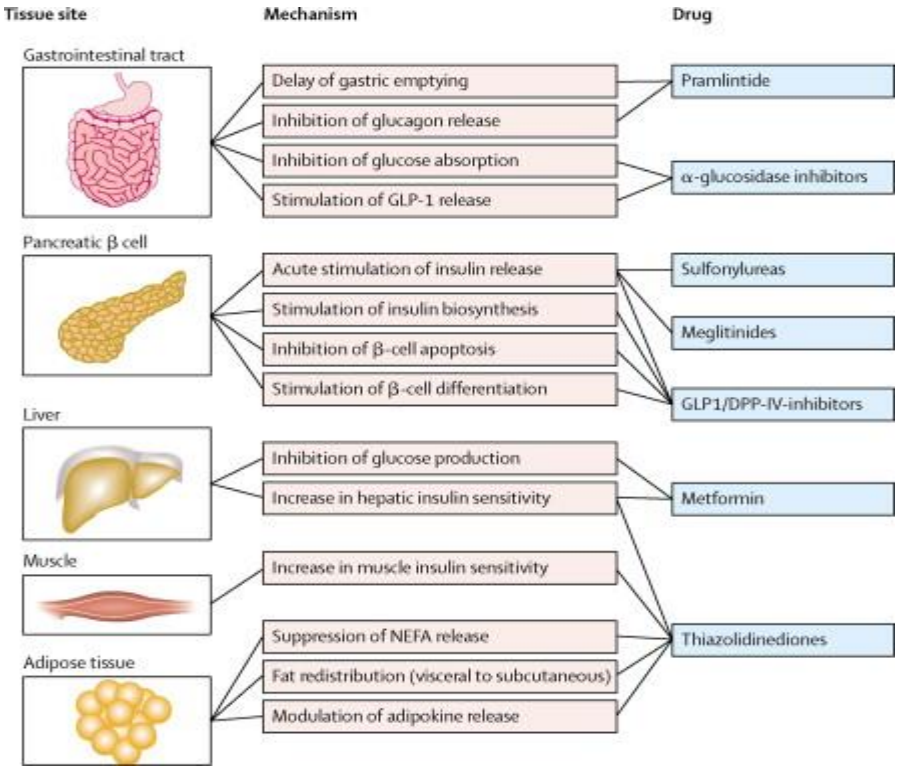

Figure 1.2. ***Pharmacological treatment of hyperglycaemia according to site of action. GLP1=glucagon-like peptide 1. DPP-IV=dipeptidyl peptidase IV.*** (Stumvoll *et al.* 2005).

Gaining control over blood glucose levels, lipid levels, and blood pressure has been identified as the primary intervention for reducing risk of CVD (Chudyk & Petrella, 2011). Although both intense blood pressure and blood glucose-lowering therapy have been shown to reduce microvascular complications in T2DM (Stratton *et al.* 2006; UK Prospective Diabetes Study Group 33 (UKPDS33), 1998), enhanced glycaemic control through the use of anti-diabetic drugs, or exogenous insulin therapy does not necessarily prevent the number of cardiovascular events (UKPDS33, 1998). Further reductions in HbA_{1c} levels and blood pressure are needed to improve long-term cardiovascular outcome (Praet & van Loon, 2007).

It is widely accepted that regular exercise contributes to glycaemic control (Boule *et al.* 2003), insulin sensitivity (Houmard *et al.* 2004) and reduced cardiovascular morbidity and mortality by improving dyslipidaemia (Balducci *et al.* 2004), hypertension (Balducci *et al.* 2004), body composition (Snowling & Hopkins, 2006) and cardiorespiratory fitness (Boule *et al.* 2004). These effects are apparent not only in the general population but also in individuals with T2DM (Wei *et al.* 2000; Hu *et al.* 2001). However, it has been proposed that the beneficial effects of exercise training on these traditional cardiovascular risk factors merely explain approximately half of the risk reduction associated with exercise (Mora *et al.* 2007). There is increasing

evidence to suggest that the additional unexplained beneficial effects may be attributable, in part, to the protective effects of exercise against disruption of vascular homeostasis, in particular, the improvements observed in endothelial function (da Silva *et al.* 2012; Weston *et al.* 2014). Such improvements can occur independently of changes in cardiovascular risk factors (Green *et al.* 2003).

It is therefore of great relevance that several studies have demonstrated both the efficacy of physical exercise in achieving a significant decrease in HbA_{1c} and the clinically relevant improvement of glycaemic control in those with T2DM (Sanz *et al.* 2010). For each 1% increase in the level of HbA_{1c} the relative risk of CVD increases by 1.18% (Selvin *et al.* 2005), whereas each 1% decrease in HbA_{1c} levels is associated with a 37% reduction in microvascular complications and a 14% reduction in myocardial infarctions (UKPDS33, 1998). Further lowering HbA_{1c} in patients with T2DM decreases the risk of developing coronary heart disease by 5-17% and all-cause mortality by 6-15% (ten Brinke *et al.* 2008). In addition, exercise training represents the only interventional strategy that has consistently been shown to improve whole body and skeletal muscle oxidative capacity (Toledeo *et al.* 2008).

However, defects in functional exercise capacity in individuals with T2DM have been consistently observed, with these individuals displaying an impaired submaximal and maximal exercise performance that is independent of obesity and present in the absence of clinically apparent cardiovascular disease (Schneider *et al,* 1984; Regensteiner *et al*. 1995, 2009; Brandenburg *et al.* 1999; Kjaer *et al.* 1999; Bauer *et al*, 2007; Gusso *et al.* 2008; Nadeau *et al.* 2009; MacAnaney *et al.* 2011a; Wilkerson *et al.* 2011). Additionally, these individuals consistently report greater levels of perceived exertion whilst exercising compared to individuals without T2DM (ND). Subsequently this could contribute to the perception of daily routine physical activity as being more difficult, ultimately encouraging a more sedentary behaviour which is associated with a worsening of cardiovascular outcomes and predicts mortality in individuals with T2DM (Huebschmann *et* al. 2009, 2015; O'Connor *et al.* 2012).

1.7 Exercise Intolerance

Physical fitness defined as exercise capacity, is a well-established independent predictor of cardiovascular and all-cause mortality among healthy individuals (Brandenburg *et al.* 1999; Fang *et al.* 2005b; Nadeau *et al.* 2009; Kokkinos *et al.* 2009; Reusch *et al*. 2013), and individuals with diabetes and/or cardiovascular disease (Kokkinos *et al.* 2009). Maximal oxygen consumption, more commonly referred to as $\dot{V}O_{2max}$, represents the functional limit of the body's ability to deliver and extract oxygen to meet the metabolic demands of vigorous

exercise (Hawkins & Wiswell, 2003). In recent decades, $\dot{V}O_{2max}$ has gained increased recognition in clinical settings and has become the accepted gold standard measure of cardiovascular fitness and exercise capacity in healthy populations (Fletcher *et al.* 2001).

$\dot{V}O_{2max}$ declines ~10% per decade in healthy individuals from the 40th year (Hawkins & Wiswell, 2003), accelerating to more than 20% per decade from the 70th year (Fleg *et al.* 2005). The age associated reduction in $\dot{V}O_{2max}$ is well documented and is influenced by several factors, some of which are modifiable (e.g. physical activity levels, changes of body composition) and others which are not; e.g. maximal heart rate (Huebschmann *et al.* 2011), which declines at a rate of 3–5% per decade, regardless of exercise training or sex demonstrating a 'primary aging' effect (Hawkins & Wiswell, 2003). Other factors thought to be instrumental in the age associated reduction in $\dot{V}O_{2max}$ are reduced muscle O_2 supply (Proctor *et al.* 1998), capillarity (Russell *et al.* 2003), endothelial function (Mueller-Delp *et al.* 2002), O_2 diffusing capacity (Hepple *et al*, 2003) mitochondrial enzyme activity (Wilkerson *et al.* 2011) and less lean body mass, with an increase in adiposity (Proctor & Joyner, 1997).

In populations presenting with diseases, often $\dot{V}O_2$ "peak" ($\dot{V}O_{2peak}$) values are reported (i.e. the highest values measured during maximum graded exercise tests). Both adolescents and adults with type 2 diabetes demonstrate a ~20% reduction in $\dot{V}O_{2peak}$, as compared to healthy controls, even when matched for age, sex, and physical activity levels (Regensteiner *et al.* 1998; Brandenburg *et al.* 1999; Nadeau *et al.* 2009; Wilkerson *et al.* 2011), and display a decreased $\dot{V}O_{2peak}$ compared to similarly obese or sedentary subjects (Brandenburg *et al.* 1999; Bauer *et al.* 2007; Nadeau *et al.* 2009; Reusch *et al.* 2013). Whilst the precise mechanisms for the abnormal maximal exercise response observed in T2DM remain to be elucidated, both central and peripheral factors have been identified as potential contributors. Left ventricular dysfunction (Fang *et al.* 2005a); impaired stroke volume reserve (Joshi *et al.* 2009); diastolic abnormalities (Poirier *et al.* 2000), reduced peak heart rate (Green *et al.* 2015) and reduced cardiac output (CO) (Roy *et al.* 1989) are indicative of perturbations in cardiac function in T2DM in response to exercise. However, findings regarding peak cardiac output responses in T2DM are somewhat contradictory. Roy *et al.* (1989) demonstrated a compromised peak CO in individuals with T2DM however, these patients had more advanced T2DM with confirmed cardiac autonomic dysfunction. On the contrary, Regensteiner *et al.* (2009) observed no differences in CO responses at rest, nor at peak incremental exercise in premenopausal women with uncomplicated T2DM when measured by direct Fick or thermodilution methods (Regensteiner *et al.* 2009).

In addition to the impairments in maximal exercise performance described above, abnormalities in submaximal performance have also been demonstrated in individuals with T2DM (Regensteiner *et al.* 1998; Brassard *et al.* 2006; Nadeau *et al.* 2009). These include a blunting of the usual rise in $\dot{V}O_2$ and heart rate (HR) per workload increment, as well as an abnormally slowed increase of oxygen uptake ($\dot{V}O_2$ kinetics) after the onset of exercise (Regensteiner *et al.* 1995, 1998; Brassard *et al.* 2006; Nadeau *et al.* 2009).

1.8 Impairments in submaximal oxygen uptake and blood flow responses

1.8.1 Impairments in $\dot{V}O_2$ kinetics

The mitochondria play a central role in energy production and cellular metabolism. Adenosine triphosphate (ATP) is the sole energy provider to human skeletal muscle, consisting of a nucleotide, adenosine, bonded to three phosphate groups. At the onset of exercise there is an immediate increase in ATP utilisation in muscle to support the cellular events associated with muscle contraction. A small intramuscular ATP store (~5 $mM.kg^{-1}$ wet weight) can provide energy to sustain a few seconds work. However these intramuscular ATP stores dwindle rapidly and thus the rate of ATP hydrolysis must be balanced by an equivalent of ATP synthesis if physical activity is to be sustained (Whipp & Mahler, 1980).

ATP production occurs via phosphorylations within the cytosol. Firstly, the Phosphocreatine (PCr)-creatine kinase (CK) system hydrolyses phosphocreatine (PCr). The PCr-CK system corresponds to high-power and low capacity ATP**.** Upon depletion of PCr, ATP levels drop significantly, necessitating further ATP generation via glycolysis; the metabolic process by which glycogen and glucose are metabolised to pyruvate and subsequently to lactate. Although glycolysis corresponds to a lower power but a higher capacity for ATP generation than that of the PCr-CK system, both of these 'substrate-level' phosphorylation routes of energy are finite, and subsequently ATP must be resynthesized from adenosine di-phosphate (ADP) and inorganic phosphate (Pi) at a rate proportional to its hydrolysis to maintain the energy balance of the cell (Whipp & Mahler, 1980; Whipp, 1987).

This resynthesis of ATP is highly dependent on oxygen and substrate availability, and is facilitated by the mitochondrial oxidative phosphorylation system. Herein, ATP is regenerated from ADP and inorganic phosphate (Pi) in association with the transfer of electrons from fuel molecules (pyruvate, free fatty acids, amino acids, glucose and glycogen) to coenzymes and finally to O_2 (Whipp & Mahler, 1980). The overall reaction sequence describing oxidative phosphorylation is;

$$½O_2 + NADH + H^+ + 3ADP + 3\ Pi \rightarrow NAD^+ + 3ATP + H_2O$$

Whilst during the early stages of exercise, oxidative phosphorylation in the mitochondria increases to meet the energy demand (as a result of increases in [ADP] and [Pi]); for sustained activity, ATP concentration is highly dependent on oxygen and substrate availability. Through the utilisation of atmospheric oxygen (O_2) and the potential energy from exogenous macronutrients; essentially, free fatty acids, glucose, and glycogen this energy source is afforded. The oxidative phosphorylation system has a very high capacity for ATP generation but a low power in comparison to the two aforementioned ATP production systems. Thus, mitochondrial oxidative phosphorylation provides up to 90% of cellular ATP, with the balance coming from substrate-level phosphorylation (Hawley & Lessard, 2007).

Oxidative metabolism is the principal means by which humans can generate energy to perform muscular work lasting more than a few minutes. Given the essential function of the mitochondrion in aerobic metabolism, mitochondria are intuitively of interest in regard to the pathophysiology of diabetes. Hence, understanding the dynamic oxygen uptake response to a bout of exercise is imperative to determine the mechanistic basis of exercise tolerance in humans, especially in diseased populations. $\dot{V}O_2$ kinetics is a marker of the ability to oxygenate the body upon exercise initiation at submaximal levels of exertion (Whipp *et al.* 1982). It describes the time course of the exponential rise of $\dot{V}O_2$ after the onset of exercise until the achievement of steady-state oxygen uptake. It is a measure of the rate at which the cardiovascular system is capable of delivering oxygen supply to skeletal muscle and the rate at which oxygen is consumed in the skeletal muscle during exercise. The observation that muscle and pulmonary O_2 uptake ($\dot{V}O_2$) increase approximately exponentially to a new steady-state after an abrupt increase in exercise which is below the lactate threshold has long been established (Whipp & Mahler, 1980), with pulmonary $\dot{V}O_2$ being used as a proxy of muscle $\dot{V}O_2$ kinetics. However, factors such as training status, age, and pathological conditions can alter the $\dot{V}O_2$ kinetics response at the onset of exercise (Xu & Rhodes, 1999). The speed at which the $\dot{V}O_2$ kinetics increase in response to an elevated muscle demand provides a unique window into understanding metabolic control (Poole *et al.* 2008a), as it determines the size of the oxygen deficit and thus affects cellular energetics, intracellular homeostasis and substrate utilisation profile (Behnke *et al.* 2001).

At the onset of constant-load exercise, adenosine triphosphate (ATP) demand increases instantaneously to meet the increased metabolic demand, however, there is some delay in matching the ATP supply required from oxidative phosphorylation. Pulmonary $\dot{V}O_2$ follows a

finite kinetic response (Linnarson, 1974; Whipp & Mahler, 1980; Barstow *et al.* 1994), for which three phases to the response of $\dot{V}O_2$ from rest to moderate-intensity (sub-lactate threshold) constant-load exercise have been identified. The response is characterised by an early time delay-like phase (Phase I) which is usually completed within the first 15-25 s of exercise (Xu & Rhodes, 1999), and precedes the monoexponential increase (Phase II), with a time constant (τ) of approximately 35 s, to achieve a steady-state $\dot{V}O_2$ (Phase III) within ~3 min in healthy individuals (Behnke *et al.* 2001; Hughson *et al*. 2001; Grassi, 2003).

These three phases of the $\dot{V}O_2$ on-response (depicted in Figure 1.3) reflect the underlying physiology of the transient phase. Phase I, also known as the cardiodynamic phase, simply reflects the circulatory transit delay from the active muscle to the lungs, with changes in the pulmonary $\dot{V}O_2$ kinetics occurring as a result of an increase in the pulmonary blood flow that does not reflect the increase in oxygen extraction from the active muscles (Whipp & Mahler, 1980). It is suggested that the increase observed in $\dot{V}O_2$ in this phase is attributed principally to an augmented cardiac output and thus pulmonary blood flow (Linnarsson, 1974; Whipp, 1987), with smaller contributions likely arising from changes in lung gas stores and mixed venous oxygen content (Casaburi *et al.* 1989).

The phase II response also referred to as the primary phase, reflects the influence of muscle metabolic change on $\dot{V}O_2$ measured at the mouth (Xu & Rhodes, 1999), with the rate of adjustment of the primary component of pulmonary oxygen uptake being considered a close reflection (±10%) of the adjustments of oxidative metabolism at the skeletal muscle level (Barstow & Molé, 1987; Grassi *et al.* 1996; Rossiter *et al.* 1999; Behnke *et al.* 2002a). Following a transit delay from the exercising muscles, this phase is initiated by the arrival of venous blood at the lung with lower oxygen content from the exercising muscle, and increased $\dot{V}O_2$ in this phase represents augmented oxygen extraction and any continued increase of pulmonary flow (Gaesser & Poole, 1996).

Phase III, reached after ~3 minutes, reflects steady-state $\dot{V}O_2$ levels below the lactate threshold (Behnke *et al.* 2001; Hughson *et al.* 2001; Grassi, 2003). The linear increase in $\dot{V}O_2$ with work rate has been observed in this phase, with a gain of 9 to 11 ml $O_2 . W^{-1} . min^{-1}$ during moderate exercise (Whipp, 1987; Whipp & Ward, 1990; Barstow *et al.* 1993; Gaesser & Poole, 1996).

In addition, the emergence of a "slow component" when exercise is performed at a work rate greater than that elicited at the lactate threshold (LT) should be acknowledged. Although the $\dot{V}O_2$ in Phase II still increases exponentially (Barstow & Mole, 1991; Paterson & Whipp, 1991), an additional component is developed slowly after ~3 min of exercise (Barstow & Mole, 1991;

Barstow, 1994; Gaesser & Poole, 1996), which depending on the exercise intensity, either delays the attainment of a steady-state $\dot{V}O_2$ causing it to increase progressively and stabilise within ~20 mins (Gaesser & Poole, 1996), or drives the $\dot{V}O_2$ to the maximum level (Poole *et al.* 1988).

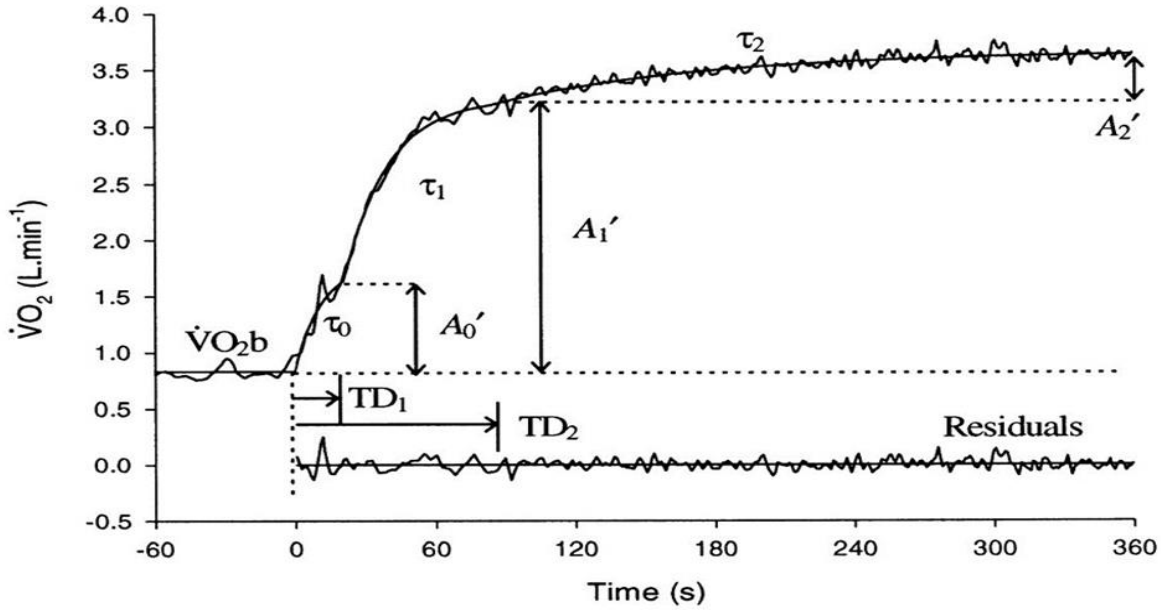

Figure 1.3. ***Schematic illustration of the exponential model that quantifies the dynamic response characteristics of $\dot{V}O_2$ kinetics from the onset of moderate- and heavy-intensity exercise. The three-exponential terms, each with a τ (time constant); TD, (time delay) and A, (amplitude), correspond to the Phase I, Phase II and slow component portions of the response. Subscript numbers represent the specific phase. $\dot{V}O_2$b is the baseline $\dot{V}O_2$ value. A_1 corresponds to the amplitude (above baseline) that Phase II $\dot{V}O_2$ attains at the onset of the slow component and A_2 is the difference between this value and the value at end-exercise.*** **(Adapted from Burnley *et al.* 2000).**

With regards to the precise kinetic profile of this response and the associated control mechanism for exercise, $\dot{V}O_2$ kinetics is still a contentious issue for which two opposing hypotheses have been proposed. The first concept attributes the finite kinetics of $\dot{V}O_2$ adjustment during on-transitions to the capacity of muscle O_2 utilisation determining the limit for the $\dot{V}O_2$ responses, independent of blood flow and delivery (Whipp & Mahler, 1980; Barstow *et al.* 1990, 1994b; Rossiter *et al.* 1999). Often referred to as 'metabolic inertia', it is thought to be a resistance within the mitochondrial oxidative enzyme system adjusting to the new metabolic requirement. The second concept suggests that the main limiting factor resides in the finite kinetics of O_2 delivery to the mitochondria of the active muscles (Hughson & Morissey, 1982; Hughson *et al.* 1993, 1996; Tschakovsky & Hughson, 1999). It is possible however, that either of these scenarios could be correct under different experimental conditions (Hughson *et al.* 2001).

These two opposing concepts continue to be debated. Consistent with the view whereby $\dot{V}O_2$ kinetics are fundamentally determined by the inertia of intramuscular metabolic processes, Rossiter *et al.* (2002) have shown similar kinetics for the rate of muscle phosphocreatine (PCr) depletion and the simultaneously measured increase in pulmonary $\dot{V}O_2$ kinetics at the onset of moderate-intensity exercise. Additional components of further resistance to the increase of $\dot{V}O_2$ have also been proposed, with the activation kinetics of key enzymes involved in oxidative metabolism being implicated (Jones & Poole, 2005). Nitric oxide (NO) production for example, is thought to compete with O_2 for the binding site of cytochrome-c oxidase (Jones *et al.* 2003b; Wilkerson *et al.* 2004b), exerting an inhibitory influence on mitochondrial respiration following the onset of exercise (Jones & Poole, 2005). Relieving this, with the inhibition of nitric oxide synthase (NOS), the enzyme responsible for the synthesis of NO; has been shown to result in a speeding of the primary phase of pulmonary kinetics during both moderate- (Jones *et al.* 2003b; 2004) and heavy-intensity exercise (Wilkerson *et al.* 2004b.) Additionally, increasing O_2 delivery to the exercising muscle by breathing hyperoxic gas mixtures or increasing muscle perfusion during moderate-intensity upright exercise did not result in a significant increase in speed of $\dot{V}O_2$ kinetics (Scheuermann & Barstow, 2003), further inferring that substrate availability does not represent a principal limitation to $\dot{V}O_2$ kinetics in healthy humans (Jones & Poole, 2005).

In contrast to this, the approximately linear relationship in steady-state reported between metabolic rate ($\dot{V}O_2$) and heart rate, cardiac output, and muscle blood flow would infer that the oxygen utilisation theory requires a pre-anticipated oxygen demand if the O_2 supply is to precisely match, or exceed, the demands placed upon it by the active muscles (Hughson *et al.* 2001). The achievement of such requires particular cardiovascular control, which can only be accomplished by superimposing feedback regulation on top of feed forward actions associated with muscle activation (Hughson *et al.* 2001). That skeletal muscle blood flow has a time course for its adaptive response similar to that of $\dot{V}O_2$ is strong evidence for this proposal (Hughson *et al*, 1996; Tschakovsky & Hughson, 1999). Studies have shown that under conditions in which O_2 delivery to the exercising muscles is less than optimal; either by altering blood flow or reducing arterial O_2 content, $\dot{V}O_2$ kinetics becomes slowed, suggesting that O_2 delivery can indeed modify $\dot{V}O_2$ kinetics (Scheuermann & Barstow, 2003).

However, regulation by a single limiting factor appears unlikely when all elements capable of influencing metabolic transitions are considered (Grassi, 2001). Instead it has been proposed that these conflicting 'inertia' concepts are actually more likely to interact in order to determine the adaptation of muscle aerobic metabolism at exercise onset under a number of common

exercise conditions (Tschakovsky & Hughson, 1999). Such interactions between O_2 utilisation and delivery are thought to be dependent on a variety of factors and to assist in distinguishing their relative roles, a "tipping point" hypothesis has been proposed (Jones & Poole, 2005). According to this hypothesis, with normal on-kinetics for O_2 delivery, $\dot{V}O_2$ on-kinetics is independent of the delivery rate. In this situation, the primary limiting factor is the O_2 utilisation rate, and further speeding of the O_2 delivery on-kinetics has no influence on the $\dot{V}O_2$ on-kinetics (Poole *et al.* 2005). However, when the O_2 delivery rate is reduced sufficiently, the hypothesis then predicts that $\dot{V}O_2$ on-kinetics will show an equitably slower response that is dependent on the O_2 delivery on-kinetics (Goodwin *et al.* 2012). It is therefore suggested that either or both of these concepts could limit $\dot{V}O_2$ on-kinetics *in vivo* (Spires *et al.*2013). The increase in muscle and pulmonary $\dot{V}O_2$ following the onset of exercise should therefore be considered to be dependent upon a complex integration of cardiovascular and metabolic factors that regulate the supply and utilisation of O_2 (Jones & Poole, 2005). Resolution of this issue is essential to understanding metabolic control in health and also for defining the mechanistic bases for the impaired $\dot{V}O_2$ kinetics found in aging (Xu & Rhodes, 1999) and in some patient populations (Poole *et al.* 2008a).

In fact, young and middle-aged individuals with T2DM have consistently demonstrated a profoundly slowed $\dot{V}O_2$ kinetics at the onset of exercise (Regensteiner *et al.* 1998; Brandenburg *et al.*1999; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012). However, this does not appear to be the case in older individuals, specifically older males. Wilkerson *et al.* (2011), observed similar $\dot{V}O_2$ kinetics responses in older males with T2DM to their age-matched healthy controls (65 ± 5 *vs.* 62 ± 6 yr). The authors attributed this finding to the long duration of T2DM (9.3 ± 3.8 yr) and the subsequent gradual development of adaptive responses in the O_2 extraction capabilities of skeletal muscle. Similarly, O'Connor *et al.* (2015) reported findings akin to those of the primary phase $\dot{V}O_2$ responses of Wilkerson *et al.* (2011) (41.1 ± 8.5 *vs.* 40.5 ± 7.8 s) in a group of older aged males (64 ± 3 *vs.* 64 ± 2 yr), with and without T2DM respectively. However, disease duration in this group was substantially less (4.1 ± 2.7 yr) than of those in the investigation by Wilkerson *et al.* (2011), thus advocating advanced age rather than disease duration as the primary influencing factor on the $\dot{V}O_2$ kinetics responses demonstrated during submaximal exercise in older individuals with T2DM.

Slowed $\dot{V}O_2$ kinetics are generally represented by a prolonged time constant of the primary phase $\dot{V}O_2$ kinetics (Huebschmann e*t al.* 2011) and mandate that individuals with T2DM will exhibit an increased oxygen deficit, thus placing greater reliance on immediate energy sources from glycogenolysis and substrate-level phosphorylation (Jones & Poole, 2005) to provide ATP

in sufficient amounts to sustain any given activity. This prolonged time constant is indicative of poorer cardiorespiratory adjustment due to either the aforementioned factors that limit the capacity of oxygen delivery to active muscles, and/or factors related to oxygen extraction and utilisation by active muscles (DeLorey *et al.* 2007). Clinically these findings are significant as they suggest a greater perturbation of intramuscular homeostasis with a reduction in [phosphocreatine, PCr], an increase in [adenosine diphosphate]$_{free}$ ([ADP]$_{free}$) and [inorganic phosphate] [Pi] (Poole *et al.* 2008a) in response to any exercise challenge, with the potential to contribute to premature muscular fatigue (Scheuermann-Freestone *et al.* 2003) and ultimately the reduced exercise intolerance frequently present in individuals with T2DM (Regensteiner *et al.* 1995, 1998). However, the precise mechanisms of such impairments responsible for the abnormal exercise response in T2DM remain to be elucidated.

Such profound impairments of the $\dot{V}O_2$ on-kinetics response as demonstrated in T2DM have been interpreted as being consistent with a decrease in oxygen delivery and decreased cardiac function, inferring that individuals with T2DM have a reduced rate of circulatory adjustment to an increase in workload (Regensteiner, 2004). Regensteiner *et al.* (1998) observed a slowed $\dot{V}O_2$ kinetics response in women with T2DM that correlated with a slowed heart rate kinetics response. On the other hand, others observed a normal response of CO during submaximal cycling in adult women with uncomplicated T2DM (MacAnaney *et al.* 2011a). O'Connor *et al.* (2012) further extended these findings showing normal absolute levels and rates of adjustment of CO in both men and women with T2DM during steady-state cycling, thereby suggesting that peripheral impairments could undermine blood flow and oxygen delivery to the skeletal muscle, thus contributing to the exercise dysfunction consistently observed within this group.

1.8.2 Impairments in blood flow responses and its mechanisms

Traditionally, the interpretation of whole-body physiological responses such as CO, pulmonary gas exchange, or fractional O_2 extraction (arteriovenous oxygen difference (a-vO_2 diff)) were accepted to be representative across body compartments. However, on the contrary, the advent of more recent technological advances has instead revealed a notable heterogeneity within the O_2 transport system from mouth to muscle to mitochondria (Koga *et al.* 2014). Whilst although some of these techniques are only applicable in animal models; such as phosphorescence quenching, and muscle intravital microscopy, they nonetheless facilitate a mechanistic interpretation of findings, by revealing what is in fact physiologically possible.

Given that both whole body and regional "fuel homeostasis" are linked by the cardiovascular system, several blood flow measurement techniques have been utilised to assess blood flow in

humans both at rest and during exercise. These include; electromagnetic flow meters (Cronestrand, 1970), indicator dilution methods (Jorfeldt & Wahren, 1971; Andersen & Saltin, 1985); namely, thermodilution and indocyanine green dye infusion, magnetic resonance imaging (MRI) (Hundley *et al.* 1996) and venous occlusion plethysmography (Joyner *et al.* 2001).

Doppler ultrasound and thermodilution are the most widely used techniques to measure muscle blood flow (Andersen & Saltin, 1985; Rådegran & Saltin, 1998; Gonzalez-Alonso *et al.* 2006; Green *et al.* 2011). The use of Doppler ultrasound has become an attractive tool to measure blood flow in specific conduit arteries in different exercise models such as the femoral artery during leg-extension exercise (Rådegran, 1997; Osada, 2004; Walther *et al.* 2006), the brachial artery during and following dynamic handgrip exercise (Tschakovsky *et al.* 1995; Shoemaker *et al.* 1996; Hughson *et al.* 1996), and the popliteal artery in calf plantar-flexion exercise (Labropoulos *et al.* 1998; Villar & Hughson, 2013) due to the non-invasive nature of its application. On the contrary, thermodilution is invasive requiring the administration of an infusate, which in turn is diluted by blood, with a corresponding change in temperature in proportion to blood flow (Casey *et al.* 2008). This technique has been used to measure perfusion in muscle both at rest and during maximal exercise (Andersen & Saltin, 1985).

These measurements of blood flow are indeed representative of systemic perfusive O_2 conductance and do not necessarily assess the transient physiological responses at the level of the microvasculature. However, local assessments of O_2 saturation and muscle oxidative capacity provide further mechanistic insights, with local estimates of blood flow traditionally been made using radioactive isotope clearance (e.g. ^{133}xenon clearance) (Lassen *et al.* 1964), and microdialysis (Rådegran *et al.* 1998). In addition, newer imaging techniques such as magnetic resonance imaging (MRI) or positron emission tomography (PET) **(**Ament *et al.* 1998; Richardson *et al.* 2001; Koga *et al.* 2014) have been adapted to study blood flow distribution in humans during small muscle mass exercise (Ament *et al.* 1998; Heinonen *et al.* 2011; 2012). Unfortunately, the application of MRI and PET demands sophisticated and expensive equipment and analysis procedures, and are thus not a feasible option in many investigations. Furthermore, these two techniques are more suitable for the assessment of steady-state responses. However, near infrared spectroscopy (NIRS) is a more affordable, non-invasive imaging technique that can be utilised to continuously monitor and quantify the oxygenation status of human tissue. Similar to phosphorescence quenching, NIRS provides a high-temporal fidelity insight into the dynamics of muscle microvascular oxy/deoxygenation across rest-exercise transitions (Heinonen *et al.* 2015), facilitating a quantitative assessment of O_2 delivery

and utilisation in the myocytes and microvessels expressed as muscle deoxygenation ([HHb+Mb]). Subsequently, NIRS has been employed in studies of clinical populations whereby O_2 delivery and/or utilisation of O_2 are implicated in the disease process.

The NIRS-derived signal of skeletal muscle oxygenation (NIRS-O_2) reflective of the absorption of light by haemoglobin (Hb) and myoglobin (Mb) depending on its state of oxygenation thereby represents the balance between the oxygen supply and consumption (Boushel *et al.* 1998). NIR light absorption changes in muscle, reflect changes in oxygenation at the level of the arterioles, venules, capillaries, and intracellular sites of O_2 transport and uptake (Chance *et al.* 1992; Boushel *et al.* 2001). The oxygenation indices obtained by NIRS are the result of the balance between O_2 delivery and $\dot{V}O_2$ at the site of interrogation, and are thus considered a surrogate for O_2 extraction (Grassi *et al.* 2003), thereby facilitating the assessment of microvascular function**.** Thus, NIRS combined with measurements of total body oxygen consumption ($\dot{V}O_2$) gained through breath-by-breath gas analysis, allows quantitative measurements to be determined from skeletal muscle thereby facilitating the assessment of two major determinants of the capacity of muscles to exercise; O_2 delivery and utilisation.

Reductions in leg blood flow have been reported in patients with T2DM during steady-state submaximal exercise in the femoral artery, both during cycling (Kingwell *et al.* 2003) and knee extension exercise (Lalande *et al.* 2008), the latter being independent of CO (Lalande *et al.* 2008), suggesting a maldistribution of active muscle blood flow at the level of the microvasculature. Indications of vascular impairments were also observed in the exercising muscle of rodent models of diabetes. Behnke *et al.* (2003) observed an uncharacteristic capillary PO_2 response displaying a transient lowering of capillary PO_2 in rat models of diabetes, thereby, limiting O_2 transport from the capillary to the myocyte during the exercise bouts (Behnke *et al.* 2003). Thus, they demonstrated a transient impairment in O_2 delivery relative to muscle O_2 uptake after the onset of exercise limiting oxygen transfer and utilisation.

More recently the effect of T2DM on the dynamic response of leg blood flow (LBF) and leg vascular conductance (LVC) in middle aged females (MacAnaney *et al.* 2011b), and middle-aged males and females (Kiely *et al.* 2014) during calf plantarflexion exercise has been investigated. A novel finding by MacAnaney *et al.* (2011b) was the observation of concomitant reductions in the dynamic response of leg vascular conductance (LVC = LBF/mean arterial pressure (MAP)) and blood flow, during high intensity (70% maximum voluntary contraction (MVC)) calf plantarflexion in females with T2DM in the presence of similar MAP. This slowed LVC kinetic response was attributed to the slower rate of increase of the second growth phase

of the hyperaemic response, whilst the first growth phase remained unaffected by T2DM. This serves to reinforce the concept of impaired vasodilatory mechanisms in the contracting skeletal muscle of T2DM. Of particular interest in this study was that T2DM did not affect the end-exercise LVC values, nor phasic amplitudes of LVC at 70% MVC, thereby indicating that during bouts of high-intensity exercise which elicit near maximal blood flow, vasodilatory mechanisms are still intact in individuals with T2DM (MacAnaney *et al.* 2011b). In further support of the impaired haemodynamic responses during calf exercise at 70% MVC in T2DM, Kiely *et al.* (2014) reported in a larger combined group of men and women with T2DM, a lesser amplitude of the first growth phase, in addition to a profound slowing of the second growth phase of the hyperaemic response compared with healthy controls during constant-load calf plantarflexion exercise. However, interestingly, the kinetics response was unaltered in this same group with T2DM during low-intensity bouts of calf plantarflexion (30% MVC), thus lending support to the notion that at light exercise intensities individuals with T2DM possibly utilise other pathways to facilitate the increased metabolic requirements (Copp *et al*. 2010).

Although the above discussed findings of an impaired hyperaemic response in T2DM pertain solely to submaximal exercise, they strengthen the argument for reduced O_2 delivery as the predominant source of impairment in $\dot{V}O_2$ control during submaximal exercise and subsequently in exercise capacity in T2DM. In an attempt to further strengthen the plausibility of impaired haemodynamic responses contributing to the reduced functional capacity of this clinical population, Kiely *et al.* (2014) investigated the maximal hyperaemic response and the slope of the hyperaemic response during a maximum graded calf-plantarflexion exercise. Results from this study revealed that individuals with T2DM obtained significantly lower peak forces (F_{peak}) (~15%) than their ND counterparts during isolated isometric calf plantarflexion, which interestingly were similar to the significant reductions of ~15% in $\dot{V}O_{2peak}$ values achieved during graded cycling exercise within these same participants. Furthermore, the reduction in peak force was accompanied by significant reductions in the maximal hyperaemic response. This also suggests that exercise tolerance appears to be affected by mechanisms limiting the performance of contracting muscles in isolation (Kiely *et al.* 2014), thereby reinforcing the role of peripheral factors influencing functional capacity in T2DM.

Although the mechanisms of this vascular control are still a subject of much debate, they have ultimately been attributed to the interplay and balance of locally induced vasodilators and sympathetically mediated vasoconstrictors (Van Teeffelen & Segal, 2006; Calbet *et al.* 2006), acting to either alter vascular tone, and/or the perfusion pressure across the capillary bed (Shoemaker & Hughson, 1999). Commencing with electrical signals on the endothelial cells

and smooth muscle, functional vasodilatory responses which govern the distribution and magnitude of blood flow are rapidly triggered in the vascular networks of healthy individuals, resulting in the subsequent sympathetic constriction of the proximal arterioles and concurrent dilation in distal arterioles, thus enabling the increased local metabolic demands be fulfilled (Segal, 2005). However, extensive evidence for macro- and microvascular dysfunction in T2DM exists (McVeigh *et al.* 1992; Williams *et al.*1996; Creager *et al.* 2003; Kingwell *et al.* 2003; Regensteiner *et al*. 2003), and could potentially account for, or part thereof, the impaired microvascular haemodynamic responses during exercise. When the strategic anatomical position of endothelial cells located between the circulating blood and the vessel wall is considered, functional abnormalities in either the endothelial cells themselves or their subjacent vascular smooth muscle cells will most likely negatively impact microvascular blood flow dynamics and the distribution of muscle blood flow during exercise.

The endothelium plays a central role in maintaining vascular homeostasis by synthesising and releasing potent vasodilators and vasoconstrictors, thus, modulating the reactivity of vascular smooth muscle (McVeigh *et al.* 1992). Dysfunction of the vascular endothelium commonly associated with T2DM (McVeigh *et al.* 1992; Regeinsteiner *et al.* 2003) predominantly pertains to a reduced NO availability. Substantial evidence that endothelium-dependent vasodilation is impaired in animal models (Oyama *et al.* 1986) and in humans with T2DM has emerged (McVeigh *et al.* 1992; Williams *et al.*1996; Kingwell *et al.* 2003). McVeigh *et al.* (1992) provided the first signs of direct evidence for endothelial dysfunction in T2DM through the demonstration of an impaired vasodilatory reaction of resistance vessels in the forearm in response to endothelium cell-dependent stimuli. Kingwell *et al.* (2003) further expanded these findings when examining the relationship between LBF responses in the femoral vein to endothelium-dependent vasodilation during cycling exercise. Following an intrafemoral artery infusion of acetylcholine, a significant reduction in LBF was observed in T2DM compared to that of their ND counterparts during a 25 min cycle bout and was consequently attributed to impaired endothelium-dependent vasodilation.

Impaired endothelium-dependent vasodilation is ultimately influenced by decreased production of vasodilators (NO and prostacyclin) and/or increased production of vasoconstrictors (prostanoids, angiotensin II, endothelin and noradrenalin) (Schalkwijk & Stehouwer, 2005). Clark (2008) reported that an imbalance in the vasoactive substances is evidenced in T2DM, whereby a decrease in NO is accompanied by an increase in endothelin-1, with vasodilation subsequently being blunted whilst contraction of the smooth muscle cells of the vasculature is being promoted.

The vascular smooth muscle, also considered to be a fundamental player in microvascular hyperaemic responses, primarily serves to govern the physical changes in peripheral vascular resistance. It is tightly regulated by several interacting signals from other cells, many of which act through their effects on NO and prostanoid formation in endothelial cells in a predominantly vasodilatory role in healthy vessels. In addition to the impaired endothelium- dependent vasodilation in T2DM, McVeigh *et al.* (1992) also identified smooth-muscle dysfunction through the demonstration of an impaired vasodilatory reaction of resistance vessels in the forearm in response to smooth muscle cell-dependent stimuli. The authors attributed this abnormal response to glyceryl trinitrate to a nitroglycerin tolerance in T2DM, thus speculating that the response to a direct-acting NO donor would not be attenuated. However, in contrast to this, Williams *et al.* (1996), observed no differences in blood flow responses following the intra-arterial administration of Verapamil, (a Ca^{2+} antagonist) in T2DM, suggesting that the functional integrity of the vascular smooth muscle is intact in uncomplicated T2DM. With Verapamil inducing uncompromised NO independent vasodilation in T2DM, it further implicates a defective NO pathway and resultant endothelial dysfunction as the primary causative factor for impaired blood flow responses in T2DM. Similarly, in the abovementioned Kingwell *et al.* (2003) study, further to the impaired endothelial-dependent blood flow response, the authors also reported normal responses in the vascular smooth muscle in response to exogenous sodium nitroprusside, a direct-acting smooth muscle relaxant. Furthermore, Williams *et al.* (1996) and more recently Kiely *et al.* (2014) observed no differences in reactive hyperaemia in the forearm in individuals with and without T2DM, further supporting the view of preserved vascular control in uncomplicated T2DM.

Moreover, these functional abnormalities are exacerbated by impairments in sympathetic nervous function. Research concerning the regulation of skeletal muscle microcirculatory perfusion typically pertains to vasodilatory mechanisms; however, a physiological phenomenon termed 'functional sympatholysis' also exists (Remensnyder *et al.* 1962). Although it is widely accepted that increased metabolic requirements in active tissues are facilitated through increased sympathetic vasoconstriction of non-active tissues, in areas such as the splanchnic and renal regions (Rowell, 1974), this exercise-induced increase in sympathetic discharge is also targeted to the active skeletal muscle (Ray & Mark, 1995). However, functional sympatholysis opposes the latter, over-riding the vasoconstriction of the smooth muscle cells of the metabolically active tissue, thereby serving to optimise the distribution of muscle blood flow to achieve metabolism-perfusion matching in exercise (Thomas, 2015).

With functional sympatholysis being manifest in the small distal arterioles within the microvasculature of the skeletal muscle (Van Teeffelen & Segal, 2006), through simultaneous constriction of the proximal vessels, it also regulates systemic arterial pressure. As such, a persistence of sympathetic vasoconstriction poses a great threat to this arrangement from the maldistribution of muscle blood flow, impaired muscle performance and subsequent exercise intolerance to an exaggerated pressor response to exercise.

It has been suggested that functional sympatholysis may be impaired in T2DM owing to the deficient state of the local metabolic factors and endothelium-derived substances being unable to over-ride the sympathetic potency (Creager *et al.* 2003). More recently however, Thaning *et al.* (2011) observed that in the presence of an uncompromised endothelium, the capacity to blunt sympathetic vasoconstriction during moderate-intensity exercise was intact in middle-aged individuals with T2DM. Further research however, into the involvement of this mechanism during exercise in the presence of confirmed endothelial dysfunction in individuals with T2DM is warranted.

1.9 Priming: an acute intervention aimed at increasing exercise tolerance

Interestingly, a prior bout of high-intensity, or "priming" exercise (PE), alters the time course of the $\dot{V}O_2$ kinetics response during a subsequent exercise bout. An acute bout of priming exercise manipulates $\dot{V}O_2$ kinetics, especially in diseased and aging populations (Poole *et al.* 2008a). As such, positive alterations have been observed in bouts of moderate- to severe-intensity exercise preceded by an acute bout of heavy intensity exercise, and have been attributed, at least in part, to the enhancement of the overall $\dot{V}O_2$ kinetics (Gausche *et al.*1989; Jones *et al.* 2003a; Bailey *et al.* 2009).

However, it must be acknowledged that the observed enhancement of $\dot{V}O_2$ kinetics following PE has been accomplished through a variety of manipulations of the parameters of this response. Gerbino *et al*. (1996) and MacDonald *et al.* (1997) reported an accelerated $\dot{V}O_2$ mean response time (MRT), via the application of a mono-exponential model of the entire response. However, more recently, the plausibility of these findings have been questioned through the application of more comprehensive modelling approaches, whereby the discrete phases of the response have been considered (Figure 1.3) through the application of a bi-exponential or tri-exponential model (Burnley *et al.* 2000, 2001, 2002a; Koppo & Bouckaert 2002; Sahlin *et al.* 2005). Consequently, in healthy, trained individuals (mean $\dot{V}O_{2max}$ > 45 $mL.kg^{-1}.min^{-1}$) the acceleration of the response was attributed to a reduction in the magnitude of the slow component with an increase in the primary amplitude of the response, whilst the τ of the primary

component remained unaffected (Burnley *et al.* 2000, 2001, 2002b; Koppo & Bouckaert 2002; Sahlin *et al.* 2005). It should be noted that when a limitation in the time constant of the primary phase presents as a consequence of O_2 delivery, such as when tilting the body from the upright to the supine or prone posture, thereby reducing perfusion pressure to active muscles (Rossiter *et al.* 2001; Jones *et al.* 2006), prior heavy-intensity exercise was evidenced to accelerate the rate of adaptation of the primary phase in the subsequent bout of primed exercise (Rossiter *et al.* 2001; Jones *et al.* 2006).

The mechanisms behind priming exercise remain a subject of considerable debate. To date, the putative mechanisms responsible for the alteration of the $\dot{V}O_2$ dynamic response presented in the literature include; elevated muscle temperature (Bishop *et al.* 2003; Mohr *et al.* 2004), increased blood flow (Bangsbo *et al*, 2001; Koppo & Bouckaert, 2001), enhanced muscle perfusion (Wilkerson *et al.* 2004a; DeLorey *et al.* 2007; Jones *et al.* 2008), heightened oxidative enzyme activity (Wilkerson *et al.* 2005; Gurd *et al.* 2006) and/or the augmentation of muscle fibre recruitment (Burnley *et al.* 2002b).

1.9.1 Muscle temperature

The well-established elevation in muscle temperature associated with prior exercise has been advocated as a potential contributor to the acceleration of the $\dot{V}O_2$ kinetics response subsequent to priming exercise (Bishop *et al.* 2003; Mohr *et al.* 2004), however, to date results are equivocal (Xu & Rhodes, 1999).

Through the use of hot water perfused pants, Koga *et al.* (1997) passively increased muscle temperature to ~38ºC in healthy individuals, compared to the control condition of ~35ºC, prior to completing a 6 min heavy-intensity (50% Δ; which is the sum of the PO at VT and 50% of the difference between the PO at VT and $\dot{V}O_{2max}$) exercise bout. The authors reported a small but significant reduction in the slow component of the $\dot{V}O_2$ response after raising the leg muscle temperature by approximately 3ºC compared to the control group, which was attributed to an elevated muscle temperature upon comparison of post-exercise temperatures (~40 *vs.* ~38ºC). Surprisingly, no increase in the primary $\dot{V}O_2$ amplitude was observed subsequent to the passive warming, considering increased muscle temperature would be expected to increase $\dot{V}O_2$ throughout exercise by the Q_{10} effect (i.e. the effect of temperature on the metabolic rate) (Jones *et al.* 2003a).

In contrast to the favourable results from Koga *et al.* (1997), Burnley *et al.* (2002a) reported reductions in the amplitude of the $\dot{V}O_2$ slow component during heavy-intensity exercise (Δ50%) subsequent to both 6 min prior heavy-intensity exercise (Δ50%) and prior sprint exercise (30 s

all out sprint), whilst passive warming of the lower limb to 42ºC, over a 40 min period had no distinct effect other than increasing the muscle temperature by ~2.6ºC. Similarly, Koppo *et al.* (2002) reported reductions in the amplitude of the $\dot{V}O_2$ slow component during heavy-intensity exercise (90% $\dot{V}O_{2peak}$) subsequent to 6 min prior heavy-intensity exercise (90% $\dot{V}O_{2peak}$) and not subsequent to passive warming of the lower limbs to the same temperature evoked by that of the priming exercise (~37ºC).

In light of these findings, it is reasonable to infer that increased muscle temperature resulting from prior exercise is unlikely to be responsible for the altered heavy-exercise $\dot{V}O_2$ kinetics responses observed subsequent to heavy-intensity priming exercise.

1.9.2 Improved oxygen availability and muscle perfusion

Consequent of the early work by Gausche *et al.* (1989) and Gerbino *et al.* (1996), it was originally proposed that an acidosis-mediated increase in O_2 delivery was the primary influencing factor in the observed accelerated $\dot{V}O_2$ kinetics response subsequent to a bout of heavy intensity priming exercise. The increased lactic acidosis resulted in greater perfusion and increased O_2 availability through a rightward shift of the oxyhaemoglobin dissociation curve. Additionally, the accumulation of this vasoactive metabolite further enhanced vasodilation in the active muscle thus, increasing bulk O_2 delivery. More recently, evidence has emerged questioning the role of residual acidosis in the altering of the metabolic and gas exchange responses to exercise.

In this regard, to determine the influence of systemic lactic acidosis on accelerated $\dot{V}O_2$ kinetics, an experimental model was implemented whereby different muscle groups are utilised during the 'priming' and 'primed' exercise bout respectively. This implies that if the accelerated $\dot{V}O_2$ kinetics response associated with priming is in fact consequent of the improved muscle blood flow and enhanced oxygen delivery from the residual lactic acidosis, then it would be expected that the $\dot{V}O_2$ response in a subsequent bout of exercise would be the same regardless of the type of priming exercise and muscle group utilised. This model implemented by many (Bohnert *et al.* 1998; Fukuba *et al.* 2002; Koppo *et al.* 2003) returned similar results; in the presence of similar levels of systemic lactic acidosis, prior heavy intensity exercise which 'primed' the same muscle group resulted in a greater reduction in the magnitude of the $\dot{V}O_2$ slow component. Specifically, during arm cranking exercise, despite an observed increase in the amplitude of the primary component, the primary time constant was not affected by prior primed arm cranking exercise (Fukuba *et al.* 2002; Koppo *et al.* 2003). Additionally, in contrast to the observed increase in haemoglobin concentration in the *vastus lateralis* subsequent to the priming bout of

leg exercise, enhanced leg muscle perfusion was not observed following prior arm cranking exercise (Fukuba *et al.* 2002).

Furthermore, if residual lactic acidosis played an instrumental role in the dynamic response of $\dot{V}O_2$ in primed heavy-intensity exercise, it would be reasonable to expect greater alterations in situations presenting with more highly elevated blood lactate concentrations. As such, Burnley *et al.* (2002a) investigated the association of reductions in the amplitude of the $\dot{V}O_2$ slow component and residual lactic acidosis via the manipulation of blood lactate concentrations though the use of two different priming protocols; 30 s maximal all-out cycling sprints, and 6 min constant-load cycling at Δ50%. Despite significantly higher blood lactate concentrations returned via the sprint cycle bouts (6.4 ± 0.4 mM *vs.* 3.4 ± 0.3 mM); reductions in the amplitudes of the $\dot{V}O_2$ slow component were similar. Collectively, these studies suggest that the metabolic acidosis or high blood lactate concentration commonly associated with an altered $\dot{V}O_2$ kinetics, are not directly implicated in the response.

However, extensive evidence exists associating heavy-intensity priming exercise with increased blood flow and oxygen extraction (Krustrup *et al.* 2001), increased HR (Bearden & Moffatt, 2001; Burnley *et al.* 2002a; Tordi *et al.* 2003), estimated CO (Tordi *et al.* 2003) and muscle oxygenation (Burnley *et al.* 2002a; Fukuba *et al.* 2002).

Interestingly, Murias *et al.* (2011c) examined the relationship between the adjustment of near infrared spectroscopy (NIRS)-derived muscle deoxygenation ([HHb+Mb]) to $\dot{V}O_2$ (Δ[HHb+Mb]/$\Delta\dot{V}O_2$) in young healthy individuals presenting with a range of slow to fast $\dot{V}O_2$ kinetics. The authors revealed a strong association ($r = 0.91$) between Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratios and $\dot{V}O_2$ time constants. Elevated ratios (>1) displaying a transient 'overshoot' relative to the subsequent steady-state were demonstrated in the groups with slower $\dot{V}O_2$ kinetics, which were progressively reduced as $\tau\dot{V}O_{2p}$ was reduced. The authors thereby claimed that when $\tau\dot{V}O_{2p}$ was in excess of 20 s, the rate of adjustment appeared to be predominantly constrained by the matching of local O_2 distribution to muscle O_2 uptake. Thus, it is considered that in individuals presenting with slower $\dot{V}O_2$ kinetics, the rate of adjustment is most likely curtailed by an impaired O_2 availability within the active tissues.

It is therefore reasonable to expect that if local muscle O_2 availability does indeed limit the adjustment of $\dot{V}O_2$ at the onset of exercise then subsequent reductions in $\tau\dot{V}O_{2p}$ following a bout of priming exercise should occur. In agreement with this concept, subsequent to a bout of heavy-intensity priming exercise an accelerated adjustment to oxidative metabolism was observed in both older, and young individuals who presented with $\tau\dot{V}O_2$ in excess of 20 s,

(Scheuermann *et al.* 2002; DeLorey *et al.* 2004b; Gurd *et al.* 2005, 2006; Chin *et al.* 2010; DeRoia *et al.* 2012) during moderate-intensity exercise.

Similarly, when muscle perfusion pressure was manipulated (i.e., reduced) carrying out exercise transitions in the prone and supine postures, thereby likely reducing muscle O_2 availability, similar findings were observed (Rossiter *et al.* 2001; Jones *et al.* 2006). Indeed, consequent to the compromising exercise positions adopted, individuals in these studies presented with relatively long time constants of the primary phase of the $\dot{V}O_2$ response (49 ± 6 and 38 ± 18 s respectively), and subsequent to the prior bout of PE, individuals demonstrated significantly reduced time constants of the primary component (41 ± 8 and 24 ± 9 s respectively).

In the study by Rossiter *et al.* (2001), individuals performed two consecutive bouts of high-intensity, square-wave knee extension exercise in the prone position. Significant reductions in the amplitude of the $\dot{V}O_2$ slow component, (expressed as a percentage of the total amplitude; 6.8 ± 4.9 vs. 2.7 ± 2.4 %) were accompanied by an acceleration of the primary phase of the $\dot{V}O_2$ kinetics response (49 ± 6 vs. 41 ± 8 s) in the subsequent high-intensity bout, thus speeding the overall $\dot{V}O_2$ kinetics response. Similarly, Jones *et al.* (2006) investigated the effects of PE in both the supine and upright cycling postures. The authors observed a 37% reduction (P<0.05) in the primary phase τ (38 s ± 18 s vs. 24 ± 9 s) during supine cycling subsequent to a bout of heavy-intensity priming exercise, albeit having no significant effect during upright cycling. Interestingly, prior exercise had no effect on the amplitude of the $\dot{V}O_2$ slow component (0.40 ± 0.29 *vs.* 0.41 ± 0.20 L.min^{-1}) in the supine position, despite significantly (P=0.006) reducing (0.45 ± 0.16 *vs.* 0.22 ± 0.14 L.min^{-1}) it in the upright position.

It can thus be inferred that the speeding of the primary $\dot{V}O_2$ kinetics pertains to exercise in circumstances whereby O_2 delivery is compromised, be that purposely manipulated (Rossiter *et al.* 2001; Jones *et al.* 2006), or as an indirect implication of a pathophysiological condition.

1.9.3 Increased activity of mitochondrial enzymes

It has been established that whilst exercising within the moderate-intensity domain (i.e. below ventilatory threshold (VT)), $\dot{V}O_2$ kinetics in healthy, trained individuals are limited by metabolic inertia (Grassi, 2001; Poole *et al.* 2008a). Thus, increased activation of mitochondrial oxidative enzyme activity has been identified as a potential favourable manipulator of $\dot{V}O_2$ kinetics. It is purported that subsequent to a bout of heavy-intensity PE, the transition to predominantly aerobic sources would be accelerated, consequent of the upregulation of rate-limiting oxidative enzymes.

A relationship between enzyme activation, substrate availability and O_2 uptake has been reported (Gurd *et al.* 2006). Pyruvate dehydrogenase (PDH); a mitochondrial matrix enzyme responsible for converting pyruvate to acetyl-CoA is thus, proposed as a possible site of metabolic inertia. Howlett *et al*, (1999) examined the effect of fully activating PDH prior to the onset of exercise via the administration of dichloroacetate (DCA). It was reported that the prior activation of PDH was associated with significantly lower lactate accumulation, slower PCr degradation and greater glycogen breakdown. Following a bout of PE, Gurd *et al.* (2006) observed an elevated baseline PDH compared to the control group which was associated with a decrease in the $\dot{V}O_2$ mean response time of moderate-intensity exercise. A similar trend was observed subsequent to priming in older populations (Gurd *et al.* 2009). The accelerated $\dot{V}O_2$ kinetics demonstrated in a subsequent bout of primed moderate-intensity exercise was coincident with a significantly higher pre-exercise PDH activity. As such, this should in turn reduce the dependency on substrate-level phosphorylation and thereby less PCr consumption and lactate accumulation would be evident (Gurd *et al*, 2006).

However, there is limited information regarding the effects of priming exercise on oxidative enzyme activity in heavy-intensity upright cycling exercise. Jones *et al.* (2004) examined the effect of severe intensity priming (Δ70%) on a subsequent bout of heavy-intensity cycling with and without the prior administration of DCA. The authors observed a trend (P=0.08) for a decreased amplitude of the $\dot{V}O_2$ slow component subsequent to the administration of DCA, however it did not affect the mean response time, nor the rate of adaptation of the primary $\dot{V}O_2$ kinetics response.

1.9.4 Motor Unit Recruitment

The recruitment of additional motor units has been implicated in the development of the $\dot{V}O_2$ slow component (Poole *et al.* 1994a; Whipp, 1994). Considering that priming exercise has been evidenced to reduce the slow component, with a concomitant increase in the primary amplitude of the response, this suggests that priming in some way may alter the pattern in which motor units are recruited (Burnley *et al.* 2002b). Based on this premise, Burnley *et al.* (2002b) measured surface electromyography (EMG) throughout two 6 min bouts of heavy-intensity cycling exercise separated by 12 min of passive recovery. Integrated electromyography (iEMG) was higher during the first 2 min of the primed compared with the unprimed bout but then remained unchanged for the remaining 4 min of the exercise bout. Consistent with their hypothesis, the primary $\dot{V}O_2$ amplitude and iEMG were increased by a similar magnitude in the primed exercise bout. As iEMG merely reflects overall motor unit recruitment and is unable to

differentiate between selective recruitment of motor units, the authors thus purported that increased motor unit recruitment with motor units of presumably similar contractile elements may be occurring at the onset of exercise.

This was supported in a later study by Di Menna *et al.* (2008) whereby the effects of PE on $\dot{V}O_2$ kinetics during upright work-to-work (w-to-w) severe-intensity cycle exercise in young (mean age = 31, yr), trained (mean $\dot{V}O_{2peak}$ = 45 ± 5 mL.kg^{-1}.min^{-1}) males were investigated. Despite the demonstration of a significantly lengthened $\tau\dot{V}O_{2p}$ in severe-intensity cycling transitions initiated from a moderate-intensity baseline (w-to-w), in comparison to those observed following the on-transition from an unloaded baseline (42 ± 15 and 33 ± 8 s), priming exercise had no discernible effect on the $\tau\dot{V}O_{2p}$ in the subsequent w-to-w transition (42 ± 15 and 42 ± 17 s). It did however significantly increase the amplitude of the primary phase whilst also reducing the amplitude of the $\dot{V}O_2$ slow component (0.47 ± 0.09 vs 0.27 ± 0.13 L.min^{-1}). Furthermore and of particular interest in this study, was the additional observation of the concomitant reduction in $\Delta iEMG_{(6-2)}$, such that the end-exercise value (min 6) was no longer different from the minute 2 value subsequent to PE, whereas during the unprimed w-to-w, there was a significant increase in $\Delta iEMG_{(6-2)}$, thus mirroring the effect of PE on the amplitude of the $\dot{V}O_2$ slow component.

Collectively, the above findings signify that the reduced slow component observed following priming exercise is potentially linked to altered motor unit recruitment patterns, such that the requirement for additional fibre activation as exercise proceeds and the associated $\dot{V}O_2$ cost of that activation are reduced (Burnley *et al.* 2002b; DiMenna *et al.* 2010c).

1.10 *Aims*

It is evident that the influence of T2DM on aerobic capacity is complex. Changes in $\dot{V}O_2$ kinetics at the onset of exercise may be related to O_2 utilisation, O_2 delivery or a combination of both. The use of near infrared spectroscopy (NIRS) provides an insight into the dynamic balance between regional O_2 delivery and utilisation at the level of the microvasculature. When used in association with measurements of pulmonary $\dot{V}O_2$, it facilitates the determination of the time course of local muscle O_2 extraction. As such this may in turn enable the identification of a potential key pathophysiological mechanism responsible for the reduced exercise capacity in individuals with T2DM. Surprisingly, the rate of deoxygenated haemoglobin responses during ramp exercise in T2DM have not been investigated.

Furthermore, it would appear that PE may possess the ability to augment functional exercise capacity in this clinical population. PE has been frequently evidenced to accelerate $\dot{V}O_2$

kinetics, thereby increasing the initial aerobic metabolism contribution to activity whilst consequently reducing anaerobic ATP provision. Subsequently, by reducing the initial oxygen deficit, time to exhaustion may be increased. To the knowledge of this investigator, the effects of priming exercise on dynamic responses of $\dot{V}O_2$ and/or [HHb+Mb] on subsequent exercise have not been assessed. Thus, given that muscle O_2 supply appears to be constrained in T2DM, an acute intervention (i.e. priming exercise) presenting such attributes could prove to be particularly useful in a clinical setting whilst also providing further insight into potential mechanisms implicated in the impaired $\dot{V}O_2$ kinetics response consistently demonstrated by these individuals.

Accordingly, the aims of the 4 experiments carried out in the present thesis were as follows:

- *Experiment 1* aimed to examine the influence of T2DM on the profile of muscle fractional O_2 extraction (estimated using deoxygenated haemoglobin) during ramp incremental cycle exercise.

- The aim of *Experiment 2* was to assess the influence of priming exercise on oxygen uptake and muscle deoxygenation kinetics during moderate-intensity cycle exercise in type 2 diabetes.

- *Experiment 3* aimed to investigate the influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise in type 2 diabetes; and

- The aim of *Experiment 4* was to investigate the influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise from an elevated baseline (i.e. from work-to-work transitions) in type 2 diabetes.

Chapter 2: The effect of type 2 diabetes in muscle deoxygenation during ramp incremental cycling exercise.

2.1 INTRODUCTION

Defects in functional exercise capacity in patients with T2DM have been consistently reported. Individuals with T2DM demonstrate impairments in maximal exercise capacity in the region of 20%, which are independent of obesity and present in the absence of clinically apparent cardiovascular disease (Regensteiner *et al.* 1995, 1998; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012). The ramp incremental exercise test to volitional fatigue provides an insight into exercise capacity, being a protocol that is frequently utilised to determine the maximal O_2 uptake ($\dot{V}O_{2max}$). Thus, it is representative of the integration of the pulmonary, cardiovascular, and muscular systems to uptake, transport and utilise O_2 respectively (Poole *et al.* 2008a). In addition, this protocol can provide key information on the adaptive capacity of individuals in non-steady state conditions as it requires the aerobic metabolism to adapt to the continuously changing conditions.

Oxidative metabolism is the dominant source of energy for active tissue with $\dot{V}O_{2max}$ being consequent to the product of whole-body perfusive (cardiac output [CO] x arterial O_2 concentration [CaO_2]) and diffusive (muscle transcapillary) O_2 conductance (Okushima *et al.* 2016). These physiological variables in O_2 utilisation are expressed within Fick's equation;

[$\dot{V}O_2$ = CO x arterial-venous O_2 difference (a-vO_{2diff})]

According to this equation, as the demand for O_2 in an active tissue increases, a linear relationship between whole body CO and $\dot{V}O_2$ per increment in exercise intensity develops (Richardson *et al.* 1993; Proctor *et al.* 1998), with a resultant hyperbolic response of (a-vO_{2diff}) (Richardson *et al.* 1993; Calbet *et al.* 2007). From rest to intense exercise, systemic a-vO_{2diff} increases hyperbolically from approximately 5 to 15 ml.dl^{-1} (Whipp & Ward, 1982; Wittenberg & Wittenberg, 1989; Stringer *et al.* 2005), with a concomitant increase in CO of ~5–6 L.min^{-1} for a 1 L.min^{-1} increase in $\dot{V}O_2$ (Astrand *et al.* 1964; McDonough & Danielson 1974; Proctor *et al.*1998). However, more recently, Spencer *et al.* (2012) challenged the linearity of whole body CO to whole body $\dot{V}O_2$ during ramp incremental exercise. The authors instead suggested it is in fact a linear a-vO_{2diff} – to – $\dot{V}O_2$ relationship which occurs, resonating earlier findings by Stringer *et al.* (2005) of a slower increase in CO at high work rates during ramp incremental exercise. Moreover, Ferreira *et al.* (2007), Boone *et al.* (2009) and Murias *et al.* (2013) defied the notion that systemic profiles mirror those at the level of the microvasculature subsequent to

investigation of the dynamic response of O_2 extraction within a local muscle, where upon identification of a nonlinear relationship between active muscle blood flow and muscle $\dot{V}O_2$ was revealed.

In healthy individuals $\dot{V}O_{2max}$ may be affected by both central and peripheral mechanisms. Although it is generally acknowledged that the $\dot{V}O_{2max}$ is limited primarily by the maximal cardiac output (Saltin, 1985), as demonstrated in Fick's equation, the $\dot{V}O_{2max}$ reached is in fact dependent upon both O_2 delivery and peripheral extraction and utilisation, which is ultimately influenced by the O_2 diffusing capacity of the active muscle. Suggested limiting factors which can influence the pathway of O_2 conductance from the mouth to the mitochondrion include age, sex, training status, and disease states (Poole *et al.* 2008a).

An age-associated decline in $\dot{V}O_{2max}$ has been well-established in humans with declines of ~10% per decade occurring in healthy individuals from the 40th year (Hawkins & Wiswell, 2003), accelerating to more than 20% per decade from the 70th year (Fleg *et al.* 2005). Evidence suggests that a reduced muscle blood flow (Poole *et al*. 2003) consequent to a decreased non-modifiable CO and combined with the maldistribution of the blood to the active tissues play an important role in age-related decreases in whole-body oxidative capacity.

Whilst the precise mechanisms for the aforementioned abnormal exercise response observed in T2DM remain to be elucidated, both central and peripheral factors have been identified as potential contributors, with such impairments in aerobic capacity often attributed to reductions in peak CO and [a-vO_2] (Green *et al.* 2015). Left ventricular dysfunction (Fang *et al.* 2005a); impaired stroke volume reserve (Joshi *et al.* 2009); diastolic abnormalities (Poirier *et al.* 2000), slower heart rate kinetics (Regensteiner *et al.* 1998), or reduced cardiac output (Roy *et al.* 1989) are indicative of perturbations in cardiac function in T2DM in response to exercise. Findings regarding peak CO responses in T2DM are somewhat contradictory. Roy *et al.* (1989) demonstrated a compromised peak cardiac output in individuals with T2DM with confirmed cardiac autonomic dysfunction. On the contrary, Baldi *et al.* (2003), observed reductions of approximately 30% in $\dot{V}O_{2peak}$ in middle-aged T2DM males and females, with peak CO values, obtained by the CO rebreathing method, remaining similar between the two groups. Regensteiner *et al.* (2009) also observed no difference in CO at peak exercise in premenopausal women with uncomplicated T2DM, when measured by direct Fick or thermodilution methods.

Impairments in peripheral O_2 delivery and extraction may also affect $\dot{V}O_{2max}$ in individuals with T2DM with reductions of 30% in $\dot{V}O_{2peak}$ accompanied by reductions of 19% in [a-vO_2], albeit being estimated from peak CO (Baldi, *et al.* 2003). Evidence of compromised peripheral

vascular function during maximal exercise in isolated muscles (Kiely *et al.* 2014), reductions in leg blood flow independent of CO (Lalande *et al.* 2008; MacAnaney *et al.* 2011b) and an impaired dynamic response of peripheral O_2 delivery to active muscles during isolated high-intensity constant-load exercise, have also been reported in T2DM. Consequently, a maldistribution of active muscle blood flow in individuals with T2DM ensues (MacAnaney *et al.* 2011b; Kiely *et al.* 2014).

Traditionally, the interpretations of whole-body physiological responses such as CO, pulmonary gas exchange, or fractional O2 extraction ($a\text{-}vO_{2diff}$) were accepted to be representative across body compartments. However, on the contrary, a notable heterogeneity within the O_2 transport system from mouth to muscle to mitochondria has instead been revealed (Koga *et al.* 2014). Thereby, given the Fick relationship is determined at pulmonary level, it is thus in fact representative of systemic fractional O_2 extraction (Benson *et al.* 2013) and as such, may not be reflective of the discrete adjustments that occur between oxygen delivery and metabolic demand at the level of the active muscle vasculature (Spencer *et al.* 2012; Murias *et al.* 2013; Okushima *et al.* 2016).

The use of near infra-red spectroscopy (NIRS) during exercise permits the non-invasive determination of a muscle's oxygenation index, by means of measuring the concentration changes in deoxygenated haemoglobin and myoglobin (Δ[HHb+Mb]), which is considered a surrogate for microvascular O_2 extraction (DeLorey *et al.* 2003). The NIRS-derived signal of skeletal muscle oxygenation (NIRS-O_2) reflective of the absorption of light by haemoglobin (Hb) depending upon its state of oxygenation, thereby represents the balance between the oxygen supply and consumption (Boushel *et al.* 1998). More specifically, an increased muscle oxygenation, represented by a reduced [HHb+Mb] signal would be indicative of an increased O_2 delivery with respect to $\dot{V}O_2$, whereas a decreased muscle oxygenation, represented by an increased [HHb+Mb] signal would be indicative of an increased $\dot{V}O_2$ with respect to O_2 delivery. In recent years, several investigators have utilised this technique to characterise the dynamic response of [HHb+Mb] during ramp incremental exercise (Ferreira *et al.* 2007; Boone *et al.* 2009; Gravelle *et al.* 2012). By facilitating the quantification of O_2 extraction, NIRS provides insights into the dynamic balance between regional O_2 delivery and utilisation at the level of the microvasculature (Spencer *et al.* 2012). Through NIRS, impaired capacity of O_2 extraction by skeletal muscle can be both detected and quantified, which may in turn represent a key pathophysiological mechanism responsible for significantly lower $\dot{V}O_{2peak}$ responses in clinical populations.

To examine the contribution of peripheral mechanisms in the impairment of exercise capacity and tolerance in T2DM we simultaneously investigated cardiorespiratory and microvascular responses to ramp incremental exercise. The primary aim of this study was to explore the influence of T2DM on the profile of local muscle fractional O_2 extraction, as indicated by the NIRS-derived Δ[HHb+Mb] response during ramp incremental cycle exercise. This may provide further insight into potential contributory mechanisms responsible for the lower $\dot{V}O_{2peak}$ consistently reported in this patient population.

2.2 METHODOLOGY

2.2.1 Participants

Seventeen individuals with uncomplicated T2DM (12 men/5 women), and 17 individuals without T2DM (ND) (12 men/5 women) volunteered to participate in this study (Table 2.1). Five female participants were premenopausal (2 T2DM and 3 ND) and five were postmenopausal (3 T2DM and 2 ND) not undergoing hormone replacement therapy (HRT). All participants were non-smokers and had not smoked during the 12-month period preceding the study. All of the patients with T2DM had a clinical history of diabetes of between 2 to 9.5 years (mean ± SD = 5.7 ± 3.7 years).

2.2.1.1 Recruitment of participants

2.2.1.1.1 Individuals without T2DM (ND)

Individuals without T2DM (ND) were recruited from the general population, through the placement of adverts in a number of large multinational companies based in Dublin City Centre/South Dublin area (Appendix 1). Contact details for the principal investigators were provided for any individuals interested in seeking further information. Individuals who sought further information received a copy of the participant information leaflet (PIL) (Appendix 2) detailing the nature of the study, testing involved, requirements for participation, as well as the potential risks and benefits associated with participation. Participants were given seven days for reflection following provision of the study information. A private meeting was offered to each potential participant to discuss the contents of the PIL and all elements of the study. Upon fulfilling the inclusion criteria (*see 2.2.1.2.2*) and having had any questions or queries surrounding any aspect of the protocol or study answered by the principal investigators, participants then signed the informed consent form (Appendix 2).

2.2.1.1.2 Individuals with type 2 diabetes (T2DM)

All participants with T2DM were recruited from the Diabetes Outpatient Clinics of St. Columcille's Hospital (Louglinstown, Co. Dublin) and St. Vincent's University Hospital (SVUH, Dublin 4). Individuals with T2DM who fulfilled the inclusion criteria (*see 2.2.1.2.1*) were initially identified during chart review by a member of the medical team in accordance with hospital regulations. Eligible patients were presented with a study flyer which included the contact details for the principal investigators and directed to contact them for further information (Appendix 3). As per the ND individuals, interested patients were provided with a copy of the PIL (Appendix 4) detailing the nature of the study, testing involved, requirements for participation, as well as the potential risks and benefits associated with participation. They too were given seven days for reflection following provision of the study information. A private meeting was offered to each patient to discuss the contents of the PIL, and all elements of the study. Having had any questions or queries surrounding any aspect of the protocol or study answered by the principal investigators participants signed the informed consent form (Appendix 4).

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

2.2.1.2 Inclusion/exclusion criteria

2.2.1.2.1 Individuals with T2DM

Individuals with T2DM were deemed suitable for inclusion in this study if they were aged between 18 to 60 years ((to exclude the confounding effects of aging, given that the diabetes-induced impairments in $\dot{V}O_2$ kinetics responses appear to be masked by older age (i.e. between 60 and 70 yr) at least in men, (O'Connor, 2015)), diagnosed with T2DM within the previous 12 years, and had adequately controlled HbA_{1c} levels (<10%). Individuals with T2DM on insulin were deemed to be ineligible. Individuals with T2DM also had to be untrained, defined as ≤ 1 bout of 60 min of moderate-intensity exercise per week for the previous six months.

In addition, each individual with T2DM had to satisfactorily complete a 12-lead electrocardiogram (ECG) exercise stress test (Bruce protocol) on a treadmill supervised by a medical practitioner in St. Columcille's Hospital. This is an established clinically validated

diagnostic tool which aids the detection of significant coronary arterial disease (CAD). The ECG complex and heart rhythm was assessed at rest, throughout the exercise protocol, and then during recovery to ensure that the heart responded appropriately to the exercise protocol. Criteria for exclusion from the study on the basis of an unsatisfactory exercise stress test were as follows: symptoms of chest discomfort consistent with angina, cardiac dysrhythmias more severe than occasional atrial or ventricular premature contractions, and flat or downsloping ST segment depression ≥0.1mV. Blood pressure (BP) and heart rate were monitored continuously throughout the protocol, with a systolic BP (SBP) in excess of 220 mmHg or a diastolic BP (DBP) in excess of 105 mmHg during exercise considered grounds for exclusion.

Additional grounds for exclusion included the existence of persistent proteinuria (urine protein >200mg/dl), high creatinine levels, SBP in excess of 170 mmHg at rest; DBP in excess of 95 mmHg at rest; the presence of diabetes complications and/or comorbid conditions including autonomic insufficiency/dysfunction, symmetrical neuropathy, abnormal cardiac function or evidence of ischaemic heart disease, angina or other cardiac or pulmonary symptoms limiting exercise performance. The absence of such comorbid conditions was established and confirmed by history, physical examination and laboratory testing by the hospital consultants.

2.2.1.2.2 Individuals without T2DM (ND)

Individuals without T2DM (ND) were deemed eligible for this study if they were aged between 18 and 60 years; were untrained, defined as ≤ 1 bout of 60 min of moderate-intensity exercise per week for the previous six months, and were free from cardiovascular disease (CVD) or any other comorbid conditions that may affect exercise performance. Each ND participant was obliged to have a medical examination by a medical doctor at the Department of Physiology, Trinity College Dublin to ensure suitability for exercise testing. This involved the completion of a medical questionnaire (Appendix 5) and a physical examination, whereby absence of comorbid conditions was established. In addition they were required to provide a fasted venous blood sample. Whole blood count, fasting plasma glucose and lipid levels, and HBA_{1c} levels were obtained from this blood sample.

2.2.1.2.3 Blood sample collection

Following a 12-hour fast, blood samples were extracted from the antecubital vein for haematological analysis. Samples for whole blood haematology determinations and HbA_{1c} were collected in lavender topped BD Vacutainers® (K_3EDTA and K_2EDTA), samples for glucose analysis in grey topped BD Vacutainers® (10mg sodium fluoride, 8 mg potassium

oxalate), and samples for lipid profiles, including triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), in gold topped BD Vacutainers® (clot activator and gel for serum separation).

HbA_{1c} samples were analysed in SVUH's biochemistry laboratory, with all other samples analysed in-house in the Department of Physiology, Trinity College Dublin, on commercially available units; ((whole blood count (Coulter Ac Tdiff: Coulter Ltd, UK), fasting blood glucose (Olympus AU640, USA) and lipid profiles (Olympus AU640, USA)).

2.2.1.2.4 Ankle brachial index (ABI)

In order to confirm eligibility for participation in the study, individuals with and without T2DM underwent an assessment to determine their ankle:brachial index (ABI). This is a ratio of the SBP measured at the ankle to that measured at the brachial artery, utilised to assess for signs and symptoms of peripheral arterial disease (PAD). Individuals were positioned in the supine position, with the arms and legs positioned at the same level of the heart for 10 mins prior to measurement. Appropriately sized standard blood pressure cuffs (Hokanson, United States), approximately 20% greater than the diameter of the extremity were placed 2.5 cm above the antecubital fossa and malleoli of both upper and lower limbs respectively. ABI was then derived from the SBP measured in the arms and legs at rest. Three measurements were made at each of the following sites and in the following order: left arm (left brachial artery), left ankle (left dorsalis pedis and left posterior tibialis), right arm (right brachial artery) and right ankle (right dorsalis pedis and right posterior tibialis). The artery in question was located via palpation and an MD6 Bidirectional Doppler wand (Hokanson, USA) applied at a 45-60º angle. The cuff was inflated progressively up to 20 mmHg suprasystolic pressure, gradually deflated until the pressure level could be detected via the Doppler wand and duly noted. Repeating the measurement on both arms has been shown to reduce standard error and bias by 30-40% (Espeland *et al.* 2008).

The right ABI was calculated as the ratio of the higher right ankle pressure divided by the highest right or left brachial pressure, with the left ABI calculated in the same manner but on the opposite side. An ABI reading of ≤ 0.90 is reported to be the most common and consensual threshold for the diagnosis of PAD with readings within the range of 0.9 and 1.3 considered to be indicative of an absence of PAD (Aboyans *et al.* 2012).

However, as a patient may be asymptomatic at rest and only experience symptoms related to compromised vascular flow when ambulating, we repeated the above protocol following the

individual performing 30 repetitions of active ankle plantarflexion exercise (i.e. heel raises). This protocol has an excellent correlation between ABI obtained after this method compared with treadmill exercise in people with claudication (Amirhamzeh *et al.* 1997; McPhail *et al.* 2001). In healthy patients a mild decrease in ABI can be expected when measured immediately after exercise cessation (Carter, 1972; Ouriel & Zarins, 1982). The ankle pressure then increases rapidly and reaches the pre-exercise values within 1 to 2 minutes (Carter, 1972). We therefore, recorded these measurements on one occasion at the sites which provided the readings for the resting ratio.

2.2.1.3 Determination of physical activity levels

All participants were classified as untrained ($\leq$1 h.week^{-1} of moderate-intensity exercise), and had not participated in a continuous exercise program in the preceding 6 months. To confirm that the participants in this study could be deemed untrained, an RT3 accelerometer (Stayhealthy Inc, California) was used to record intensity, frequency and duration aspects of activity as well as the total volume of activity accumulated (Rowlands *et al.* 2004) and The Low Level Physical Activity Recall questionnaire (LOPAR) was used to subjectively estimate physical activity levels (Appendix 6).

2.2.1.3.1 RT3 Tri-axial accelerometers

Accelerometers function by way of integrating a filtered digitized acceleration signal over a user-specified time interval known as an epoch. At the end of each epoch, the summed value or activity count is written to memory (Trost *et al.* 2005). The RT3 accelerometer has been established as an effective tool for the assessment of physical activity in adults and children (Rowlands *et al.* 2004), being validated against oxygen uptake in both children and adults over a range of activities (Welk *et al.* 2000; Powell *et al.* 2003), as well as heart rate monitoring in children (Welk & Corbin, 1995). The RT3 measures the accelerations of movement and integrates activity from three planes; the mediolateral (x), anteriorposterior (y) and vertical (z) to yield a vector magnitude (Hussey *et al.* 2007), and depending on the selected mode of operation, applies a particular time sampling mechanism to enable the capture of intensity, frequency and duration information.

It has been estimated that between three and four days of monitoring is required to obtain a reliable estimate of physical activity based on the vector sum (Coleman & Epstein, 1998). Individuals in the present study were therefore required to wear the RT3 unit on their right hip for five consecutive days during all waking hours (except whilst bathing or swimming). The

RT3 was attached to a belt using its integral belt clip. We utilised a pre-set mode (mode 3), whereby the time sampling interval (epoch) is set at 1 minute and accumulated activity counts on individual axes are converted to a digital representation for processing. Output is then expressed as mean counts per minute for each activity (i.e. activity count) which is stored in the unit's memory chip and saved for download to a PC for analysis and interpretation (Eston *et al.* 1998).

Accelerometry data were assessed for counts.min^{-1} for vector magnitude. With previously defined cut-off values used to categorise minutes of physical activity: inactive (0-100 counts.min^{-1}), light (101-970 counts.min^{-1}) (Healy, 2007), moderate (971-2333 counts.min^{-1}) and vigorous (2334+ counts.min^{-1}) (Rowlands *et al*. 2004). The accumulated physical activity counts obtained for 5 days were averaged to give a mean output for 1 day, with the total time spent in each category being expressed in h.day^{-1}.

2.2.1.3.2 The Low Level Physical Activity Recall questionnaire (LOPAR)

We also utilised the Low Level Physical Activity Recall questionnaire (LOPAR) to subjectively estimate physical activity levels in participants in the study (Appendix 6). The LOPAR questionnaire has previously been used by other investigators to test activity levels in participants with T2DM (Regensteiner *et al.* 1998; Brandenburg *et al.* 1999). Furthermore, it has been validated in patients with PAD (Kriska *et al.* 2006) as evidenced by correlations with maximal treadmill walking time ($r = 0.46$, $P<0.01$), with changes in maximal treadmill walking time after exercise training ($r = 0.46$, $P<0.05$), and with energy expenditure during home-based exercise measured by Vitalog microcomputer ($r = 0.50$, $P<0.05$).

Individuals were required to recall their daily activity for the previous seven days, categorised into work, homebased and leisure physical activities. 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 individual by multiplying the amount of time spent performing an activity by the MET value of that activity.

2.2.2 Experimental design

2.2.2.1 Study overview

ND participants were required to visit the cardiovascular performance laboratory in the Department of Physiology, Trinity College Dublin on one occasion, whilst all T2DM patients were required to visit the exercise testing facility in St. Columcille's Hospital on one occasion. All premenopausal participants were tested during the mid-follicular phase (days 5-12) of the menstrual cycle, which was self-determined. Participants were asked to refrain from consuming alcohol, caffeine and non-prescribed nutritional supplements in the 24 hours prior to testing and to limit their exercise to normal activities of daily living.

During this visit, initially anthropometric data was collected, followed by the measurement of the aortic pulse wave velocity (PWV). Participants then performed a ramp incremental cycling test to exhaustion. Following a 5 min passive recovery period, participants finally completed a 'confirmatory' high-intensity cycling bout (*see 2.2.2.2.3).*

2.2.2.2 Visit to the cardiovascular laboratory

2.2.2.2.1 Anthropometry and pulse wave velocity

An anthropometrical evaluation was performed upon each individual's visit to the laboratory. Stature was measured to 0.01 m using a SECA^{TM} Stadiometre (SECA Ltd, Germany) and body mass (kg) was determined using a set of SECA^{TM} digital scales (SECA Ltd, Germany). Body mass index (BMI) was then calculated as body mass (kg) / height squared (m^2). Waist and hip circumferences (cm) were measured in duplicate with an anthropometric tape while the subjects were wearing light clothing. Waist circumference was measured at the minimum circumference between the iliac crest and the rib cage. Hip circumference was measured at the maximum protuberance of the buttocks. The waist:hip ratio (WHR) was then calculated: waist measurement (cm) / hip measurement (cm).

The aortic pulse wave velocity (PWV), which represents the propagation velocity of pressure waves is a direct measure of aortic stiffness (Laurent *et al.* 2001), and was measured through the non-invasive method of applanation tonometry (SphygmoCor, AtCor Medical, Sydney, Australia) at the carotid and femoral arteries. PWV measures the speed of the arterial pressure waves travelling along the aortic and aorto-iliac pathway. In brief, aortic PWV was measured by sequentially recording ECG-gated carotid and femoral artery waveforms. The measurement

of the tonometry transit distance was obtained via an anthropometric tape measuring the surface of the body connecting the carotid site with the suprasternal notch and then subsequently, the suprasternal notch with the femoral site. Carotid-femoral transit time estimates the average aortic distensibility, thus PWV was calculated using the mean time difference and the arterial path length between the two measuring sites with the SphygmoCor software (AtCor Medical). Considering most compliance resides in the aorta, this estimate is closely related to total arterial compliance (Schram *et al.* 2004).

2.2.2.2.2 Ramp incremental cycle test to exhaustion

Participants performed a ramp incremental cycling test to exhaustion in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, The Netherlands) with appropriate adjustments made to the ergometer seat and handle bar position for each participant (*Figure 2.1a*). Exercise was performed initially for 2 min at a baseline resistance of 10W (i.e. 'unloaded' cycling) to reduce excess internal work (Boone *et al.* 2008). This was followed by 10/15 $W.min^{-1}$ increments in females (ND, *n*=0/5; T2DM, *n*=2/3 respectively) or 10/15/20/25 $W.min^{-1}$ increments in males (ND, *n*=0/2/8/2; T2DM, *n*=1/1/9/1 respectively) depending on activity levels of participants, and were brought to volitional exhaustion. Pedal frequency was held constant at an individually selected cadence between 60-75 revolutions per minute (rpm). This cadence was maintained throughout all further testing protocols. Failure in a test was determined as a drop in cadence exceeding 5 rpm for >3 s. Peak workload was determined according to the point of termination of the test. HR was continuously monitored while pulmonary oxygen uptake ($\dot{V}O_2$), pulmonary carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER: $\dot{V}CO_2/\dot{V}O_2$) were recorded on a breath-by-breath (BbB) basis. Peak HR was defined as the highest heart rate attained within the last 15 s of the point of termination of the test.

As it is frequently reported that some individuals do not evidence a definitive plateau of the $\dot{V}O_2$ – work rate relationship on this test, secondary criteria based upon measurements of the respiratory exchange ratio (RER), maximal heart rate or blood [lactate] (Poole *et al.* 2008b) are often relied upon to corroborate a maximum effort (Astrand & Rodahl, 1986; Rossiter *et al.* 2006; Poole *et al.* 2008b). However, such criteria have been called into disrepute (Poole *et al.* 2008b), thus, the utilisation of a subsequent confirmatory high-intensity constant load bout *(see section 2.2.2.2.3)* has frequently been implemented (Day *et al.* 2003; Rossiter *et al.* 2006; Murias *et al*. 2010b).

In the present study all participants demonstrated a plateau in $\dot{V}O_2$ during the confirmatory tests *(see section 2.2.2.2.3)*, and moreover the $\dot{V}O_2$ values recorded during this confirmatory test were not different to those obtained during the ramp incremental cycle test. Thus, we were certain that in the present study, all participants achieved a $\dot{V}O_{2max}$.

2.2.2.2.3 Confirmatory test

Within five minutes of completion of the ramp incremental cycle test, participants performed a high-intensity, constant-load cycling bout to exhaustion at an intensity equivalent to 85% of the peak power output achieved in the ramp test. This protocol was performed to confirm the attainment of $\dot{V}O_{2max}$ in the prior graded exercise test whilst also facilitating the non-invasive determination of maximal cardiac output via the inert rebreathing technique (Innocor; Innovision, Denmark). Participants were instructed to indicate, via the raising of their hand when they felt they were ~30 s from exhaustion. At this point, individuals were verbally encouraged to continue and within 30 s the measurement of CO was carried out.

2.2.3 Equipment and techniques

2.2.3.1 Cardiometabolic unit, heart rate monitor and pulse oximeter

A HR monitor (Polar S610i, Polar Ltd, Finland) was secured around the participant's chest with a chest strap, and a pulse oximeter (Innocor; Innovision, Denmark) clipped on to the middle finger of the left hand to monitor arterial O_2 saturation (SpO_2). A nose-clip and a silicone mouth piece which was attached to a filter and subsequently to the rebreathing valve unit (RVU) of the cardiometabolic unit (Innocor, Innovision, Denmark) were used. This allowed for the gas exchange and ventilatory variables to be analysed on a breath-by-breath (BbB) basis throughout the protocol ($\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$). Prior to each test the cardiometabolic unit was calibrated. This involved calibration of the flowmeter using a 3 L syringe (Hans Rudolph, Kansas City, MO), flow-gas delay via a specific breathing technique performed by the investigator following a graphical tachymeter on the visual display unit, and finally the oxygen sensor, by exposing the sample line to the atmospheric air. Both the oxygen sensor and photoacoustic gas analyser require multi-point calibration to be performed by the manufacturer periodically (6-12 months).

2.2.3.2 Blood pressure

Beat-to-beat systolic and diastolic blood pressure was continuously monitored throughout the exercise protocol using the volume clamp method at the level of the finger (Finometer, Finepress Medical Systems B.V. the Netherlands). MAP was calculated from systolic and diastolic pressures (MAP: 0.33 systolic BP + 0.66 diastolic BP). Peak BP was expressed as the highest 15-second mean pressures obtained before the participant's volitional termination of the test.

2.2.3.3 Rating of perceived exertion (RPE)

Rates of perceived exertion (RPE) were obtained using a Borg scale of 6 to 20 (Borg, 1970) as a subjective measure of exercise tolerance (Appendix 7) at the end of each minute of the ramp test. Prior to commencing the incremental ramp protocol, participants were familiarised with the scale and instructed to point to the number on the scale reflective of perceived exertion at time of prompting.

2.2.3.4 Near infrared spectroscopy (NIRS) and subcutaneous fat layer of the vastus lateralis

Local muscle deoxygenation (Δ[HHb+Mb]) profiles of the right quadricep's *vastus lateralis* (VL) muscle were made with near infrared spectroscopy (NIRS) (Hamamatsu Niro 200Nx; Hamamatsu Photonics, Hamamatsu, Japan) throughout the ramp incremental exercise protocol. The *vastus lateralis* is a dominant locomotor muscle during cycling (Laplaud *et al*. 2006; Okushima *et al.* 2016) thereby being a suitable choice for examining exercise-induced changes in active muscle oxygenation.

Optodes were placed on the belly of the muscle, (5-8 cm above the lateral femoral condyle), parallel to the major axis of the thigh *(Figure 2.1b)*. The system consists of both an emission probe that carries NIR light from the laser diodes and a detector probe. The inter-optode spacing between the emitter and receiver was 3 cm. The depth of the measured area was estimated to be approximately one-half the distance between the emitter and the receiver (~1.5 cm). Findings from Monte Carlo simulation studies of NIR light propagation, in respect to the scattering and absorption characteristics of the skin and muscle for NIR light, have deemed an emitter-detector spacing of 2 cm to be an acceptable distance for NIR light to travel in order to reach the site of interrogation, when the subcutaneous thickness is ≤ 15 mm (Matsushita *et al.* 1998). In the present study, we determined the thickness of the skin and adipose tissue at the site of the optode placement via 2D ultrasound operating in B-mode (Zonare Ultra Smart Cart, Software version 4.7, USA). The skin under the probes was shaven as required. The optodes were housed in a black rubber holder and secured on the skin surface with bi-adhesive tape and then covered with a dark elastic bandage, which served the two-fold purpose of minimising extraneous movement and the intrusion of stray light throughout the exercise protocol. Three laser diodes (λ = 735, 810, and 850 nm) were pulsed in rapid succession and the light returning from the tissue was detected by the photodiode for online estimation and display of the concentration changes from the resting baseline of Δ[HHb+Mb]. Changes in light intensities were recorded continuously at 2 Hz and transferred to a computer for later analysis.

Near-infrared spectroscopy (NIRS) is a unique, powerful, non-invasive imaging technique that can be used to continuously monitor and quantify the oxygenation status of human tissue. The theoretical basis of NIRS, limitations, and its use in exercise measurements have been described in detail elsewhere (Mancini *et al.* 1997; McCully & Hamaoka *et al.* 2000; Ferrari *et al.* 2004). In brief, NIRS is based on the relative ease with which the light of the near-infrared region of

the spectrum (wavelength 700 -1000 nm) passes through biological tissues, including skin, bone and muscle (Boushel & Piantadosi, 2000). Three main categories of NIR light spectrometers exist: continuous wave (CW NIRS), time domain and frequency domain. For the purpose of this study, spatially resolved spectroscopy (SRS) was used, a spectrometer which employs the properties of CW NIRS. The principles of operation and algorithms utilised have also been described in detail elsewhere (Mancini *et al.* 1997; McCully & Hamaoka *et al.* 2000; Ferrari *et al.* 2004). Briefly, NIR light is transmitted from an emitting optode through the biological tissue in a spherical pattern at an established intensity and at two or more wavelengths surrounding the isosbestic point to a light-detecting photodiode which measures the intensity of the exiting light (Bakker *et al.* 2012). As mentioned, the penetration depth of the emitted light is considered to be approximately half the distance between the two optodes (Kalliokoski *et al.* 2006). The path length taken by the reflected light cannot be readily quantified (Mancini *et al.* 1994) therefore SRS provides an estimate of this path length.

Chromophores are light-absorbing compounds in tissue within the near-infrared range (Jöbsis, 1977), some of which whose light absorption capabilities are oxygenation status dependent. The primary chromophores of interest in skeletal muscle are oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb), and determining the concentration of these variables provides an insight into the oxygenation state of the tissue. In a scattering medium like tissue, quantification of NIRS signal can be challenging, however, SRS has been established as being one of the most reliable (Delpy & Cope, 1997). In addition to scattering, the attenuation of the emitted light can also be related to the change in chromophore concentration or absorption (Ferrari *et al.* 1992). However, the effects of both parameters are considered using a modified Beer-Lambert law into which a path-length factor is incorporated to correct for a scattering of photons in the tissue (Van Beekvelt *et al.* 2001; Bakker *et al.* 2012)

$$A = \varepsilon[c]LB + G$$

where A is the absorption of light expressed as optical density, ε the extinction coefficient of the chromophore, [c] the chromophore concentration, L the distance between the point of light entry and exit (optode separation), B the path length resulting from scatter in the tissue and G is a lumped parameter related to tissue and optode geometry. The extinction coefficient, which describes how strongly a chromophore absorbs light at a particular wavelength, can be estimated easily by direct measurement. However, although highly desirable, the ability to estimate path length B in real time is somewhat impractical and instead an average path length factor is applied. Therefore values for B are only approximate and vary to some extent with

changes in absorption by tissue chromophores. Values of *G* are also difficult to determine, hence absolute concentration values have been difficult to determine accurately (Boushel & Pintadosi, 2000). Regardless of the concerns in measuring path length and geometry, their contributions to light attenuation in tissues can generally be assumed to be constant for a fixed optode position (Boushel & Pintadosi, 2000). Consequently, NIR signals monitored do not yield absolute levels of tissue oxygenation (Wilson *et al.* 1989) but can only be viewed in relative terms, with changes in O_2 saturation being expressed relative to the overall change in the signal from a baseline 'zero-set' value. Hence, by quantifying changes in [HbO_2] and [HHb+Mb] from an arbitrary baseline, changes in muscle O_2 delivery and extraction at the level of the microcirculation can be attained to provide an index of O_2 extraction during exercise in humans (Ferreira *et al.* 2007).

Each chromophore has a unique absorption spectrum, where the specific extinction coefficient (ε) is expressed as a function of the wavelength (Horecker, 1942; Pellicer & Bravo, 2011). The absorbance of HHb and HbO_2 peak at 760 nm and 850 nm respectively, with the isosbestic point for both occurring at 798 nm (Chance *et al.* 1992; Mancini *et al.* 1994). The NIR absorption spectrum of Mb overlaps with that of Hb, and are indistinguishable by NIRS, therefore measurements are based on the principle that the signal obtained by NIRS, reflects changes in the oxygenation of the tissue that are attributed to the desaturation of both Hb and Mb (De Blasi *et al.* 1993; Grassi *et al.* 2003). However, despite the presence of similar absorption spectra, estimates of relative contribution from both chromophores are conflicting. The ratio of [Hb] to [Mb] has been reported to be >5 in human skeletal muscle (Mancini, 1997), with Mb contributing to light absorption changes by ~10% (Chance *et al.* 1992; Mancini *et al.* 1994). Thus these findings therefore suggest that the interpretation of the NIRS derived signals can be attributed predominantly to the oxygen status of Hb (Kallioloski *et al.* 2006). On the contrary, Hb has been proposed to contribute as little as ~10-20% (Seiyama *et al.* 1988; Manfredini *et al.* 2009; Marcinek *et al.* 2007) to the signal. As such, interpretation of the NIRS derived signal cannot be attributed predominantly to the oxygen status of Hb. Whilst an appreciable limitation of this technique is acknowledged, for the remainder of this study, reference will be made to [Hb+Mb] accordingly.

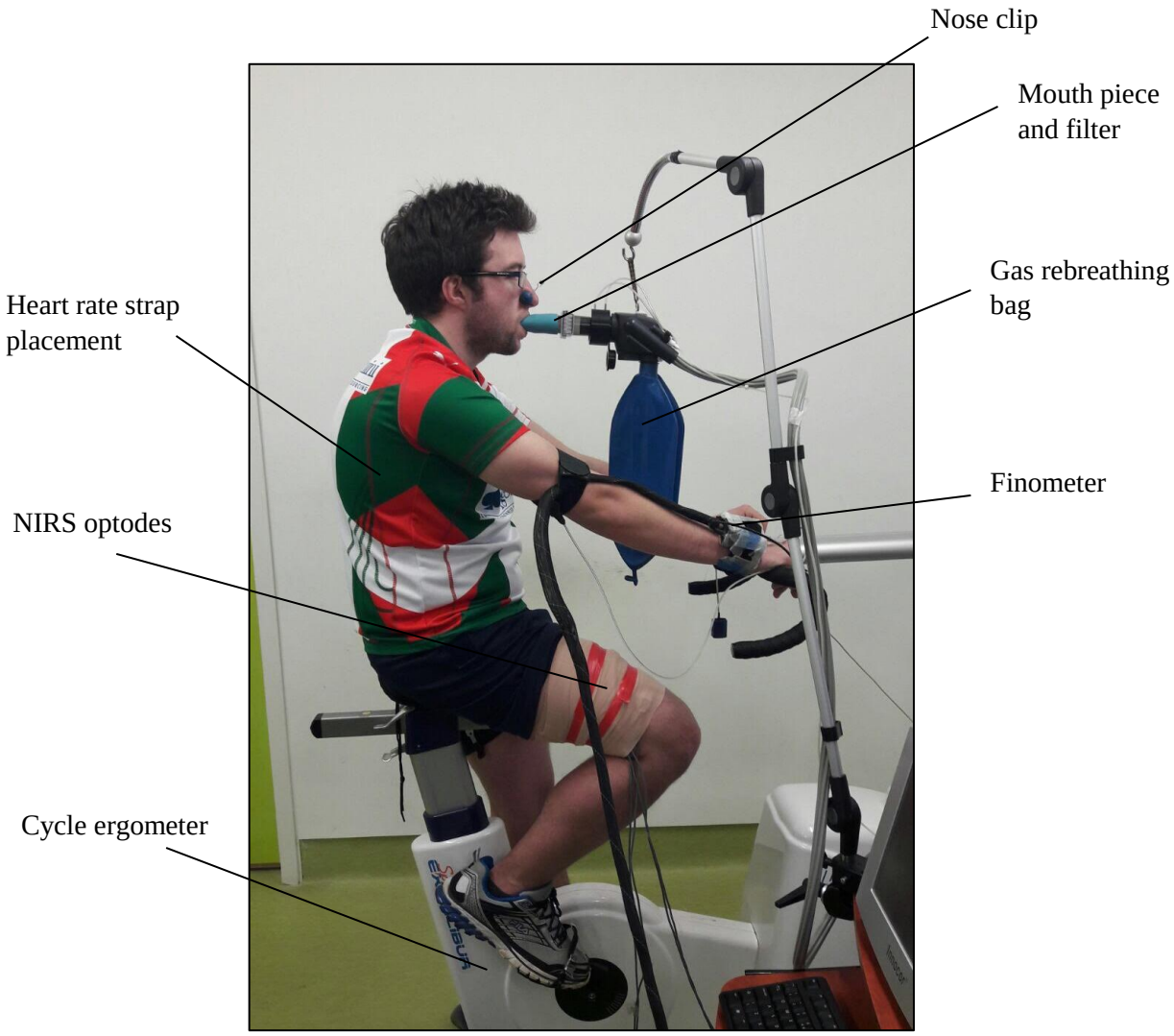

Figure 2.1a. Illustration of the experimental set-up for the ramp incremental cycling protocol.

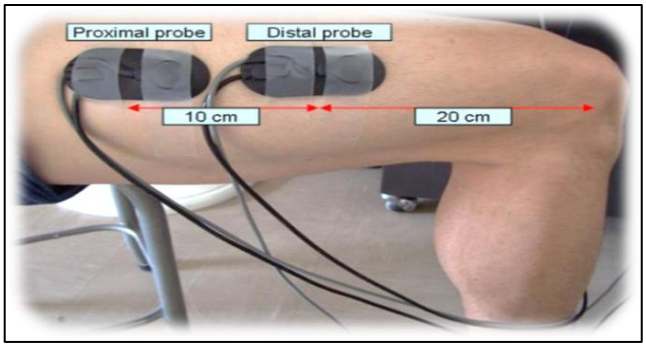

Figure 2.1b. Placement position of the NIRS optodes

2.2.3.5 Maximal cardiac output (CO)

Maximal CO was determined non-invasively via the inert gas rebreathing technique (Innocor, Innovision, Denmark) during the confirmatory cycling bout at 85% of the peak power achieved in the ramp test. Prior to the onset of this confirmatory test, participants completed two bouts of rebreathing in 'demo' mode in order to familiarise themselves with the technique. The rebreathing technique requires individuals to maintain a consistent breathing frequency of ~18-20 breaths per minute in synchrony with a graphical tachymeter on the visual display unit and through verbal prompting as necessary by the operator. The rebreathing manoeuvre is then performed over a 15 s period (5-6 breaths) employing an estimated gas volume of ~300% of each participant's predicted tidal volume.

Inert gas rebreathing is an established non-invasive method to measure pulmonary blood flow, which has been validated against the well-established invasive methods of dye dilution, thermodilution and direct Fick (Gabrielsen *et al.* 2002; Peyton & Thompson, 2004; Agostoni *et al.* 2005). The system consists of a three-way respiratory valve with a mouthpiece and a rebreathing bag connected to a fast responding infrared photoacoustic gas analyser. This technique compares the reduction in the concentration of a blood-soluble gas with that of an insoluble gas in a closed rebreathing system. Thus, the participant breathes through a hermetically closed circuit system containing an oxygen rich gas mixture of 0.5% nitrous oxide (N_2O, blood soluble), 0.1% sulphur hexafluoride (SF_6, blood insoluble), 15% O_2 and 5% CO_2 in a 4-L runner bag. Gas concentrations are measured continuously and simultaneously over a 5-6 breath interval at the level of the mouth via the photoacoustic analyser. The total system volume (i.e. including that of the lungs, valve, and rebreathing bag) is calculated from the remaining concentration of blood-insoluble SF_6 after equilibration.

The pulmonary blood flow estimated with this apparatus is considered to be a direct indicator of the total pulmonary blood flow participating in gas exchange. With the alveolar-capillary N_2O gradient enabling N_2O to pass freely between alveolus and capillary blood, the absence of N_2O in the precapillary blood can thus be assumed. Consequently, the rate of N_2O disappearance from the alveolus can be considered to be directly proportional to the flow of blood perfusing the ventilated parts of the lungs. Thus, taken together, the total system volume determined via SF_6 mixing and the rate of N_2O disappearance from the alveolus after SF_6 equilibration (generally after 2-3 breaths) permit calculation of pulmonary blood flow. This is deemed equal to CO in the absence of a significant intrapulmonary shunt (Gabrielsen *et al.*

2002). Assuming an adequate rebreathing manoeuvre is performed by the individual whereby full N_2O clearance is achieved, CO can be determined.

However, given that across the spectrum of human oxidative potential (i.e., basal or resting $\dot{V}O_2$ up to $\dot{V}O_{2max}$) CO increases with a slope (S) between 5 and 6 litre/litre $\dot{V}O_2$ (with an intercept (I) of 5-6 litres.min^{-1} (Rowell, 1993; Poole, 1997) further validation of CO measurements are possible. Accordingly, the approximate proportionality between cardiac output and $\dot{V}O_2$ across changes in $\dot{V}O_2$ can be determined via the application of the following equations, irrespective of methods utilised to measure CO (Poole & Jones, 2012);

Eqn 1) $CO = S.\ \dot{V}O_2 + I$

thus,

Eqn 2) $CO\ (L.min^{-1}) = 5.5 \times \dot{V}O_2 + 5.5$

Although, whilst the inert gas rebreathing technique has indeed been validated against direct Fick, dye- and thermo-dilution methods, it is important to appreciate that all techniques have measurement errors. And whilst we endeavoured to prevent the occurrence of both technical and human errors when participants were performing the rebreathing manoeuvre, the supplementary calculation of the approximate proportionality between CO and $\dot{V}O_2$ across changes in $\dot{V}O_2$ simply served to identify any non-physiologically sound CO values generated by the cardiometabolic unit. Accordingly, any such values were subsequently excluded, and thus did not confound the results presented herein.

2.2.4 Data analysis

2.2.4.1 $\Delta\dot{V}O_2/\Delta PO$

The rate of change (Δ) in oxygen consumption ($\dot{V}O_2$) relative to power output (PO) during ramp incremental exercise is considered an important parameter in estimating exercise tolerance in clinical populations. It is dependent on the cardiovascular system to deliver and the muscle capacity to extract oxygen (Hansen *et al.* 1987), and therefore, can reflect the limited capacity of aerobic metabolism to adjust to the non-steady state conditions incurred during an incremental ramp protocol. The BbB $\dot{V}O_2$ data were averaged over 15 s intervals and plotted as a function of work rate to reflect the increase in aerobic metabolism ($\Delta\dot{V}O_2$) for each increase in power output (ΔPO). From this plot, the $\dot{V}O_2$ slopes, S_1 and S_2 (the $\Delta\dot{V}O_2/\Delta PO$ slope below and above VT, respectively) were calculated by linear regression (Barstow *et al.* 2000). The baseline $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measure in the final 60 s of the 'unloaded' phase.

2.2.4.2 Muscle deoxygenation

The NIRS-derived signal was normalised such that the unloaded exercise baseline value was adjusted to zero ('zero set'), and thus the NIRS data are presented as a relative change from the baseline- to the end-exercise values. Given the uncertainty of the optical path length in the *vastus lateralis* at rest and during exercise, Δ[HHb+Mb] data are presented as normalised delta units. Prior to analysis, NIRS data were averaged to give 1 s intervals and time aligned with the cardiorespiratory data. The second-by-second [HHb+Mb] data was averaged by applying a five-point moving average and then normalised to the peak amplitude of the response (%Δ[HHb+Mb]), such that 0% represents the mean-steady state value of the last 30 s of the unloaded cycling and 100% represents the highest mean value of the last 30 s of any work rate. In contrast to the exponential increase in [HHb+Mb] observed during the step-transition to constant steady-state exercise, representative of the NIRS kinetic response; the [HHb+Mb] response during ramp incremental exercise varies as a function of continuous dynamic adjustments to changes in workload (Ferreira *et al.* 2007; Spencer *et al.* 2012; Belotti *et al.* 2013; Fontana *et al.* 2015; Keir *et al.* 2015). Thus, the [HHb+Mb] response dynamics were expressed in relation to power output (PO), in both absolute (PO) and relative (% PO) terms prior to curve fitting. However, for the benefit of the present study it is more appropriate to consider the [HHb+Mb] dynamic response in relation to relative exercise intensity. By making direct comparisons between healthy controls and individuals with T2DM at the same absolute work rate misleading conclusions would be likely.

To characterise the profile of %Δ[HHb+Mb] during ramp incremental exercise, previous investigators (Ferreira *et al.* 2007; Boone *et al.* 2009, 2010; Di Menna *et al*. 2010a) have favoured the utilisation of a sigmoid regression analyses model. More recently however Spencer *et al.* (2012) argued the accuracy of such application, suggesting that the sigmoid function attempts to characterise the overall observed response, and therefore, does not accurately portray the physiological responses occurring during ramp incremental exercise. Alternatively upon visual inspection, Spencer *et al.* (2012) suggested that the profile may be more accurately described as consisting of three distinct phases. Initially at the onset of the protocol there is little adjustment in %Δ[HHb+Mb] relative to increases in work rate. This indicates that adequate O_2 delivery relative to utilisation occurs in the early stages of ramp incremental exercise. However, the authors determined that this delayed increase in %Δ[HHb+Mb] was not well-characterised by a linear function, owing to its limited duration, and subsequently too few data points to characterise the response. Following this, however, an approximately linear increase in %Δ[HHb+Mb] relative to changes in work rate ensues, representing an increased reliance on O_2 extraction relative to metabolic demand. This culminates in the third stage with the demonstration of a plateau in %Δ[HHb+Mb] despite the continued increase in work rate, indicating that a point of maximum O_2 extraction occurs towards the end of ramp incremental exercise (Spencer *et al*. 2012). The break point (*BP)* represents the *x* value at the "breaking point" between the two linear segments, reflecting the point of maximum O_2 extraction which occurs at the threshold between the severe- and heavy-intensity exercise domains. The profile of %Δ[HHb+Mb] is therefore best characterised by a piecewise function including two linear segments; the 'double-linear model' (Vieth, 1989), as opposed to a sigmoid regression.

In the present study individual profiles were therefore plotted as a function of PO and %PO and characterised by a piecewise 'double linear' regression function to establish the slope of increase of deoxygenation ($Slope_1$), plateau as maximal exercise was approached ($Slope_2$), and the break point (*BP*) located between the increasing deoxygenation and its plateau. The double-linear function was applied using SigmaPlot 12.0 (Systat Software, Point Richmond, CA), of the form:

$$y = a + b * x – c * (x-d)*f$$

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

where a and b represent the y-intercept and slope of the first linear function, d is the time delay or *BP* where the segments intersect, with the slope of the second linear function being calculated from the parameter estimates of b and c.

2.2.4.3 Ventilatory threshold and respiratory compensation point

The ventilatory threshold (VT) defined as the exercise level at which $\dot{V}_E/\dot{V}O_2$ exhibits a systematic exponential increase without a concomitant increase in $\dot{V}_E/\dot{V}CO_2$ (Wasserman *et al.* 1973), was estimated identifying the break point in the plot of $\dot{V}CO_2$ against $\dot{V}O_2$ (Beaver *et al.* 1986; Amann *et al.* 2006). The VT relates to the first non-linear increases of $\dot{V}_E$ and $\dot{V}CO_2$, due to the buffering of H^+ in response to the systematic increase of blood lactate above resting values (Wasserman *et al.* 1973).

The respiratory compensation point (RCP) defined as the second non-linear increase of $\dot{V}_E$ and $\dot{V}CO_2$, corresponds to the minimal work rate at which the increase in $\dot{V}_E/\dot{V}O_2$ is accompanied by an increase of $\dot{V}_E/\dot{V}CO_2$ (Wasserman & McIlroy, 1964). This is mainly explained by the occurrence of an acidosis (pH decrease) as bicarbonate is overwhelmed by the increasing production of lactate (Vallier *et al.* 2000). It is widely accepted as the point demarcating the boundary between heavy- and severe-intensity exercise domains.

2.2.4.4 Relationship between [HHb+Mb]-BP, RCP and VT

The underlying mechanisms for the breakpoint between the two linear phases in Δ[HHb+Mb] (Δ[HHb+Mb]-*BP*) at high intensities have not yet been established. However, strong associations have been demonstrated between Δ[HHb+Mb]-*BP* and the RCP (Murias *et al.* 2013; Boone *et al.* 2015), thus alluding to a relationship between ventilatory responses at heavier intensity exercise and local muscle O_2 transport/extraction. In the present study, in an attempt to further characterise [HHb+Mb]-*BP* comparisons were made with RCP and VT, with all indices expressed relative to peak power output.

2.2.5 Statistical analysis

Statistical analysis was performed using the software SigmaPlot version 12.0 (Systat Software, Point Richmond, CA). Prior to analysis, normal Gaussian distribution of the data was assessed using the Shapiro-Wilk's test. Physical and physiological responses between groups were compared using the unpaired Student's t-test for parametric analyses, or the Mann-Whitney U test for non-parametric analyses. Linear correlation was used to assess the relationship between the Δ[HHb+Mb]-*BP* and the RCP and VT respectively. Statistical significance was accepted at a P value ≤ 0.05. All values are expressed as means ± standard deviation (SD) or as median and interquartile ranges for data that were deemed not normally distributed.

The key variable is the rate of increase in [HHb+Mb] in relation to the relative change in workload (PO). An indicator of the rate of change in [HHb+Mb] can be obtained by calculation of the Δ [HHb+Mb]/%PO slope of the first linear component when the response is fitted using a bi-linear function. Recently published studies comparing young, healthy, male vs. female participants and exploring the effects of different pedal rates have reported average slope values of 1.2 ± 0.3 (Mean ± SD) and the difference between groups/conditions was 0.4. Thus, given that the estimated standard deviation (SD) of each population is 0.3, and that the minimum difference we wish to detect as significant would be 0.4, the minimum sample size needed to detect a significant effect at $\beta=0.05$ and $\alpha=0.05$ (90% power) for a t-test power calculation design based on 2 groups, is 13 individuals.

2.3 RESULTS

2.3.1 Participants

2.3.1.1 Physical characteristics

Physical characteristics for the participants are presented in Table 2.1. Anthropometrical measurements did not significantly differ between groups, with the exception of WHR, whereby individuals with T2DM displayed a significantly greater WHR ($P\leq0.05$) compared to the ND controls. Individual anthropometrical measurements are displayed in Appendix 8. No differences were observed in ABI, BP or the subcutaneous fat layer of the *vastus lateralis* between groups (Table 2.1). However, individuals with T2DM displayed a significantly faster PWV compared with ND controls, albeit falling within normative ranges for this clinical measurement (<10 ms^{-1}) (VanBortel *et al.* 2012; Mancia *et al.* 2013).

Table 2.1. ***Anthropometrical data, PWV, ABI and resting BP for ND and T2DM individuals.***

	ND (*n* =17)	**T2DM (*n* =17)**
Sex (male, female)	12, 5	12, 5
Age (yr)	44 ± 8	48 ± 7
Height (m)	1.72 ± 0.10	1.73 ± 0.10
Weight (kg)	91.1 ± 13.8	95.8 ± 18.3
BMI ($kg.m^{-2}$)	30.8 ± 3.5	31.9 ± 4.8
WHR (a.u)[a]	0.94 ± 0.08*	1.02 ± 0.07
Fat layer VL (mm)[b]	7.8 ± 4.5	5.9 ± 1.6
Pre exercise ABI (a.u.)[c]	1.14 ± 0.16	1.08 ± 0.09
Post exercise ABI (a.u.)[c]	*1.13 (0.23)*	*1.10 (0.22)*
PWV (ms^{-1})[d]	6.7 ± 1.4*	8.6 ± 1.7
SBP (mmHg)[e]	126 ± 13	129 ± 12
DBP (mmHg)[e]	77 ± 8	80 ± 8

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. BMI, body mass index; WHR, waist:hip ratio; ABI, ankle:brachial index; PWV, pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). a=14 (ND) and 17 (T2DM); b= 13 (ND) and 15 (T2DM); b=11 (ND) and 14 (T2DM); c=13 (ND) and 16 (T2DM); d=11(ND) and 14 (T2DM); e=14(ND) and 17(T2DM).

2.3.1.2 Haematological parameters and prescriptive medications

Mean haematological parameters and prescriptive medications are presented in Tables 2.2 and 2.3 respectively. As expected, participants with T2DM displayed significantly higher HbA_{1c} and fasting plasma glucose levels ($P<0.001$). They also had significantly higher total cholesterol ($P<0.05$) than the ND controls, with no differences between HDL and LDL distribution, and a tendency for significantly different triglyceride levels.

Table 2.2. ***Haematological parameters for ND individuals and individuals with T2DM.***

	ND	**T2DM**
HbA_{1c} (%)[a]	*5.1 (0.5)***	*6.8 (0.9)*
FPG ($mmol.L^{-1}$)[b]	*3.9 (0.4)***	*7.4 (2.9)*
Total cholesterol ($mmol.L^{-1}$)[c]	3.6 ± 0.9*	4.7 ± 1.2
LDL-C ($mmol.L^{-1}$)[d]	2.0 ± 0.7	2.2 ± 0.7
HDL-C ($mmol.L^{-1}$)[e]	1.2 ± 0.2	1.35 ± 0.3
Triglycerides ($mmol.L^{-1}$)[f]	*1.1 (0.9)*†	*1.5 (1.3)*

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. HbA_{1c}, glycosylated haemoglobin; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). **Significantly different than T2DM ($P \leq 0.001$). †Tendency towards a difference than T2DM ($P \leq 0.10$). a=7 (ND) and 15 (T2DM); b=10 (ND) and 13 (T2DM); c=10 (ND) and 12 (T2DM); d=10 (ND) and 10 (T2DM); e=12 (ND) and 10 (T2DM); f=10 (ND) and 13 (T2DM).

Table 2.3. ***Prescriptive medications for ND and T2DM individuals.***

	ND (*n* = 17)	T2DM (*n* = 17)
Anti-hypertensives		
Angiotensin converting enzyme inhibitor		3
Angiotensin II receptor blocker		2
Aspirin	1	4
Calcium channel blocker		4
Statins	2	7
Hypoglycaemic medications		
Oral hypoglycaemics		15
Subcutaneous hypoglycaemics		1
Sulphonylureas		3

2.3.1.3 Physical activity levels

Group mean activity levels are presented in Tables 2.4 and 2.5 for accelerometry and LOPAR data respectively. Individuals with T2DM displayed significantly higher activity counts for levels of light activity over the course of the 5-day period; however, periods of moderate and vigorous activity levels obtained from RT3 accelerometers as well as results from the LOPAR questionnaire were similar between the groups.

Table 2.4. ***Group mean activity levels based on the number of hours per day as determined by RT3 accelerometers.***

	ND (*n*=13)	T2DM (*n*=6)
Inactive (h.day^{-1})	18.93 ± 1.33	17.96 ± 1.22
Light (h.day^{-1})	4.21 ± 1.03*	5.55 ± 1.0
Moderate (h.day^{-1})	0.67 ± 0.38	0.44 ± 0.28
Vigorous (h.day^{-1})	*0.19 (0.23)*	*0.06 (0.09)*

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Inactive, <100 counts.min^{-1}; Light, 101 - 970 counts.min^{-1}; Moderate, 971-2333 counts.min^{-1}; Vigorous, >2333 counts.min^{-1}.* Significantly different than T2DM ($P \leq 0.05$).

Table 2.5. ***Group mean activity levels based on the number of METS per hour per week as determined by the LOPAR questionnaire.***

	ND (*n*=10)	**T2DM (*n*= 10)**
LOPAR (MET.hr^{-1}.wk^{-1})	*165 (91)*	*125 (75)*

Median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups.

2.3.2 Performance data from ramp incremental cycling test

2.3.2.1 Physiological responses

The physiological responses for both groups at peak exercise are displayed in Table 2.6 whereas responses at VT and RCP are displayed in Table 2.7.

2.3.2.1.1 Peak responses

$\dot{V}O_{2max}$ normalised to kilograms of body weight was significantly (P<0.05) reduced in individuals with T2DM compared with the ND controls, representing a 21% reduction in peak exercise capacity. In absolute terms (L.min^{-1}) $\dot{V}O_{2max}$ also tended to be lower in the group with T2DM, although it did not reach statistical significance (P=0.06). In addition, they displayed a tendency towards lower peak PO (P=0.08) at exhaustion, with no differences in time to failure. RER and RPE were similar between the T2DM and the ND groups at peak exercise, indicating both groups made their maximal effort during the ramp incremental protocol. Individuals with T2DM displayed a significantly lower CO (P<0.05) with a tendency towards a lower peak HR (P<0.10).

2.3.2.1.2 Responses at RCP

The group with T2DM displayed a significantly lower normalised $\dot{V}O_2$ (mL.kg^{-1}.min^{-1}) as well as a lower percentage $\dot{V}O_{2max}$ (P<0.05) at RCP. In absolute terms (L.min-1) $\dot{V}O_2$ also tended (P<0.10) to be lower in the group with T2DM. Additionally individuals with T2DM displayed a tendency toward lower percentage PO_{peak} (P<0.10).

2.3.2.1.3 Responses at VT

The group with T2DM displayed a significantly lower PO at VT (P<0.05).

Table 2.6. ***Peak physiological responses.***

	ND (*n*=17)	T2DM (*n*=17)
$\dot{V}O_{2max}$ (mL.kg^{-1}.min^{-1})	*27.5 (8.5)**	*22.8 (6.8)*
$\dot{V}O_{2max}$ (L.min^{-1})	2.60 ± 0.58†	2.18 ± 0.65
Peak PO (W)	*196 (107.5)*†	*186 (106)*
Peak HR (beats.min^{-1})^{a}	*175 (27)*†	*164 (35)*
Age predicted HR (beats.min^{-1})	176 ± 8	172 ± 7
Peak RER (a.u.)	1.13 ± 0.06	1.16 ± 0.08
Peak SBP (mmHg)^{b}	170 ± 23	187 ± 19
Peak DBP (mmHg)^{b}	103 ± 16	103 ± 23
Peak MAP (mmHg)^{b}	126 ± 17	137 ±24
Peak CO (L.min^{-1})^{c}	15.37 ± 2.34	12.08 ± 1.97
Peak RPE (a.u.)	*19 (3)*	*18 (3)*
TTF (s)	682 ± 105	682 ± 134

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P\leq0.05$). †Tendency towards a difference than T2DM ($P\leq0.10$). a=12 (ND) and 16 (T2DM); b=9 (ND) and 12 (T2DM); c=6 (ND) and 9 (T2DM).

Table 2.7. ***Physiological responses at VT and RCP.***

	ND (*n*=17)	T2DM (*n*=16)
$\dot{V}O_2$ @ VT (mL.kg^{-1}.min^{-1})	19.6 ±4.5	16.2 ± 2.9
$\dot{V}O_2$ @ VT (L.min^{-1})	1.78 ± 0.44	1.55 ± 0.47
$\dot{V}O_2$ @ VT (%)	69 ± 9	73 ± 10
PO @ VT (W)	125 ± 45*	95 ± 34
PO @ VT (%)	58 ± 12	57 ± 10
$\dot{V}O_2$ @ RCP (mL.kg^{-1}.min^{-1})	24.8 ± 4.8*	20.2 ± 3.3
$\dot{V}O_2$ @ RCP (L.min^{-1})	2.25 ± 0.49†	1.93 ± 0.57
$\dot{V}O_2$ @ RCP (%)	87 ± 5*	91 ± 6
PO @ RCP (W)	173 ± 50†	146 ± 42
PO @ RCP (%)	81 ± 7	82 ± 8

Values are mean ± SD. An intolerance of the mouth piece and/or face mask for one individual with T2DM, precluded the calculation of the above parameters.*Significantly different than T2DM ($P\leq0.05$). †Tendency towards a difference than T2DM ($P\leq0.10$).

2.3.2.2 $\Delta\dot{V}O_2/\Delta PO$

The rate of change in $\dot{V}O_2$ relative to power output ($\Delta\dot{V}O_2/\Delta PO$) was not significantly different during the ramp incremental exercise between the T2DM and ND groups with no observed differences in slopes pre (P=0.14) or post (P=0.34) VT. Group mean slope parameters of this response are presented in Table 2.8.

Table 2.8. *Slopes revealed by linear fit of $\Delta\dot{V}O2/\Delta PO$ during the ramp incremental test.*

Slope *(S)*	**ND (*n*=17)**	**T2DM (*n*=16)**
S_1 (mL.min^{-1}.W^{-1})	*9.70 (1.48)*	*9.17 (1.98)*
S_2 (mL.min^{-1}.W^{-1})	*10.28 (2.40)*	*9.40 (3.99)*

Median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. S_1 and S_2 slope of linear regression between $\dot{V}O_2$ and PO before and after VT, respectively.

2.3.2.3 NIRS-derived [HHb+Mb] response dynamics

Group mean parameter estimates from the double linear model of the %Δ[HHb+Mb] profile as a function of normalised power output (%PO) or absolute PO(W) are displayed in Tables 2.9 and 2.10 respectively. The slope of the first linear regression function ($Slope_1$) used to establish the dynamic adjustment of [HHb+Mb] normalised to PO was significantly steeper in participants with T2DM than the ND controls. *Figure 2.2* displays representative profiles of the modelled [HHb+Mb] response dynamics during ramp incremental exercise for an individual without, and an individual with T2DM when expressed as a function of relative power output (PO%).

2.3.2.4 Relationship between [HHb+Mb]-BP, RCP and VT

In an attempt to further characterise [HHb+Mb]-*BP*, comparisons were made with RCP and VT, with all indices expressed relative to peak power output. Statistical analysis revealed no significant difference between the [HHb+Mb]-*BP* and RCP in both the ND and T2DM groups (81 ± 12 *vs.* 81 ± 7 %, and 78 ± 11 *vs.* 82 ± 8 %, respectively), whereas the [HHb+Mb]-*BP* was significantly different (P<0.05) from the VT in both groups. However, these indices were not correlated in either group ([HHb+Mb]-*BP* and RCP; ND, r=0.20; T2DM, r=0.05 and [HHb+Mb]-*BP* and VT; ND, r=0.25; T2DM, r=0.30).

Table 2.9. ***Parameter estimates for the %Δ[HHb+Mb] profile for both groups plotted as a function of normalised PO(%) during the ramp incremental test.***

Slope *(S)*	**ND (*n*=13)**	**T2DM (*n*=14)**
Slope$_1$	*1.15 (0.25)**	*1.36 (0.45)*
Slope$_2$	0.28 ± 0.73	-0.09 ± 0.83
BP (%)	81 ± 11	77 ± 11

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. NIRS data proved to be unreliable for 7 participants; (4 ND and 3 T2DM) and thus, were excluded from the analysis. *Slope*$_1$ and *Slope*$_2$ of linear regression before and after breakpoint (*BP)* respectively. *Significantly different than T2DM ($P \leq 0.05$).

Table 2.10. ***Parameter estimates for of the %Δ[HHb+Mb] profile for both groups plotted as a function of absolute PO(W) during the ramp incremental test.***

Slope *(S)*	**ND (*n* = 13)**	**T2DM (*n* = 14)**
Slope$_1$	*0.58 (0.40)*	*0.68 (0.59)*
Slope$_2$	*0.14 (0.67)*	*0.03 (0.17)*
BP (W)	183 ± 50	151 ± 48

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. *Slope*$_1$ and *Slope*$_2$ of linear regression before and after breakpoint (*BP)* respectively.

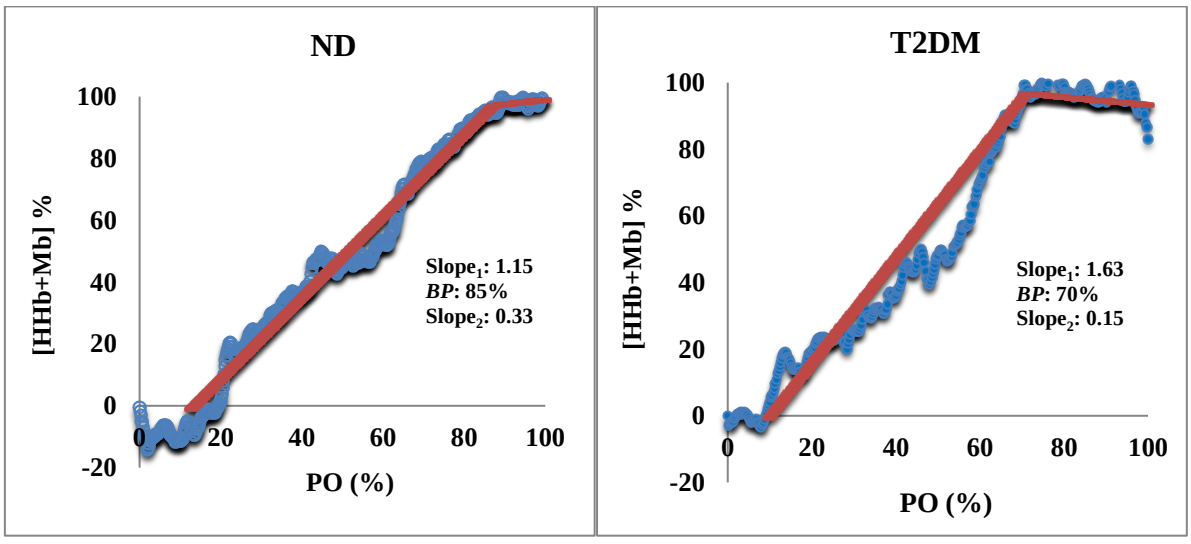

Figure 2.2. Representative profiles of the modelled [HHb+Mb] response dynamics during ramp incremental exercise for an individual without, and an individual with T2DM when expressed as a function of relative power output (PO%). Double-linear regression models superimposed on the data; associated coefficients are included on each panel (pre-BP above, post-BP below).

2.4 DISCUSSION

The present study examined for the first time the influence of T2DM on the profile of muscle fractional oxygen extraction, as indicated by the NIRS-derived Δ[HHb+Mb] response during ramp incremental exercise. The main finding of this study was that;

1. The first slope of the double linear regression function used to establish the dynamic adjustment of [HHb+Mb] was significantly (P<0.05) steeper in participants with T2DM than ND controls (1.14 ± 0.21 *vs.* 1.48 ± 0.46), however a similar Δ[HHb+Mb]-*BP* relative to PO% was observed between the groups (81 ± 11 and 77 ± 11, %).

Our findings of a 21% lower $\dot{V}O_{2max}$ ($mL.min^{-1}.kg^{-1}$) and lower exercise capacity in T2DM compared with their ND counterparts are consistent with the approximate 20% reduction in $\dot{V}O_{2max}/\dot{V}O_{2peak}$ previously reported in adults with T2DM (Regensteiner *et al.* 1998; Baldi *et al.* 2003; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012). However, the influence of T2DM on aerobic capacity is complex, with multiple physiological mechanisms serving as potential culprits for such reductions in functional capacity in this clinical population.

In the literature, impaired aerobic capacity is often attributed to reductions in peak CO and [a-vO_2] (Green *et al*. 2015.) Central O_2 delivery may very well limit functional capacity because of limitations in cardiac function and subsequent blood flow. In the present study, individuals with T2DM displayed a significant reduction in peak CO (15.37 ± 2.34 *vs.* 12.08 ± 1.98 $L.min^{-1}$; P<0.05) as well as a tendency for a significantly reduced peak HR (170 ± 15 *vs.* 162 ± 15; P=0.074) compared to their respective ND counterparts. It is thus plausible to speculate that such reductions may have at least in part contributed to the impaired exercise response.

Given the pattern of change in [a-vO_2] can be estimated through the use of the deoxy-haemoglobin signal from NIRS (DeLorey *et al.* 2003; Grassi *et al.* 2003; Ferreira *et al.* 2005b), we employed a similar technique to measure changes in tissue deoxygenation (Δ[HHb+Mb]) within the microvasculature of the *vastus lateralis* muscle during the ramp protocol. As changes in Δ[HHb+Mb] are considered a surrogate for microvascular O_2 extraction (DeLorey *et al.* 2003), we wanted to explore the possibility of uncharacteristic local muscle fractional O_2 extraction contributing to the reduced $\dot{V}O_{2max}$ in T2DM, and thus, NIRS was utilised to facilitate the identification of a possible mismatch between O_2 delivery and utilisation in the active musculature of T2DM individuals.

Accelerated active muscle deoxygenation depicts an increased dependence on O_2 extraction in providing adequate $\dot{V}O_2$ at a given work rate. In the present study, the steeper primary slope

(1.48±0.46 *vs.* 1.14 ± 0.21, $P<0.005$) of the bi-linear regression (HHb% as a function of PO %) observed in T2DM is suggestive of a reduced capacity to increase peripheral O_2 delivery amidst increasing O_2 demands (Rissanen *et al.* 2015). As observed in rodent models with T2DM (Behnke *et al.* 2002b), the reported greater Δ[HHb+Mb] responses in T2DM compared with ND rodents at any given absolute PO may be associated with a lower microvascular PO_2, and thereby lower O_2 diffusion to the muscle mitochondria. It is noteworthy that at near peak levels of PO, however, active muscle deoxygenation occurred at a similar rate (0.28 ± 0.73 *vs.* -0.09 ± 0.83, $P=0.240$) implying that a balance in O_2 delivery and utilisation in both groups as maximal aerobic capacity was reached.

These findings are in accordance with the theoretical interpretation that a reduced perfusion pressure and hence muscle O_2 availability leads to a reduction in $\dot{V}O_{2max}$ and exercise tolerance (Hughson *et al.* 1996). This concept has consistently been supported by studies whereby O_2 availability during exercise is manipulated via adopting the supine posture (Hughson *et al.* 1996; MacDonald *et al.* 1998; Koga *et al,* 1999) with demonstrations of impaired $\dot{V}O_2$ kinetics (Hughson *et al.* 1993; MacDonald *et al*. 1997; Jones *et al,* 2006) and reductions in $\dot{V}O_{2max}$ (Koga *et al.* 1999; Jones *et al.* 2006; Egaña *et al.* 2007). Furthermore, in a more recent study, DiMenna *et al.* (2010a) reported a greater reliance on fractional O_2 extraction during ramp incremental exercise in the supine posture compared to the upright position, with the demonstration of an increased %Δ[HHb+Mb] response which progressively increased relative to increasing exercise intensity (%PO). This was displayed by the sigmoidal %Δ[HHb+Mb]/PO(%) profile, as proposed by Ferreira *et al.* (2007) in both the upright and supine postures. Interestingly, the authors reported that the slope of the sigmoid was approximately doubled for supine cycling compared to the upright posture, thus indicating significantly greater oxygen extraction for the same power output.

At the other end of the spectrum, a rightward shift of the %Δ[HHb+Mb] pattern relative to percentage peak power was displayed by a symmetrical sigmoid model in trained male cyclists during upright ramp incremental cycling exercise when compared to less trained physically active males (Boone *et al*. 2009). This altered profile of Δ[HHb+Mb] is indicative of an enhanced muscle blood flow/oxygen uptake relationship with increased aerobic fitness, i.e., a greater muscle perfusion relative to metabolic demand. The authors attributed these findings to an increased oxidative capacity and/or higher percentage of slow-twitch fibres, both of which are typically characteristic of endurance trained cyclists.

With evidence of a concomitant imbalance in local muscle O_2 utilisation and microvascular blood flow during ramp exercise in T2DM, attention is directed to peripheral factors in an attempt to determine the potential mechanisms behind these responses.

2.4.1 Blood flow

Simultaneous increases in systemic CO and arterial pressure, with reductions in blood supply to non-active tissues have been well established as the primary mechanisms in facilitating the increased O_2 requirement of active tissues during exercise of increasing intensities.-Moreover, the combined interaction between active muscles and their microvascular supply is paramount if such metabolic requirements are to be fulfilled. During intense aerobic exercise, muscle blood flow at the level of the microcirculation can increase by a factor of 100 (Thomas & Segal, 2004) and is facilitated via an interplay between arteriolar, capillary and venular segments in response to local and regional metabolic demand. This response is regulated by the functional interactions between the skeletal muscle fibres and the respective smooth muscle cells, endothelial cells and neural projections (Segal, 2005).

Pulmonary $\dot{V}O_2$ (Andersen & Saltin, 1985; Day *et al.* 2003) and muscle blood flow (Andersen & Saltin, 1985; Richardson *et al.* 1995) increase in a linear fashion with PO and subsequent ATP requirements during ramp incremental exercise. In recognition of the differing regulatory events acting at local levels of the microvasculature and at regional levels, Murias *et al.* (2013) explored their subsequent influence on the delivery and extraction of O_2 during ramp incremental exercise. Through the simultaneous measurement of the dynamic adjustment of systemic (open circuit acetylene) and peripheral blood flow and O_2 extraction (NIRS) in 20 healthy young individuals, Murias and colleagues (2013) ascertained that the linear relationship existing between systemic blood flow and metabolic demand during progressive exercise is not in fact a replicated at the level of the active musculature. Instead, whilst exercising within the 'moderate'-intensity domain, a nonlinear increase in local blood flow to the active muscle concomitant with a rapid increase of muscle deoxygenation as $\dot{V}O_2$ progressively increased was observed. However, upon entering the heavy-intensity domain whilst progressing to maximal exercise, a plateau of local deoxygenation was reached.

The notion that peripheral blood flow and hence O_2 delivery are indeed governed by different mechanisms at the level of the microvasculature, combined with strong evidence of impaired peripheral vascular function, and herewith, peripheral O_2 delivery to active muscles in T2DM could potentially provide an important insight to the impaired $\dot{V}O_{2max}$, as well as $\dot{V}O_2$ kinetics response reported in T2DM. The impaired kinetics response of $\dot{V}O_2$ demonstrated during

submaximal exercise in T2DM, serves as an important potential determinant of early onset fatigue and exercise intolerance (Hughson & Tschakovosky, 1999). Profound impairments in the primary phase of this response, observed during moderate-intensity exercise in T2DM (Regensteiner *et al.* 1998; Brandenburg *et al.* 1999; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012), are reflective of poorer cardiorespiratory adjustment in this cohort and thus, it is all the more plausible that the control of $\dot{V}O_2$ in T2DM is reliant on the factors governing O_2 delivery and/or extraction at a microvascular level.

The strong evidence in favour of an attenuated hyperaemic and haemodynamic response in this clinical population should be considered in combination with the primary finding from the present study, whereby individuals with T2DM displayed an increased dependence on O_2 extraction in providing adequate $\dot{V}O_2$ at a given work rate. Thus, an impaired vascular function, consequent to a compromised vasodilatory response to metabolic stimulus and subsequent reduced tissue perfusion may contribute to the observed limitation of $\dot{V}O_2$ and exercise performance in T2DM. However, it is pertinent to acknowledge a potentially impaired cardiac function and subsequent regional O_2 delivery, consequent to the significant reduction in CO (P>0.05) in this group with T2DM. It is thus plausible to speculate that these substantial reductions may have at least in part contributed to the impaired exercise response.

2.4.2 Skeletal muscle

Given its sheer size as an organ, skeletal muscle is also likely to play an integral role in the impairments of the exercise response in T2DM. Skeletal muscle is composed of a mixture of fibre types: type I, IIa and IIb, each possessing very different metabolic characteristics thus, differ in their energy requirements for cellular function. These fundamental characteristics dictate their relative contribution to the production of energy, be that from PCr-CK, glycolysis or oxidative phosphorylation (Gehlert *et al.* 2012). "Metabolic flexibility" being the systemic capacity to alternate between substrates for ATP production according to supply and demand (Kelley *et al.* 1999) is permitted as a result of these differing fibre types. However, individuals with T2DM have been reported to experience a high degree of metabolic inflexibility (Kelley *et al.* 1999), being incapable of alternating between free fatty acids or glucose due to defects in the pathways controlling energy homeostasis. This contributes greatly to the further development of insulin resistance within the skeletal muscle (Storlien *et al.* 2004).

Type I fibres are associated with increased oxidative enzyme expression and are thus, both metabolically and mechanically efficient while the converse applies to type IIb fibres (Storlien *et al.* 2004). However, structural changes have been observed in the skeletal muscles of T2DM,

with reductions in mitochondrial content (Ritov *et al.* 2005; Boushel *et al.* 2007) and functional capacity (Kelley *et al.* 2002; Ritov *et al.* 2005) as well as alterations in muscle fibre type (Marin *et al.* 1994) being reported.

The mitochondria constitute the major site for oxidative phosphorylation upon which skeletal muscle is heavily reliant. With the vast majority of mitochondria found in slow twitch type 1 and type IIa muscle fibres they possess a high capacity for oxidative metabolism. Through enzyme measurements of oxidative capacity, Kelley *et al.* (2002) demonstrated that individuals with T2DM display substantial reductions of approximately 30% in mitochondrial size, even when corrected for fibre type, as well as a reduction of approximately 40% in the mitochondrial respiratory complex I activity. This dictates a compromised skeletal-muscle mitochondrial bioenergetics capacity, thereby potentially impacting exercise capacity through influencing the $\dot{V}O_2$ dynamic response.

Furthermore, it is well recognised that blood flow to skeletal muscles is also governed by its composition of different fibre types (Kindig & Poole, 1998). The enhanced ability for endothelium-dependent vasodilation is attributed to the highly oxidative type I fibres in addition to a lower sympathetic mediated vasoconstriction at rest (Delp & Armstrong, 1988). However, they also possess a reduced capacity to moderate sympathetic vasoconstriction "sympatholysis" during muscle contraction (Thomas *et al.* 1994). With increasing work rate progressive recruitment of muscle fibres from primarily type I to a mixed type I/type II pool is typically observed as exercise intensity or duration increases (Gollnick *et al.* 1974). In both isolated rat preparations and human muscle, fibres positioned higher in the recruitment hierarchy have expressed a greater fractional O_2 extraction to achieve a given rate of oxidative metabolism during exercise (Behnke *et al.* 2003, McDonough *et al.* 2005, Ferreira *et al.* 2006). With type 1 fibres having a higher microvascular O_2 pressure, and possessing a higher capillary-to-myocyte O_2 driving pressure, they subsequently rely less on fractional O_2 extraction to attain a given $\dot{V}O_2$ (Behnke *et al.* 2003; McDonough *et al.* 2005). This indicates that muscles composed primarily of slow-twitch fibres demonstrate a faster adjustment and matching of microvascular blood flow relative to muscle O_2 utilisation, compared with fast twitch fibres.

However, muscle fibre distribution has shown to be altered in individuals with T2DM, possessing a lower proportion of Type I muscle fibres relative to Type IIb (Marin *et al.* 1994) with an approximate 2-fold increase in type IIb fibres (Mogensen *et al.* 2007). It is possible that such an alteration in muscle fibre type, can also contribute to the earlier recruitment of the higher order fibres and thus a greater reliance on fractional O_2 extraction in the present study.

These type II fibres possess a lower capillary-to-myocyte O_2 driving force compared to their slow oxidative antagonists at rest and during submaximal exercise intensities (Behnke *et al.* 2003; McDonough *et al.* 2005). Thus the metabolic consequence of the earlier recruitment of these fibres will be a lower intracellular energy state (with a reduction in [ATP]/[ADP]. [Pi]; NAD/NADH, and [PCr]) required to maintain $\dot{V}O_2$, subsequently contributing to their lower resistance to fatigue (Ferreira *et al.* 2006). It is therefore reasonable to speculate that the potentially altered muscle fibre recruitment in our T2DM cohort may possibly have contributed to the increased reliance on fractional oxygenation observed.

However, the above postulation was not corroborated with the demonstration of similar $\Delta\dot{V}O_2/\Delta PO$ slopes in individuals with T2DM and the ND controls in the present study. It should be acknowledged that this may be due to the fact that these slopes pertain to pulmonary $\dot{V}O_2$, and thus reflect the culmination of muscle $\dot{V}O_2$ and $\dot{V}O_2$ from the rest of the body, as well as convective O_2 transport and changes in lung gas stores (Barstow & Molé, 1987).

2.4.3 Conclusion

It should be noted that a large body of evidence exists supporting the concept of increased arterial stiffness in T2DM (Cruickshank *et al*. 2002; Schram *et al*. 2004; Stehouwer *et al*. 2008). T2DM has been demonstrated to be independently associated with lower aortic distensibility when compared with age-, sex-, comorbidities- (Lee *et al*. 2007; van der Meer *et al*. 2007) MAP-, HR- and BMI- matched controls (Schram *et al*. 2004). We thereby acknowledge that observations in the present study may have been influenced by a diminished distensibility of individuals' central and/or peripheral vasculature. However, although individuals with T2DM displayed significantly higher PWV values than their respective ND counterparts (8.6 ± 1.7 *vs.* 6.7 ± 1.4 ms^{-1}) we are unable to comment with certainty on the clinical significance of these findings.

A fixed threshold value of $12ms^{-1}$ was proposed for carotid-femoral PWV in the 2007 European Society of Hypertension (ESH) and European Society of Cardiology (ESC) hypertension guidelines (Mancia *et al*. 2007). This was deemed a conservative estimate of significant alterations of aortic function in middle-aged hypertensive individuals, with PWV values above this threshold, increasing the predictive value for fatal and non-fatal CV events in these individuals (Mancia *et al.* 2013) These reference values were determined as a function of age and BP between populations, as were the reported values in the present study.

More recently however, this threshold has been questioned, as it was based on published epidemiological studies, and could not account for other factors which can influence PWV; the traditional major CV risk factors (Boutouyrie *et al*. 2010) and the non-standardisation of measurement techniques applied (VanBortel *et al.* 2012). Subsequently, in a collaborative study based on a large European population (11,092 individuals) reference values were proposed whereby gender, dyslipidaemia and smoking status were accounted for and further categorised according to age and BP (Boutouyrie *et al*. 2010). The PWV values reported in the present study also lie within the normative ranges presented by Boutouyrie and colleagues (2010) when categorised according to age and the normal BP range (≥ 120/80 and <130/85 mmHg), and high-normal BP range (≥130/85 and <140/90 mmHG).

Additionally, in a more recent expert consensus statement by VanBortel and colleagues (2012), the authors proposed that the threshold value be adjusted to $10ms^{-1}$ providing arguments for the requirement of a standardised measurement procedure to be implemented whereby only 80% of the direct carotid-femoral distance should be considered. Subsequently, this lower cut-of threshold has been proposed in the more recent 2013 ESH/ESC hypertension guidelines (Mancia *et al.* 2013).

Furthermore, it has been demonstrated that many routinely prescribed anti-hypertensive medications reduce arterial stiffness, with renin-angiotensin-aldosterone system (RAAS) inhibitors, such as ACE inhibitors and angiotensin II receptor blockers (ARBs), being widely suggested to have a BP independent effect on arterial stiffness (Asmar *et al.* 2002; Laurent & Boutouyrie, 2007 Karalliedde *et al*. 2008). Currently, (RAAS) inhibitors are the first-line drugs for hypertension treatment in T2DM patients, which was also the case for many of the participants in the present study (Table 2.3), and thus, we cannot account for the potential impact these medications may have had on the PWV values recorded. Thereby, we acknowledge that important differences in absolute PWV values exist, and therefore the clinical significance of the PWV results presented in this study should be interpreted cautiously.

Although the findings from this present study, do not fully explain the observed defect in exercise capacity in diabetes, they do offer an insight into potential contributory mechanisms. The demonstration of a greater rate of oxygen extraction for a given increase in PO suggest that a reduced O_2 delivery is an important underlying cause of exercise intolerance during a maximum graded test in T2DM.

Chapter 3: Influence of priming exercise on oxygen uptake and muscle deoxygenation kinetics during moderate-intensity cycling in type 2 diabetes.

3.1 INTRODUCTION

Extensive evidence exists that young and middle-aged individuals with uncomplicated T2DM demonstrate a profound slowed oxygen uptake ($\dot{V}O_2$) kinetics at the onset of submaximal exercise (Regensteiner *et al.* 1995, 1998; Brandenburg *et al.* 1999; Brassard *et al.* 2006; Nadeau *et al.* 2009; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012, 2015) represented by a prolonged time constant of the primary phase of $\dot{V}O_2$ kinetics ($\tau\dot{V}O_{2p}$). This mandates that individuals with T2DM will exhibit an increased oxygen deficit, thus placing greater reliance on non-oxidative sources for energy production to provide ATP in sufficient amounts to sustain any given activity (Jones & Poole, 2005). Clinically these findings are significant as they suggest a greater perturbation of intracellular homeostasis (Poole *et al.* 2008a) in response to any exercise challenge with the potential to contribute to premature muscular fatigue (Scheuermann-Freestone *et al.* 2003) and hence contribute to the reduced exercise intolerance consistently demonstrated by individuals with T2DM (Regensteiner *et al.* 1995, 1998; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012).

In T2DM whereby the existence of a prolonged $\dot{V}O_2$ time constant is reflective of poorer cardiorespiratory adjustment, the subsequent exercise intolerance has been attributed to dysfunction at several steps of the O_2 transport pathway. Consequently, contradictory evidence exists surrounding the influence and role of the factors involved. On one hand, the presence of left ventricular dysfunction (Fang *et al.* 2005a), impaired stroke volume reserve (Joshi *et al.* 2009), diastolic abnormalities (Poirier *et al.* 2000), slower heart rate kinetics (Regensteiner *et al.* 1998) and reduced cardiac output (Roy *et al.* 1989) are suggestive of perturbations in cardiac function in T2DM in response to exercise. On the other hand, a NIRS-derived transiently increased skeletal muscle deoxygenation response displayed by individuals with T2DM (Bauer *et al.* 2007) upon initiation of submaximal cycling exercise has been attributed to a relative mismatch in muscle O_2 delivery to $\dot{V}O_2$ during the early stages of submaximal exercise. This, in combination with evidence of an impaired O_2 delivery to active muscles such as; compromised peripheral vascular function (MacAnaney *et al.* 2011b; Kiely *et al.* 2014), reductions in leg blood flow independent of reductions in CO (Lalande *et al.* 2008; MacAnaney *et al.* 2011b), or blunted endothelium-dependent vasodilation (McVeigh *et al.* 1992; Kingwell *et al.* 2003; Lalande *et al.* 2008), suggest the maldistribution of active muscle blood flow in this

clinical population (Kingwell *et al.* 2003; Lalande *et al.* 2008; MacAnaney *et al.* 2011b; Kiely *et al.* 2014).

Traditionally, studies examining these implicating factors have relied on measurements of bulk blood flow in the large conduit arteries delivering blood to the exercising limbs, combined with the sampling of arterial and venous blood at discrete time points to calculate the (a-vO_{2diff}) (DeLorey *et al.* 2003). However, with the Fick relationship being determined at pulmonary level, this is representative of systemic fractional O_2 extraction (Benson *et al*. 2013) and not that of the discrete adjustments that occur between oxygen delivery and metabolic demand at the level of the active muscle vasculature (Spencer *et al.* 2012; Murias *et al.* 2013; Okushima *et al.* 2016). Subsequently, in recent years, several investigators have employed near infra-red spectroscopy (NIRS), to assess the non-invasive determination of a muscle's oxygenation index by means of the concentration changes in deoxygenated haemoglobin and myoglobin ([HHb+Mb]) thereby, being considered a surrogate for microvascular O_2 extraction (DeLorey *et al.* 2003). By facilitating the quantification of O_2 extraction, NIRS provides insights into the dynamic balance between regional O_2 delivery and utilisation at the level of the microvasculature (Spencer *et al.* 2012).

Heavy priming exercise (PE) has been identified as an intervention that may influence both intracellular metabolic processes and improved muscle O_2 delivery, especially in diseased and aging populations (Poole *et al.* 2008a). Significant reductions have been demonstrated in the primary $\dot{V}O_2$ time constant in older individuals; a population consistently demonstrating slowed $\tau\dot{V}O_{2p}$ responses (Poole & Musch, 2010; Murias & Paterson, 2015), when moderate-intensity exercise is preceded by a bout of heavy-intensity priming exercise (Scheuermann *et al.* 2002; DeLorey *et al.* 2004b; DeRoia *et al.* 2012). Similar improvements have also been observed following priming exercise in healthy young individuals that present with an initially slow $\dot{V}O_2$ kinetics response ($\tau\dot{V}O_{2p}$ in excess of 20 s) (Gerbino *et al.* 1996; DeLorey *et al.* 2004b; Gurd *et al.* 2005, 2006; Chin *et al.* 2010; Murias *et al.* 2011c). This may potentially be explained by an association between this time constant of $\dot{V}O_2$ and that of the splitting of phosphocreatine (~20 s), and hence, it is unlikely that any additional improvement of local O_2 delivery in those participants with $\tau\dot{V}O_{2p}$ < 20 s will further influence the rate of adjustment of $\dot{V}O_2$ kinetics (Jones & Poole, 2005).

The mechanisms contributing to the enhanced $\dot{V}O_2$ dynamic response observed in submaximal exercise subsequent to a bout of heavy-intensity priming exercise remain a subject of considerable debate (Tschakovsky & Hughson, 1999; Jones & Poole, 2005; Poole *et al.* 2008a).

Purported mechanisms however, include increased blood flow (Bangsbo *et al.* 2001; Koppo & Bouckaert, 2001), enhanced muscle perfusion (Wilkerson *et al.* 2004a; DeLorey *et al.* 2007; Jones *et al.* 2008), heightened oxidative enzyme activity (Wilkerson *et al.* 2005; Gurd *et al.* 2006) and/or O_2 extraction (Krustrup *et al.* 2001; DeLorey *et al.* 2007).

Interestingly, in a study by Murias *et al.* (2011c) whereby the relationship between the adjustment of muscle deoxygenation to $\dot{V}O_2$ in young healthy individuals presenting with a range of slow to fast $\dot{V}O_2$ kinetics was explored, a strong association (r =0.91) occurred between Δ[HHb+Mb]/$\Delta\dot{V}O_2$ and $\dot{V}O_2$ time constants. Elevated ratios (>1) of Δ[HHb+Mb]/$\Delta\dot{V}O_2$ were demonstrated in the groups with slower $\dot{V}O_2$ kinetics displaying a transient 'overshoot' relative to the subsequent steady-state exercise which was progressively reduced as $\tau\dot{V}O_{2p}$ was reduced. The authors claimed that when $\tau\dot{V}O_{2p}$ was in excess of 20 s the rate of adjustment appeared to be predominantly constrained by the matching of local O_2 distribution to muscle O_2 uptake. Thus, it is considered that in individuals presenting with slower $\dot{V}O_2$ kinetics, the rate of adjustment is most likely curtailed by an impaired O_2 availability within the active tissues.

Such favourable manipulation of the $\dot{V}O_2$ kinetics response via heavy-intensity priming exercise particularly in the aging population is very promising. Thus, with the substantial commonality which exists between T2DM and aging in terms of exercise tolerance, functional aerobic capacity and the putative constraining mechanisms implicated in the same, the aim of the present study was to investigate the influence of priming exercise and T2DM on oxygen uptake and muscle deoxygenation kinetics during submaximal exercise.

In keeping with the accumulated evidence from the literature, we therefore hypothesised that:

1) A bout of heavy priming exercise would speed the adjustment of the primary phase of the $\dot{V}O_2$ kinetics response in a subsequent bout of moderate-intensity cycling exercise in individuals with T2DM.
2) The reduction in the effective time constant of the $\dot{V}O_2$ kinetics response would be due to a better matching of microvascular O_2 delivery to utilisation, as reflected by a smaller NIRS-derived muscle haemoglobin deoxygenation (Δ[HHb+Mb]) to $\dot{V}O_2$ ratio (i.e. reduced Δ[HHb+Mb]/$\Delta\dot{V}O_2$) throughout the exercise on-transient.

3.2 METHODOLOGY

3.2.1 Participants

Twelve individuals with uncomplicated T2DM (7 males/5 females) and twelve individuals without T2DM (ND) (7 males/5 females) volunteered to participate in this study (Table 3.1). Eight of the individuals with T2DM, and eleven of the ND controls also participated in *Experiment 1*. Four female participants were premenopausal (2 T2DM and 2 ND) and six were postmenopausal (3 T2DM and 3 ND) not undergoing HRT. All participants were non-smokers and had not smoked during the 12-month period preceding the study. All of the patients with T2DM had a clinical history of diabetes between 2 to 10 years (mean ± SD = 5.9 ± 4.2 yrs).

3.2.1.1 Recruitment of participants

3.2.1.1.1 Individuals without T2DM (ND)

As per section 2.2.1.1.1

3.2.1.1.2 Individuals with T2DM

As per section 2.2.1.1.2

3.2.1.2 Inclusion /exclusion criteria

3.2.1.2.1 Individuals with T2DM

As per section 2.2.1.2.1

3.2.1.2.2 Individuals without T2DM (ND)

As per section 2.2.1.2.2

3.2.1.2.3 Blood sample collection

As per section 2.2.1.2.3

3.2.1.2.4 Ankle brachial index (ABI)

As per section 2.2.1.2.4

3.2.1.3 Determination of physical activity levels

As per section 2.2.1.3

3.2.1.3.1 RT3 Tri-axial accelerometers

As per section 2.2.1.3.1

3.2.1.3.2 The Low Level Physical Activity Recall questionnaire (LOPAR)

As per section 2.2.1.3.2

3.2.2 **Experimental design**

3.2.2.1 Study overview

Individuals without T2DM (ND) were required to visit the cardiovascular performance laboratory in the Department of Physiology, Trinity College Dublin on two separate occasions, whilst all individuals with T2DM were required to visit the exercise testing facility in St. Columcille's Hospital on two occasions. All premenopausal participants were tested during the mid-follicular phase (days 5-12) of the menstrual cycle, which was self-determined. All participants were asked to refrain from consuming alcohol, caffeine and non-prescribed nutritional supplements in the 24 hours prior to testing, and to limit their exercise to normal activities of daily living.

3.2.2.1.1 Visit 1 overview

During this visit, initially anthropometric data was collected, followed by the measurement of the aortic pulse wave velocity (PWV). Participants then performed a ramp incremental cycling test to exhaustion to enable the determination of $\dot{V}O_{2max}$ and ventilatory threshold (VT) (*as per section 2.2.4.3*). Following a 5 min passive recovery period, participants finally completed a 'confirmatory' high-intensity cycling bout (*as per section 2.2.2.2.3).*

3.2.2.1.2 Visit 2 overview

From the previously completed ramp incremental cycling test the work rates required for this protocol were calculated. A power output (PO) equivalent to 80% of the PO at VT (moderate-intensity), and a PO corresponding to 50% delta (Δ50%; the sum of the PO at VT and 50% of the difference between the PO at VT and $\dot{V}O_{2max}$) were determined.

3.2.2.2 Visit 1 to the cardiovascular laboratory

3.2.2.2.1 Anthropometry and pulse wave velocity

As per section 2.2.2.2.1

3.2.2.2.2 Ramp incremental cycle test to exhaustion

Participants performed a ramp incremental cycling test to exhaustion in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, The Netherlands) with appropriate adjustments made to the ergometer seat and handle bar position for each participant. Exercise was performed initially for 2 min at a baseline resistance of 10W (i.e. 'unloaded' cycling), to reduce excess internal work (Boone *et al.* 2008). This was followed by 10/15 $W.min^{-1}$ increments in females (*n*=2/8) or 15/20/25 $W.min^{-1}$ increments in males (*n*=5/8/1) respectively depending on activity levels of participants, and were brought to volitional exhaustion. Pedal frequency was held constant at an individually selected cadence between 60-75 revolutions per minute (rpm). This cadence was maintained throughout all further testing protocols. Failure in a test was determined as a drop in cadence exceeding 5 rpm for >3 s. Peak workload was determined according to the point of termination of the test. HR was continuously monitored while pulmonary oxygen uptake ($\dot{V}O_2$), pulmonary carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER; $\dot{V}CO_2/\dot{V}O_2$) were recorded on a breath-by-breath (BbB) basis. Peak HR was defined as the highest heart rate attained within the last 15 s of the point of termination of the test.

As it is frequently reported that some individuals do not evidence a definitive plateau of the $\dot{V}O_2$-work rate relationship on this test, secondary criteria based upon measurements of the RER, maximal heart rate or blood [lactate] (Poole *et al.* 2008) are often relied upon to corroborate a maximum effort (Astrand & Rodahl, 1986; Rossiter *et al.* 2006; Poole *et al.* 2008). However, such criteria have been called into disrepute (Poole *et al.* 2008), thus, the utilisation of a subsequent confirmatory high-intensity constant load bout *(see section 2.2.2.2.3)* has frequently been implemented (Day *et al.* 2003; Rossiter *et al.* 2006; Murias *et al*. 2010).

In the present study all participants demonstrated a plateau in $\dot{V}O_2$ during the confirmatory tests, and moreover the $\dot{V}O_2$ values recorded during this confirmatory test were not different to those obtained during the ramp incremental cycle test. Thus, we were certain that in the present study, all participants achieved a $\dot{V}O_{2max}$.

3.2.2.2.3 Confirmatory test

Within five minutes of completion of the ramp incremental cycle test, participants performed a high-intensity, constant-load cycling bout to exhaustion at an intensity equivalent to 85% of the peak power output achieved in the ramp test. This protocol was performed to confirm the attainment of $\dot{V}O_{2max}$ in the prior graded exercise test whilst also facilitating the non-invasive

determination of maximal cardiac output via the inert rebreathing technique (Innocor; Innovision, Denmark). Participants were instructed to indicate via the raising of their hand when they felt they were ~30 s from exhaustion. At this point, individuals were verbally encouraged to continue and within 30 s the measurement of CO was carried out.

3.2.2.3 Visit 2 to the cardiovascular laboratory

3.2.2.3.1 Priming effect on moderate-intensity cycling exercise

Individuals performed four bouts of constant-load, moderate-intensity cycling at 80% VT. Two of these constant-load bouts were completed without prior priming exercise (Mod A) and two bouts were undertaken preceded by a priming exercise (PE) at an intensity of Δ50% (Mod B). The duration of each step transition was 6 min and each transition was preceded by a 3 min 'baseline' cycling period at 10W. Changes in PO were initiated as a step function without a warning to the individual. There was a 12 min rest period between each of the cycling bouts, except following the first primed moderate intensity bout (Mod B). In this instance, participants remained seated in a chair for 45 min. This is the advocated time for physiological restoration following a bout of heavy intensity exercise, thus having no effect on $\dot{V}O_2$ kinetics during subsequent exercise (Burnley *et al.* 2006a). *Figure 3.1* displays a schematic representation of the protocol.

3.2.2.4 Cardiac output responses

Cardiac output was determined non-invasively during the fifth minute of the unprimed moderate-intensity cycling bout via the inert gas rebreathing technique (Innocor; Innovision, Denmark; *see section 2.2.3.5).*

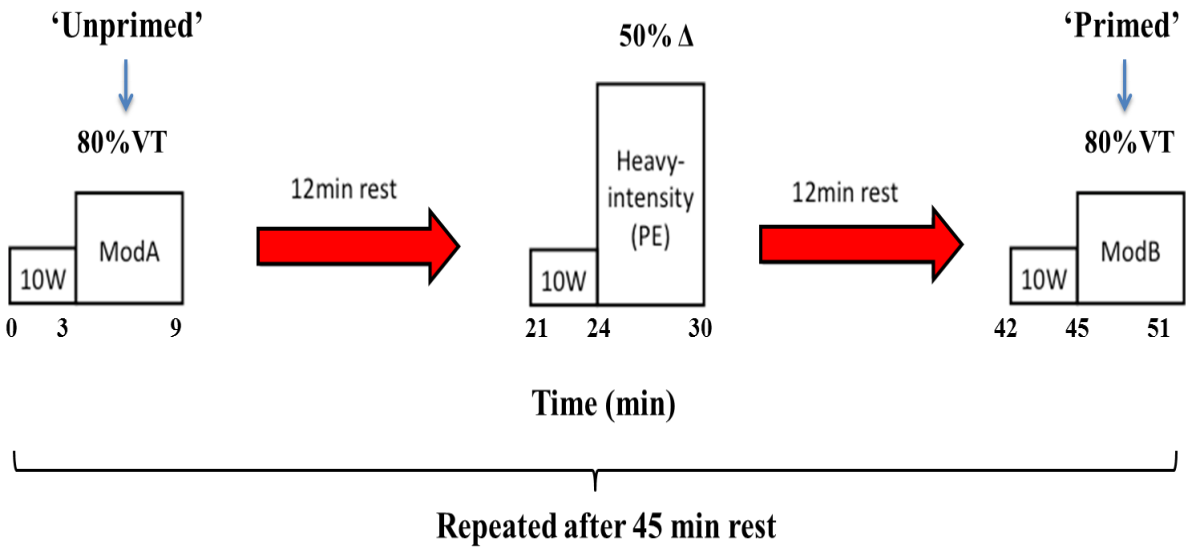

Figure 3.1 ***Schematic representation of the protocol.***

3.2.3 Equipment and techniques

3.2.3.1 Cardiometabolic unit, heart rate and pulse oximeter

As per section 2.2.3.1

3.2.3.2 Near infrared spectroscopy (NIRS) and subcutaneous fat layer of the vastus lateralis

As per section 2.2.3.4

3.2.4 Data analysis

3.2.4.1 Physiological responses at peak and VT

As per sections 2.2.2.2.2 and 2.2.4.3, respectively.

3.2.4.2 80% VT data analysis

3.2.4.2.1 $\dot{V}O_2$ Kinetics

The $\dot{V}O_2$ to moderate-intensity exercise (<VT) is well described by a mono-exponential function (Whipp & Wasserman, 1972) (Equation 1), whereby $\dot{V}O_2$ rises rapidly to attain a steady-state (Phase III) within ~2 mins (*section 1.8.1*). However, for exercise above the VT, attainment of a steady-state in $\dot{V}O_2$ is delayed due to the emergence of a second component; the

$\dot{V}O_2$ slow component *(section 1.8.1)* and is therefore better described by a bi-exponential function (Whipp *et al.*1982) (Equation 2).

Any exponential function has an amplitude and a time constant (τ), which reflects the time required for the attainment of 63% of the total amplitude (Figure 1.3). The exponential nature of the Phase II $\dot{V}O_2$ response can thus be described with the following equation:

Equation 1 $$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A[1\text{-}e^{-(t\text{-}TD)/\tau}]$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time *t*; $\dot{V}O_2$ baseline is the mean $\dot{V}O_2$ before the commencement of the step transition to the higher-intensity work rate; A is the steady-state amplitude of the increase in $\dot{V}O_2$, and $[1\text{-}e^{-(t\text{-}TD)/\tau}]$ is the exponential function describing the rate at which $\dot{V}O_2$ is rising towards the steady-state amplitude. In this exponential function, TD represents the time delay before the start of the exponential term and τ is the time constant.

By the same token, the $\dot{V}O_2$ response to heavy-intensity exercise (>VT) is better represented by a bi-exponential function (Whipp *et al.*1982) (*Figure 1.3)* of the form:

Equation 2 $$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p[1\text{-}e^{-(t\text{-}TD_p)/\tau_p)}]\cdot F1 + A_s[1 - e^{-(t - TD_s)/\tau_s)}]\cdot$$

where A_p and A_s are the amplitudes of the $\dot{V}O_2$ primary and slow components respectively, TD_p and TD_s are the independent time delays before the commencement of the respective primary and slow components, and τ_p and τ_s are the time constants for the primary and slow components, respectively.

In this experiment, the breath by breath $\dot{V}O_2$ data for each moderate-intensity transition were linearly interpolated to provide second-by-second values and time aligned such that time 0 represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, "average" response for each individual and further time-averaged into 5 s bins to provide a single time-averaged response per individual. The first 20 s of data after the onset of exercise were deleted in an attempt to avoid inclusion of data points from the cardiodynamic phase, thus ensuring that the Phase II component is being manifest**.** The model parameters of the $\dot{V}O_2$ on-transient kinetic response were thus determined from 20-360 s of the step transition via a weighted least-squares nonlinear regression procedure (TableCurve 2D, Systat) in which the best fit was defined by minimization of the residual sum of squares. Data points lying outside the 95% prediction interval during the initial fit of a model were excluded, being attributed to aberrant events, e.g. coughing.

Eight participants revealed an apparent $\dot{V}O_2$ slow component, suggesting that the ventilatory threshold was overestimated in these participants, and instead exercise was performed in the heavy-intensity domain. Accordingly, the averaged and smoothed response for each participant was suitably fitted to either the abovementioned monoexponential function (Equation 1) or biexponential function (Equation 2) as follows:

Equation 1 $$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p[1\text{-}e^{-(t\text{-}TD_p)/\tau_p)}]\cdot F1$$

Equation 2 $$\dot{V}O_2(t) = \dot{V}O_2 \text{ baseline} + A_p[1\text{-}e^{-(t\text{-}TD_p)/\tau_p)}]\cdot F1 + A_s[1 - e^{-(t - TD_s)/\tau_s)}]\cdot F2$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time *t*; $\dot{V}O_2$ baseline $_{is}$ the mean $\dot{V}O_2$ in the final 30 s of unloaded cycling; $A_p - A_{s,}$ are the amplitudes of the increase in $\dot{V}O_2$ of the primary and slow component phases respectively; $TD_p - TD_s$ are the phase delays, and $\tau_p - \tau_s$ are the time constants, representing the duration of time for which $\dot{V}O_2$ increases to a value equivalent to 63% of the amplitude. The conditional expressions F1-F2 limit the fitting of the phase to the period at and beyond the time delay associated with that phase.

Parameter estimates of the best-fit function were used and only estimates representing the primary phase are presented. Whilst the presence of a slow component was detected in 8 participants, the presence of this phase does not appear to significantly affect the parameter estimates of the earlier phases (Wilkerson *et al.* 2004b).

The steady-state $\dot{V}O_2$ response or the absolute primary component amplitude, referred to as $\dot{V}O_2$ End A was calculated using the following equation:

Equation 3 $$\text{End A} = \text{baseline } \dot{V}O_2 + A_p[1\text{-}e^{-(t\text{-}TD_p)\tau_p)}]$$

Additionally, the functional "gain" of the primary $\dot{V}O_2$ response, reflective of the increase in $\dot{V}O_2$ per unit increase in power output was computed by the following formula:

Equation 4 $$\dot{V}O_2 \text{ gain} = (\text{End A} - \text{Baseline } \dot{V}O_2 / (\text{PO @80\% VT} - 10\text{W})$$

3.2.4.2.2 Muscle deoxygenation [HHb+Mb] kinetics

Local muscle deoxygenation (Δ[HHb+Mb]) profiles of the right quadriceps *vastus lateralis* muscle were made with near infrared spectroscopy (NIRS) (Hamamatsu Niro 200Nx; Hamamatsu Photonics, Hamamatsu, Japan) throughout the exercise protocol *(as per section 2.2.3.4).* To provide information on muscle oxygenation throughout the protocol, we also modelled the [HHb+Mb] response to exercise. As per the $\dot{V}O_2$ data, the NIRS-derived Δ[HHb+Mb] data for each transition was linearly interpolated to provide second-by-second values and time aligned. Data from each transition were ensemble-averaged to yield a single, average response for each individual, and further time-averaged into 5 s bins to provide a single time-averaged response per individual.

A time delay at the onset of exercise occurs in the [HHb+Mb] profile before it increases with an exponential like time course (DeLorey *et al.* 2003) which has been interpreted to reflect a tight coupling between muscular O_2 uptake and local O_2 delivery (DeLorey *et al.* 2003; Grassi *et al.* 2003). This was determined in the present study via visual inspection as a systematic increase above the pre-transition level. [HHb+Mb] data were fitted from the first data point at the end of this TD to 180 s with a monoexponential model of the form in Eqn 1 (as per $\dot{V}O_2$; *section 3.2.4.2.1*) to determine the time course of muscle deoxygenation. The shorter fitting window of 180 s was selected to counteract the reported variations in the [HHb+Mb] signal, which typically present between 180-240 s from exercise onset, from impacting the fitting of the on-transient response whilst permitting the reaching of a steady-state (Ferreira *et al.* 2005a, 2005b; Murias *et al.* 2011a, 2011b). Visual inspection of the NIRS-derived HHb signal and analysis of least squares residuals suggested that fitting with a monoexponential model would reasonably estimate the time course of muscle deoxygenation during the time period corresponding to the primary phase of the $\dot{V}O_2$ response (DeLorey *et al.* 2003).

The time course for the increase in Δ[HHb+Mb] can be described by the τΔ[HHb+Mb], however, the time course for the overall change of the Δ[HHb+Mb] for the primary response phase, referred to as the effective τ'Δ[HHb+Mb], was determined from the sum of the time delay and τ [HHb+Mb] from the onset of exercise.

The end amplitude of the response, referred to as [HHb+Mb] End A_p was calculated using Eqn 3 (as per $\dot{V}O_2$; *section 3.2.4.2.1).*

3.2.4.2.3 Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio

Additionally, the parameter estimate of [HHb+Mb] amplitude, derived from the monoexponential fit, was used to determine the Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio during this phase of the response. As the [HHb+Mb] signal does not measure microvascular blood flow or O_2 delivery, this is instead an index of the degree of O_2 extraction required for a given increment in $\dot{V}O_2$, thus, reflecting the dynamic balance between O_2 delivery and utilisation (Murias & Paterson, 2015).

For the calculation of the Δ[HHb+Mb]/Δ$\dot{V}O_2$ index (Murias *et al.* 2010a, 2011b) the individual second-by-second Δ[HHb+Mb] and $\dot{V}O_2$ data were firstly normalised (from 0%, corresponding to the pre-transition 10W baseline value to 100% reflecting the post-transition steady-state response). Then, Δ[HHb+Mb] and $\dot{V}O_2$ were time aligned by left-shifting the normalised $\dot{V}O_2$ data by 20 s, accounting for the duration of the cardiodynamic phase, to ensure that the onset of exercise coincided with the beginning of the primary phase of $\dot{V}O_2$ (Murias *et al.* 2011b). Although previous investigations by Murias *et al* (2011b) identified potential limitations associated with the determination of this cut-off point, overall it was deemed a reasonable representation for the group tested. The normalised and time aligned data was then further averaged into 5 s bins.

The overall Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio for the adjustment during the exercise on-transient was derived for each individual as the mean value from 20-120 s into the transition. The commencement point of 20 s was selected as it is representative of the region where the Δ[HHb+Mb] and $\dot{V}O_2$ signals meet, with the 120 s end point indicative of the time point at which a steady-state value of 1.0 had been achieved by the Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio. Values>1.0 represent a time period whereby during the exercise transition there was a greater reliance on fractional O_2 extraction compared with the exercise steady-state (values=1.0), thus reflecting a poorer local O_2 delivery relative to muscle O_2 utilisation in the area of NIRS interrogation (Murias *et al.* 2011b).

3.2.5 **Statistical analysis**

Statistical analysis was performed using the software SigmaPlot version 12.0 (Systat Software, Point Richmond, CA). Prior to analysis, normal Gaussian distribution of the data was assessed using the Shapiro-Wilk's test. Physical and peak physiological responses between groups were compared using the unpaired Student's t-test for parametric analyses, or the Mann-Whitney U test for non-parametric analyses. The kinetic parameter estimates for $\dot{V}O_2$ and [HHb+Mb] during moderate-intensity exercise were analysed by using a two-way repeated measures ANOVA (primed/unprimed condition and diabetes as the main effects). In the cases of significant differences obtained from the repeated measures ANOVA, post hoc Tukey tests were performed. Statistical significance was accepted at a P value ≤ 0.05. All values are expressed as means ± standard deviation (SD) or as median and interquartile ranges for data that were deemed not normally distributed.

The key variable is the rate of change of the primary phase of oxygen uptake ($\dot{V}O_{2p}$). An indicator of the rate of change in $\dot{V}O2$ can be obtained by calculation of its time constant (τ). The τ is the time required to achieve ~63 % of the difference between the baseline and the plateau of that phase. Recently published studies in our laboratory and others, which, included data for both individuals with T2D and age-matched healthy controls, revealed τ values of ~35 ± 7 s (Mean ± SD) during cycling at 80% VT. The difference between unprimed and primed τ values (based on studies comparing older vs young healthy participants, or studies comparing the postural effects; upright vs supine cycling, in young participants) was 10 s. Thus, given the estimated standard deviation (SD) of each population is 7 s, and that the minimum difference we wish to detect as significant would be 10 s, the minimum sample size needed to detect a significant effect at $\beta=0.05$ and $\alpha=0.05$ (90% power) for an ANOVA power calculation design based on 2 groups, is 11 subjects.

3.3 RESULTS

3.3.1 Participants

3.3.1.1 Physical Characteristics

Physical characteristics for the participants are presented in Table 3.1 Anthropometrical measurements did not significantly differ between groups, with the exception of WHR, whereby individuals with T2DM displayed a significantly greater WHR ($P<0.05$) compared to the ND controls. Individual anthropometric measurements are displayed in (Appendix 9). No differences were observed in ABI, or subcutaneous fat layer of the *vastus lateralis* (VL). However, individuals with T2DM displayed a significantly faster PWV compared with the ND controls.

Table 3.1. ***Anthropometrical data, PWV, ABI and resting BP for ND and T2DM individuals.***

	ND (***n*** **=12)**	**T2DM** (***n*** **=12)**
Sex (male, female)	7, 5	7, 5
Age (yr)	44 ± 9	48 ± 8
Height (m)	1.68 ± 0.07	1.69 ± 0.08
Weight (kg)	86.0 ± 12.0	91.7 ± 18.6
BMI (kg.m^{-2})	30.4 ± 4.1	32.1 ± 5.6
WHR (a.u)	0.91 ± 0.08*	1.02 ± 0.09
Fat layer VL (mm)[a]	8.3 ± 4.5	6.5 ± 1.7
Pre exercise ABI (a.u.)	1.15 ± 0.17	1.09 ± 0.09
Post exercise ABI (a.u.)	*1.17 (0.24)*	*1.19 (0.18)*
PWV (ms^{-1})[b]	6.4 ± 1.4*	9.2 ± 2.1
SBP (mmHg)[c]	126 ± 13	124 ± 13
DBP (mmHg)[c]	79 ± 9†	73 ± 7

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. BMI, body mass index; WHR, waist:hip ratio; ABI, ankle:brachial index; PWV, pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P\leq0.05$). †Tendency towards a difference than T2DM ($P\leq0.10$). a=12 (ND) and 10 (T2DM); b=11 (ND) and 10 (T2DM); c= 12 (ND) and 11 (T2DM).

3.3.1.2 Haematological parameters and prescriptive medications

Mean haematological parameters and prescriptive medications are presented in Tables 3.2 and 3.3 respectively. As expected, individuals with T2DM displayed significantly higher HbA_{1c} and fasting plasma glucose levels ($P<0.001$). They also had significantly higher total cholesterol and triglycerides ($P<0.05$) than the ND controls, with no differences between HDL-C and LDL-C distribution.

Table 3.2. ***Haematological parameters for ND and T2DM individuals.***

	ND	**T2DM**
HbA_{1c} (%)[a]	*5.1 (0.5)***	*6.8 (1.4)*
FPG (mmol.L^{-1})[b]	*4.0 (0.7)***	*7.3 (4.1)*
Total cholesterol (mmol.L^{-1})[c]	*3.4 (1.6)**	*4.7 (1.2)*
LDL-C (mmol.L^{-1})[d]	*1.6 (1.3)*	*2.3 (1.3)*
HDL-C (mmol.L^{-1})[d]	1.3 ± 0.3	1.2 ± 0.2
Triglycerides (mmol.L^{-1})[d]	*1.1 (2.5)**	*1.8 (0.7)*

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. HbA_{1c}, glycosylated haemoglobin; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P\leq0.05$). ** Significantly different than T2DM ($P\leq0.001$). a=7 (ND) and 11 (T2DM); b=9 (ND) and 10 (T2DM); c=8 (ND) and 9 (T2DM); d=9 (ND) and 7 (T2DM).

Table 3.3. ***Prescriptive medications for ND and T2DM individuals.***

	ND (*n* = 17)	**T2DM (*n* = 17)**
Anti-hypertensives		
Angiotensin converting enzyme inhibitor		3
Angiotensin II receptor blocker		2
Aspirin	1	3
Calcium channel blocker		3
Statins	2	5
Hypoglycaemic medications		
Oral hypoglycaemics		10
Subcutaneous hypoglycaemics		2
Sulphonylureas		1

3.3.1.3 Physical activity levels

Group mean activity levels are presented in Tables 3.4 and 3.5 for accelerometry and LOPAR data respectively. Individuals with T2DM displayed significantly (P=0.05) higher activity counts for levels of light activity over the course of the 5-day period; however, periods of inactive/sedentary behaviour were comparable between the two groups, as were the results returned via the LOPAR questionnaire.

Table 3.4. ***Group mean activity levels based on the number of hours per day as determined by RT3 accelerometers.***

	ND (***n*** **= 9)**	**T2DM** (***n*** **=5)**
Inactive (h.day^{-1})	19.20 ± 1.20	17.76 ± 0.83
Light (h.day^{-1})	*4.28 (1.11)**	*5.98 (1.97)*
Moderate (h.day^{-1})	0.63 ± 0.4	0.65 ± 0.58
Vigorous (h.day^{-1})	*0.19 (0.29)*	*0.06 (0.17)*

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Inactive, <100 counts.min^{-1}; Light, 101-970 counts.min^{-1}; Moderate, 971-2333 counts.min^{-1}; Vigorous, >2333 counts.min^{-1}.*Significantly different than T2DM (P≤0.05)

Table 3.5. ***Group mean activity levels based on the number of METS per hour per week as determined by the LOPAR questionnaire.***

	ND (***n*** **=11)**	**T2DM** (***n*** **= 9)**
LOPAR (MET.hr^{-1}.wk^{-1})	*152 (82)*	*126 (128)*

Median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups.

3.3.2 Performance Data from Ramp Incremental Cycling Test

3.3.2.1 Physiological responses

The physiological responses for both groups at peak exercise, and at VT are displayed in Table 3.6. Peak $\dot{V}O_2$ ($\dot{V}O_{2max}$) normalised to kilograms of body weight was significantly (P=0.008) reduced in individuals with T2DM compared with the ND controls, representing a 22% reduction in peak exercise capacity. In absolute terms ($L.min^{-1}$), $\dot{V}O_{2max}$ also tended to be lower in the group with T2DM, although it did not reach statistical significance (P=0.10). In addition, they displayed a strong tendency toward significance for peak PO at exhaustion (P=0.06), with no differences in time to failure (P=0.70). RER values were similar between the T2DM group and the ND group respectively at peak exercise (P=0.75) indicating both groups made their maximal effort during the ramp incremental protocol.

Table 3.6. ***Physiological responses at peak exercise and VT.***

	ND (*n* = 12)	**T2DM (*n* = 12)**
$\dot{V}O_{2max}$ ($mL.kg^{-1}.min^{-1}$)	27.6 ± 6*	21.4 ± 4.1
$\dot{V}O_{2max}$ ($L.min^{-1}$)	2.35 ± 0.5†	1.97 ± 0.6
Peak PO (W)	188 ± 46†	151± 46
Peak HR ($beats.min^{-1}$)	169 ± 16	164 ± 15
Age predicted HR ($beats.min^{-1}$)	176 ± 9	172 ± 8
Peak RER (a.u)	1.15 ±0.07	1.13 ± 0.08
Peak CO ($L.min^{-1}$)[a]	14.26 ± 1.74	13.26 ± 2.01
TTF (secs)	638 ± 86	656 ± 119
$\dot{V}O_2$ @ VT ($mL.kg^{-1}.min^{-1}$)	*16.73 (7.81)*	*14.93 (5.71)*
$\dot{V}O_2$@ VT ($L.min^{-1}$)	1.55 ± 0.38	1.48 ± 0.46
PO @ VT (W)	102 ± 35	86 ± 31

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). a = 5 (ND) and 5 (T2DM).

3.3.3 Performance data from 80% VT

3.3.3.1 $\dot{V}O_2$ kinetics

The parameter estimates of the kinetics analysis of the $\dot{V}O_2$ response for both ND and T2DM individuals with (Mod B) and without (Mod A), a prior bout of priming exercise are presented in Table 3.7. At baseline, the parameter estimates for the primary component of the kinetics response were similar between groups, albeit individuals with T2DM displaying a tendency (P<0.10) for a higher baseline $\dot{V}O_2$ value and slower ($\tau\dot{V}O_{2p}$).

PE however, resulted in a significantly altered $\dot{V}O_{2p}$ kinetic response in both groups, with significantly (P<0.001) elevated baseline $\dot{V}O_2$ values and a reduced $\tau\dot{V}O_{2p}$ (P<0.05) in the subsequent moderate-intensity exercise bout.

The adaptation of $\dot{V}O_2$ at the onset of moderate-intensity exercise prior and subsequent to priming exercise respectively, for representative individuals with and without T2DM are illustrated in *Figure. 3.2*.

Table 3.7. ***Dynamic response characteristics of oxygen uptake for unprimed (Mod A) and primed (Mod B) moderate-intensity exercise in individuals with and without T2DM.***

	Mod A Unprimed		**Mod B Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	12	12	12	12
$\dot{V}O_2$ baseline, L.min^{-1}	0.77 ± 0.10†	0.91 ± 0.25	0.82 ± 0.10‡	0.98 ± 0.23‡
$\dot{V}O_2$ A_p, L.min^{-1}	0.68 ± 0.31	0.55 ± 0.25	0.72 ± 0.35	0.50 ± 0.20
$\dot{V}O_2$ end A_p , L.min^{-1}	1.46 ± 0.35	1.46 ± 0.42	1.54 ± 0.39‡	1.49 ± 0.40‡
$\tau\dot{V}O_{2p}$, s	35 ± 9†	43 ± 14	28 ± 10‡	34 ± 11‡
$\dot{V}O_2$ gain, mL.min^{-1}.W^{-1}	9.34 ± 2.14	9.40 ± 2.16	9.79 ± 2.05	8.66 ± 1.32

Values are means ± SD. Dynamic response characteristics of oxygen uptake (VO2) during unprimed and primed cycling exercise at 80% ventilatory threshold (VT) in individuals with and without T2DM. A_p, amplitude; TD, time delay; τ_p, time constant of the primary response. ‡Significantly different between Mod A and Mod B conditions within the group (P≤0.05). *Significantly different than T2DM (P≤0.05). †Tendency towards a difference than T2DM (P≤0.10). $^{\#}$Tendency towards a difference than unprimed (P≤0.10).

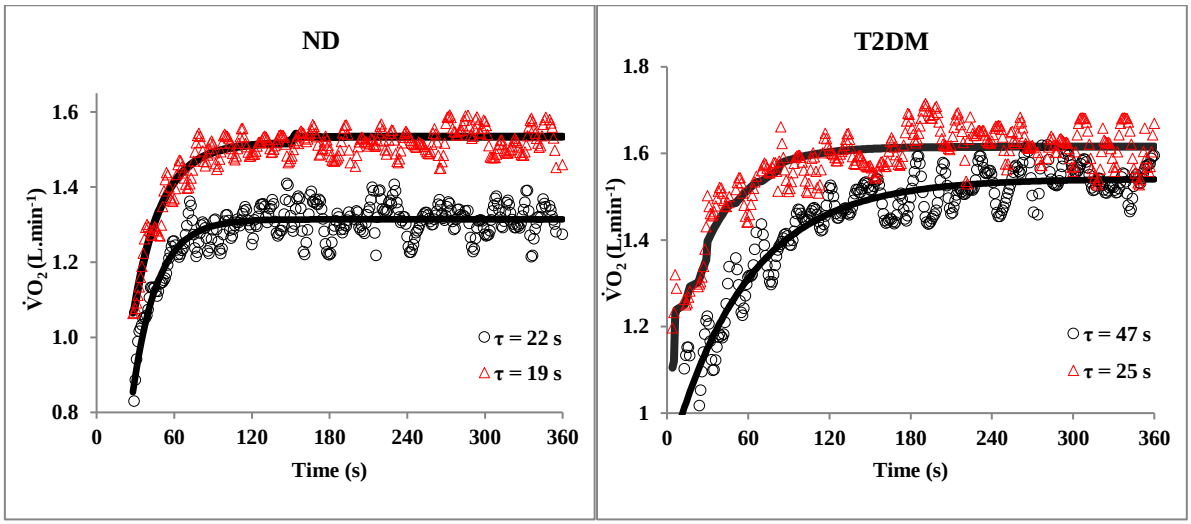

Figure 3.2. Oxygen uptake responses for representative ND and T2DM individuals for unprimed (○) and primed (Δ) bouts of moderate-intensity exercise. The continuous lines of best fit illustrate the primary phase of the oxygen uptake ($\dot{V}O_2$) response. $\tau\dot{V}O_{2p}$ values are included in each panel. Note the faster $\tau\dot{V}O_{2p}$ response in the primed bout in both ND and T2DM individuals**.**

3.3.3.2 Muscle Deoxygenation Kinetics and Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio

Kinetic parameters for Δ[HHb+Mb] and Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratios are displayed in Table 3.8. Baseline values and amplitudes for Δ[HHb+Mb] were similar between groups and between the primed and unprimed conditions. The NIRS-derived [HHb+Mb] pre-transition period (TD) was significantly longer (P<0.05) in the T2DM group during both conditions. After the time delay, [HHb+Mb] increased to a steady-state in each individual, with the estimated time constant being similar between groups in both conditions. Despite the longer TD in individuals with T2DM, the overall time course for muscle deoxygenation (effective response time), determined as the [HHb+Mb]TD + τ was similar between groups in both conditions.

The overall Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio was calculated to indicate the degree of O_2 extraction relative to steady-state values for a given increment in $\dot{V}O_2$ during each moderate-intensity transition. The overall Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio displayed a strong trend (P=0.06) for a priming effect in both groups, but it did not reach significance. In addition, the rate of adjustment for $\tau\dot{V}O_2$ was significantly slower than that of the effective response time (τ + Td) [HHb+Mb] in the unprimed bout in both ND and T2DM (P=0.06 and P=0.01 respectively), but not during the subsequent primed bout (P=0.64 and P=0.12). Thus, whereas the steady-state reliance on O_2 extraction for a given $\dot{V}O_2$ may have been similar, the initial on-transient in Mod A displayed an "overshoot" in the Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio that was ameliorated during the primed bout (Figure 3.4), which is indicative of a greater proportional contribution of O_2 extraction. The adaptation of Δ[HHb+Mb] at the onset of moderate-intensity exercise prior and subsequent to

priming exercise respectively, for representative ND and T2DM individuals are illustrated in Figure 3.3.

Table 3.8. ***Parameters of the Δ[HHb+Mb] kinetics and Δ[HHb+Mb]/ΔV̇O2 in Mod A and Mod B in ND and T2DM individuals***

	Mod A Unprimed		Mod B Primed	
	ND	T2DM	ND	T2DM
n	12	12	12	12
Δ[HHb+Mb] baseline, a.u.	-68.8 ± 45.2	-15 ± 40.8	-43 ± 65.6	3.7 ± 56.2
Δ[HHb+Mb] A_p, a.u.	57.4 ± 48.0	92.5 ± 61.7	57.7 ± 43.1	97.0 ± 66.6
Δ[HHb+Mb] TD, s	11 ± 3*	13 ± 5	11 ± 2*	13 ± 2
τΔ[HHb+Mb], s	14 ± 7	15 ± 8	18 ± 7	14 ± 6
τ'Δ[HHb+Mb], s	26 ± 5	29 ± 6	29 ± 8	28 ± 6
Normalised Δ[HHb+Mb]/ΔV̇O2	1.04 ± 0.12	1.18 ± 0.17	1.01 ± 0.12#	1.05 ± 0.15#
HHb End A_p, a.u.	-11.4 ± 69.8	77.6 ± 88.7	14.7 ± 76.5	100.7 ± 106.3

Values are means ± SD. Dynamic response of muscle deoxygenation [HHb+Mb] during unprimed and primed cycling exercise at 80% ventilatory threshold (VT) in individuals with and without T2DM. A_p, amplitude; TD, calculated time delay of [HHb+Mb] response; τ, time constant of the primary response; τ', effective response time (TD + τ); a.u., arbitrary units. Normalised Δ[HHb+Mb]/ΔV̇O2 ratio, calculated as the 20- to 120-s average of the normalised Δ[HHb+Mb]/ΔV̇O2.. *Significant difference between ND and T2DM ($P \leq 0.05$). #Tendency towards a difference than unprimed ($P \leq 0.10$)

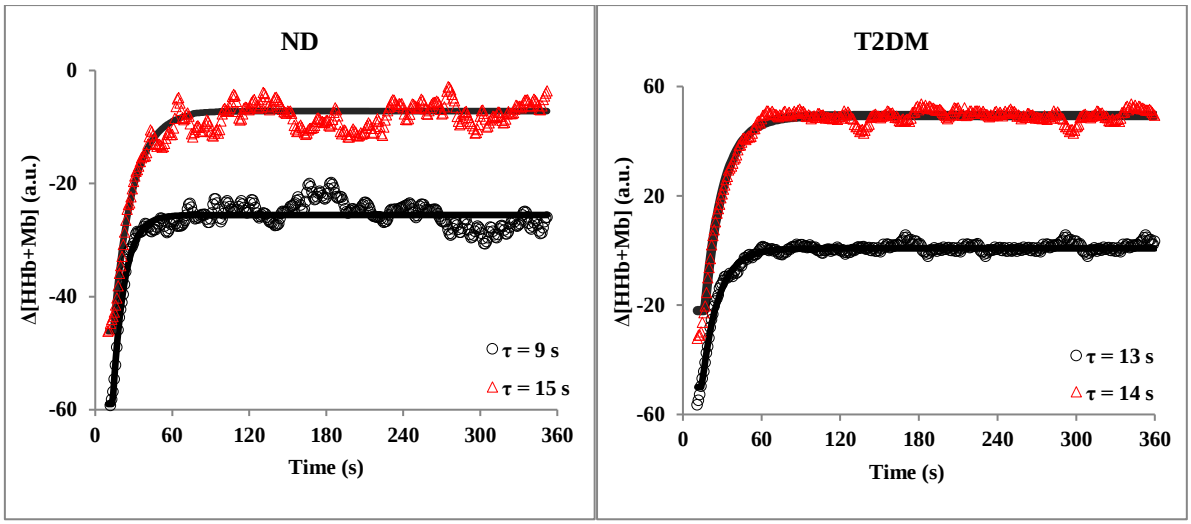

Figure 3.3. Deoxygenated haemoglobin [HHb+Mb] experimental data for representative individuals for unprimed (○) and primed (Δ) bouts of moderate-intensity exercise. The continuous lines of best fit indicate the exponential fitting of the experimental data.

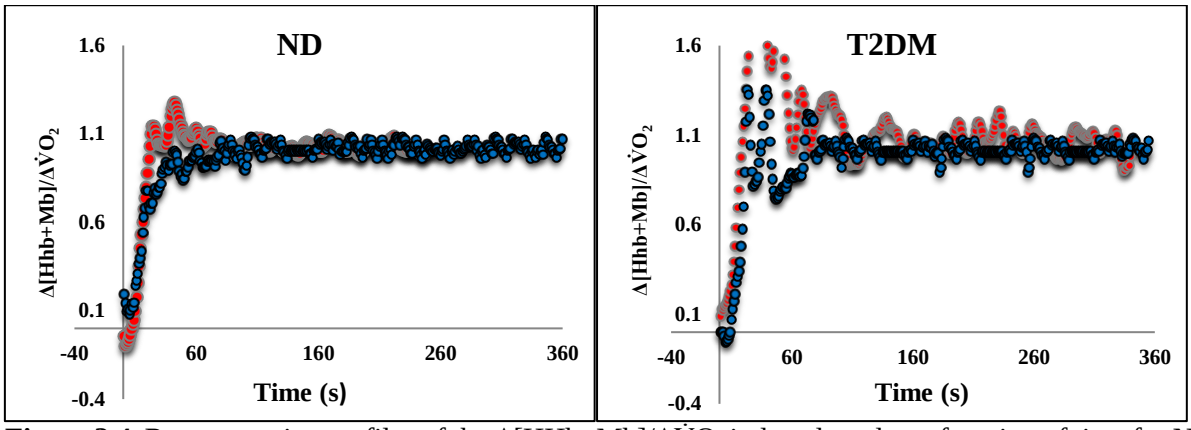

Figure 3.4. Representative profiles of the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ index plotted as a function of time for ND and T2DM individuals at the onset of moderate-intensity exercise prior (Mod A, red circles) and subsequent to priming (Mod B, blue circles) exercise. Note how priming exercise reduces the on-transient 'overshoot' in Δ[HHb+Mb]/$\Delta\dot{V}O_2$.

3.3.3.3 Cardiac output and heart rate responses

Heart rate responses for ND and T2DM individuals during moderate-intensity exercise with and without a prior bout of priming exercise and CO responses during the unprimed exercise bout are presented in Table 3.9. Resting HR was significantly elevated before the moderate-intensity cycling after PE (P<0.001) with a tendency for a main diabetes effect (P=0.10). Additionally, PE significantly elevated end exercise HR ($P\leq$0.001). A trend for significance in delta HR was observed subsequent to PE (P=0.08) with a tendency for a main diabetes effect (P=0.06). There were no significant differences observed in CO between groups during the unprimed moderate-intensity bout (P=0.69).

Table 3.9. ***Baseline cardiac output responses and heart rate responses for unprimed and primed moderate-intensity exercise.***

	Mod A Unprimed		**Mod B Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	7	7	7	7
Resting HR, beats.min^{-1a}	92 ± 18†	110 ± 10	104 ± 13†‡	120 ± 12‡
End-exercise HR, beats.min^{-1a}	127 ± 18	132 ± 11	136 ± 18‡	141 ± 13‡
ΔHR, beats.min^{-1a}	35 ± 14†	23 ± 7	32 ± 12$^{\dagger\#}$	22 ± 7$^{\#}$
CO, L.min^{-1b}	11.72 ± 3.25	11.15 ± 2.76		

Values are means ± SD. CO, cardiac output; HR, heart rate. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P\leq$0.05). ‡Significantly different between Mod A and Mod B conditions within the group ($P\leq$0.05). †Tendency towards a difference than T2DM ($P\leq$0.10). $^{\#}$Tendency towards a difference than unprimed ($P\leq$0.10) a = 12 (ND) and 11 (T2DM); b= 12 (ND) and 8 (T2DM)

3.4 DISCUSSION

The present study investigated the effect of a prior bout of heavy-intensity priming cycling exercise on the temporal relationship between the adaptation of muscle O_2 consumption and delivery, as reflected by the primary $\dot{V}O_2$ response and NIRS-derived deoxygenation of the *vastus lateralis* muscle respectively, during the on-transient of a subsequent bout of moderate-intensity cycling exercise in T2DM. The main findings were that;

1. Consistent with our first hypothesis, priming exercise increased the adjustment of the primary phase of the $\dot{V}O_2$ kinetics response in a subsequent bout of moderate-intensity cycling exercise in T2DM.
2. The reduction in the effective time constant of the $\dot{V}O_2$ kinetics response was likely due to a better matching of microvascular O_2 delivery to utilisation, with the elimination of the "overshoot" in the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio during the on-transient exercise response. Additionally, the overall magnitude of the change in the absolute $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio displayed a near significant reduction following the priming exercise (P=0.06).

3.4.1 $\dot{V}O_2$ kinetics

Our finding of a 22% lower $\dot{V}O_{2max}$ ($mL.min^{-1}.kg^{-1}$) and lower exercise capacity in individuals with T2DM compared with the ND control group is consistent with the approximate 20% reduction in $\dot{V}O_{2max}$ previously reported in adults with T2DM (Regensteiner *et al.* 1998; Baldi *et al.* 2003; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012). Similarly, the magnitude of difference in the $\tau\dot{V}O_{2p}$ between the groups with and without T2DM is also consistent with the literature, although it did not reach statistical significance (P=0.11). It is likely that interindividual variability in the responses precluded the attainment of statistical significance

The demonstration of a faster adaptation of pulmonary $\dot{V}O_2$ during the on-transition to moderate-intensity exercise in middle aged individuals both with and without T2DM ($\tau\dot{V}O_{2p}$ in excess of 20 s), when preceded by an acute bout of heavy-intensity exercise is consistent with the literature surrounding priming exercise in young adults presenting with slow $\tau\dot{V}O_{2p}$ (> 20 s) as well as untrained older adults (Scheuermann *et al.* 2002; DeLorey *et al.* 2004b; Gurd *et al.* 2009; DeRoia *et al.* 2012). The findings of investigations in older individuals were consistent with the views that the dynamic response of $\dot{V}O_2$ in older adults is likely constrained by a slower rate of O_2 delivery (DeLorey *et al.* 2004b; DeRoia *et al.* 2012) to the exercising muscle and/or by metabolic activation (Scheuermann *et al.* 2002; Gurd *et al.* 2009).

For instance, DeLorey *et al*. (2004b) investigated the effects of heavy-intensity priming exercise (Δ50%) on $\dot{V}O_2$ and muscle deoxygenation kinetics during subsequent moderate-intensity exercise in older (mean age = 68, yr) healthy adults presenting with an unprimed $\tau\dot{V}O_{2p}$ of ~38 s. Based on the premise that a prior bout of heavy-intensity exercise enhances muscle blood flow (Gerbino *et al.* 1996), the authors hypothesised that this intervention would accelerate $\dot{V}O_2$ kinetics in older adults and that HR would be elevated prior to the onset and throughout the subsequent moderate-intensity cycling bout indicating improved muscle perfusion and O_2 delivery. Thus, in accordance with previous work by DeCort *et al.* (1991) and Yoshida & Whipp (1994), DeLorey *et al.* (2004b) utilised the adaptation of HR to indirectly assess the rate of adaptation of cardiac output and thus, muscle O_2 delivery. A significantly elevated HR before the onset of the subsequent submaximal exercise bout was observed in the older adults (τHR 47 ± 24 *vs*. 51 ± 31 s) implying an increase in muscle O_2 delivery. In addition, a significant speeding of $\dot{V}O_2$ kinetics was observed, consistent with the notion that muscle O_2 delivery does indeed impose a limitation to $\dot{V}O_2$ kinetics in older adults. Similarly, in the present study, resting HR was significantly elevated in both groups prior to the initiation of the primed submaximal exercise bout and thus, was indicative of enhanced perfusion in both groups.

More recently DeRoia *et al.* (2012) carried out a similar study on 54 healthy older males (mean age = 66, yrs), which yielded similar results. Heavy-intensity (Δ50%) PE accelerated the overall $\dot{V}O_2$ kinetics response, owing to a shorter $\tau'\dot{V}O_{2p}$ (39 ± 7 *vs*. 36 ± 4 s). In accordance with DeLorey *et al*. (2004b), DeRoia and colleagues (2012) also observed a significantly elevated HR before the onset of the subsequent submaximal exercise bout (111 ± 117 *vs.* 120 ± 24 beats.min^{-1}). However, they also measured CO, to which PE evidenced no discernible influence on the adjustment of bulk O_2 delivery (12.6 ± 2.5 *vs.*12.5 ± 2.5 L.min^{-1}).

The above findings of DeLorey *et al.* (2004b) and DeRoia *et al.* (2012) were somewhat akin to those of an earlier, albeit smaller investigation by Scheuermann *et al.* (2002). In this study the authors reported an accelerated $\dot{V}O_2$ kinetics in a bout of moderate-intensity (80% VT) cycling exercise preceded by a prior bout of heavy-intensity exercise (Δ50%) in 8 healthy older (mean age = 65, yr) males. However, the authors reported the presence of a constrained HR kinetic response in the older individuals in the subsequent primed submaximal bout (Scheuermann *et al.* 2002), most likely reflective of changes in the autonomic control of HR. Nonetheless, the authors purported that the already elevated baseline HR and consequent muscle blood flow and perfusion may have been sufficient to fulfil the demands of the exercise-induced increase in mitochondrial respiration. They also acknowledged that the accelerated $\dot{V}O_2$ kinetics response, in the presence of the slowed HR kinetics however, may implicate the involvement of

intracellular biochemical processes in limiting the rate of O_2 utilisation at the onset of exercise (Scheuermann *et al.* 2002).

The likelihood of such observations by Scheuermann *et al.* (2002) being related to a combination of enhanced muscle perfusion and O_2 delivery with prior activation of mitochondrial enzyme activity was strengthened in later investigation by Gurd *et al.* (2009). In this study, Gurd and colleagues (2009) examined the priming effect of heavy-intensity (Δ50%) exercise on $\dot{V}O_2$ kinetics, pyruvate dehydrogenase (PDH) activation, muscle metabolite contents, and muscle deoxygenation in older adults (mean age = 70, yr) during a subsequent moderate-intensity (90% VT) cycling exercise bout. Significant reductions were observed in the τVO_{2p} (39 ± 14 *vs.* 29 ± 5 s) during the primed moderate-intensity bout, which were accompanied by a reduction in PCr breakdown, a higher pre-exercise muscle PDH activity at baseline and higher muscle contents of free ADP and P_i, whilst NIRS-derived muscle oxygenation and deoxygenation kinetics were elevated and unchanged respectively. Thus, the significant improvement in oxidative phosphorylation was thereby considered to reflect an integrative upregulation in the provision of all oxidative substrate via attenuating the delay in activating rate-limiting mitochondrial enzymes and providing substrate including O_2 for mitochondrial oxidative phosphorylation. Thereby, the authors concluded that the constrained $\dot{V}O_2$ kinetics consistently observed in older adults can most likely be attributed to impaired metabolic activation in combination with reduced O_2 availability.

We can therefore allude that the demonstration of an accelerated τVO_{2p} concomitant with an elevated baseline HR subsequent to heavy-intensity PE in individuals with T2DM in the present study may be attributed to the enhanced muscle perfusion and O_2 delivery associated with PE.

3.4.2 NIRS-derived muscle deoxygenation kinetics

In the present study, whereby the NIRS-derived [HHb+Mb] signal was accepted as a surrogate for fractional O_2 extraction, the changes observed in the NIRS derived [HHb+Mb] were indicative of the balance between O_2 availability and utilisation in the microvasculature within the region of NIRS interrogation. In an earlier study by Bauer *et al.* (2007) whereby the [HHb+Mb] response at the onset of moderate-intensity (85% VT) was investigated in middle-aged (mean age = 47, yr) individuals with T2DM, significant reductions in $\tau\dot{V}O_{2p}$ (ND; 34.2 ± 8.2 *vs.*T2DM; 43.8 ± 9.6 s) in the presence of similar HR kinetics (45 ± 16 *vs.* 51 ± 14 s) were demonstrated. However, of particular interest in this study, was the occurrence of a distinct overshoot of [HHb+Mb] above the level for steady-state upon initiation of the moderate-intensity exercise bout in the individuals with T2DM.

In the present study, despite the demonstration of a significant reduction in the $\tau\dot{V}O_{2p}$ consequent of the primed exercise bout, the dynamic responses of Δ[HHb+Mb] were predominantly similar between individuals with and without T2DM in both exercise conditions. However, a longer time-delay was evident in individuals with T2DM at the onset of both exercise conditions. Although this delay before an increase in muscle oxygen consumption has traditionally been interpreted to reflect a tight coupling between muscular O_2 uptake and local O_2 delivery during this interval (DeLorey *et al.* 2003; Grassi *et al.* 2003), it has also been deemed to be reflective of a metabolic inertia, whereby the activation of mitochondrial respiration and muscle O_2 consumption is constrained relative to the onset of exercise (DeLorey *et al.* 2003). Although the existence of such a delay has been refuted by several investigators (McCreary *et al.* 1996; Rossiter *et al.* 1999; Whipp *et al.* 1999) observing an almost instantaneous monoexponential reduction in PCr; a surrogate for muscle O_2 consumption (Whipp *et al.* 1999), via magnetic resonance spectroscopy, it is feasible to consider that the locus of control during the initial 15-20 s of exercise onset resides intracellularly. Nevertheless, in agreement with previous studies on older adults (DeLorey *et al.* 2004b, Gurd *et al.* 2009; De Roia *et al.* 2012), we may have expected a reduced TD following the priming bout consequent of the purportedly faster activation of mitochondrial enzymes and/or of PDH (Gurd *et al.* 2008, 2009). Furthermore, it would have been reasonable to expect a slower τΔ[HHb+Mb], representative of the slower adjustment of muscle O_2 consumption to the faster adjustment of O_2 delivery, consistent with an enhanced muscle blood flow and O_2 delivery subsequent to a prior bout of heavy-intensity exercise (DeLorey *et al.* 2004b, Gurd *et al.* 2006, 2009). However, in accordance with the observations reported by DeRoia *et al.* (2012), despite the observed elevation in baseline HR following the prior bout of heavy-intensity exercise, thereby inferring an enhanced local muscle perfusion, τΔ[HHb+Mb] remained unchanged in the present study.

3.4.3 Δ [HHb+Mb]/Δ$\dot{V}O_2$

Combined with measurements of pulmonary $\dot{V}O_2$, we calculated the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio, an index indicative of the degree of O_2 extraction required for a given increment in $\dot{V}O_2$ (DeRoia *et al.* 2012; Murias *et al.* 2010a, 2011b). Previously, through the application of this measure, older adults as well as young and middle aged adults presenting with $\tau\dot{V}O_{2p}$ in excess of 20 s (Murias *et al.* 2011c) exhibited an elevated or an overshoot in the $\Delta[HHb+Mb]/\Delta\dot{V}O_2$ ratio. This increased reliance on O_2 extraction during the on-transient of exercise has been attributed to an impaired blood flow distribution at the level of the muscle microvasculature.

Particularly in our population of people with T2DM, we expected the demonstration of this 'overshoot' or elevated Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio throughout the unprimed bout of submaximal exercise, which subsequently would be reduced, if not abolished, in the preceding primed bout if O_2 delivery was indeed a limiting factor (Murias *et al.* 2011b). As expected, the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio displayed an overshoot which was more evident in T2DM (relative to the steady-state ratio of 1.0). This overshoot in the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio was then almost abolished by the prior bout of heavy-intensity exercise in the subsequent bout of submaximal exercise, owing to a significant reduction in the $\tau\dot{V}O_{2p}$ and unchanged τ'Δ[HHb+Mb]. This thereby strengthened our initial hypothesis that following an acute priming intervention, a reduction in the effective time constant of the $\dot{V}O_2$ kinetics response in middle-aged individuals with uncomplicated T2DM would be due to a better matching of microvascular O_2 delivery to utilisation.

3.4.4 Conclusion

As previously expressed in *Experiment 1 (section 2.4.3)*, we are unable to comment with confidence on the clinical implications of the significantly higher PWV (6.4 ± 1.4 *vs.* 9.2 ± 2.1 ms^{-1}) demonstrated by individuals with T2DM in the present study compared to the ND controls. Thus, we acknowledge that observations in the present study may have been influenced by the presence of increased arterial stiffness in the vasculature of the individuals with T2DM. In addition, it would be remiss not to acknowledge that in the present study individuals with T2DM displayed a tendency (P=0.06) for a higher resting heart rate than that of the ND controls (110 ± 10 vs 92 ± 18 bpm respectively). Whilst a higher resting heart rate is also associated with factors such as obesity, higher blood pressure and reduced physical activity (Zhang & Kesteloot, 1999; Zhang *et al.* 2010); for which individuals in this particular study displayed no significant differences, it is also associated with a proatherosclerotic lipid profile. Indeed, individuals with T2DM in this study displayed significantly higher total cholesterol and triglycerides (P<0.05) than the ND controls. Moreover, a faster resting heart rate is also a characteristic feature of cardiac autonomic neuropathy (CAN) (Vinik *et al.* 2013); a dysfunction in the autonomic nervous system which presents in one third of individuals with T2DM (Vinik & Ziegler, 2007). Whilst traditionally, CAN has been considered a chronic complication of long-term T2DM, emerging evidence suggests that neuropathic complications present from as early as the onset of T2DM (Vinik *et al.* 2013).

CAN results by impairment of the autonomic nerve fibres regulating heart rate, cardiac output, myocardial contractility, cardiac electrophysiology and the constriction and dilation of blood

vessels, and therefore leads to abnormalities in cardiovascular dynamics (Vinik *et al.* 2013). The first manifestation of diabetic CAN is attributable to damage of the vagus nerve; which is responsible for nearly 75% of parasympathetic activity (Pop-Bushi, 2010) and subsequently resting tachycardia ensues as the sympathetic activity becomes dominant (Low *et al.* 2004). The progressive damage of the autonomic balance is signified by additional symptoms, such as postural hypotension, a significant decrease in heart rate variability (HRV), and of particular interest in this situation, an intolerance to exercise (Ziegler, 1994).

Given that etiological factors associated with diabetic CAN are broad with the inclusion of poor glycaemic control, longer diabetes duration, increased age, female sex and greater body mass index (Vinik & Ziegler, 2007), current protocols of best practise recommend that individuals with T2DM presenting with an increased risk of having CAN should be tested for cardiac stress before undertaking an exercise program. Accordingly, as addressed in *section 2.2.1.2.1*, all individuals with T2DM in the current study were required to satisfactorily complete a 12-lead ECG exercise stress test to ensure that the heart responded appropriately to exercise prior to being deemed suitable for this study.

Nonetheless, the demonstration of a faster adaptation of the $\dot{V}O_2$ kinetics response while maintaining similar [HHb+Mb] kinetic responses, coupled with the finding of a substantially reduced Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio in individuals with T2DM in a bout of submaximal exercise when preceded by an acute 6 min bout of heavy-intensity exercise is encouraging. These findings are in accordance with those observed in young and older individuals in response to an endurance training intervention (Murias *et al.* 2010a, 2011b). The facilitation of such a substantially decreased reliance on fractional O_2 extraction by an acute "one-off" bout of exercise is difficult to explain when one considers the myriad of potential influencing physiological factors. However, the combination of a near-significant reduction in this Δ[HHb+Mb]/$\Delta\dot{V}O_2$ overshoot during the on-transient relative to steady-state, subsequent to the acute intervention, strengthens the notion of an enhanced blood flow distribution at the level of the muscle microvasculature. Although it is acknowledged that $\dot{V}O_2$ represents oxygen consumption in the whole muscle, compared to the [HHb+Mb] signal which is reflective of the region of NIRS interrogation; this observation would suggest that a bout of priming exercise adequately enhanced local perfusion to meet the increased metabolic requirements via a slower O_2 extraction rate at the level of the microvasculature.

The accumulated data in the present study was compatible with that observed in older adults, whereby a bout of heavy-intensity priming exercise, prior to moderate-intensity exercise was

demonstrated to benefit oxidative metabolism, with a concomitant improvement in the local matching of O_2 delivery to utilisation. We acknowledge these data do not exclude the possibility that the basic control to $\dot{V}O_2$ kinetics resides within intracellular factors, but due to the non-invasive nature of this study, we are unable to comment with certainty on the mechanisms of these observations. However, in accordance with Gerbino *et al.* (1996), we can thus allude, that muscle perfusion and/or muscle O_2 delivery was enhanced subsequent to a bout of heavy-intensity priming exercise in the present study. Collectively the favourable manipulation in the rate of adaptation of the $\dot{V}O_2$ kinetics response, concomitant with an elevated baseline HR and the subsequent amelioration of the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ overshoot during the on-transient of submaximal exercise in middle-aged individuals with uncomplicated T2DM, highlights the potential that lies within the acute intervention of heavy-intensity priming exercise.

Chapter 4: Influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise in type 2 diabetes.

4.1 INTRODUCTION

Upon initiation of constant-load exercise within the moderate-intensity domain, (i.e., corresponding to power outputs below the ventilatory threshold (VT)), the oxygen uptake response of the primary phase ($\dot{V}O_{2p}$) is mono-exponential, with steady-state reached within two-three minutes (Wasserman *et al.* 1973). However, whilst exercising in the heavy-intensity domain, (i.e., at exercise intensities above the VT), an additional component, termed the "slow component" is superimposed onto the primary phase of the kinetics response. It generally presents between 80-100 s as a gradually developing component rise in $\dot{V}O_2$ (i.e. with an absence of, or delayed, steady-state $\dot{V}O_2$) (Barstow 1994; Whipp 1994b) to an extent greater than that expected from the $\dot{V}O_2$-work rate relationship (Whipp, 1974; Paterson & Whipp, 1991), and is thus indicative of an increasing inefficiency in the active muscles as time progresses and fatigue develops (Zoladz & Korzeniewski, 2001). The slow component culminates with either $\dot{V}O_2$ increasing to maximal levels, the obtainment of a delayed plateau, or the termination of the exercise bout (Poole *et al.* 1991; Zoladz & Korzeniewski, 2001). Although the mechanisms responsible for the manifestation of this response remain a topic of great debate, theories proposed to date, such as altered fibre type recruitment (Krustrup *et al.* 2004), blood lactate accumulation (Burnley *et al.* 2002a) or increased muscle temperature (Koga *et al.* 1997) remain inadequate and are therefore subject to ongoing research. Nonetheless, general agreement exists surrounding the significance of the slow component, reflecting a gross inefficiency whereby the energy cost of activity is increasing despite a constant submaximal power output (Poole *et al.* 1991; Zoladz & Krozeniewski, 2001).

Subsequently, the slow component of oxygen uptake is of considerable interest with its putative link to fatigue, and thus much research has focused on whether the magnitude of this slow component can be reduced. Although endurance training has been demonstrated to alter the $\dot{V}O_2$ kinetic response at heavy-intensity exercise, consequent of a reduction in the magnitude of the slow component (Carter *et al.* 2000), interestingly, an acute bout of heavy intensity priming exercise has been advocated as a favourable manipulator of $\dot{V}O_2$ kinetics. In fact, in healthy active individuals the acceleration of the response was attributed to a reduction in the magnitude of the slow component with an increase in the amplitude of the primary phase whilst the time constant of the primary component remained unaffected (Burnley *et al.* 2000, 2001, 2002a, 2002b; Koppo & Bouckaert 2002; Sahlin *et al.* 2005). It should be noted that when a

limitation in the time constant of the primary phase presented as a consequence of central O_2 delivery, such as when exercising in the supine position (Rossiter *et al.* 2001; Jones *et al.* 2006), prior heavy-intensity exercise was evidenced to accelerate the rate of adaptation of the $\tau\dot{V}O_{2p}$ response in the subsequent bout of heavy-intensity primed exercise (Rossiter *et al.* 2001; Jones *et al.* 2006).

Nonetheless it seemingly suggests that prior heavy exercise may favourably alter $\dot{V}O_2$ kinetics, increasing the initial aerobic metabolism contribution to activity while consequently reducing anaerobic ATP provision. Subsequently, by reducing the initial oxygen deficit, time to exhaustion may be increased, which may be of particular significance for clinical populations presenting with constrained $\dot{V}O_2$ kinetics at the onset of submaximal exercise, as is the case in T2DM.

Extensive evidence exists that young and middle-aged individuals with uncomplicated T2DM demonstrate a profoundly slowed $\dot{V}O_2$ kinetics at the onset of moderate-intensity (<VT) submaximal exercise (Regensteiner *et al.* 1995; Regensteiner *et al.* 1998; Brandenburg *et al.* 1999; Brassard *et al.* 2006; Nadeau *et al.* 2009; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012) represented by a prolonged time constant of the primary phase of $\dot{V}O_2$ kinetics ($\tau\dot{V}O_{2p}$). However, less evidence exists for impaired $\dot{V}O_2$ kinetics during heavy-intensity (>VT) cycling exercise in individuals with T2DM. MacAnaney *et al.* (2011a) reported similar time constants of the primary phase of the response in middle-aged females with uncomplicated T2DM when compared to their overweight and lean counterparts, whilst Regensteiner *et al.* (1998), observed a trend for slower $\tau\dot{V}O_{2p}$ in middle-aged women with uncomplicated T2DM (56 ± 21, s) compared to their ND overweight and lean counterparts (41±8 and 43 ± 8, s respectively). However, it is noteworthy that the magnitude of the lengthening in the time constants reported by the Regensteiner group in the heavy-intensity domain (~23%) in the individuals with T2DM is consistent with the impairments reported within the moderate-intensity exercise domain. Nonetheless, these findings are still clinically significant as they suggest a greater perturbation of intracellular homeostasis (Poole *et al.* 2008a) in response to any exercise challenge, with the potential to contribute to premature muscular fatigue (Scheuermann-Freestone *et al.* 2003) and hence contribute to the reduced exercise intolerance consistently demonstrated by individuals with T2DM (Regensteiner *et al.* 1995, 1998; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012).

Whilst the precise mechanisms responsible for the constrained dynamic response in this clinical population remain to be elucidated, a limitation in O_2 delivery and distribution to the active muscles has been identified as a key instrumental factor, with central and/or peripheral

mechanisms likely. Proposed central mechanisms, although not exclusively, include; left ventricular dysfunction (Fang *et al.* 2005a), impaired stroke volume reserve (Joshi *et al.* 2009), diastolic abnormalities (Poirier *et al.* 2000); constrained heart rate kinetics (Regensteiner *et al.* 1998), and reduced cardiac output (Roy *et al.* 1989). Whereas, evidence of impaired peripheral mechanisms include; compromised peripheral vascular function (MacAnaney *et al.* 2011b; Kiely *et al.* 2014), reductions in leg blood flow independent of reductions in CO (Lalande *et al.* 2008; MacAnaney *et al.* 2011b), blunted endothelium-dependent vasodilation (McVeigh *et al.* 1992; Kingwell *et al.* 2003; Lalande *et al.* 2008), and the maldistribution of active muscle blood flow (Kingwell *et al.* 2003; Lalande *et al.* 2008; MacAnaney *et al.* 2011b; Kiely *et al.* 2014).

Thus given that muscle O_2 supply appears to be constrained in T2DM, and heavy-intensity priming exercise has been advocated as an intervention that may enhance muscle O_2 delivery, we tested the hypothesis that priming exercise (PE) would increase the speed of the adjustment of the primary phase ($\tau\dot{V}O_{2p}$) of pulmonary oxygen uptake and/or reduce the amplitude of the slow component of $\dot{V}O_2$ during high-intensity cycling in T2DM. Our specific hypotheses were that; 1) the time constants of the primary phase of the $\dot{V}O_2$ kinetics during heavy-intensity exercise would be significantly reduced by PE in T2DM, 2) that the amplitude of the slow component during the heavy-intensity bout would also be reduced by PE in T2DM, but that 3) PE would have little influence on the [HHb+Mb] kinetics response during the heavy-intensity cycling bout in individuals with T2DM.

4.2 METHODOLOGY

4.2.1 *Participants*

Twelve individuals with uncomplicated T2DM (8 men/4 women), and twelve individuals without T2DM (ND) (8 men/4 women) volunteered to participate in this study (Table 4.1). Nine of the individuals with T2DM, and eleven of the ND controls also participated in *Experiment 1*. Four female participants were premenopausal (2 T2DM and 2 ND) and four were postmenopausal (2 T2DM and 2 ND) not undergoing HRT. All participants were non-smokers and had not smoked during the 12-month period preceding the study. All of the patients with T2DM had a clinical history of diabetes between 2 – 10 years (mean ± SD = 5.9 ± 4.0 years).

4.2.1.1 *Recruitment of participants*

4.2.1.1.1 Individuals without T2DM (ND)

As per section 2.2.1.1.1

4.2.1.1.2 Individuals with T2DM

As per section 2.2.1.1.2

4.2.1.2 Inclusion/exclusion criteria

4.2.1.2.1 Individuals with T2DM

As per section 2.2.1.2.1

4.2.1.2.2 Individuals without T2DM (ND)

As per section 2.2.1.2.2

4.2.1.2.3 Blood sample collection

As per section 2.2.1.2.3

4.2.1.2.4 Ankle brachial index (ABI)

As per section 2.2.1.2.4

4.2.1.3 Determination of physical activity levels

As per section 2.2.1.3

4.2.1.3.1 RT3 Tri-axial accelerometers

As per section 2.2.1.3.1

4.2.1.3.2 The Low Level Physical Activity Recall questionnaire (LOPAR)

As per section 2.2.1.3.2

4.2.2 Experimental design

4.2.2.1 Study overview

Participants without T2DM (ND) were required to visit the cardiovascular performance laboratory in the Department of Physiology, Trinity College Dublin on two separate occasions, whilst all T2DM patients were required to visit the exercise testing facility in St. Columcille's Hospital on two occasions. All premenopausal participants were tested during the mid-follicular phase (days 5-12) of the menstrual cycle, which was self-determined. All participants were asked to refrain from consuming alcohol, caffeine and non-prescribed nutritional supplements in the 24 hours prior to testing, and to limit their exercise to normal activities of daily living.

4.2.2.1.1 Visit 1 overview

During this visit, initially anthropometric data was collected, followed by the measurement of the aortic pulse wave velocity (PWV). Participants then performed a ramp incremental cycling test to exhaustion to enable the determination of $\dot{V}O_{2max}$ and the estimated ventilatory threshold (VT) (*as per section 2.2.4.3*). Following a 5 min passive recovery period, participants finally completed a 'confirmatory' high-intensity cycling bout (*as per section 2.2.2.2.3).*

4.2.2.1.2 Visit 2 overview

From the previously completed ramp incremental cycling test the work rates required for this protocol were calculated. A power output corresponding to 50% delta (Δ50%; the sum of the PO at VT and 50% of the difference between the PO at VT and $\dot{V}O_{2max}$) was determined.

4.2.2.2 Visit 1 to the cardiovascular laboratory

4.2.2.2.1 Anthropometry and pulse wave velocity

As per section 2.2.2.2.1

4.2.2.2.2 Ramp incremental cycle test to exhaustion

Participants performed a ramp incremental cycling test to exhaustion in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, The Netherlands) with appropriate adjustments made to the ergometer seat and handle bar position

for each participant. Exercise was performed initially for 2 min at a baseline resistance of 10W (i.e., 'unloaded' cycling), to reduce excess internal work (Boone *et al.* 2008). This was followed by 10/15 W.min^{-1} increments in females (*n*=2/6) or 15/20/25 W.min^{-1} increments in males (*n*=5/7/4) respectively depending on activity levels of participants, and were brought to volitional exhaustion. Pedal frequency was held constant at an individually selected cadence between 60-75 revolutions per minute (rpm). This cadence was maintained throughout all further testing protocols. Failure in a test was determined as a drop in cadence exceeding 5 rpm for >3 s. Peak workload was determined according to the point of termination of the test. HR was continuously monitored while pulmonary oxygen uptake ($\dot{V}O_2$), pulmonary carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER: $\dot{V}CO_2$ / $\dot{V}O_2$) were recorded on a breath-by-breath (BbB) basis. Peak HR was defined as the highest heart rate attained within the last 15 s of the point of termination of the test.

As it is frequently reported that some individuals do not evidence a definitive plateau of the $\dot{V}O_2$-work rate relationship on this test, secondary criteria based upon measurements of the respiratory exchange ratio (RER), maximal heart rate or blood [lactate] (Poole *et al.* 2008b) are often relied upon to corroborate a maximum effort (Astrand & Rodahl, 1986; Rossiter *et al.* 2006; Poole *et al.* 2008b). However, such criteria have been called into disrepute (Poole *et al.* 2008b), thus, the utilisation of a subsequent confirmatory high-intensity constant load bout *(see section 2.2.2.2.3)* has frequently been implemented (Day *et al.* 2003; Rossiter *et al.* 2006; Murias *et al*. 2010b).

In the present study all participants demonstrated a plateau in $\dot{V}O_2$ during the confirmatory tests, and moreover the $\dot{V}O_2$ values recorded during this confirmatory test were not different to those obtained during the ramp incremental cycle test. Thus, we were certain that in the present study, all participants achieved their $\dot{V}O_{2max.}$

4.2.2.2.3 Confirmatory test

Within five minutes of completion of the ramp incremental cycle test, participants performed a high-intensity, constant-load cycling bout to exhaustion at an intensity equivalent to 85% of the peak power output achieved in the ramp test. This protocol was performed to confirm the attainment of $\dot{V}O_{2max}$ in the prior graded exercise test whilst also facilitating the non-invasive determination of maximal cardiac output via the inert rebreathing technique (Innocor; Innovision, Denmark). Participants were instructed to indicate via the raising of their hand when they felt they were ~30 s from exhaustion. At this point, individuals were verbally encouraged to continue and within 30 s the measurement of CO was carried out.

4.2.2.3 Visit 2 to the cardiovascular laboratory

4.2.2.3.1 Priming effect on heavy-intensity cycling exercise (Δ50%)

Individuals performed four bouts of constant-load, heavy-intensity cycling at Δ50%. Two of these constant-load bouts were completed without prior priming exercise (Δ50% unprimed) and two bouts were undertaken preceded by priming exercise (PE) at an intensity of Δ50% (Δ50% primed). Exercise was performed continuously; the duration of each step transition was 6 min, and each transition was preceded by a 3 min baseline cycling period of 10W. Changes in WR were initiated as a step function without a warning to the individual. There was a 12 min rest period between each of the cycling bouts, except following the first primed heavy-intensity bout. In this instance, participants remained seated in a chair for the duration of a 45 min passive rest period. This is the advocated time for physiological restoration following a bout of heavy intensity exercise, thus having no effect on $\dot{V}O_2$ kinetics during subsequent exercise (Burnley *et al.* 2006a). Figure 4.1 displays a schematic representation of the protocol.

4.2.2.3.2 Cardiac output responses

Cardiac output was determined non-invasively during the third minute of the unprimed heavy-intensity cycling bout via the inert gas rebreathing technique (Innocor; Innovision, Denmark; *see section 2.2.3.5*).

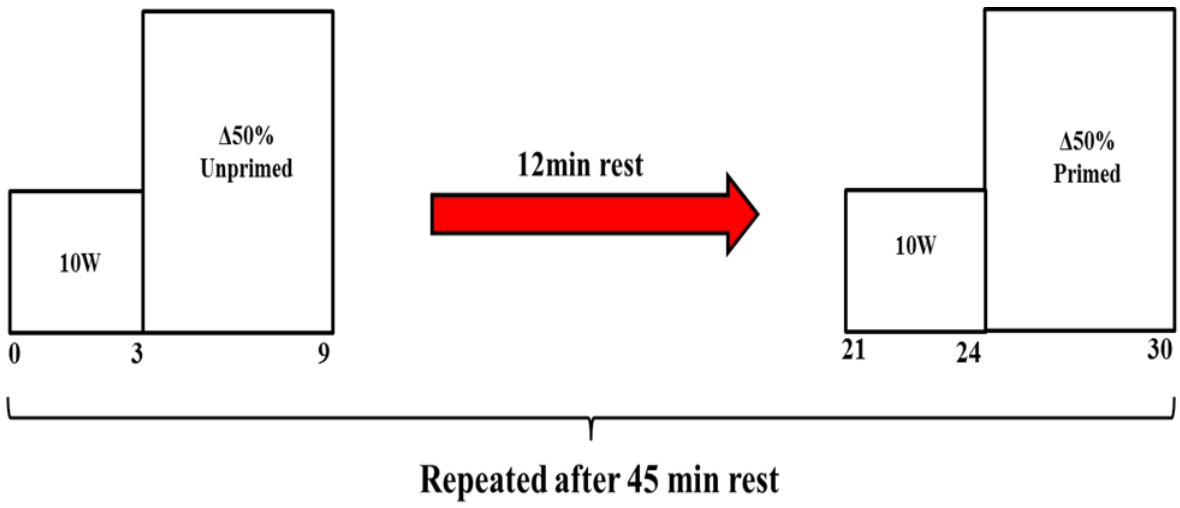

Figure. 4.1 ***Schematic representation of the protocol***

4.2.3 Equipment and techniques

4.2.3.1 Cardiometabolic unit, heart rate and pulse oximeter

As per section 2.2.3.1

4.2.3.2 Near infrared spectroscopy (NIRS) and subcutaneous fat layer of the vastus lateralis

As per section 2.2.3.4

4.2.4 Data analysis

4.2.4.1 *Physiological responses at peak and VT*

As per sections 2.2.2.2.2 and 2.2.4.3 respectively.

4.2.4.2 *Δ50%data analysis*

4.2.4.2.1 $\dot{V}O_2$ kinetics

The breath by breath $\dot{V}O_2$ data for each transition were linearly interpolated to provide second-by-second values and time aligned such that time 0 represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, average response for each individual and further time-averaged into 5 s bins to provide a single time-averaged response per individual. The first 20 s of data after the onset of exercise were deleted in an attempt to avoid inclusion of data points from the cardiodynamic phase. The model parameters of the $\dot{V}O_2$ on-transient kinetic response were thus determined from 20 – 360 s of the step transition via a weighted least-squares nonlinear regression procedure (TableCurve 2D, Systat) in which the best fit was defined by minimization of the residual sum of squares. Data points lying outside the 95% prediction interval during the initial fit of a model were excluded, being attributed to aberrant events, e.g. coughing. Thus, the averaged and smoothed response for each participant was fitted to a biexponential function (*Equation 1*) as follows:

Equation 1 $$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1- e^{-(t-TD_p)/\tau_p)}]\cdot F1 + A_s[1 - e^{-(t-TD_p)/\tau_s)}]\cdot F2$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time *t*; $\dot{V}O_2$ baseline is the mean $\dot{V}O_2$ in the final 30 s of unloaded cycling; $A_p - A_s$, are the amplitudes of the increase in $\dot{V}O_2$ for the primary and slow component phases; $TD_p - TD_s$ are the phase delays, and $\tau_p - \tau_s$ are the time constants, defined as the duration of time for which $\dot{V}O_2$ increases to a value equivalent to 63%

of the amplitude. The conditional expressions F1-F2 limit the fitting of the phase to the period at and beyond the time delay associated with that phase.

The absolute primary component amplitude, referred to as (absolute A_p) was calculated using the following formula:

Equation 2 Absolute A_p = baseline $\dot{V}O_2 + A_p[1- e^{- (t-TD_p)/\tau_p)}]$

The functional "gain" of the primary $\dot{V}O_2$ response, reflective of the increase in $\dot{V}O_2$ per unit increase in power output was computed by the following formula:

Equation 3 Primary $\dot{V}O_2$ gain = (Absolute A_p – Baseline $\dot{V}O_2$ / (PO @ 50%Δ – 10W)

The absolute "gain" of the entire $\dot{V}O_2$ response, was calculated in the same manner, with the end-exercise $\dot{V}O_2$ defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. Finally, the mean response time (MRT) was calculated to provide information on the "overall" $\dot{V}O_2$ kinetics during the heavy-intensity exercise bout, with no distinction made for the various phases of the response. This was calculated through the fitting of a monoexponential curve from the onset to the end of the heavy-intensity exercise bout.

Equation 4 $\dot{V}O_2(t) = \dot{V}O_{2baseline} + A[1- e^{- (t-TD)/\tau)}]$

4.2.4.2.2 Muscle deoxygenation [HHb+Mb] kinetics

Local muscle deoxygenation (Δ[HHb+Mb]) profiles of the right quadriceps *vastus lateralis* muscle were made with near infrared spectroscopy (NIRS) (Hamamatsu Niro 200Nx; Hamamatsu Photonics, Hamamatsu, Japan) throughout the exercise protocol *(as per section 2.2.3.4).* To provide information on muscle oxygenation throughout the protocol, we also modelled the [HHb+Mb] response to exercise. As per the $\dot{V}O_2$ data, the NIRS-derived Δ[HHb+Mb] data for each transition was linearly interpolated to provide second-by-second values and time aligned. Data from each transition were ensemble-averaged to yield a single, average response for each individual, and further time-averaged into 5 s bins to provide a single time-averaged response per individual.

A time delay at the onset of exercise occurs in the [HHb+Mb] profile before it increases with an exponential like time course (DeLorey *et al.* 2003). This was determined in the present study via visual inspection as a systematic increase above the pre-transition level. [HHb+Mb] data were fitted from the first data point at the end of this TD to 360 s with a biexponential model

of the form in Eqn 1 (as per $\dot{V}O_2$; *section 4.2.4.2.1*) to determine the time course of muscle deoxygenation.

The time course for the increase in Δ[HHb+Mb] can be described by the τΔ[HHb+Mb], however, the time course for the overall change of the Δ[HHb+Mb] for the primary response phase, referred to as the effective τ'Δ[HHb+Mb], was determined from the sum of the time delay and τ[HHb+Mb] from the onset of exercise.

The absolute primary component amplitude, referred to as [HHb+Mb] absolute A_p, was calculated using Eqn 2 (as per $\dot{V}O_2$; *section 4.2.4.2.1*).

The final end amplitude of the response, referred to as Absolute [HHb+Mb] end A, was calculated from the sum of the absolute primary component amplitude and the amplitude of the slow component.

Finally, the mean response time (MRT) was calculated to provide information on the "overall" [HHb+Mb] kinetics during the heavy-intensity exercise bout, with no distinction made for the various phases of the response. This was calculated through the fitting of a monoexponential curve of the form in Eqn 4 (as per $\dot{V}O_2$; *section 4.2.4.2.1*), from the onset to the end of the heavy-intensity exercise bout.

4.2.5 Statistical analysis

Statistical analysis was performed using the software SigmaPlot version 12.0 (Systat Software, Point Richmond, CA). Prior to analysis, normal Gaussian distribution of the data was assessed using the Shapiro-Wilk's test. Physical characteristics and peak physiological responses between groups were compared using the unpaired Student's t-test for parametric analysis, or the Mann-Whitney U test for non-parametric analysis. The kinetic parameter estimates for $\dot{V}O_2$ and [HHb+Mb] during heavy-intensity exercise were analysed by using a two-way repeated measures ANOVA (primed/unprimed condition and diabetes as the main effects). In the case of significant differences obtained from the repeated measures ANOVA, post hoc Tukey tests were performed. Statistical significance was accepted at a P value ≤ 0.05. All values are expressed as means ± standard deviation (SD) or as median and interquartile ranges for data that were deemed not normally distributed.

The key variable is the rate of change of the primary phase of oxygen uptake ($\dot{V}O_{2p}$). An indicator of the rate of change in $\dot{V}O_2$ can be obtained by calculation of its time constant (τ).

The τ is the time required to achieve ~63 % of the difference between the baseline and the plateau of that phase. Recently published studies in our laboratory and others, which, included data for both individuals with T2D and age-matched healthy controls, revealed τ values of ~35 ± 7 s (Mean ± SD) during cycling at 80% VT. The difference between unprimed and primed τ values (based on studies comparing older *vs* young healthy participants, or studies comparing the postural effects; upright *vs* supine cycling, in young participants) was 10 s. Thus, given the estimated standard deviation (SD) of each population is 7 s, and that the minimum difference we wish to detect as significant would be 10 s, the minimum sample size needed to detect a significant effect at β=0.05 and α=0.05 (90% power) for an ANOVA power calculation design based on 2 groups, is 11 subjects.

4.3 RESULTS

4.3.1 Participants

4.3.1.1 Physical characteristics

Physical characteristics for the participants are presented in Table 4.1. Anthropometrical measurements did not significantly differ between groups, with the exception of WHR, whereby T2DM individuals displayed a significantly greater WHR (P<0.05) compared to their ND counterparts. Individual anthropometric measurements are displayed in (Appendix 10). No differences were observed in ABI, or subcutaneous fat layer of the vastus *lateralis* (VL). However, individuals with T2DM displayed a significantly faster PWV (P<0.05), and a tendency for a lower DBP (P=0.01) compared with their ND counterparts.

Table 4.1. ***Anthropometrical data, PWV, ABI and resting BP for ND and T2DM individuals.***

	ND (***n*** **=12)**	**T2DM** (***n*** **=12)**
Sex (male, female)	8, 4	8, 4
Age (yr)	*38 (18)*	*43 (13)*
Height (m)	1.70 ± 0.07	1.71 ± 0.08
Weight (kg)	*86 (16)*	*93 (39)*
BMI (kg.m^{-2})	31 ± 4	31 ± 5
WHR (a.u)[a]	0.93 ± 0.06*	1.01 ± 0.08
Fat layer VL (mm)[a]	8.6 ± 4.2	6.3 ± 1.7
Pre exercise ABI (a.u.)	*1.17(0.30)*	*1.09 (0.13)*
Post exercise ABI (a.u.)	1.15 ± 0.13	1.12 ± 0.12
PWV (ms^{-1})[b]	6.3± 1.4*	9.1 ± 2.0
SBP (mmHg)	128 ± 11	124 ± 12
DBP (mmHg)	80 ± 8†	74 ± 10

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. BMI, body mass index; WHR, waist:hip ratio; ABI, ankle:brachial index; PWV, pulse wave velocity. SBP; systolic blood pressure; DBP, diastolic blood pressure. Some variables have missing values and the sample sizes with codes are shown below *Significantly different than T2DM (P≤0.05). †Tendency towards a difference than T2DM (P≤0.10). a=12 (ND) and 11 (T2DM); b=10 (ND) and 11 (T2DM).

4.3.1.2 Haematological parameters and prescriptive medications

Mean haematological parameters and prescriptive medications are presented in Tables 4.2 and 4.3 respectively. As expected, individuals with T2DM displayed significantly higher HbA1c and fasting plasma glucose levels (P≤0.001). They also had significantly higher total cholesterol and triglycerides (P<0.05) than the ND controls, with no differences between HDL-C and LDL-C distribution.

Table 4.2. ***Haematological parameters for ND and T2DM individuals.***

	ND	**T2DM**
HbA_{1c} (%)[a]	*5.1 (0.4)*[**]	*6.9 (2.8)*
FPG (mmol.L^{-1})[b]	*3.90 (0.70)*[**]	*7.00 (4.80)*
Total cholesterol (mmol.L^{-1})[c]	3.59 ± 0.85[*]	5.08 ± 1.29
LDL-C (mmol.L^{-1})[d]	*1.63 (1.30)*[†]	*2.65 (1.23)*
HDL-C (mmol.L^{-1})[e]	*1.2 (0.45)*	*1.03 (0.24)*
Triglycerides (mmol.L^{-1})[c]	*1.10 (0.65)*[*]	*1.90 (1.19)*

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. HbA_{1c}, glycosylated haemoglobin; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Some variables have missing values and the sample sizes with codes are shown below. [*]Significantly different than T2DM (P≤0.05). [**]Significantly different than T2DM (P≤0.001). [†]Tendency towards a difference than T2DM (P≤0.10). a=6 (ND) and 11 (T2DM); b=9 (ND) and 11 (T2DM); c=9 (ND) and 8 (T2DM); d=9 (ND) and 6 (T2DM); e=9 (ND) and 7 (T2DM).

Table 4.3. ***Prescriptive medications for ND and T2DM individuals.***

	ND (n = 12)	**T2DM (n = 12)**
Anti-hypertensives		
Angiotensin converting enzyme inhibitor		3
Angiotensin II receptor blocker		2
Aspirin	1	3
Calcium channel blocker		3
Statins	2	6
Hypoglycaemic medications		
Oral hypoglycaemics		10
Subcutaneous hypoglycaemics		2
Sulphonylureas		2

4.3.1.3 Physical Activity Levels

Group mean activity levels are presented in Tables 4.4 and 4.5 for accelerometry and LOPAR data respectively. Physical activity behaviour was comparable between the two groups, when assessed via both accelerometry and the LOPAR questionnaire, although individuals with T2DM displayed a tendency (P=0.06) for greater levels of inactivity.

Table 4.4. ***Group mean activity levels based on the number of hours per day as determined by RT3 accelerometers.***

	ND (n=10)	**T2DM (n=4)**
Inactive (h.day^{-1})	19.50 ± 1.51†	17.75 ± 0.96
Light (h.day^{-1})	3.68 ± 1.25	5.38 ± 1.28
Moderate (h.day^{-1})	0.63 ± 0.40	0.77 ± 0.60
Vigorous (h.day^{-1})	0.24 ± 0.21	0.10 ± 0.14

Values are mean ± SD. Inactive, <100 counts.min^{-1}; Light, 101-970 counts.min^{-1}; Moderate, 971-2333 counts.min^{-1}; Vigorous, >2333 counts.min^{-1}. †Tendency towards a difference than T2DM (P≤0.10).

Table 4.5. ***Group mean activity levels based on the number of METS per hour per week as determined by the LOPAR questionnaire.***

	ND (n=10)	**T2DM (n=8)**
LOPAR (MET.hr^{-1}.wk^{-1})	153 ± 55	138 ± 88

Values are mean ± SD

4.3.2 Performance data from ramp incremental cycling test

4.3.2.1 Physiological responses

The physiological responses for both groups at peak exercise, and at VT are displayed in Tables 4.6. Maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$) normalised to kilograms of body weight was significantly (P<0.05) reduced in individuals with T2DM compared with the ND controls, representing a 23% reduction in peak exercise capacity. In absolute terms (L.min^{-1}), $\dot{V}O_{2max}$ also tended to be lower in the group with T2DM, although it did not reach statistical significance (P=0.08). In addition, they displayed a strong tendency toward significance for peak PO (P=0.06) at exhaustion, with no differences in time to failure. RER was similar between the T2DM group

and the ND group respectively at peak exercise, indicating both groups made their maximal effort during the ramp incremental protocol.

Table 4.6. ***Physiological responses at peak exercise and VT.***

	ND (*n* = 12)	**T2DM (*n* = 12)**
$\dot{V}O_{2max}$ (mL.kg^{-1}.min^{-1})	28.3 ± 6.3*	21.9 ± 3.9
$\dot{V}O_{2max}$ (L.min^{-1})	2.47 ± 0.50†	2.04 ± 0.60
Peak PO (W)	198 ± 51†	157 ± 50
Peak HR (beats.min^{-1})^{a}	167 ± 15	163 ± 14
Age predicted HR (beats.min^{-1})	*181 (18)*	*177 (13)*
Peak RER (a.u.)	1.15 ± 0.08	1.12 ± 0.07
Peak CO (L.min^{-1})^{b}	14.75 ± 1.97	13.38 ± 1.98
TTF (secs)	643 ± 86	653 ± 119
$\dot{V}O_2$ @ VT (mL.kg^{-1}.min^{-1})	18.72 ± 5.10	15.94 ± 3.19
$\dot{V}O_2$@ VT (L.min^{-1})	1.63 ± 0.39	1.48 ± 0.46
PO @ VT (W)	107 ± 39	88 ± 33

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample sizes with codes are shown below.*Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). a=11 (ND) and 12 (T2DM); b=6 (ND) and 5 (T2DM).

4.3.3 Performance Data from Δ50%

4.3.3.1 $\dot{V}O_2$ kinetics

The parameter estimates of the kinetic analysis of the $\dot{V}O_2$ response for both ND and T2DM individuals with and without a prior bout of priming exercise are presented in Table 4.7. At baseline, the parameter estimates for the primary component of the kinetic response were similar between groups, albeit individuals with T2DM displaying a tendency (P=0.094) for a lower amplitude of the response. A slow component was apparent in all individuals in both exercise bouts, being significantly lower in individuals with T2DM (P<0.05) but with no significant differences in the onset of same. Additionally, end $\dot{V}O_2$ was significantly lower, and MRT was significantly longer in individuals with T2DM (P<0.05).

PE however, resulted in significantly (P<0.001) elevated baseline $\dot{V}O_2$ values, with a reduced τ_p in the subsequent exercise bout. The primary amplitude (A_p) displayed a tendency to be elevated subsequent to PE (P=0.1). However, the absolute $\dot{V}O_2$ amplitude at the end of the primary phase, was significantly (P<0.05) increased after prior heavy exercise, due in part to

the elevated baseline $\dot{V}O_2$. The amplitude of the $\dot{V}O_2$ slow component (A_s), although not significantly altered (P=0.110) by prior heavy exercise, it was substantially greater in individuals with T2DM with its magnitude being reduced by 42% compared to 2% in the ND group. Additionally, there was a significant (P<0.001) reduction in the MRT of the overall $\dot{V}O_2$ response subsequent to the prior heavy exercise, with a significant interaction (diabetes x priming; P<0.001) demonstrating that the longer MRT in the unprimed condition in T2DM was not evident following PE.

The adaptation of $\dot{V}O_2$ at the onset of heavy-intensity exercise, with and without a prior bout of priming exercise for a representative ND and T2DM individual is illustrated in Figure 4.2.

Table 4.7. ***Dynamic response characteristics of oxygen uptake for unprimed and primed heavy-intensity exercise.***

	Δ50% Unprimed		**Δ50% Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	12	12	12	12
$\dot{V}O_2$ baseline, L.min^{-1}	0.86 ± 0.12	0.86 ± 0.15	0.93 ± 0.16‡	0.89±0.17‡
$\dot{V}O_2$ A_p, L.min^{-1}	1.20 ± 0.44†	0.93 ± 0.41	1.27 ± 0.40$^{\#}$	0.97±0.41$^{\#}$
$\dot{V}O_2$ absolute A_p, L.min^{-1}	2.06 ± 0.51	1.79 ± 0.48	2.21 ± 0.46‡	1.87±0.51‡
$\dot{V}O_2$ τ_p, s	31 ± 5	37 ± 10	29 ± 7‡	31±9‡
$\dot{V}O_2$ A_s, L.min^{-1}	0.36 ± 0.18*	0.26 ± 0.15	0.35 ± 0.19*	0.15±0.07
$\dot{V}O_2$ TD_s, s	125 ± 24	145 ± 47	133 ± 43	144±48
Primary $\dot{V}O_2$ gain, mL.min^{-1}.W^{-1}	8.5 ± 1.5	9.0 ± 1.2	8.1 ± 1.7$^{\#}$	8.5±1.5$^{\#}$
Absolute $\dot{V}O_2$ gain, mL.min^{-1}.W^{-1}	11.0 ± 1.6	10.6 ± 2.4	11.4 ± 1.4	10.0±1.7
End $\dot{V}O_2$, L.min^{1}	2.37 ± 0.58*	1.99 ± 0.51	2.53 ± 0.57*‡	2.00±0.50
$\dot{V}O_2$ MRT, s	55 ± 7*	75 ± 10	48 ± 6‡	55±14‡

Values are means ± SD. Dynamic response characteristics of oxygen uptake ($\dot{V}O_2$) during unprimed and primed cycling exercise at 50% delta (Δ) in individuals with T2DM and ND controls. A_p and A_s, amplitudes; TD_p and TD_s, time delays; τ_p and τ_s, time constants; for primary and slow component phases. ‡Significantly different between primed conditions within the group (P≤0.05). *Significantly different than T2DM (P≤0.05). †Tendency towards a difference than T2DM (P≤0.10). $^{\#}$Tendency towards a difference from unprimed (P≤0.10).

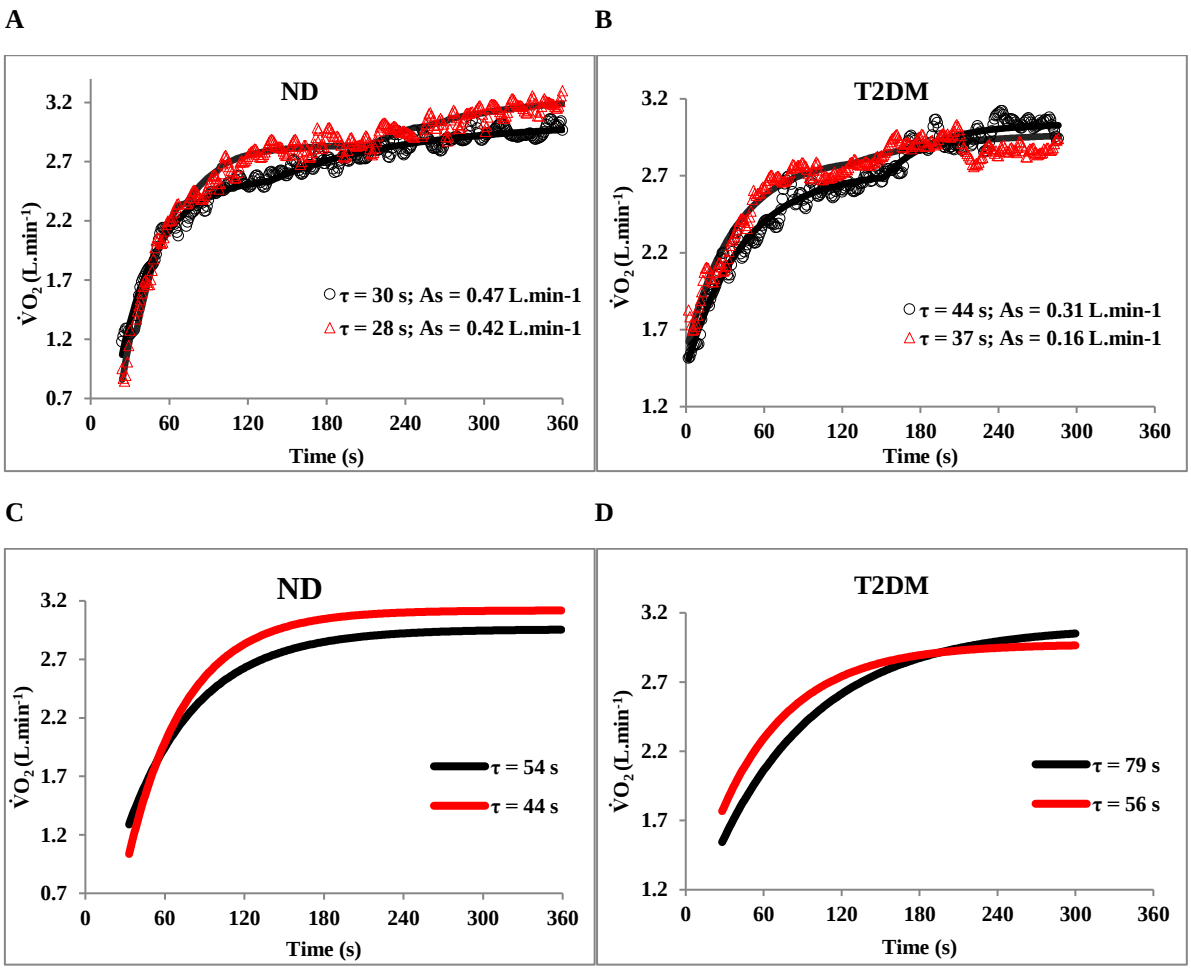

Figure 4.2. Oxygen uptake responses for representative ND and T2DM individuals for unprimed (○) and primed (Δ) bouts of heavy-intensity exercise. The continuous lines of best fit illustrate the primary and slow component phases of the oxygen uptake ($\dot{V}O_2$) response. $\tau\dot{V}O_{2p}$ and amplitudes of the slow component (A_s) are included in panels A & B, with MRT's included in panels C & D.

4.3.3.2 Muscle deoxygenation [HHb+Mb] kinetics

Kinetic parameters for Δ[HHb+Mb] for both ND and T2DM individuals with and without a prior bout of priming exercise are displayed in Table 4.8. At baseline, the parameter estimates for the [HHb+Mb] kinetics response were similar between groups, albeit individuals with T2DM displaying a tendency (P=0.09) for a higher amplitude of the slow component (A_s), and a significantly (P<0.05) shorter MRT.

PE resulted in significantly (P<0.001) elevated baseline levels for Δ[HHb+Mb]. PE however did not alter the NIRS-derived ([HHb+Mb] pre-transition period (TD_p) of the primary component of the response, the magnitude of change in Δ[HHb+Mb] (A_p), nor the time constant of this response. Furthermore the overall time course for muscle deoxygenation, (effective response time), determined as the ([HHb+Mb] TD + τ was similar between groups. Nonetheless, the absolute [HHb+Mb] amplitude at the end of the primary phase ([HHb+Mb] absolute A_p),

as well as the end amplitude of the entire response (Absolute [HHb+Mb] end A) was increased in individuals with T2DM subsequent to priming exercise

The adaptations of [HHb+Mb] during the on-transient of heavy-intensity (Δ50%) exercise for a representative ND and T2DM individual are presented in Figure 4.3.

Table 4.8. Kinetic parameters for Δ[HHb+Mb] for unprimed and primed heavy-intensity exercise.

	Δ50% Unprimed		**Δ50% Primed**	
	ND	T2DM	ND	T2DM
n	12	12	12	12
Δ[HHb+Mb] Baseline, a.u.	-49.8 ± 34.8	-56.5 ± 62.0	-32 ± 37.3‡	-9.6 ± 52.0‡
Δ[HHb+Mb] A_p, a.u.	88.5 ± 83.2	125.3 ± 74.3	85.2 ± 83.4	145.4 ± 83.8
Δ[HHb+Mb] TD_p, s	11 ± 4	13 ± 3	12 ± 6	12 ± 3
Δ[HHb+Mb]τ_p, s	13 ± 8	12 ± 5	14 ± 6	13 ± 4
Δ[HHb+Mb]τ', s	24 ± 8	25 ± 6	25 ± 9	24 ± 5
Δ[HHb+Mb] absolute A_p, a.u.	38.8 ± 108.0	68.9 ± 99.5	53.3 ± 114.0#	135.8 ± 106.0‡
Δ[HHb+Mb] A_s, a.u.	24.3 ± 22.4†	26.2 ± 25.8	24.9 ± 22.0	15.0 ± 15.6#
Δ[HHb+Mb] TD_s, s	107 ± 53	138 ± 49	104 ± 40	150 ± 77
Δ[HHb+Mb]τ_s, s	91 ± 71	74 ± 59	80 ± 65‡	53 ± 53‡
Δ[HHb+Mb]MRT, s	53 ± 46*	33 ± 27	53 ± 41*	16 ± 6
Absolute Δ[HHb+Mb] end A, a.u.	63.1 ± 106.0	84.1 ± 101.0	76.1 ± 106.9	144.6 ± 101.4‡

Values are means ± SD. Dynamic response of muscle deoxygenation [HHb+Mb] during unprimed and primed cycling exercise at 50% delta (Δ) in individuals with T2DM and ND controls. A_p and A_s, amplitudes; TD_p and TD_s, calculated time delay; τ_1 and τ_2, time constants; of the primary and slow component phases. τ', effective response time (TD + τ); MRT, mean response time; a.u. arbitrary units. ‡Significantly different between primed conditions within the group ($P \leq 0.05$). *Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). #Tendency towards a difference from unprimed ($P \leq 0.10$).

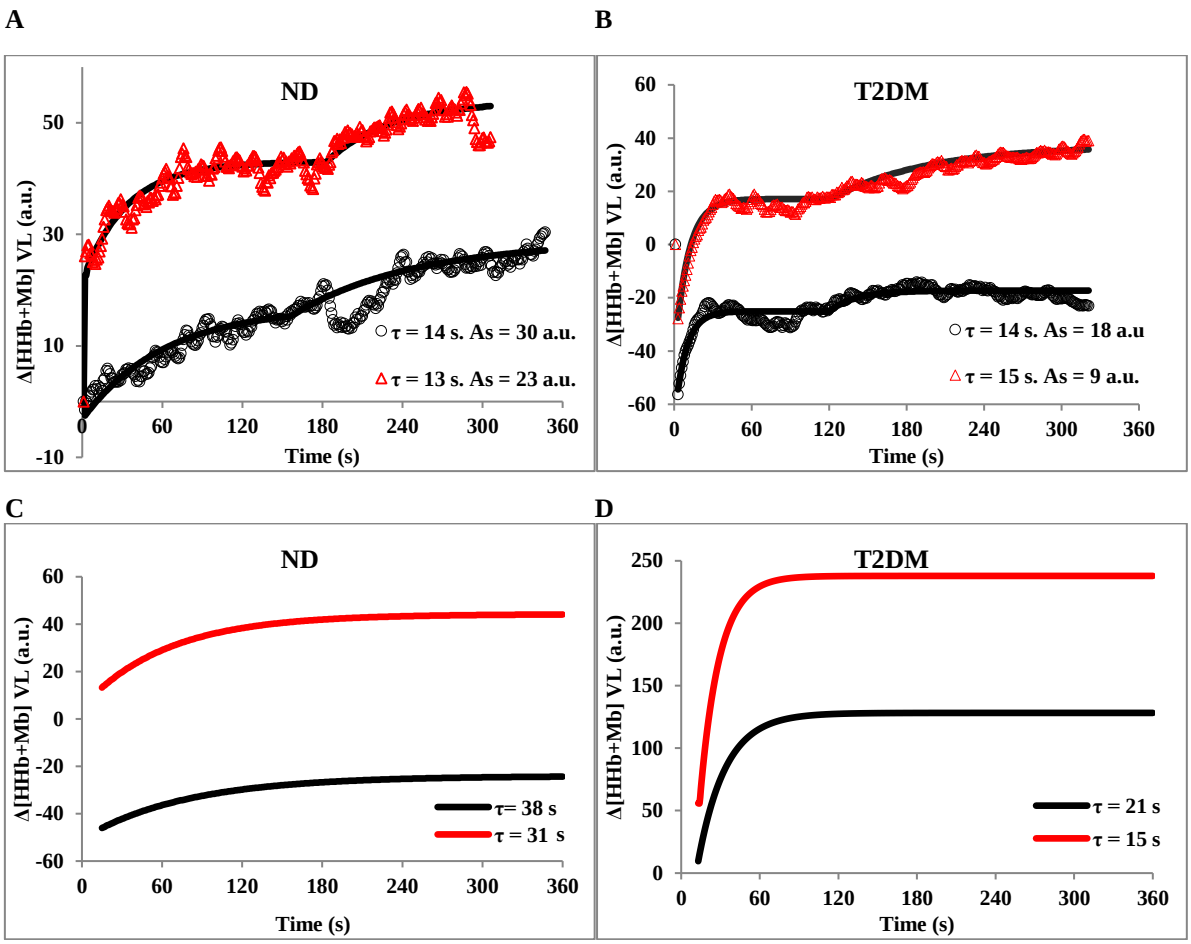

Figure 4.3. Deoxygenated haemoglobin [HHb+Mb] experimental data for representative ND and T2DM individuals for unprimed (○) and primed (Δ) bouts of heavy-intensity exercise. The continuous lines of best fit illustrate the primary and slow component phases of the [HHb+Mb] response. τ[HHb+Mb] and amplitudes of the[HHb+Mb] slow component are included in panels A & B, with MRT's included in panels C & D.

4.3.3.3 Cardiac output and heart rate responses

Heart rate responses for both ND and T2DM during heavy-intensity exercise (Δ50%) with and without a prior bout of priming exercise, and CO responses during the unprimed bout are presented in Table 4.9. Individuals with T2DM displayed significantly lower CO values to that of their respective ND counterparts (P<0.05). No significant differences were revealed for heart rate responses.

Table 4.9 ***Heart rate responses to heavy-intensity exercise and cardiac output at Δ50%***

	Δ50% Unprimed		**Δ50% Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	12	12	12	12
Baseline HR, beats.min^{-1}[(a)]	100 ± 12	110 ± 13	106 ±10	119 ± 13
End HR, beats.min^{-1}[(a)]	157 ±16	158 ± 12	162 ±15	161 ±13
ΔHR, beats.min^{-1}[(a)]	57 ± 11	48 ± 6	56 ±11	42 ± 10
Baseline CO, L.min^{-1}[(b)]	15.6 ± 4.2*	11.8 ± 2.2	-	-

Values are means ± SD. Baseline cardiovascular parameters in unprimed and primed cycling exercise at 50% delta (Δ) in individuals with T2DM and ND controls. HR, heart rate. CO, cardiac output. Some variables have missing values and the sample sizes with codes are shown below *Significantly different than T2DM ($P \leq 0.05$). a=11 (ND) and 9 (T2DM); b=11 (ND) and 8 (T2DM).

4.4 DISCUSSION

The present study investigated the effect of a prior bout of heavy-intensity "priming" cycling exercise (PE) during subsequent heavy-intensity cycling in individuals with T2DM. We hypothesised that PE would increase the speed of adjustment of the primary phase (τ_p) of pulmonary oxygen uptake ($\dot{V}O_2$) and/or reduce the amplitude of the slow component of $\dot{V}O_2$ during heavy-intensity cycling given that muscle O_2 supply appears to be limited in T2DM. The principal findings of this study were that;

1. In agreement with our first hypothesis, priming exercise increased (P<0.05) the primary phase of the $\dot{V}O_2$ kinetics response in a subsequent bout of heavy-intensity cycling exercise in individuals with T2DM.
2. In contrast with our second hypothesis, the amplitude of the $\dot{V}O_2$ slow component was not significantly reduced by PE (P=0.1). However, despite not reaching statistical significance, the magnitude of this reduction was ~42% in individuals with T2DM compared to ~2% in their ND counterparts.
3. A significant (P<0.001) reduction in the MRT of the overall $\dot{V}O_2$ response subsequent to the prior heavy-intensity exercise was observed with a significant interaction (diabetes x priming; P<0.001), thereby demonstrating that the longer MRT in the unprimed condition in T2DM was not evident following PE.

In accordance with previous investigators (Koppo & Bouckaert 2001; Burnley *et al.* 2000), the overall acceleration of the $\dot{V}O_2$ kinetics response in the present study can be, at least partially, attributed to the substantially reduced amplitude of the $\dot{V}O_2$ slow component. It is likely that interindividual variability in the responses precluded the attainment of statistical significance. Interestingly, the observed reduction in the amplitude of the slow component in the present study was accompanied by a putatively uncharacteristic acceleration of the primary phase of the $\dot{V}O_2$ kinetics response. This is in contrast to findings by the majority of previous investigators, whereby the acceleration of the overall $\dot{V}O_2$ response was attributed to a reduction in the amplitude of the slow component, concomitant with an unaltered time constant of the primary component (Burnley *et al.* 2000, 2002a, 2002b 2006a; Bearden & Moffatt, 2001; Scheuermann *et al.* 2001; Koppo & Bouckaert 2002). However, on the contrary, it would appear that this is only applicable to healthy and relatively active (mean $\dot{V}O_{2max}$ >45mL.kg^{-1}.min^{-1}) individuals exercising in the upright position where O_2 delivery is not a limiting factor, and is in fact circumstance dependent. This contravening notion is derived from the observations by Rossiter *et al.* (2001) and Jones *et al.* (2006) whereby participants were required to exercise in

the prone and supine positions respectively. In these positions, muscle perfusion pressure is likely reduced and muscle O_2 availability might be compromised.

Specifically, in the study by Rossiter and colleagues, (2001) individuals performed two consecutive bouts of high-intensity, square-wave, knee extension exercise in the prone position. Significant reductions in the amplitude of the $\dot{V}O_2$ slow component, (expressed as a percentage of the total amplitude; 6.8 ± 4.9 *vs.* 2.7 ± 2.4 %) were accompanied by an acceleration of the primary phase of the $\dot{V}O_2$ kinetics response (49 ± 6 *vs.* 41 ± 8 s) in the subsequent high-intensity bout, thus speeding the overall $\dot{V}O_2$ kinetics response. Similarly, Jones *et al.* (2006) investigated the effects of PE in both the supine and upright cycling postures. The authors observed a 37% reduction (P<0.05) in $\tau\dot{V}O_{2p}$ (38 s ± 18 s *vs.* 24 ± 9 s) during supine high-intensity cycling subsequent to a bout of heavy-intensity priming exercise, albeit having no significant effect during upright cycling. Interestingly, prior exercise had no effect on the $\dot{V}O_2$ amplitude of the slow component phase (0.40 ± 0.29 *vs.* 0.41 ± 0.20, $L.min^{-1}$) in the supine position, despite significantly (P < 0.05) reducing (0.45 ± 0.16 *vs.* 0.22 ± 0.14 $L.min^{-1}$) it in the upright position. However, it should be noted that within this study, participants exercised at the same absolute work rate in both exercise postures; equating to 50% and 72% delta during upright and supine exercise respectively. The authors acknowledged the possibility that the difference in relative exercise intensity may have influenced muscle fibre recruitment patterns in both the first and second bouts of exercise. Greater muscle fatigue in the first bout of supine exercise likely imposed an additional requirement for the recruitment of higher order fibres during subsequent exercise to maintain power output. As a result, the desired enhanced muscle perfusion facilitated by PE may have been tempered by the greater residual fatigue, obligating a greater or earlier recruitment of lower efficiency fibres after the onset of subsequent exercise (Jones *et al.* 2006).

In addition, it is interesting that the mechanisms behind the altered overall $\dot{V}O_2$ kinetics response demonstrated by the ND individuals in the present study subsequent to priming exercise differed from those in the upright posture of the above mentioned study by Jones *et al.* (2006). This was most likely a direct consequence of the different relative fitness levels of the two groups in these two studies, whereby the ND individuals in the present study demonstrated inferior $\dot{V}O_{2max}$ results to the more active individuals in the posture related study by Jones *et al.* (2006) (~28 *vs.* ~42 $mL.kg^{-1}.min^{-1}$) and thus displayed a slower rate of adaptation in the primary phase of $\dot{V}O_2$ kinetics prior to PE (31 ± 5 *vs.* 29 ± 10 s). Nonetheless, it can still be inferred that the speeding of the primary $\dot{V}O_2$ kinetics mainly pertains to exercise in circumstances whereby

O_2 delivery is compromised, be that purposely manipulated (Rossiter *et al.* 2001; Jones *et al.* 2006), or as an indirect implication of the pathophysiological features of T2DM.

Additionally, during high-intensity exercise subsequent to PE an increase in the amplitude of the $\dot{V}O_2$ primary component has been consistently demonstrated (Burnley *et al.* 2002a, 2002b, 2006a; Fukuba *et al.* 2002), albeit only in the presence of an adequate recovery interval (approximately 9-10 min) whereby the restoration of the baseline $\dot{V}O_2$ has been facilitated (Bearden & Moffatt, 2001; Burnley *et al.* 2001, 2002a, 2002b). Earlier investigations by Burnley *et al.* (2000), Rossiter *et al.* (2001) and Scheuermann *et al.* (2001) showed an unchanged net primary amplitude following PE, most likely as a consequence of an elevated baseline $\dot{V}O_2$. In an attempt to address this issue, Burnley *et al.* (2001) later extended the duration of the original 6 min passive recovery period between the two bouts of exercise to 12 min. This was based on the premise that the recovery of the concentration of lactate in the blood has a half-time of approximately 15-20 min (Weltman *et al.* 1979) and thus, the residual acidosis should be more than adequate to still favourably influence $\dot{V}O_2$ kinetics. Indeed, favourable adaptations were observed in the subsequent post PE bout of heavy-intensity exercise after 12 min of rest. A return to pre-exercise baseline $\dot{V}O_2$ values was followed by a consistent and significant increase in the primary amplitude of the $\dot{V}O_2$ response, accompanied by a reduction in the slow component amplitude, albeit with an unaltered primary time constant (Burnley *et al.* 2001). Thus, in the present study we provided a 12 min passive recovery period in between high-intensity cycling bouts to facilitate the restoration of baseline $\dot{V}O_2$ levels. However, baseline $\dot{V}O_2$ levels remained significantly elevated ($P<0.001$) upon initiation of the primed bout implying that recovery was incomplete and subsequently a significant increase in the amplitude of this response was not demonstrated, although a trend ($P=0.1$) was revealed.

Our findings are consistent with the literature whereby prior heavy exercise results in a speeding of the overall $\dot{V}O_2$ kinetics, consequent of the combination of reduction in the $\dot{V}O_2$ slow component and an accelerated rate of adaptation of the primary phase. Our original hypothesis was based on the premise that a prior-bout of heavy-intensity exercise would accelerate the overall $\dot{V}O_2$ kinetics response, consequent to the enhanced muscle blood flow associated with this intervention (Gerbino *et al.* 1996) considering muscle O_2 supply appears to be constrained in individuals with T2DM.

Such findings certainly reinforce the efficacy for the application of heavy-intensity PE as an acute intervention to accelerate the $\dot{V}O_2$ response in situations where O_2 delivery is limited, such as when the perfusion pressure is manipulated by tilting the body from the upright to

supine posture, or in many chronic conditions, such as T2DM. As previously mentioned, strong evidence exists in favour of an attenuated hyperaemic and haemodynamic response in T2DM. Individuals with T2DM have evidenced compromised peripheral vascular function (Kiely *et al.* 2014), reductions in submaximal leg blood flow independent of CO (Lalande *et al.* 2008; MacAnaney *et al.* 2011b), and an impaired dynamic response of peripheral O_2 delivery to active muscles (MacAnaney *et al.* 2011b; Kiely *et al.* 2014). This would be consistent with the observations of MacDonald *et al.* (1998), whereby despite muscle blood flow kinetics being faster than $\dot{V}O_2$ kinetics in both supine and upright knee extension and knee flexion exercise, the constrained $\dot{V}O_2$ kinetics demonstrated during exercise in the supine position was accompanied by a relatively slower leg blood flow (LBF) measured in the femoral artery (Supine; 39.7 ± 3.8 and 27.6 ± 3.9 s; Upright; 29.3 ± 3.0 and 17.3 ± 4.0 s) respectively. These findings suggest that O_2 supply at the onset of exercise may alter metabolic control and limit the rate of increase in muscle $\dot{V}O_2$. However, on the contrary, in a later study (Fukuba *et al.* 2002) exploring the effects of PE on the temporal profiles of $\dot{V}O_2$ and LBF at the femoral artery during heavy-intensity (~75% PO_{peak}) supine bi-lateral knee extension exercise, an accelerated $\dot{V}O_2$ kinetics was not accompanied by faster muscle blood flow kinetics ($\dot{V}O_2$; 72.1 ± 14.3 *vs.* 60.6 ± 13.3 s; LBF; 39.2 ± 16.1 *vs.* 40.2 ± 15.7 s). These findings are inconsistent with the notion that circulatory dynamics govern the adjustment of pulmonary $\dot{V}O_2$ during high-intensity exercise. As such, it may be possible that the maldistribution of blood flow may be the primary limiting factor of the reduced hyperaemia observed during supine exercise and/or T2DM as opposed to the bulk muscle O_2 delivery *per se*. Combining the notion that peripheral blood flow and hence, O_2 delivery, are governed by different mechanisms at systemic level and at the level of the microvasculature with evidence of impaired peripheral vascular function and oxygen delivery to active muscles in T2DM, PE may have resulted in a more favourable distribution of blood flow within the microvasculature of the active muscle units within the working muscle. An acute bout of endurance exercise (Murias *et al.* 2012) has been demonstrated to improve endothelium-dependent vascular responsiveness (increased % vasorelaxation response, and rate of vasorelaxation). Thus given that within the vascular tree, the distal arterioles exhibit a faster vasorelaxation response to that of the more proximally located ones (Roseguini *et al.* 2010) it is plausible that PE may facilitate more rapid endothelium-dependent changes at the level of the microvasculature.

In the present study despite the demonstration of an accelerated $\dot{V}O_2$ kinetics response consequent to the prior priming bout, the overall dynamic responses of Δ[HHb+Mb] remained predominantly similar. This suggests that the overall acceleration of the $\dot{V}O_2$ kinetics response

was due to an increased muscle blood flow, and/or improvement in the perfusion of local muscle to metabolic rate, rather than an increased reliance on muscle fractional O_2 extraction (DiMenna *et al.* 2010c). Subsequent to the priming heavy-intensity exercise, a trend (P<0.10) for a significantly reduced Δ[HHb+Mb] slow component was revealed with the demonstration of a 42% reduction in the amplitude of this response. This further supports the notion of a greater O_2 delivery relative to utilisation subsequent to a prior bout of heavy-intensity exercise in T2DM.

4.5 CONCLUSION

As previously acknowledged in *Experiment* 1 *(section 2.4.3)*, important differences in absolute PWV values exist, and therefore the clinical significance of the PWV results demonstrated in this study should be interpreted cautiously. However, we acknowledge that the significantly higher PWV (6.3 ± 1.4 *vs.* 9.1 ± 2.0 ms^{-1}) demonstrated by individuals with T2DM in the present study compared to individuals without, may have contributed to the observations reported upon within. Nonetheless, in light of the aforementioned pathophysiological features pertaining to T2DM, important roles for peripheral vascular sequelae resulting in the maldistribution of active muscle blood flow can certainly be argued. Thus, where the dynamics of muscle or limb blood flow may be faster than $\dot{V}O_2$ kinetics, those of capillary blood may not (Womack *et al.* 2009), and thus the insidious effects of T2DM on $\dot{V}O_2$ kinetics may occur in the absence of an impediment to the normal bulk hyperaemic control process. Furthermore with the existing evidence that heavy-intensity priming exercise is associated with increased muscle oxygenation (Ward *et al.* 1994; Burnley *et al.* 2002a, Fukuba *et al.* 2002) and muscle blood flow (Bangsbo *et al.* 2001; Krustrup *et al.* 2001), it remains plausible that bulk blood flow and/or local muscle blood distribution were enhanced in individuals with T2DM in the present study subsequent to the priming exercise intervention. The favourable manipulation of the overall $\dot{V}O_2$ kinetics response via the acceleration of the primary time constant and the substantial reduction in the slow component in the presence of an unaltered muscle deoxygenation profile once again emphasises the potential that lies within the acute intervention of heavy-intensity priming exercise for individuals with T2DM.

Chapter 5: Influence of priming exercise on pulmonary oxygen uptake and muscle deoxygenation kinetics during heavy-intensity cycle exercise from an elevated baseline in type 2 diabetes.

5.1 INTRODUCTION

Upon initiation of constant-load exercise in the heavy-intensity domain from rest or "unloaded" pedalling, the time constant for the $\dot{V}O_2$ kinetics response has been demonstrated to be either similar (Özyener *et al.* 2001) or longer (Koppo *et al.* 2004) than those demonstrated during moderate-intensity exercise. The manifestation of a "slow component" between 80-100 s dictates that $\dot{V}O_2$ is elevated above that expected from the $\dot{V}O_2$-work rate relationship (Paterson & Whipp, 1991). Interestingly, however, upon initiation of constant-load exercise from an elevated baseline work rate to a higher-intensity work rate, referred to as work-to-work (w-to-w) additional anomalies in the dynamic response of $\dot{V}O_2$ kinetics are revealed (Hughson & Morrisey, 1982; Brittain *et al.* 2001; Wilkerson & Jones, 2007). Specifically, a significantly longer time constant and an increased functional gain of the primary phase are elicited during the transition to a higher intensity work rate, be that in the upper region of the moderate-intensity (Hughson & Morrisey, 1982; Brittain *et al.* 2001), or the heavy-intensity exercise domain (Wilkerson & Jones, 2006, 2007; DiMenna *et al.* 2008, 2009a, 2010b, 2010c). Interestingly, a progressive lengthening of the time constant of the primary phase was demonstrated whereupon initiation of the transition occurred from a baseline of light-, moderate-, or heavy-intensity exercise respectively (Wilkerson & Jones, 2006).

The governing factors regulating the altered $\dot{V}O_2$ kinetics in w-to-w are complex, being attributed to either one, or a combination of the following putative mechanisms: an elevated baseline heart rate (HR), an elevated baseline metabolic rate (i.e. $\dot{V}O_2$), and/or an elevated baseline work rate (Wilkerson & Jones, 2006; DiMenna *et al.* 2010b, 2010c). An elevated HR at the onset of exercise culminates in slower HR kinetics as a direct consequence of the slower sympathetic activation of the HR subsequent to prior exercise as opposed to the rapid parasympathetic withdrawal upon transitioning from rest-to-work (Brittain *et al.* 2001). As such a considerable limitation is imposed on central O_2 delivery, thus potentially contributing to the slowing of $\dot{V}O_2$ kinetics. Alternatively a raised baseline metabolic rate may constrain cellular respiration in the already active muscle fibres consequent to a potential combination of an increased free ADP concentration ([ADP]), Pi concentration ([Pi]) and muscle [H^+], a reduction in [PCr], [ATP], and less negative Gibbs free energy of ATP hydrolysis (ΔG_{ATP}), and/or Ca^{2+} concentration ([Ca^{2+}] (DiMenna *et al.* 2010b; Nederveen *et al.* 2017). Collectively, such a

disruption to the metabolic environment prior to the onset of the exercise transition could mandate a constrained $\dot{V}O_2$ kinetics response.

Finally, the altered $\dot{V}O_2$ dynamic response observed during w-to-w, as per the aforementioned transition to heavy–intensity exercise from an unloaded work-rate (as per *Experiment 3*), may also be attributed to the recruitment of higher-order (type II) muscle fibres to meet the augmented metabolic demand (Whipp, 1994b; Barstow *et al.* 1996). It is recognised that muscle fibres positioned higher in the recruitment hierarchy have a higher threshold for activation, are less oxidative and more glycolytic with a reduced capillary density (Bottinelli & Reggiani, 2000). As such these fibres are faster to contract, but at the same time faster to fatigue. The kinetic features of the intramuscular [PCr] response to exercise are considered to be similar to those of $\dot{V}O_{2p}$ (Barstow *et al.* 1994; Rossiter *et al.* 1999, 2001), thus the lengthened $\tau\dot{V}O_{2p}$ displayed in w-to-w transitions during upright cycle exercise occurring simultaneously with a substantially lengthened τ and greater reduction in [PCr] for a given increase in work rate (Jones *et al.* 2008), is indeed suggestive of a greater reliance on the recruitment of higher-order muscle fibres. Therefore, based on Henneman's "size principle" a transition from an elevated baseline work rate should predict an even greater contribution from higher-order fibres to meet the augmented muscle force requirements during the transitions to a higher work rate (Henneman & Mendell, 1981; Wilkerson & Jones, 2006; DiMenna *et al.* 2010c). Thus, the subsequent imposition of a greater reliance on substrate phosphorylation with the concomitant reliance and on-going recruitment of type II fibres to attain the given $\dot{V}O_2$ is likely to be evidenced.

Although few studies have explored the effects of heavy-intensity priming exercise on the $\dot{V}O_2$ dynamic response during w-to-w transitions (DiMenna *et al.* 2008, 2009a, 2009b, 2010b, 2010c), similar findings to studies investigating the effects of PE on subsequent high-intensity upright cycling exercise bouts from baseline transitions have been reported. Specifically, PE was demonstrated to be ineffective at reducing $\tau\dot{V}O_{2p}$ while the amplitudes of the primary phase and slow component were increased and reduced respectively, with a subsequent overall speeding of the $\dot{V}O_2$ kinetics as demonstrated by a reduction in the MRT (DiMenna *et al*. 2008). It should be noted however, that when a limitation in the time constant of the primary phase presents as a consequence of impaired O_2 delivery, such as when exercising in the supine position (Rossiter *et al.* 2001; Jones *et al.* 2006), prior heavy-intensity exercise has been evidenced to accelerate the rate of adaptation of the $\dot{V}O_2$ primary response in the subsequent bout of both heavy-intensity exercise (Rossiter *et al.* 2001; Jones

The mechanisms behind "priming" exercise on subsequent high-intensity exercise with or without an elevated baseline remain a subject of considerable debate (Tschakovsky & Hughson, 1999; Jones & Poole, 2005; Poole *et al.* 2008a). Purported mechanisms however, include increased blood flow (Bangsbo *et al.* 2001; Koppo & Bouckaert, 2001), enhanced muscle perfusion (Wilkerson *et al.* 2004a; DeLorey *et al.* 2007; Jones *et al.* 2008), heightened oxidative enzyme activity (Wilkerson *et al.* 2005; Gurd *et al.* 2006) and/or O_2 extraction (Krustrup *et al.* 2001; DeLorey *et al.* 2007) and the augmentation of muscle fibre recruitment (Burnley *et al.* 2002b).

Nonetheless it is suggested that prior heavy-intensity exercise may favourably alter $\dot{V}O_2$ kinetics increasing the initial aerobic metabolic contribution to activity while consequently reducing anaerobic ATP provision. Subsequently, by reducing the initial oxygen deficit, time to exhaustion may be increased which may be of particular significance for clinical populations presenting with constrained $\dot{V}O_2$ kinetics at the onset of submaximal exercise, as is the case in T2DM. Thus given that muscle O_2 supply appears to be constrained in T2DM, combining a priming exercise intervention with the w-to-w model (i.e. further slowing the $\tau\dot{V}O_{2p}$ of the heavy-intensity bout by initiating this exercise bout from an elevated baseline) may provide further insight into potential mechanisms implicated in the impaired $\dot{V}O_2$ kinetics response consistently demonstrated by these individuals.

The purpose of the present study was to investigate the influence of PE on $\dot{V}O_2$ and muscle deoxygenation kinetics during moderate-to-heavy intensity (w-to-w) cycling exercise transitions in individuals with T2DM. By manipulating the baseline metabolic rate, the investigation of the interaction between recruitment of discrete sections of the muscle fibre pool and muscle O_2 delivery on $\dot{V}O_2$ kinetics during cycle exercise is facilitated.

Our specific hypotheses were that PE would speed the adjustment of the primary phase of the $\dot{V}O_2$ kinetics response and reduce the amplitude of the slow component in the subsequent heavy-intensity bout of the exercise transition in individuals with T2DM. Furthermore, this favourable manipulation of the $\dot{V}O_2$ kinetics response would occur in the absence of alterations in estimated fractional O_2 extraction.

5.2 METHODOLOGY

5.2.1 *Participants*

Seven individuals with uncomplicated T2DM (3 men/4 women), and seven individuals without T2DM (ND) (3 men/4 women) volunteered to participate in this study (Table 5.1). One of the individuals with T2DM, and six of the ND controls also participated in *Experiment 1*. Three female participants were premenopausal (1 T2DM and 2 ND) and five were postmenopausal (3 T2DM and 2 ND) not undergoing HRT. All participants were non-smokers and had not smoked during the 12-month period preceding the study. All of the patients with T2DM had a clinical history of diabetes between 3-11 years (mean ± SD = 7.2 ± 4.2 years).

5.2.1.1 *Recruitment of participants*

5.2.1.1.1 Individuals without T2DM (ND)

As per section 2.2.1.1.1

5.2.1.1.2 Individuals with T2DM

As per section 2.2.1.1.2

5.2.1.2 Inclusion/exclusion criteria

5.2.1.2.1 Individuals with T2DM

As per section 2.2.1.2.1

5.2.1.2.2 Individuals without T2DM (ND)

As per section 2.2.1.2.2

5.2.1.2.3 Blood sample collection

As per section 2.2.1.2.3

5.2.1.2.4 Ankle brachial index (ABI)

As per section 2.2.1.2.4

5.2.1.3 Determination of physical activity levels

As per section 2.2.1.3

5.2.1.3.1 RT3 Tri-axial accelerometers

As per section 2.2.1.3.1

5.2.1.3.2 The Low Level Physical Activity Recall questionnaire (LOPAR)

As per section 2.2.1.3.2

5.2.2 Experimental design

5.2.2.1 Study overview

Participants without T2DM (ND) were required to visit the cardiovascular performance laboratory in the Department of Physiology, Trinity College Dublin on two separate occasions, whilst all T2DM patients were required to visit the exercise testing facility in St. Columcille's Hospital on two occasions. All premenopausal participants were tested during the mid-follicular phase (days 5-12) of the menstrual cycle, which was self-determined. All participants were asked to refrain from consuming alcohol, caffeine and non-prescribed nutritional supplements in the 24 hours prior to testing, and to limit their exercise to normal activities of daily living.

5.2.2.1.1 Visit 1 overview

During this visit, initially anthropometric data was collected, followed by the measurement of the aortic pulse wave velocity (PWV). Participants then performed a ramp incremental cycling test to exhaustion to enable the determination of $\dot{V}O_{2max}$ and the estimated ventilatory threshold (VT) (*as per section 2.2.4.3*). Following a 5 min passive recovery period, participants finally completed a 'confirmatory' high-intensity cycling bout (*as per section 2.2.2.2.3).*

5.2.2.1.2 Visit 2 overview

From the previously completed ramp incremental cycling test the work rates required for this protocol were calculated. A power output equivalent to 80% of the PO at VT (moderate-intensity), and a PO corresponding to 50% delta (Δ50%; the sum of the PO at VT and 50% of the difference between the PO at VT and $\dot{V}O_{2max}$) were determined.

5.2.2.2 Visit 1 to the cardiovascular laboratory

5.2.2.2.1 Anthropometry and pulse wave velocity

As per section 2.2.2.2.1

5.2.2.2.2 Ramp incremental cycle test to exhaustion

Participants performed a ramp incremental cycling test to exhaustion in an upright position on an electrically braked cycle ergometer (Excalibur Sport; Lode B.V., Groningen, The Netherlands) with appropriate adjustments made to the ergometer seat and handle bar position for each participant. Exercise was performed initially for 2-min at a baseline resistance of 10W (i.e. 'unloaded' cycling), to reduce excess internal work (Boone *et al.* 2008). This was followed by 10/15 W.min^{-1} increments in females (*n*=3/5) or 15/20 W.min^{-1} increments in males (*n*=2/4) respectively depending on activity levels of participants, and were brought to volitional exhaustion. Pedal frequency was held constant at an individually selected cadence between 60-75 revolutions per minute (rpm). This cadence was maintained throughout all further testing protocols. Failure in a test was determined as a drop in cadence exceeding 5 rpm for >3 s. Peak workload was determined according to the point of termination of the test. HR was continuously monitored while pulmonary oxygen uptake ($\dot{V}O_2$), pulmonary carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER: $\dot{V}CO_2/\dot{V}O_2$) were recorded on a breath-by-breath (BbB) basis. Peak HR was defined as the highest heart rate attained within the last 15 s of the point of termination of the test.

As it is frequently reported that some individuals do not evidence a definitive plateau of the $\dot{V}O_2$-work rate relationship on this test, secondary criteria based upon measurements of the RER, maximal heart rate or blood [lactate] (Poole *et al.* 2008b) are often relied upon to corroborate a maximum effort (Astrand & Rodahl, 1986; Rossiter *et al.* 2006; Poole *et al.* 2008b). However, such criteria have been called into disrepute (Poole *et al.* 2008b), thus, the utilisation of a subsequent confirmatory high-intensity constant load bout *(see section 2.2.2.2.3)* has frequently been implemented (Day *et al.* 2003; Rossiter *et al.* 2006; Murias *et al*. 2010b). In the present study all participants demonstrated a plateau in $\dot{V}O_2$ during the confirmatory tests, and moreover the $\dot{V}O_2$ values recorded during this confirmatory test were not different to those obtained during the ramp incremental cycle test. Thus, we were certain that in the present study, all participants achieved a $\dot{V}O_{2max.}$

5.2.2.2.3 Confirmatory test

Within five minutes of completion of the ramp incremental cycle test, participants performed a high-intensity, constant-load cycling bout to exhaustion at an intensity equivalent to 85% of the peak power output achieved in the ramp test. This protocol was performed to confirm the attainment of $\dot{V}O_{2max}$ in the prior graded exercise test whilst also facilitating the non-invasive

determination of maximal cardiac output via the inert rebreathing technique (Innocor; Innovision, Denmark). Participants were instructed to indicate via the raising of their hand when they felt they were ~30 s from exhaustion. At this point, individuals were verbally encouraged to continue and within 30 s the measurement of CO was carried out.

5.2.2.3 Visit 2 to the cardiovascular laboratory

5.2.2.3.1 Priming effect on work-to-work cycling exercise (w-to-w)

Individuals performed four bouts of constant-load, heavy-intensity cycling at Δ50% each commencing from an elevated baseline (80% VT). The protocol consisted of 4 separate bouts of cycling exercise, each involving; 3 min of "unloaded" cycling at 10W, 6 min of moderate-intensity cycling (80% VT) followed immediately by 6 min of heavy-intensity cycling (w-to-w). Two of these w-to-w bouts were completed without prior priming exercise (w-to-w unprimed) and two bouts were undertaken preceded by priming exercise at an intensity of Δ50% (w-to-w primed). Exercise was performed continuously with changes in PO initiated as a step function without a warning to the individual. There was a 12 min rest period between each of the cycling bouts, except following the first primed w-to-w bout. In this instance, participants remained seated in a chair for the duration of a 45 minute passive rest period. This is the advocated time for physiological restoration following a bout of heavy intensity exercise, thus having no effect on $\dot{V}O_2$ kinetics during subsequent exercise (Burnley *et al*. 2006a). Figure 5.1 displays a schematic representation of the protocol.

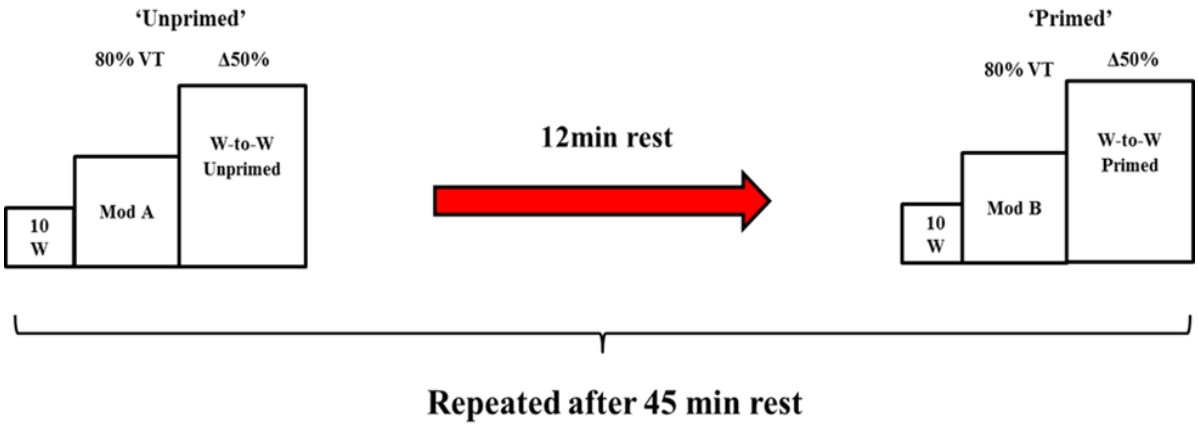

Figure. 5.1 ***Schematic representation of the work-to-work protocol.***

5.2.3 Equipment and techniques

5.2.3.1 Cardiometabolic unit, heart rate and pulse oximeter

As per section 2.2.3.1

5.2.3.2 Near infrared spectroscopy (NIRS) and subcutaneous fat layer of the vastus lateralis

As per section 2.2.3.4

5.2.4 Data analysis

5.2.4.1 *Physiological responses at peak and VT*

As per sections 2.2.2.2.2 and 2.2.4.3 respectively.

5.2.4.2 *Δ50% data analysis*

5.2.4.2.1 $\dot{V}O_2$ kinetics

The breath by breath $\dot{V}O_2$ data for each transition were linearly interpolated to provide second-by-second values and time aligned such that time 0 represented the onset of exercise. Data from each transition were ensemble-averaged to yield a single, average response for each individual and further time-averaged into 5 s bins to provide a single time-averaged response per individual. The first 20 s of data after the onset of exercise were deleted in an attempt to avoid inclusion of data points from the cardiodynamic phase. The model parameters of the $\dot{V}O_2$ on-transient kinetic response were thus determined from 20 – 360 s of the step transition via a weighted least-squares nonlinear regression procedure (TableCurve 2D, Systat) in which the best fit was defined by minimization of the residual sum of squares. Data points lying outside the 95% prediction interval during the initial fit of a model were excluded, being attributed to aberrant events, e.g. coughing. Thus, the averaged and smoothed response for each participant was fitted to a biexponential function (*Equation 1*) as follows:

Equation 1 $$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A_p[1- e^{-(t-TD_p)/\tau_p)}]\cdot F1 + A_s[1 - e^{-(t - TD_p)/\tau_s)}]\cdot F2$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time *t*; $\dot{V}O_2$ baseline is the mean $\dot{V}O_2$ measured over the final 60 s of the moderate-intensity cycling exercise preceding the step transition to heavy-intensity cycling exercise; $A_p - A_s$, are the amplitudes of the increase in $\dot{V}O_2$ for the primary and slow component phases; $TD_p - TD_s$ are the phase delays, and $\tau_p - \tau_s$ are the time constants, defined as the duration of time for which $\dot{V}O_2$ increases to a value equivalent

to 63% of the amplitude. The conditional expressions F1-F2 limit the fitting of the phase to the period at and beyond the time delay associated with that phase.

The absolute primary component amplitude, referred to as (absolute A_p) was calculated using the following formula:

Equation 2 Absolute A_p = baseline $\dot{V}O_2 + A_p[1- e^{- (t-TD_p)/\tau_p)}]$

The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of heavy-intensity exercise.

Finally, the mean response time (MRT) was calculated to provide information on the "overall" $\dot{V}O_2$ kinetics during the heavy-intensity exercise bout, with no distinction made for the various phases of the response. This was calculated through the fitting of a monoexponential curve (Eqn 3) from the onset to the end of the heavy-intensity exercise bout.

Equation 3 $\dot{V}O_2(t) = \dot{V}O_{2baseline} + A[1- e^{- (t-TD)/\tau_)}]$

5.2.4.2.2 Muscle deoxygenation [HHb+Mb] kinetics

Local muscle deoxygenation (Δ[HHb+Mb]) profiles of the right quadriceps *vastus lateralis* muscle were made with near infrared spectroscopy (NIRS) (Hamamatsu Niro 200Nx; Hamamatsu Photonics, Hamamatsu, Japan) throughout the exercise protocol *(as per section 2.2.3.4).* To provide information on muscle oxygenation throughout the protocol, we also modelled the [HHb+Mb] response to exercise. As per the $\dot{V}O_2$ data, the NIRS-derived Δ[HHb+Mb] data for each transition was linearly interpolated to provide second-by-second values and time aligned. Data from each transition were ensemble-averaged to yield a single, average response for each individual, and further time-averaged into 5 s bins to provide a single time-averaged response per individual.

A time delay at the onset of exercise occurs in the [HHb+Mb] profile before it increases with an exponential like time course (DeLorey *et al.* 2003) which has been interpreted to reflect a tight coupling between muscular O_2 uptake and local O_2 delivery (DeLorey *et al.* 2003; Grassi *et al.* 2003). This was determined in the present study via visual inspection as a systematic increase above the pre-transition level. [HHb+Mb] data were fitted from the first data point at the end of this TD to 360 s with a biexponential model of the form in Eqn 1 (as per $\dot{V}O_2$; *section 5.2.4.2.1*) to determine the time course of muscle deoxygenation.

The time course for the increase in Δ[HHb+Mb] can be described by the τΔ[HHb+Mb], however, the time course for the overall change of the Δ[HHb+Mb] for the primary response

phase, referred to as the effective τ'Δ[HHb+Mb], was determined from the sum of the time delay and τ[HHb+Mb] from the onset of exercise.

The absolute primary component amplitude, referred to as [HHb+Mb] absolute A_p, was calculated using Eqn 2 (as per $\dot{V}O_2$; *section 5.2.4.2.1*).

The final end amplitude of the response, referred to as Absolute [HHb+Mb] end A, was calculated from the sum of the absolute primary component amplitude and the amplitude of the slow component.

Finally, the mean response time (MRT) was calculated to provide information on the "overall" [HHb+Mb] kinetics during the heavy-intensity exercise bout, with no distinction made for the various phases of the response. This was calculated through the fitting of a monoexponential curve of the form in Eqn 3 (as per $\dot{V}O_2$; *section 5.2.4.2.1*), from the onset to the end of the heavy-intensity exercise bout.

5.2.4.3 *80% VT data analysis*

5.2.4.3.1 $\dot{V}O_2$ kinetics

As per Experiment 2, section 3.2.4.2.1

5.2.4.3.2 Muscle deoxygenation [HHb+Mb] kinetics

As per Experiment 2, section 3.2.4.2.2

5.2.4.3.3 Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio

As per Experiment 2, section 3.2.4.2.3

5.2.5 Statistical analysis

Statistical analysis was performed using the software SigmaPlot version 12.0 (Systat Software, Point Richmond, CA). Prior to analysis, normal Gaussian distribution of the data was assessed using the Shapiro-Wilk's test. Physical characteristics and peak physiological responses between groups were compared using the unpaired Student's t-test for parametric analyses, or the Mann-Whitney U test for non-parametric analyses. The kinetic parameter estimates for $\dot{V}O_2$ and Δ[HHb+Mb] during both the moderate- and heavy-intensity exercise bouts of the w-to-w protocol were analysed by using a two-way repeated measures ANOVA (primed/unprimed condition and diabetes as the main effects). In the case of significant differences obtained from

the repeated measures ANOVA, post hoc Tukey tests were performed. Statistical significance was accepted at a P value ≤ 0.05. All values are expressed as means ± standard deviation (SD) or as median and interquartile ranges for data that were deemed not normally distributed.

The key variable is the rate of change of the primary phase of oxygen uptake ($\dot{V}O_{2p}$). An indicator of the rate of change in $\dot{V}O_2$ can be obtained by calculation of its time constant (τ). The τ is the time required to achieve ~63 % of the difference between the baseline and the plateau of that phase. Recently published studies in our laboratory and others, which, included data for both individuals with T2D and age-matched healthy controls, revealed τ values of ~37 ± 7 s (Mean ± SD) during cycling at 50% Delta. The difference between unprimed and primed τ values (based on studies comparing the postural effects; upright vs supine cycling, in young participants) was 10 s. Thus, given that the estimated standard deviation (SD) of each population is 7 s and that the minimum difference we wish to detect as significant would be 10 s, the minimum sample size needed to detect a significant effect at β=0.05 and α=0.05 (70% power) for an ANOVA power calculation design based on 2 groups, is 7 subjects.

5.3 RESULTS

5.3.1 Participants

5.3.1.1 Physical Characteristics

Physical characteristics for the participants are presented in Table 5.1. Anthropometrical measurements did not significantly differ between groups, with the exception of WHR, whereby individuals with T2DM displayed a trend for a greater WHR (*P*=0.06) compared to the ND controls. Individual anthropometric measurements are displayed in (Appendix 11). No differences were observed in ABI, or subcutaneous fat layer of the *vastus lateralis* (VL). However, individuals with T2DM displayed significantly faster PWV compared with the ND controls, albeit falling within normative ranges for this parameter (<10 ms^{-1}) (VanBortel *et al.* 2012; Mancia *et al.* 2013).

Table 5.1. ***Anthropometrical data, PWV, ABI and resting BP for ND and T2DM individuals.***

	ND (*n* =7)	**T2DM (*n* =7)**
Sex (male, female)	3,4	3, 4
Age (yr)	41 ± 10	46 ± 8
Height (m)	1.65 ± 0.06	1.68 ± 0.08
Weight (kg)	*80 (11.3)*	*76 (43.5)*
BMI (kg.m^{-2})	*30 (4)*	*28 (10)*
WHR (a.u)[a]	0.89 ± 0.06†	0.98 ± 0.10
Fat layer VL (mm)[a]	*12.7 (10.2)*	*6.5 (2.8)*
Pre exercise ABI (a.u.)	*1.10 (0.18)*	*1.07 (0.10)*
Post exercise ABI (a.u.)	1.19 ± 0.13	1.11 ± 0.10
PWV (ms^{-1})[b]	6.0 ± 1.3*	8.3 ± 1.3
SBP (mmHg)	124 ± 10	118 ± 7
DBP (mmHg)	78 ± 7*	70 ± 5

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. BMI, body mass index; WHR, waist:hip ratio; ABI, ankle:brachial index; PWV, pulse wave velocity; SBP, systolic blood pressure; DBP, diastolic blood pressure. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). a=7 (ND) and 6 (T2DM); b=6 (ND) and 6 (T2DM).

5.3.1.2 Haematological parameters and prescriptive medications.

Mean haematological parameters and prescriptive medications are presented in Tables 5.2 and 5.3 respectively. As expected, individuals with T2DM displayed significantly higher HbA_{1c} and fasting plasma glucose levels ($P \leq 0.05$). They also demonstrated significantly higher total cholesterol levels ($P \leq 0.05$), in addition to a tendency for higher levels of triglycerides ($P=0.07$) than the ND controls, albeit no differences between HDL-C and LDL-C distribution.

Table 5.2. ***Haematological parameters for ND individuals and individuals with T2DM.***

	ND	**T2DM**
HbA_{1c} (%)[a]	*5.1 (0.2)**	*6.5(1.4)*
FPG (mmol.L^{-1})[b]	4.4 ± 0.8*	7.1 ± 1.8
Total cholesterol (mmol.L^{-1})[c]	3.9 ± 0.9*	4.2 ± 0.7
LDL (mmol.L^{-1})[c]	2.1 ± 0.9	2.4 ± 0.8
HDL (mmol.L^{-1})[c]	1.2 ± 0.2	1.1 ± 0.1
Triglycerides (mmol.L^{-1})[c]	1.12 ± 0.5†	2.3 ± 1.3

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. HbA_{1c}, glycosylated haemoglobin; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). a=4 (ND) and 5 (T2DM); b=6 (ND) and 5 (T2DM); c=6 (ND) and 3 (T2DM)

Table 5.3. ***Prescriptive medications for ND individuals and individuals with T2DM.***

	ND (*n* = 7)	T2DM (*n* = 7)
Anti-hypertensives		
Angiotensin converting enzyme inhibitor		2
Angiotensin II receptor blocker		1
Aspirin	1	3
Calcium channel blocker		2
Statins	1	2
Hypoglycaemic medications		
Oral hypoglycaemics		7
Subcutaneous hypoglycaemics		1
Sulphonylureas		1

5.3.1.3 Physical activity levels

Group mean activity levels are presented in Tables 5.4 and 5.5 for accelerometry and LOPAR data respectively. Physical activity behaviour was similar between the two groups, when assessed via both accelerometry and the LOPAR questionnaire, although individuals with T2DM displayed a tendency (P=0.07) for greater levels of light physical activity.

Table 5.4. ***Group mean activity levels based on the number of hours per day as determined by RT3 accelerometers.***

	ND (*n* = 6)	T2DM (*n* =2)
Inactive (h.day^{-1})	19.16 ± 1.68	17.78 ± 1.36
Light (h.day^{-1})	3.84 ± 1.12†	5.83 ± 1.08
Moderate (h.day^{-1})	0.73 ± 0.50	0.35 ± 0.23
Vigorous (h.day^{-1})	*0.20 (0.25)*	*0.04 (0.06)*

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Inactive, <100 counts.min^{-1}; Light, 101-970 counts.min^{-1}; Moderate, 971-2333 counts.min^{-1}; Vigorous, >2333 counts.min^{-1}. †Tendency towards a difference than T2DM ($P \leq 0.10$).

Table 5.5. ***Group mean activity levels based on the number of METS per hour per week as determined by the LOPAR questionnaire.***

	ND (*n* =6)	T2DM (*n* = 4)
LOPAR (MET.hr^{-1}.wk^{-1})	169 ± 62	188 ±108

Values are mean ± SD

5.3.2 Performance data from ramp incremental cycling test

5.3.2.1 Physiological responses

The physiological responses for both groups at peak exercise, and at VT are displayed in Table 5.6. Maximum $\dot{V}O_2$ ($\dot{V}O_{2max}$) normalised to kilograms of body weight was significantly (P<0.05) reduced in individuals with T2DM compared with the ND controls, representing a 25% reduction in peak exercise capacity. In addition, a tendency toward significance for absolute ($\dot{V}O_{2max}$) (P=0.08) was displayed. RER was similar between the ND and T2DM group respectively at peak exercise, indicating both groups made their maximal effort during the ramp incremental protocol. Additionally, individuals with T2DM displayed a tendency for a significantly lower relative $\dot{V}O_2$ (P=0.09) and PO (P=0.10) at VT.

Table 5.6. ***Physiological responses at peak exercise and VT.***

	ND (*n* = 7)	**T2DM (*n* = 7)**
$\dot{V}O_{2max}$ (mL.kg^{-1}.min^{-1})	27.9 ± 7.6*	20.9 ± 1.6
$\dot{V}O_{2max}$ (L.min^{-1})	2.31 ± 0.50†	1.80 ± 0.51
Peak PO (W)	180 ± 55	138 ± 45
Peak HR (beats.min^{-1})^{a}	170 ± 16	167 ± 13
Age predicted HR (beats.min^{-1})	179 ± 10	175 ± 8
Peak RER (a.u)	1.12 ± 0.08	1.10 ± 0.08
Peak CO (L.min^{-1})^{b}	13.35 ± 2.01	11.84 ± 4.06
TTF (secs)	659 ± 104	636 ± 113
$\dot{V}O_2$ @ VT (mL.kg^{-1}.min^{-1})	19.63 ± 5.83†	15.32 ± 2.13
$\dot{V}O_2$@ VT (L.min^{-1})	1.64 ± 0.43	1.31 ± 0.30
PO @ VT (W)	104 ± 44†	73 ± 17

Mean ± SD values are shown in normal font for variables which were normally distributed; whereas median (and interquartile range) values are shown in italic font for variables which showed significant skewness and were not normally distributed in one or both groups. Some variables have missing values and the sample sizes with codes are shown below.*Significantly different than T2DM (P≤0.05). †Tendency towards a difference than T2DM (P≤0.10). a = 6 (ND) and 7 (T2DM); b = 4 (ND) and 5 (T2DM).

5.3.3 Performance data from work-to-work exercise (Δ50%)

5.3.3.1 $\dot{V}O_2$ kinetics

The parameter estimates of the kinetics analysis of the $\dot{V}O_2$ response for both ND and T2DM during the work-to-work on-transition with and without a prior bout of priming exercise are presented in Table 5.7. At baseline, the parameter estimates of the $\dot{V}O_2$ kinetics response were similar between groups.

PE however, resulted in a significant ($P \leq 0.05$) reduction in the amplitude of the $\dot{V}O_2$ slow component (A_s), by ~40% in both groups, accompanied by a significant ($P=0.001$) reduction in the MRT. Additionally, baseline $\dot{V}O_2$ values displayed a tendency ($P=0.06$) to be elevated subsequent to PE in both groups.

The adaptation of $\dot{V}O_2$ at the onset of the heavy-intensity exercise bout of the w-to-w transitions with and without a prior bout of priming exercise for a representative ND and T2DM individual is illustrated in Figure 5.2.

Table 5.7. ***Dynamic response characteristics of oxygen uptake for unprimed and primed work-to-work heavy-intensity exercise in individuals with and without T2DM.***

	Δ50% Unprimed		**Δ50% Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	7	7	7	7
$\dot{V}O_2$ baseline, L.min^{-1}	1.50 ± 0.51	1.30 ± 0.27	1.56 ± 0.58$^{\#}$	1.31 ± 0.27$^{\#}$
$\dot{V}O_2$ A_p, L.min^{-1}	0.53 ± 0.15	0.40 ± 0.18	0.55 ± 0.16	0.42 ± 0.22
$\dot{V}O_2$ absolute A_p, L.min^{-1}	2.03 ± 0.63	1.70 ± 0.45	2.11 ± 0.67	1.73 ± 0.48
$\dot{V}O_2$ τ_p, s	42 ± 11	54 ± 14	41 ± 11	42 ± 17
$\dot{V}O_2$ A_s, L.min^{-1}	0.20 ± 0.18	0.13 ± 0.15	0.12 ± 0.11‡	0.08 ± 0.10‡
$\dot{V}O_2$ TD_s, s	132 ± 53	119 ± 41	137 ± 55	128 ± 46
End $\dot{V}O_2$, L.min^{-1}	2.27 ± 0.6	1.78 ± 0.47	2.22 ± 0.56	1.77 ± 0.46
$\dot{V}O_2$ MRT, s	65 ± 8	72 ± 10	54 ± 14‡	53 ± 18‡

Values are means ± SD. Dynamic response characteristics of oxygen uptake ($\dot{V}O_2$) during unprimed and primed w-to-w cycling exercise at 50% delta (Δ) in individuals with and without T2DM. A_p and A_s, amplitudes; TD_p and TD_s, time delays; τ_p and τ_s, time constants; for primary and slow component phases. ‡Significantly different between unprimed and primed conditions within the group ($P \leq 0.05$). *Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). $^{\#}$Tendency towards a difference than unprimed ($P \leq 0.10$).

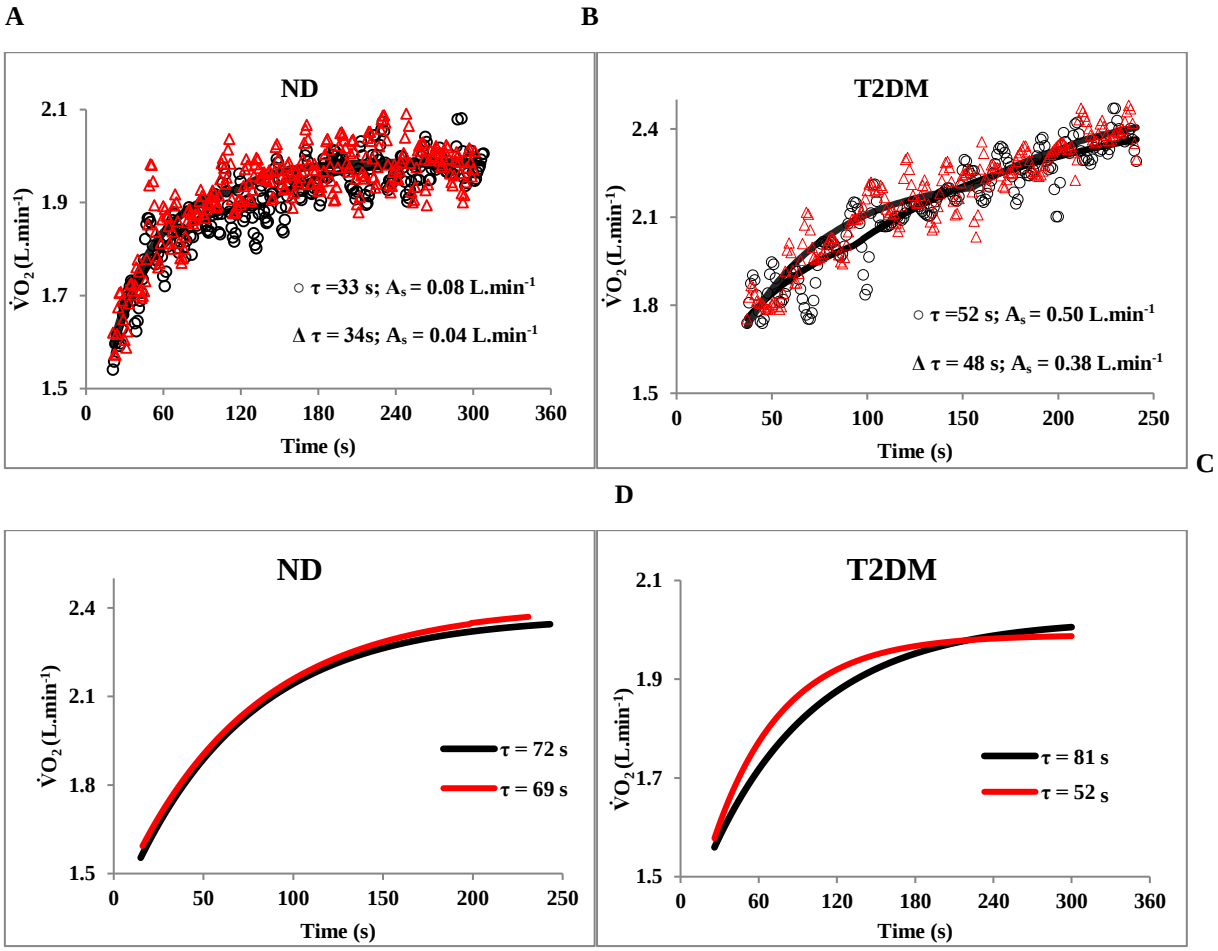

Figure 5.2. Oxygen uptake responses for a representative ND and T2DM individual for unprimed (○) and primed (Δ) bouts of the heavy-intensity exercise bout of the w-to-w transition. The continuous lines of best fit illustrate the primary and slow component phases of the oxygen uptake ($\dot{V}O_2$) response. $\tau\dot{V}O_{2p}$ and amplitudes of the slow component are included in panels A & B, with MRT's **included** in panels C & D.

5.3.3.2 Muscle deoxygenation kinetics

Kinetic parameters for Δ[HHb+Mb] for both ND and T2DM during the work-to-work on-transition with and without a prior bout of priming exercise are presented in Table 5.8 At baseline, the parameter estimates for the Δ[HHb+Mb] kinetic response were similar between groups, albeit individuals with T2DM displaying a tendency (P=0.09) for a higher amplitude of the primary response (A_p).

PE resulted in significantly (P<0.001) elevated baseline levels for Δ[HHb+Mb]. PE however did not alter the NIRS-derived ([HHb+Mb] pre-transition period (TD_p) of the primary component of the response, the magnitude of change in Δ[HHb+Mb] (A_p), nor the time constant of this response. Furthermore the overall time course for muscle deoxygenation, (effective response time), determined as the ([HHb+Mb] TD + τ was similar between groups. Nonetheless,

the absolute [HHb+Mb] amplitude at the end of the primary phase ([HHb+Mb] absolute A_p), as well as the end amplitude of the entire response (Absolute [HHb+Mb] end A) was significantly increased subsequent to priming exercise (P<0.05). Additionally, a tendency was displayed (P=0.09) for a reduction in the [HHb+Mb] MRT subsequent to PE in both groups.

The adaptation of [HHb+Mb] during the on-transient of the heavy-intensity bout of the w-to-w transition with and without a prior bout of priming exercise for a representative ND and T2DM individual is illustrated in Figure 5.3.

Table 5.8. ***Kinetic parameters for Δ[HHb+Mb] for unprimed and primed work-to-work exercise heavy-intensity exercise.***

	Δ50% Unprimed		**Δ50% Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	**7**	**7**	**7**	**7**
Δ[HHb+Mb] Baseline, a.u.	-0.2 ± 150.6	61.8 ± 92.3	17.3 ± 108.8‡	100.9 ± 103.6‡
Δ[HHb+Mb] A_p, a.u.	16.2 ± 12.8 †	34.3 ± 19.0	17.7 ± 16.9†	29.0 ± 13.6
Δ[HHb+Mb] TD_p, s	8.53 ± 4	11 ± 7	13 ± 9	10 ± 8
Δ[HHb+Mb] τ_p , s	25 ± 22	23 ± 13	17 ± 9	20 ± 9
Δ[HHb+Mb] τ', s	32 ± 23	34 ±19	30 ±12	30 ± 7
Δ[HHb+Mb] absolute A_p,	16.0 ± 103.3	96.1 ± 110.2	34.9 ± 109.8‡	130.4 ± 110.6‡
Δ[HHb+Mb] A_s, a.u.	5.3 ± 6.2	8.8 ± 6.2	7.1 ± 6.9	5.7 ± 6.05
Δ[HHb+Mb] TD_s, s	112 ± 50	89 ± 56	121 ± 78	86 ± 41
Δ[HHb+Mb]MRT, s	47 ± 41	52 ± 32	32 ± 22#	37 ± 24#
Absolute [HHb+Mb] end A, a.u.	21.3 ± 102.8	104.9 ± 109.9	41.0 ± 106.8‡	136 ± 109.2‡

Values are means ± SD. Dynamic response of muscle deoxygenation [HHb+Mb] during unprimed and primed cycling exercise at 50% delta (Δ) in individuals with and without T2DM. A_p and A_s, amplitudes; TD_p and TD_s, calculated time delay; τ_p and τ_s, time constants; of the primary and slow component phases. τ', effective response time (TD + τ); MRT, mean response time; a.u. arbitrary units. ‡Significantly different between unprimed and primed conditions within the group (P≤0.05). *Significantly different than T2DM (P≤0.05). †Tendency towards a difference than T2DM (P≤0.10). #Tendency towards a difference from unprimed (P≤0.10).

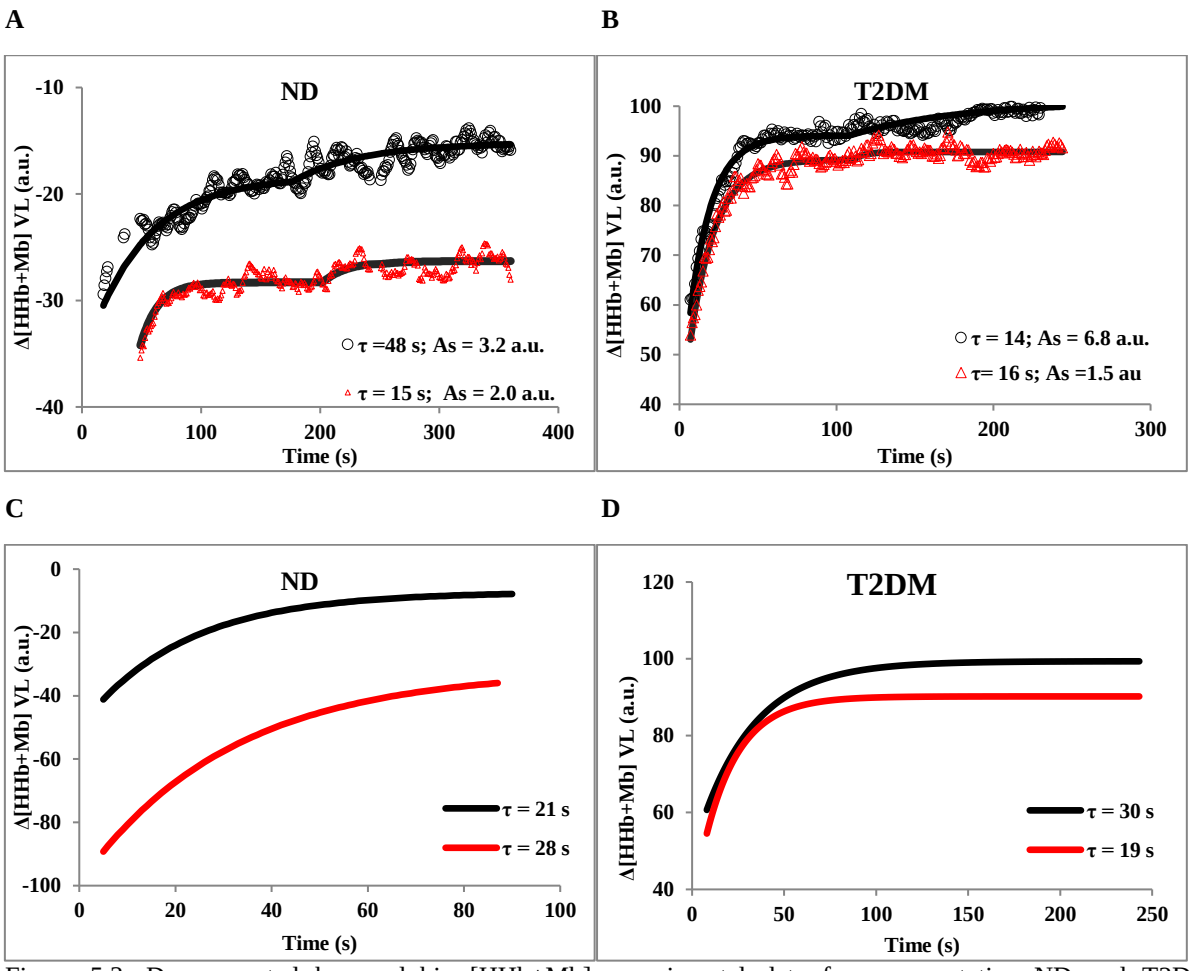

Figure 5.3. Deoxygenated haemoglobin [HHb+Mb] experimental data for representative ND and T2DM individuals for unprimed (○) and primed (Δ) bouts of the heavy-intensity exercise bout of the w-to-w transitions. The continuous lines of best fit illustrate the primary and slow component phases of the [HHb+Mb] response. τ[HHb+Mb] and amplitudes of the[HHb+Mb] slow component are included in panels A & B, with MRT's included in panels C & D.

5.3.3.3 Cardiac output and heart rate responses

Baseline CO, and heart rate responses for both ND and T2DM during the work-to-work on-transition with and without a prior bout of priming exercise are presented in Table 5.9 At baseline, individuals with T2DM displayed similar CO values to that of the ND controls. Baseline HR was significantly elevated before the w-to-w cycling transition in both groups, and consequently, delta HR was reduced significantly subsequent to PE (P<0.05) albeit to a lesser degree in individuals with T2DM with a main diabetes effect in the primed bout (P=0.01).

Table 5.9. ***Baseline cardiac output responses, and heart rate responses for unprimed and primed heavy-intensity work-to-work exercise.***

	Δ50% Unprimed		Δ50% Primed	
	ND	**T2DM**	**ND**	**T2DM**
n	7	7	7	7
Baseline HR, beats.min^{-1}	135 ± 16	138 ± 10	145 ± 16‡	143 ± 9‡
End-exercise HR, beats.min^{-1}	167 ± 19	159 ± 10	169 ± 15	162 ± 10
ΔHR, beats.min^{-1}	32 ± 8*	21 ± 7	22 ± 7*‡	19 ± 5‡
Baseline CO, L.min^{-1a}	14.52 ± 3.12	12.5 ± 2.88		

Values are means ± SD. CO, cardiac output; HR, heart rate. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). ‡Significantly different between primed and unprimed conditions within the group ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). a=6 (ND) and 5 (T2DM).

5.3.4 Performance data from 80% VT

5.3.4.1 $\dot{V}O_2$ kinetics

The parameter estimates of the kinetic analysis of the moderate-intensity $\dot{V}O_2$ response for both ND and T2DM individuals with and without a prior bout of priming exercise are presented in Table 5.10. At baseline, the parameter estimates of the kinetics response were similar between groups, albeit individuals with T2DM displaying a significantly slower time constant of the response ($\tau\dot{V}O_2$). Additionally, individuals with T2DM displayed a tendency (P=0.1) for a shorter TD.

PE however, resulted in significantly elevated baseline $\dot{V}O_2$ values (P<0.05) and thus, an elevated end amplitude of the response for both groups, whilst also displaying a tendency for an accelerated $\tau\dot{V}O_{2p}$ (P=0.08) in the subsequent moderate-intensity exercise bout. Additionally end $\dot{V}O_2$ values were significantly increased by PE in both groups (P<0.05).

Table 5.10. ***Dynamic response characteristics of oxygen uptake for unprimed (Mod A) and primed (Mod B) moderate-intensity exercise.***

	Mod A Unprimed		**Mod B Primed**	
	ND	**T2DM**	**ND**	**T2DM**
n	7	7	7	7
$\dot{V}O_2$baseline, L.min^{-1}	0.73 ± 0.13	0.80 ± 0.16	0.79 ±.11‡	0.87 ± 0.17‡
$\dot{V}O_2$ A, L.min^{-1}	0.66 ± 0.40	0.46 ± 0.13	0.71 ± 0.48	0.44 ± 0.11
$\dot{V}O_2$ End A, L.min^{-1}	1.39 ± 0.49	1.26 ± 0.26	1.5 ± 0.53‡	1.31 ± 0.24‡
$\dot{V}O_2$ TD, s	15 ± 6†	7 ±11	16 ± 3	11 ± 9
$\tau\dot{V}O_2$, s	30 ± 4*	39 ± 11	24 ± 7$^{\#}$	33 ± 13$^{\#}$
$\dot{V}O_2$ gain, mL.min^{-1}.W^{-1}	8.6 ± 2.5	9.7 ± 2.2	9.1 ± 2.9	9.3 ± 1.2
End $\dot{V}O_2$, L.min^{-1}	1.39 ± 0.48	1.27 ± 0.25	1.50 ± 0.59‡	1.31 ± 0.25‡

Values are means ± SD. Dynamic response characteristics of oxygen uptake ($\dot{V}O_2$) during unprimed and primed cycling exercise at 80% ventilatory threshold (VT) in individuals with and without T2DM. TD, time delay; τ, time constant of the response. ‡Significantly different between unprimed and primed conditions within the group ($P \leq 0.05$). *Significantly different than T2DM ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). $^{\#}$Tendency towards a difference from unprimed ($P \leq 0.10$).

5.3.4.2 Muscle deoxygenation kinetics and Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio

Kinetic parameters for Δ[HHb+Mb] and Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratios are displayed in Table 5.11.

Parameter estimates were similar between groups at baseline, albeit individuals with T2DM displaying a tendency for a higher baseline [HHb+Mb] (P=0.09).

PE however, resulted in significantly elevated amplitudes of the response (P<0.05) in both groups and thus, an elevated end amplitude of the overall response. Additionally a significantly shorter TD was observed subsequent to the prior priming exercise bout (P<0.05). As per *Experiment 2 (see section 3.2.4.2.3)*, the overall Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio was calculated to indicate the degree of O_2 extraction relative to steady-state values for a given increment in $\dot{V}O_2$ during each moderate-intensity transition. The overall Δ[HHb+Mb]/Δ$\dot{V}O_2$ ratio displayed a trend ($P \leq 0.10$) for a priming effect in both groups, albeit not reaching significance.

Table 5.11. ***Parameters of the Δ[HHb+Mb] kinetics and Δ[HHb+Mb]/ΔV̇O₂ for unprimed (Mod A) and primed (Mod B) moderate-intensity exercise.***

	Mod A Unprimed		Mod B Primed	
	ND	T2DM	ND	T2DM
n	7	7	7	7
Δ[HHb+Mb] baseline, a.u.	-54.21 ± 51.03†	-0.28 ± 47	-49.61 ± 81	9.8 ± 58
Δ[HHb+Mb] A, a.u.	43.7 ± 0.56	61.9 ± 40	57.06 ± 6‡	79.75 ± 53‡
Δ[HHb+Mb] End A, a.u.	-10.5 ± 78.6	61.7 ± 75.2	7.5 ± 90.7‡	89.6 ± 92.3‡
Δ[HHb+Mb] TD, s	15 ± 3	16 ± 4	10 ± 7‡	14 ± 2‡
τΔ[HHb+Mb], s	16 ±14	17 ± 7	21 ± 9	20 ± 8
τ'Δ[HHb+Mb], s	31 ± 11	33 ± 5	31 ± 7	34 ± 5
Normalised Δ[HHb+Mb]/ΔV̇O₂	1.06 ± 0.23	1.24 ± 0.33	0.94 ± 0.11#	1.02 ± 0.18#

Values are means ± SD. Dynamic response of muscle deoxygenation [HHb+Mb] during unprimed and primed cycling exercise at 80% ventilatory threshold (VT) in individuals with and without T2DM. TD, calculated time delay of HHb response; τ, time constant of the response; τ', effective response time (TD + τ); a.u., arbitrary units; Normalised Δ[HHb+Mb]/ΔV̇O₂ ratio, calculated as the 20- to 120-s average of the normalised Δ[HHb+Mb]/ΔV̇O₂. ‡Significantly different between Mod A and Mod B conditions within the group ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). #Tendency towards a difference from unprimed ($P \leq 0.10$).

5.3.4.3 Cardiac output and heart rate responses

Heart rate responses for both ND and T2DM during moderate-intensity exercise with and without a prior bout of priming exercise and CO responses during the unprimed exercise are presented in Table 5.12. During the unprimed bout, individuals with T2DM tended to show (P=0.06) lower CO responses compared to their respective ND counterparts. Resting HR was significantly elevated before the moderate-intensity cycling after PE (P<0.001) with a main diabetes effect (P=0.05). Additionally, PE significantly elevated end exercise HR (P≤0.001). A trend for significance in delta HR was observed subsequent to PE (P=0.10) with a main diabetes effect (P=0.039).

Table 5.12. ***Baseline cardiac output responses and heart rate responses for unprimed and primed moderate-intensity exercise.***

	Mod A Unprimed		Mod B Primed	
	ND	**T2DM**	**ND**	**T2DM**
n	7	7	7	7
Baseline HR, beats.min^{-1}	99 ± 14*	114 ± 9	111 ± 14‡	122 ± 9‡
End-exercise HR, beats.min^{-1}	135 ± 16	138 ± 10	145 ± 16‡	143 ± 9‡
ΔHR, beats.min^{-1}	36 ± 14*	25 ± 6	34 ± 12$^{\#}$	21 ± 7$^{\#}$
Baseline CO, L.min^{-1a}	13.92 ± 2.87†	9.63 ± 3.14		

Values are means ± SD. CO, cardiac output; HR, heart rate. Some variables have missing values and the sample sizes with codes are shown below. *Significantly different than T2DM ($P \leq 0.05$). ‡Significantly different between Mod A and Mod B conditions within the group ($P \leq 0.05$). †Tendency towards a difference than T2DM ($P \leq 0.10$). *Significantly different than T2DM ($P \leq 0.05$). $^{\#}$Tendency towards a difference from unprimed ($P \leq 0.10$). a = 6 (ND) and 4 (T2DM).

5.4 DISCUSSION

The present study investigated the effect of heavy-intensity "priming" exercise (PE) on pulmonary $\dot{V}O_2$ and muscle deoxygenation kinetics during subsequent moderate-to-heavy intensity (w-to-w) cycling exercise transitions in individuals with T2DM. The principal findings were as follows:

3. In partial agreement with our first hypothesis, despite the rate of adjustment of the primary phase of the $\dot{V}O_2$ kinetics response ($\tau\dot{V}O_{2p}$) of the heavy-intensity cycling bout not being significantly reduced by PE (P=0.14), a reduction of ~22% was observed in individuals with T2DM, with a much smaller reduction of ~2% among the ND controls.
4. Consistent with our second hypothesis, PE significantly (P<0.05) reduced the amplitude of the slow component during the heavy-intensity exercise bout of the w-to-w transitions in T2DM.
5. A significant (P<0.05) reduction in the MRT of the overall $\dot{V}O_2$ response during w-to-w cycling exercise transitions subsequent to PE was observed in T2DM.

5.4.1 Heavy-intensity exercise

Our results demonstrate that PE induced a faster overall $\dot{V}O_2$ kinetics response in the subsequent high-intensity bout during the w-to-w cycling transitions in individuals with T2DM. In accordance with the literature, individuals in the present study demonstrated a substantially longer τ (ND, 42 ± 11 s; T2DM, 54 ± 14 s) of the primary phase of the $\dot{V}O_2$ kinetics response upon initiation of heavy-intensity cycling from an elevated baseline work rate (w-to-w) compared to that demonstrated upon transitioning from an unloaded baseline (ND, 31 ± 5 s; T2DM, 37 ± 10 s; see Table 4.7, *Experiment* 3) (Hughson & Morrisey, 1982; Brittain *et al.* 2001; Wilkerson & Jones, 2006, 2007; DiMenna *et al.* 2008, 2009a, 2009b, 2010c). It is worth noting that 6 of 7 ND individuals and 2 out of 7 individuals with T2DM who participated in the present study also took part in *Experiment 3*. $\tau\dot{V}O_{2p}$ values pertaining to this small subset of participants were similar to the aforementioned values for both w-to-w and unloaded transitions to heavy-intensity exercise, (40 ± 10 s, ND and 61 ± 29 s, T2DM and 31 ± 8 s, ND and 38 ± 7 s, T2DM respectively).

In the present study the performance of a prior PE bout resulted in a substantial reduction in the time constant of the primary $\dot{V}O_2$ kinetics during the heavy-intensity bout of the w-to-w transitions in individuals with T2DM (~22%) compared to their ND counterparts (~2%), albeit not significant (main effect PE, P=0.140). It is likely that interindividual variability in response

precluded the attainment of statistical significance. Interestingly, this enhanced rate of adaptation of the time course of the kinetics response demonstrated by the individuals with T2DM during the primed w-to-w in T2DM was subsequently on a par with that demonstrated by the ND controls (42 ± 17 and 41± 11 s, respectively).

The above findings strengthen the notion of a constrained rate of adaptation of the primary $\dot{V}O_2$ kinetics response as a consequence of impaired O_2 delivery. In circumstances whereby muscle O_2 availability does in fact limit the adjustment of $\dot{V}O_2$ at the onset of exercise, then subsequent reductions in $\tau\dot{V}O_{2p}$ following a bout of PE can be expected (Poole & Musch, 2010). This was evidenced by Di Menna *et al.* (2010c) in a study whereby participants were required to exercise in the supine position, thus compromising muscle perfusion pressure. The authors explored the effects of PE on $\dot{V}O_2$ kinetics during severe-intensity supine cycle exercise in young (mean age = 35, yr) active males (mean $\dot{V}O_{2max}$ = 51 mL.kg^{-1}.min^{-1}). Consistent with the literature, $\tau\dot{V}O_{2p}$ was significantly lengthened by ~50% in severe-intensity supine compared with upright cycling transitions initiated from a moderate-intensity baseline. Interestingly the $\tau\dot{V}O_{2p}$ values evidenced in this w-to-w posture-related study bear a close resemblance to those observed in the present w-to-w study. The $\tau\dot{V}O_{2p}$ demonstrated in the upright cycling position was similar to that of the individuals without T2DM in the present study (38 ± 10 and 42 ± 11, s respectively), whereas the lengthened $\tau\dot{V}O_{2p}$ demonstrated whilst cycling in the supine position precisely matched that displayed by individuals with T2DM (54 ± 19 and 54 ± 14, s, respectively). Moreover, subsequent to priming exercise, the magnitude of the improvements were similar in both studies; the supine $\tau\dot{V}O_{2p}$ being reduced to similar values as evidenced in the unprimed upright posture (34 ± 9 and 38 ± 10, s respectively), whilst individuals with T2DM displayed substantially reduced $\tau\dot{V}O_{2p}$ in line with the ND controls (42 ± 17 and 41 ± 11, s respectively). These findings suggest that a prior bout of heavy-intensity priming exercise counteracted the adverse effects of T2DM on $\tau\dot{V}O_{2p}$ within a high-intensity bout during subsequent w-to-w cycling exercise.

In an earlier study, Di Menna *et al.* (2008) investigated the effects of PE on $\dot{V}O_2$ kinetics during upright w-to-w severe-intensity cycle exercise in young (mean age = 31, yr) active males (mean $\dot{V}O_{2max}$ = 45 mL.kg^{-1}.min^{-1}). Despite the demonstration of a significantly lengthened $\tau\dot{V}O_{2p}$ in severe-intensity cycling transitions initiated from a moderate-intensity baseline (w-to-w) in comparison to those observed on-transition from an unloaded baseline (42 ± 15 and 33 ± 8 s), no significant differences were observed between the $\tau\dot{V}O_{2p}$ in the unprimed and primed w-to-w transitions to severe-intensity exercise (42 ± 15 and 42 ± 17, s). These results indicate that the slower primary phase kinetics observed during moderate-to-severe exercise transitions are

not related to a muscle O_2 delivery limitation in healthy young individuals. Notably, the findings reported by these authors are in accordance with the findings presented in the majority of other studies centred on the influence of PE during transitions from unloaded pedalling to heavy- or severe-intensity upright cycle exercise in healthy young individuals (Burnley *et al.* 2000, 2001, 2002a, 2002b; Fukuba *et al.* 2002; Jones *et al.* 2006, 2008; Scheuermann *et al.* 2001; Wilkerson & Jones, 2007).

As such, the potential that lies within heavy-intensity PE as an acute intervention to accelerate the $\dot{V}O_2$ response in situations where O_2 delivery is inadequate deserves further recognition. In light of the aforementioned emergence of evidence favouring a diminished hyperaemic and haemodynamic response in T2DM, the above findings are in accordance with the view that PE may have resulted in a more appropriate distribution of blood flow within the microvasculature of the working muscle.

In the upright cycling w-to-w study by DiMenna *et al.* (2008), despite PE not influencing the lengthened $\tau\dot{V}O_{2p}$ (42 ± 15 *vs.* 42 ± 17, s) PE did however significantly reduce the $\dot{V}O_2$ amplitude of the slow component (0.47 ± 0.09 *vs.* 0.27 ± 0.13, $L.min^{-1}$) of the severe-intensity bout, subsequently shortening the overall MRT of the $\dot{V}O_2$ response (80 ± 24 *vs.* 63 ± 18, s). Furthermore during the unprimed severe-intensity bout of the w-to-w transitions, iEMG increased significantly between minutes 2 and 6, whilst subsequent to PE iEMG responses were no longer different between end-exercise (min 6) and min 2 of the bout.

These results imply that the slower primary phase $\dot{V}O_2$ kinetics observed during w-to-w upright cycle exercise transitions in young healthy individuals are not related to a muscle O_2 delivery limitation. Moreover, they signify that the reduced slow component observed following priming exercise is potentially linked to altered motor unit recruitment patterns, such that the requirement for additional fibre activation as exercise proceeds and the associated $\dot{V}O_2$ cost of that activation are reduced (Burnley *et al.* 2002b; DiMenna *et al.* 2010c). This was supported in a later study by the same research group, whereby subsequent to 6 min of high-intensity prone knee-extension exercise, a blunting of the ΔiEMG during the subsequent w-to-w transition was observed, concomitant with a significant reduction in the amplitude of the [PCr] slow component resulting in a 20% reduction in [PCr] degradation. The reduced iEMG response suggests that delayed-onset fibre activation may be reduced as a result of PE and may be responsible for the observed reductions in the [PCr] and $\dot{V}O_2$ slow components (Burnley *et al.* 2002b; DiMenna *et al.* 2008; Bailey *et al.* 2009). Alternatively, another plausible explanation suggested by the authors is that of an enhanced muscle O_2 delivery and a more homogenous

distribution of intramuscular blood flow consequent to PE, decreasing the anaerobic contribution. Subsequently, the rate at which fatigue developed would be reduced, thus, substantially alleviating the reliance on the recruitment of additional motor units, ultimately culminating in a reduction of the O_2 cost of exercise (DiMenna *et al.* 2008).

In contrast to these iEMG adaptations during w-to-w upright exercise subsequent to PE in the supine posture, a significantly greater mean iEMG was evidenced at the end of both unprimed and primed w-to-w cycling exercise bouts than that displayed at minute 2. The authors attributed this response to a different pattern of muscle fibre activation (DiMenna *et al.* 2010c) incurred whilst exercising in the supine position.

In the present study in addition to the significantly accelerated $\tau\dot{V}O_{2p}$, the amplitude of the $\dot{V}O_2$ slow component was significantly reduced following PE in participants with T2DM (0.13 ± 0.15 *vs.* 0.08 ± 0.10, $L.min^{-1}$). Thus, these results are also consistent with the notion that slower $\dot{V}O_2$ kinetics during w-to-w transitions to heavy-intensity exercise may be related to factors residing within the contracting muscle; specifically the metabolic properties of the population of muscle fibres recruited to meet the augmented force demands upon transitioning to a heavy-intensity work rate from an elevated metabolic rate (Jones *et al.* 2008). Furthermore, considering an altered muscle fibre distribution has been evidenced in individuals with T2DM (Marin *et al.* 1994), with reports showing a 2-fold increase in type IIb fibres (Mogensen *et al.* 2007) it is thus likely that the priming induced reduction in the amplitude of the slow component may also be potentially related to altered motor unit recruitment patterns, such that the requirement for additional fibre activation as exercise proceeds and the associated $\dot{V}O_2$ cost of that activation are reduced.

In the present study with the NIRS-derived HHb signal accepted as a surrogate for fractional O_2 extraction, the changes in the NIRS derived deoxy HHb concentration (Δ[HHb+Mb]) were considered to be indicative of the balance between O_2 availability and utilisation in the microvasculature within the region of NIRS interrogation. Despite the demonstration of a significantly accelerated MRT of the $\dot{V}O_2$ kinetics response during the w-to-w exercise consequent to the prior priming bout (72 ± 10 *vs.* 53 ± 18, s), the dynamic responses of Δ[HHb+Mb] were not accelerated. However, it should be noted that after the PE, the Δ[HHb+Mb] MRT tended to be faster in individuals with T2DM (52 ± 32 *vs.* 37 ± 24, s). This suggests that the dynamics of muscle O_2 utilisation were enhanced consequent to a priming induced increase in muscle blood flow and/or improved muscle perfusion to metabolic rate, and to a lesser extent to the exertion of an increased reliance on muscle fractional O_2 extraction.

5.4.2 Moderate-intensity exercise

During the unprimed moderate-intensity cycling, individuals with T2DM displayed a significantly longer $\tau\dot{V}O_{2p}$ than the ND controls (30 ± 4 *vs.* 39 ± 11 s, respectively). Interestingly, in contrast to findings from the w-to-w element of this study, both groups demonstrated similar trends for a reduction in the time constant of the primary phase of the $\dot{V}O_2$ response, falling in the region of ~15 to 20% subsequent to PE (P<0.10). This is consistent with previous findings in a larger number of participants in *Experiment 2* and several previous studies, whereby when unprimed $\tau\dot{V}O_{2p}$ is in excess of 20 s, the rate of adjustment is likely constrained by the matching of local O_2 distribution to muscle O_2 uptake as evidenced by subsequent reductions in $\tau\dot{V}O_{2p}$ following PE (Gerbino *et al.* 1996; DeLorey *et al.* 2004a, 2004b; Gurd *et al.* 2005, 2006; Chin *et al.* 2010; Murias *et al.* 2011b). Additionally, and also in accordance with previous studies, this is further corroborated with reductions in the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio (Murias *et al*. 2011b; DeRoia *et al*. 2012) as detailed below.

In the present study, baseline HR was significantly elevated prior to the initiation of the primed bout of moderate-intensity exercise compared with the unprimed bout in both ND individuals (99 ± 14 *vs.*111 ± 14 beats.min^{-1}) and individuals with T2DM (114 ± 9 *vs.* 122 ± 9 beats.min^{-1}) and thus, was indicative of enhanced O_2 perfusion in both groups. As such, it would have been reasonable to expect a slower τ[HHb+Mb], representative of the slower adjustment of muscle O_2 consumption to the faster adjustment of O_2 delivery, consistent with an enhanced muscle blood flow and O_2 delivery subsequent to a prior bout of heavy intensity exercise (DeLorey *et al.* 2004b, Gurd *et al.* 2006, 2009). Conversely, despite the elevated baseline HR, τ[HHb+Mb] remained unchanged. However, combined with measurements of pulmonary $\dot{V}O_2$, the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio demonstrated an "overshoot" (i.e. the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio was elevated) throughout the unprimed bout of submaximal exercise; and a trend (P=0.10) for the elimination of this "overshoot" throughout the primed exercise on-transient. Thus, consistent with results from *Experiment* 2, the speeding of the $\dot{V}O_2$ kinetics response was attributed to a better matching of microvascular O_2 delivery to utilisation.

In conclusion, it would appear that the reduction in the MRT of the overall $\dot{V}O_2$ response during the heavy-intensity bouts during w-to-w transitions facilitated by the performance of prior heavy-intensity exercise in ND individuals was accrued by motor unit recruitment and the subsequent reduction in the $\dot{V}O_2$ slow component. In individuals with T2DM the speeding of the $\dot{V}O_2$ MRT following PE, appeared to be consequent to a combination of increased O_2 delivery and potentially enhanced motor unit recruitment. Both groups demonstrated similar

reductions in the time constant of the primary phase of the $\dot{V}O_2$ response in the moderate-heavy-intensity exercise transitions subsequent to PE, likely facilitated by enhanced blood flow delivery and muscle perfusion, attributed to a prior bout of heavy-intensity priming exercise.

5.4.3 Limitations

It should be noted that the priming exercise bout utilised within this study was not a single high-intensity bout, but instead a w-to-w bout. However, preliminary pilot work data suggest that the addition of a 6 min moderate-intensity exercise bout to the priming high-intensity bout does not in fact affect the subsequent $\dot{V}O_2$ or Δ[HHb+Mb] kinetic response profiles of the w-to-w exercise transition.

In addition it should be acknowledged that despite having sufficient power (70%) in the *n* number for this study the variation was greater in individuals with T2DM. Thus, we attribute the lack of statistical power herein to the calculation of the n number based on previous experimental studies which utilised healthy individuals in either posture and/or aging related investigations, which we posited to be representative of the T2DM clinical population.

Chapter 6: General discussion

Limitations in functional exercise capacity are frequently evidenced in individuals with uncomplicated T2DM, demonstrated during exercise at both maximal and submaximal levels. The consistent demonstration of reductions in $\dot{V}O_{2max}$ in the region of 20% is of particular importance, as it highlights the premature cardiovascular aging effect of T2DM. A defect of this magnitude has the potential to contribute to the tenacious excess cardiovascular and all-cause mortality observed in this clinical population. Indeed, whilst $\dot{V}O_{2max}$ is traditionally considered the sentinel parameter of integrated cardiovascular function, it is instructive to consider the determinants of $\dot{V}O_{2max}$ when attempting to gain further insights into the mechanisms by which T2DM compromises oxygen transport. Subsequently, the constrained $\dot{V}O_2$ adaptations observed in individuals with T2DM whilst exercising at submaximal levels is of great clinical significance. The speed of $\dot{V}O_2$ kinetics may in fact be of greater prognostic value in T2DM than $\dot{V}O_{2max}$, given the perturbations that ensue in intracellular homeostasis during exercise consequent of impaired $\dot{V}O_2$ dynamics are likely to contribute to premature muscular fatigue. Indeed, evidence exists to suggest that individuals with T2DM experience exercise, even at relatively low levels, to be more strenuous than individuals without T2DM, reporting greater psychological perception of effort (Huebschmann *et al.* 2009, 2015).

The present study primarily focused on the contribution of peripheral mechanisms whilst attempting to gain a further insight into the mechanisms underlying the exercise intolerance in T2DM. As such, cardiorespiratory and estimated microvascular responses were simultaneously investigated during graded ramp incremental cycle exercise and throughout the on-transient of cycle exercise in the moderate- and heavy- intensity domain when preceded by a priming exercise bout. The following section will briefly review the main findings of each experimental chapter, and then provide an overall discussion and interpretation of these findings in the context of the current literature.

In light of the consistent demonstration of an impaired exercise tolerance in T2DM, for which the precise mechanisms remain unknown, in *Experiment 1* we simultaneously investigated cardiorespiratory and microvascular responses to ramp incremental exercise. The primary aim of this experiment was to investigate the influence of T2DM on the profile of muscle fractional O_2 extraction during ramp incremental cycle exercise as estimated using the NIRS-derived deoxygenated haemoglobin signal (Δ[HHb+Mb]). Individuals with T2DM displayed a steeper primary slope of the bi-linear regression of relative [HHb+Mb] as a function of relative PO, thus indicative of a reduced capacity to increase peripheral O_2 delivery amidst increasing O_2

demands. The increased reliance on fractional O_2 extraction demonstrated by these individuals may be associated with a reduced ability to deliver or redistribute O_2 to the exercising tissue, and/or a reduced oxidative capacity.

Pulmonary $\dot{V}O_2$ kinetics are profoundly slowed in young and middle-aged individuals with T2DM at the onset of moderate-intensity exercise (Regensteiner *et al.* 1995, 1998; Brandenburg *et al.* 1999; Brassard *et al.* 2006; Nadeau *et al.* 2009; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012, 2015). Through the measurement of the NIRS-derived Δ[HHb+Mb] in association with $\Delta\dot{V}O_2$ in *Experiment 2* we determined the time course of local muscle O_2 extraction of the *vastus lateralis* and estimated microvascular O_2 delivery to utilisation ratio (Δ[HHb+Mb]/$\Delta\dot{V}O_2$) during unprimed and primed moderate-intensity cycling exercise. Individuals with T2DM demonstrated an accelerated rate of adjustment of the primary phase of the $\dot{V}O_2$ kinetics response, but not in Δ[HHb+Mb] kinetics in a bout of moderate-intensity cycling exercise when preceded by a prior bout of heavy-intensity priming exercise. Furthermore, this was accompanied by the elimination of an "overshoot" in the Δ[HHb+Mb]/$\Delta\dot{V}O_2$ ratio during the on-transient exercise response, thereby reflecting a superior matching of microvascular O_2 delivery to utilisation consequent of the priming exercise bout.

In circumstances whereby constrained $\dot{V}O_2$ kinetics present during heavy-intensity exercise as a consequence of limited O_2 delivery, such as when cycling in the supine posture (Jones *et al.* 2006) priming exercise has been evidenced to accelerate the rate of adaptation of the response in a subsequent bout of heavy-intensity exercise. *Experiment 3* explored this priming exercise on subsequent heavy-intensity exercise in T2DM. Priming exercise facilitated an acceleration of the primary phase of the $\dot{V}O_2$ kinetics response in the subsequent bout of heavy-intensity exercise in individuals with T2DM. Additionally, a substantial reduction in the amplitude of the $\dot{V}O_2$ slow component also ensued. Moreover, the overall dynamic responses of Δ[HHb+Mb] remained predominantly similar, albeit displaying a trend in the primed heavy-intensity exercise bout for a reduction in the amplitude of the Δ[HHb+Mb] slow component. Collectively, these findings strengthen the notion of a superior O_2 delivery relative to utilisation as a direct consequence of priming exercise in subsequent heavy-intensity exercise in individuals with T2DM.

Work-to-work transitions have been acknowledged to afford superior insight into the factors which potentially influence the control of $\dot{V}O_2$ kinetics. However, the governing factors regulating the altered $\dot{V}O_2$ kinetics in work-to-work are more complex. In *Experiment 4* the

effect of priming exercise on subsequent work-to-work transitions was assessed in T2DM. Priming exercise favourably altered the overall $\dot{V}O_2$ kinetics profile in the subsequent heavy-intensity bout during w-to-w cycling transitions in both individuals with and without T2DM, albeit via different mechanisms. In the cohort without T2DM, the acceleration was most likely accrued by enhanced motor unit recruitment and the subsequent reduction in the amplitude of the $\dot{V}O_2$ slow component. In individuals with T2DM, this was more likely achieved via a combination of increased O_2 delivery and enhanced motor unit recruitment. Furthermore, this was accompanied by an unaltered overall dynamic response of Δ [HHb+Mb], with the [HHb+Mb] MRT tending to be slower subsequent to priming exercise in T2DM. This suggests that in the group with T2DM, the dynamics of muscle O_2 utilisation was enhanced consequent to a priming-induced increase in muscle blood flow and/or improved muscle perfusion to metabolic rate, as opposed to the exertion of an increased reliance on muscle fractional O_2 extraction.

Collectively, the findings from the four experiments within this thesis are consistent with the current literature concerning the exercise intolerance demonstrated by individuals with T2DM. In each of the four experiments, significant reductions in $\dot{V}O_{2max}$ were displayed by the individuals with T2DM which are in accordance with the ~20% reduction in $\dot{V}O_{2max}$ consistently reported in this clinical population (Regensteiner *et al.* 1998; Baldi *et al.* 2003; MacAnaney *et al.* 2011a; O'Connor *et al.* 2012). Additionally, these individuals also displayed impairments in the $\dot{V}O_2$ kinetics response during moderate- and heavy-intensity submaximal exercise. Whilst it has been established that $\dot{V}O_{2max}$ is inarguably constrained by the capacity of the O_2 transport system in its entirety (Rowell, 1993; Poole 1997; Wagner *et al.* 1997; Basset & Howley, 2000), this is not the case for $\dot{V}O_2$ kinetics. Instead, the presiding belief for the primary locus of control of $\dot{V}O_2$ kinetics in healthy, young individuals is within the muscle mitochondrion, and regulated predominantly by oxidative energy system inertia (Poole & Jones, 2012). However, of particular interest is the displacement of this locus of control upstream of the contracting myocytes into the O_2-transport pathway in diseased populations. Therefore understanding and appreciating the interdependence between perfusive (cardiac output/blood flow) and diffusive (transmembrane O_2 flux, O_2 extraction) conductance during maximal and submaximal exercise is of paramount importance to resolving the mechanistic bases for the decreased exercise tolerance observed in T2DM. Indeed, whilst the reduced exercise capacity in individuals with T2DM may be the consequence of a complex array of pathophysiological changes, with both central and peripheral contributory mechanisms

acknowledged, the collective findings from the four experimental chapters direct our attention to those of the peripheral nature.

Interestingly, a common misconception exists surrounding the mirroring of the bulk (perfusive) O_2 delivery and that within the microcirculation at the interface of capillary-to-myocyte (diffusive) O_2 flux. On the contrary, through the utilisation of animal models, the dynamic blood flow response at the level of the microvasculature in healthy muscle tissue has been evidenced to be slower than that measured upstream in the feed artery, and in fact closely approximates $\dot{V}O_2$ kinetics (Behnke *et al.* 2001; Ferreira *et al.* 2005b; Harper *et al.* 2006.) Furthermore, substantial heterogeneities of O_2 delivery-to-O_2 utilisation ($QO_2/\dot{V}O_2$) at the level of the microvasculature have been revealed at both intra- and inter-muscular sites, (Koga *et al.* 2014). As such, critical pathologically-induced alterations in muscle blood flow redistribution during exercise in individuals with T2DM may be masked by the obtainment of mere measures of CO or even limb blood flow in the conduit vessel (Poole *et al.* 2012). Accordingly it is all the more pertinent that techniques capable of following the dynamics of muscle microvascular oxygenation/deoxygenation across rest-exercise transitions in human (NIRS) and animal (intravital microscopy and phosphorescence quenching) muscle are exploited, thus exposing the interrelationship between $QO_2/\dot{V}O_2$ heterogeneities and function/dysfunction.

The regional $QO_2/\dot{V}O_2$ ratio plays a critical role in metabolic regulation as it governs the upstream driving pressure (i.e., microvascular Po_2) essential for achieving a given blood-myocyte O_2 flux, whilst also being an important determinant of intramyocyte Po_2 (Behnke *et al.* 2001; 2002a; McDonough *et al.* 2005; Heinonen *et al.* 2015). Interestingly, it has been evidenced that the primary increase in blood-myocyte O_2 flux during the exercise on-transition occurs via increased RBC flux and haematocrit within already flowing capillaries (Poole *et al.* 2007; 2008c; 2011). Thus, whilst individuals with T2DM have demonstrated a diminished ability to increase CO during exercise (Roy *et al.* 1989), although not always (Regensteiner *et al.* 2009), extensive evidence also exists to suggest a maldistribution of active muscle blood flow at the level of the microvasculature occurs in this clinical population. The elegant explorative work of Padilla and colleagues (2006, 2007) revealed significant impairments in the haemodynamic responses supporting RBC flux in muscle at rest and during contractions in rodent models with T2DM; the Goto-Kakizaki (GK) rat, considered to be highly representative of the T2DM state in humans (Goto *et al.* 1975). Specifically, via the utilisation of intravital microscopy to examine the microcirculation of the spinotrapezius muscle, reductions in the percentage of flowing capillaries (~30%), concomitant with impaired capillary red blood cell haemodynamics in the resting spinotrapezius muscle of GK rat were reported (Padilla *et al.*

2006) and collectively deemed responsible for a significant reduction in QO_2 of ~70%. Additionally, phosphorescence quenching revealed reductions in the driving pressure for O_2 ($P_{mv}O_2$) into the myocyte in the resting spinotrapezius muscle of these rodent models (Padilla *et al.* 2007), thereby implicating vascular dysfunction for the diminished resting QO_2 in T2DM. Moreover, upon the on-transition from rest to electrically stimulated muscle contraction, the kinetic profile of $P_{mv}O_2$ was substantially altered in the GK rat (Padilla *et al.* 2007) with a precipitous reduction in $P_{mv}O_2$, accompanied by an 'undershoot', before a delayed attainment of steady-state in the spinotrapezius muscle of the GK rat.

$P_{mv}O_2$ is therefore representative of the balance between muscle QO_2 and $\dot{V}O_2$ within the microcirculation (Behnke *et al.* 2001; 2002a; 2002b; 2003) and provides a measure of the driving pressure from O_2 diffusion from the blood to the contracting muscle. Consequently such profiles in the T2DM rodent model are symptomatic of an impaired QO_2 and thus indicative of a substantial impairment in muscle microcirculatory function in T2DM. Although this GK rat model of T2DM provides a unique insight into the microcirculatory disturbances accompanying this all-too-prevalent disease, it is acknowledged that the specific influence of structural and functional impediments on muscle diffusing capacity in humans with T2DM would undoubtedly be of great interest, however, this is presently infeasible in contracting human skeletal muscle. Nonetheless, given a decrease in $P_{mv}O_2$ will ultimately affect O_2 exchange, an impaired haemodynamic response can certainly be posited as a potential mechanistic basis for the diminished $\dot{V}O_2$ kinetics response and greater O_2 deficit that is symptomatic of individuals with T2DM at the onset of submaximal exercise .

Vascular dysfunction has been identified as a likely culprit for the diminished QO_2, with impairments in blood flow associated with compromised responses to endothelium-dependent and independent vasodilators in the vasculature of the GK rat (Sandu *et al*. 2000; Witte *et al*. 2003) and in individuals with T2DM (McVeigh *et al*. 1992; Williams *et al*. 1996; Kingwell *et al.* 2003). As such, given that blood flow to the capillary is governed at the arteriolar level (Delp & Laughlin, 1998; Pohl *et al.* 2000), the impairments in RBC and capillary haemodynamics may be associated with this compromised vasomotor control. Indeed, reductions in leg blood flow during steady-state cycling (Kingwell *et al.* 2003), knee extension (Lalande *et al.* 2008) and calf plantarflexion (Kiely *et al.* 2014) exercise have also been evidenced in the human clinical population, in addition to impairments in the dynamic response of leg blood flow and leg vascular conductance during high-intensity calf plantarflexion exercise (MacAnaney *et al.* 2011b; Kiely *et al.* 2014), being reported. Combined with the NIRS-derived, transiently increased skeletal muscle deoxygenation response displayed by individuals with T2DM at the

onset of moderate-intensity submaximal cycling exercise (Bauer *et al.* 2007), and indeed by individuals with T2DM in *Experiment 2* within, the argument is strengthened for reduced O_2 delivery as the predominant source of impairment in $\dot{V}O_2$ control during submaximal exercise and subsequently in exercise capacity. The consequence of a reduced P*mv*$o_2$in the exercising muscle of an individual with T2DM mandates that fractional O_2 extraction in the exercising muscle would have to increase further in an effort to achieve a given $\dot{V}O_2$, as was evidenced in *Experiments 1&2*, thus intensifying the decreases in intracellular Po_2 to a level below that found in healthy muscle (Padilla *et al*. 2006). Accordingly, as the speed of muscle O_2 delivery becomes limiting both muscle and pulmonary gas exchange and their kinetics are impaired (Poole & Jones, 2012).

It is acknowledged that only one muscle, namely the *spinotrapezius* and *vastus lateralis* was examined, in both the abovementioned GK rodent model studies (Padilla *et al*. 2006, 2007) and within the 4 experimental chapters of this thesis respectively. It is however, pertinent that a spectrum of heterogeneity exists in the structural and functional properties in human muscle (Koga *et al.* 2014; Okushima *et al.* 2015), which may affect the P*mv*o_2 responses found both within and among other muscles (Behnke *et al.* 2003; McDonough *et al.* 2005). Given that human skeletal muscle is composed of a mixture of fibre-types, each possessing diverse metabolic characteristics (Barstow *et al.* 1996; Pringle *et al.* 2003), which are stratified both among and within muscles (Henneman & Mendall, 1981) it is not surprising that this has been related to discrete features of the $\dot{V}O_2$ kinetics response. For instance, it is established that oxidative fibres have a greater capacity for endothelium-dependent vasodilation (Delp & Armstrong, 1988; Delp *et al*. 1997), a lower resting sympathetic vasoconstrictor tone (Thomas *et al*. 1994) and a higher P*mv*o_2 at rest and during muscle contractions (Behnke *et al*. 2003; McDonough *et al.* 2005), with low oxidative fibres, evincing greater α1-mediated vasoconstrictor responsiveness (Koga *et al.* 2014). As such, highly oxidative muscle fibres can receive an impressive blood flow, reported to be in excess of 5 litres $kg^{-1}min^{-1}$, whereas blood flow to the low oxidative highly glycolytic muscle fibres is confined to a small fraction of this (Poole & Jones, 2012). Indeed, whilst, microvascular blood flow relative to $\dot{V}O_2$, irrespective of fibre type has been evinced to increase with the same slope (i.e., S ~5-6 $litres.min^{-1}$) as for CO and whole muscle(s) (Ferreira *et al.* 2006) the intercept is much lower for the low oxidative muscle fibres. This translates to a greater reliance on fractional O_2 extraction even at low $\dot{V}O_2$'s such that P*mv*o_2 is lower and falls more rapidly and to a greater extent than seen for the highly oxidative fibres (McDonough *et al.* 2005; Ferreira *et al.* 2006; Poole *et al.* 2008a; Poole & Jones, 2012).

Therefore, in the presence of an impaired vascular function, whereby a reduction in perfusive and/or convective blood flow exists, a redistribution of blood flow away from oxidative tissues toward more glycolytic regions occurs. Furthermore, when the P*mv*o_2 is lowered, the exercising muscles are incapable of spatially distributing O_2 to meet the local energy requirements at the level of the microvasculature (Bowen *et al.* 2013; Heinonen *et al.* 2015). Thus, combined with the notion that muscle fibre distribution has been shown to be altered in individuals with T2DM, possessing a lower proportion of Type I muscle fibres relative to Type IIb (Marin *et al.* 1994; Mogensen *et al.* 2007), it is all the more plausible that an earlier recruitment of the higher order fibres may occur in our T2DM cohort. Given these type II fibres may have a slower rate of Qm (cardiac output to muscle) increase following the onset of exercise, they are most likely forced to rely more heavily on fractional O_2 extraction. Thus the metabolic consequence of the earlier recruitment of these fibres will be a lower intracellular energy state required to maintain $\dot{V}O_2$, subsequently contributing to their lower resistance to fatigue (Ferreira *et al.* 2006). It is therefore reasonable to speculate that the potentially altered muscle fibre recruitment in our T2DM cohort may have contributed to the increased reliance on fractional oxygen extraction and, consequently, a severely diminished P*mv*o_2, whereby blood–myocyte O_2 flux, and subsequently pulmonary, and thus muscle $\dot{V}O_2$ kinetics were impaired.

However, it should be acknowledged that anatomically, microvascular units (i.e., terminal arteriole and dependent capillaries) are not spatially synchronised with discrete motor units and their fibres, but instead, may supply two or more fibres, each potentially having a very different $\dot{V}O_2$ (Koga *et al.* 2014). As such, it can be expected that extremes of micro-mismatch of O_2 delivery and $\dot{V}O_2$ within contracting muscles will occur. Unfortunately, such extremes are in fact concealed from the resolution of present technology (Koga et al. 2014), specifically surface EMG, thus caution should be employed when attempting to account for muscle recruitment patterns, when interpreting O_2 delivery-$\dot{V}O_2$ relationships.

Nevertheless, it is evident that a low $QO_2/\dot{V}O_2$ ratio in individuals with T2DM mandates a diminished exercise capacity. It is therefore of particular interest as to what degree a potentially higher $QO_2/\dot{V}O_2$ ratio as a consequence of priming exercise may be beneficial in terms of augmenting $\dot{V}O_2$ kinetics and subsequently exercise tolerance. Indeed priming exercise consistently induced favourable adaptations in the overall $\dot{V}O_2$ kinetics response during moderate and heavy-intensity exercise initiated with, and without, an elevated baseline in individuals with T2DM throughout the experimental chapters. This effect was accompanied by a significantly accelerated primary time constant of $\dot{V}O_2$ in transitions to moderate- and heavy-intensity exercise, and/or a reduction in the amplitude of the $\dot{V}O_2$ slow component, mostly

evident during the w-to-w transitions. The former is consistent with the view, whereby an acceleration of the primary phase of the $\dot{V}O_2$ kinetics response only presents in circumstances within which $\dot{V}O_2$ kinetics is likely constrained by a limitation in O_2 delivery. Furthermore, the overall unaltered NIRS-derived muscle deoxygenation profiles following the priming exercise in all 3 constant load cycling experiments, further indicates that a bout of priming exercise adequately enhanced local perfusion to meet the increased metabolic requirements at the level of the microvasculature. As such, and concomitant with the consistent favourable manipulation of the $\dot{V}O_2$ kinetics response in the subsequent bout of exercise, it is reasonable to speculate that an enhanced O_2 delivery was the likely mechanism behind the priming intervention in *Experiments 2* and *3* with a substantial tendency in *Experiment 4.* Combining the notion that peripheral blood flow and hence O_2 delivery are governed by different mechanisms at both systemic level and at the level of the microvasculature, with evidence of impaired vascular function and O_2 delivery to active muscles in T2DM, it is plausible that priming exercise may have resulted in a more favourable distribution of blood flow of the active muscle units within the working muscle.

Whilst the present study is not reporting on the evaluation of muscle activation, it would be remiss not to acknowledge the potential contribution of altered motor unit recruitment patterns subsequent to priming exercise. In light of the well-established concept of the emergence of the $\dot{V}O_{2sc}$ arising predominantly from within the exercising muscles during heavy intensity exercise (Shinohara & Moritani, 1992; Poole *et al.* 1994; Whipp, 1994; Saunders *et al.* 2000; Perrey *et al.* 2001), findings from Experiment 4 strengthen the notion that the slower $\dot{V}O_2$ kinetics during heavy-intensity exercise is indeed related to a combination of fatigue-related processes necessitating the recruitment of additional motor units and/or fibres and also metabolic processes occurring within the population of muscle fibres already recruited (Vanhatalo *et al.* 2011; Poole & Jones, 2012). Accordingly, the observed priming-induced reduction in the amplitude of the $\dot{V}O_{2sc}$ may be potentially related to altered motor unit recruitment patterns, such that the requirement for additional fibre activation as exercise proceeds and the associated $\dot{V}O_2$ cost of that activation are reduced. Thus, it would appear that in individuals with T2DM, a combination of increased O_2 delivery and enhanced motor unit recruitment are responsible for the favourable priming related $\dot{V}O_2$ manipulations in subsequent heavy-intensity exercise, particularly when initiated from an elevated baseline.

Collectively, the accumulated data in the present study demonstrate that an acute bout of heavy-intensity priming exercise, prior to moderate- and heavy- intensity exercise was revealed to beneficially affect oxidative metabolism, with a concomitant improvement in the local

matching of $Q\dot{O}_2/\dot{V}O_2$. By reducing the initial oxygen deficit and consequently reducing anaerobic ATP provision, time to exhaustion may be increased. This is of particular clinical significance for individuals with T2DM presenting with a constrained $\dot{V}O_2$ kinetics at the onset of submaximal exercise.

Although the findings from the present study do not fully explain the observed defect in exercise capacity in T2DM, they do offer an insight into potential contributory mechanisms. The demonstration of a greater rate of oxygen extraction for a given increase in PO by individuals with T2DM during a maximum ramp graded test suggest that a reduced O_2 delivery is an important underlying cause of exercise intolerance in this clinical population. Further substantiating the diminished O_2 delivery in T2DM, is the acceleration of the primary $\dot{V}O_2$ kinetics response in the T2DM cohort at moderate- and heavy-intensity exercise (with and without an elevated baseline), subsequent to priming exercise. Whilst this was attributed to a heightened O_2 delivery to the exercising tissue as a function of increased HR (Bearden & Moffatt, 2001; Burnley *et al.* 2002a; Tordi *et al.* 2003) and thus CO (Tordi *et al.* 2003) neither muscle oxygenation (Ward *et al.* 1994; Burnley *et al.* 2002a; Fukuba *et al.* 2002) nor muscle blood flow (Krustrup *et al.* 2001) were directly evaluated in this study. Thus the collective findings from this body of work do not dismiss a possible role for (regional) muscle O_2 insufficiency for the diminished exercise tolerance in individuals with T2DM. However, given the acceleration of the overall $\dot{V}O_2$ kinetics response subsequent to heavy-intensity priming exercise occurred in the presence of an unaltered [HHb+Mb] response, we can thus allude that the primary response of $\dot{V}O_2$ across a metabolic transient is largely regulated by factors more proximal to the contracting myocytes. In light of the pathophysiological features pertaining to T2DM, it is reasonable to speculate that whilst the dynamics of muscle or limb (perfusive) blood flow may be faster than $\dot{V}O_2$ kinetics, those at the level of the microvasculature (diffusive) may not. Therefore, the insidious effects of T2DM on $\dot{V}O_2$ kinetics may still occur in the presence of only a minor impediment to the normal bulk hyperaemic control process. As such, the notion that factors beyond the heart substantially contribute to the diminished exercise tolerance of this clinical population is strengthened.

However, it should be acknowledged that T2DM is by no means a "unitary" disease. Although an exacerbated $Q\dot{O}_2/\dot{V}O_2$ mismatch at the level of the microvasculature is evinced in individuals with T2DM, and consequently muscle and pulmonary gas exchange and their kinetics are impaired, substantial heterogeneity exist within individuals with T2DM themselves. For instance, whilst $\tau\dot{V}O_2$ is indeed a fundamental parameter of aerobic performance (Whipp, 1970; Whipp *et al.* 1981) vast differences in individual $\tau\dot{V}O_2$ was demonstrated within this cohort of

individuals with T2DM. And indeed, whilst such variability may have influenced statistical power at times (*Experiment 4)*, it is more pertinent that such inter-individual heterogeneity is recognised and acknowledged, given how this could in fact conceal the extent of the O_2 deficit, and the resultant metabolic stress, as returned from mean group responses. As such, further exploration and consideration of such variation and thus how the disease process may impact muscle $QO_2/\dot{V}O_2$ matching in single individuals with T2DM is warranted. Such endeavours could proffer a crucial insight into more specific mechanistic components of physiological dysfunction in this all-to-prevalent disease and the implications of same may be revealed. Moreover, this may assist in the development of combative therapeutic countermeasures and ultimately enhance the quality of life and conceivably life expectancy in this clinical population.

Limitations

Although the inert gas rebreathing technique has been validated against direct Fick, dye- and thermo-dilution methods (Gabrielsen *et al.* 2002; Peyton & Thompson, 2004; Agostoni *et al.* 2005) and despite our endeavours to prevent the occurrence of both technical and/or human errors upon execution of the gas rebreathing manoeuvre, some implausible maximal CO values were returned. Such values were subsequently excluded, and therefore, CO values presented herein pertain to smaller sample sizes which are acknowledged accordingly within Tables 2.6; 3.6; 4.6; 5.6. Furthermore, the supplementary calculation of the approximate proportionality between CO and $\dot{V}O_2$ across changes in $\dot{V}O_2$ *(as per section 2.2.3.5)* revealed systematically higher (~35%) CO estimates than values generated by the cardiometabolic unit. In light of such findings, CO results presented herein should be interpreted cautiously.

Whilst limitations exist within the NIRS technology utilised in this present study, they pertain specifically to functional limitations within NIRS itself. However, it remains the only available approach to interrogate the microcirculation during whole-body exercise in intact humans. The primary limitation, although already identified and addressed accordingly in *section 2.2.3.4*, is associated with the inability to make direct comparisons of absolute concentration and changes in Δ[HHb+Mb] between individuals with T2DM and individuals without. Thus, it is pertinent that the NIRS-derived [HHb+Mb] signal does not measure microvascular blood flow or O_2 delivery, and the haematocrit is not known. However, whilst our analysis relates solely to [HHb+Mb], this variable is considered to be insensitive to blood volume changes, and possesses a time course akin to fractional O_2 extraction determined by phosphorescence quenching in carefully isolated rodent preparations (Koga *et al.* 2012).

A further consideration is that our findings are limited to the single area assessed directly beneath the optodes, and thus it is acknowledged that evaluating one muscle, *vastus lateralis*, cannot provide a full picture of the skeletal muscle blood flow response to exercise. Evidence suggests that within the depth of a muscle, there is a range of heterogeneity in both structural; pertaining to vascularity and fibre type, (Johnson *et al.* 1973) and functional; relating to fibre recruitment, vascular control, blood flow (Behnke *et al.* 2003; McDonough *et al.* 2005; Koga *et al.* 2011), and muscular properties (Okushima *et al.* 2015). Indeed, there is some evidence that the *vastus lateralis* of healthy individuals has a higher fraction of type II fibres and a lower blood flow than other regions in the quadriceps femoris (Kalliokoski *et al.* 2006). Thus, data acquisition may be primarily arising from type II muscle fibres, whereas exercise performed in the moderate-intensity domain would typically result in the preferential recruitment of type I fibres. Moreover, considerable variability for NIRS-derived muscle deoxygenation, notionally akin to $P\bar{v}O_2$, and thus the distribution of $Q\dot{O}_2/\dot{V}O_2$, has been evidenced both between *vastus lateralis* and *rectus femoris* as well as within the superficial and deep compartmental fibres of *rectus femoris*, during constant load cycling (Koga *et al*. 2007; Saitoh *et al.* 2009; Prieur *et al.* 2010; Chin *et al.* 2011; Okushima *et al.* 2015). Although Okushima *et al.* (2015) attributed these findings to muscle activation patterns, the potential impact that vascular and metabolic control properties could have was also acknowledged. However on the contrary, some investigators observed minimal (Koga *et al.* 2007), to no differences (duManoir *et al*. 2010) in spatial heterogeneities in patterns of muscle deoxygenation within the quadriceps. In addition, Kime *et al.* (2005) and Boone *et al.* (2009) have reported similar muscle deoxygenation patterns between the distal and proximal portions of the *vastus lateralis* during ramp incremental exercise.

Furthermore, it is accepted that adipose tissue thickness at the site of measurement has the potential to influence NIRS measurements through its effect on the scattering properties of the tissue. Whilst we determined the thickness of the skin and adipose tissue at the site of the interrogation via 2D ultrasound operating in B-mode, and no significant differences were revealed in any of the experiments, substantial inter-variability existed within the cohort of individuals with T2DM. Accordingly, such heterogeneity could have altered the NIRS profiles. Thus, in light of such findings, we cannot ignore the fact that disparities may exist between and within muscles within muscle groups, and therefore this may have impacted our findings.

Future studies

For the development of therapeutic strategies aimed at enhancing the quality of life and plausibly life expectancy in this clinical population our attention is drawn to the very promising recent developments in the treatment of exercise intolerance in patients with chronic heart failure (CHF). A commonality among CHF and T2DM is an exercise intolerance which includes a reduced $\dot{V}O_{2max}$ and slowed $\dot{V}O_2$ kinetics, being intricately linked to structural and functional aberrations in the O_2 transport pathway. Whilst CHF compromises almost every facet of the O_2 transport pathway, infringing on both perfusive and diffusive O_2 transport, interestingly many of the deviations more proximal to the skeletal muscle are akin to those evinced in T2DM. Specifically, within skeletal muscles in CHF, reductions in mitochondrial oxidative enzyme activity and volume density, concomitant with a mitochondrial dysfunction (Hambrecht *et al.* 1995; Delp *et al.* 1997; Gielen *et al.* 2005; Esposito *et al.* 2010) and a reduced proportion of capillaries that support RBC flux at rest and during contractions (Poole *et al.* 2012) have been revealed. Furthermore, the bioavailability of NO is greatly diminished in both conditions, due in part to an impaired NOs function (Hirai *et al.* 1995; Copp *et al.* 2012; Ferguson *et al.* 2016). As such the functional consequences coalesce at the level of the microcirculation, whereby the ability to spatially distribute QO_2 relative to $\dot{V}O_2$ within and between muscles is significantly curtailed (Poole *et al.* 2012).

Intriguing evidence has emerged advocating therapeutic treatments which aim to augment NO bioavailability, serve to raise the $QO_2/\dot{V}O_2$ ratio, speed $\dot{V}O_2$ kinetics and subsequently improve exercise performance in CHF (Sperandio *et al.* 2012*;* Zamani *et al*. 2015). This has been achieved via the acute pharmacological inhibition of muscle cGMP-specific phosphodiesterase-5 (PDE_5) (Loughney *et al.* 1998) by sildenafil intake (Guazzi, 2008) and by the supplementation of dietary nitrate (NO_3) (Kenjale *et al.* 2011; Ferguson *et al.* 2016; Zamani *et al.* 2016).

Sperandio and colleagues (2012) reported that oversignalling of the NO pathway by PDE_5 inhibition through a single dose of sildenafil, enhanced the on-transient $QO_2/\dot{V}O_2$ matching at the microcirculatory level and thus accelerated the $\dot{V}O_2$ kinetics independent of CO. Given the lack of effect of sildenafil on CO, the improvement in blood flow to and within skeletal muscles can be attributed to the specific vasoactive features of NO. By the same token, it has been reported that augmenting NO bioavailability through dietary nitrate supplementation improved exercise capacity (Zamani *et al*. 2015) and muscle contractile function (Coggan *et al.* 2015) in patients with CHF. Initial impressive explorative work from Ferguson and colleagues (2013a; 2013b; 2015) in healthy rodent models revealed that dietary NO_3 supplementation increased

exercising skeletal muscle BF and raised the $\dot{Q}O_2/\dot{V}O_2$ ratio, within fast-twitch, but not slow-twitch, skeletal muscles. However, more recently the same group observed no preferential fibre-type effects in the significantly increased skeletal muscle blood flow in rodent models with CHF following five days of dietary NO_3 supplementation. Instead, an improved vascular function was demonstrated in the full range of muscle fibre types in CHF (Ferguson *et al*. 2016).

Whilst it is well established that chronic exercise training increases the speed of pulmonary and muscle $\dot{V}O_2$ kinetics in T2DM, the therapeutic potential of acutely increasing NO bioavailability to improve the dynamic coupling of $\dot{Q}O_2/\dot{V}O_2$ at the level of the microcirculation remains unexplored in T2DM. Thus the efficacy of an acute intervention whereby the $\dot{Q}O_2/\dot{V}O_2$ relationship can be manipulated to augment blood-to-myocyte O_2 flux, with the consequent enhancement of oxidative metabolism is extremely encouraging.

Collectively, the encouraging findings from these emerging studies and those of priming exercise demonstrated herein highlight the efficacy for an acute intervention to selectively augment skeletal muscle blood flow distribution during exercise in T2DM. Perhaps a therapeutic strategy in which exercise, dietary and pharmacological interventions are combined to target the NO pathway may provide the most effective means of improving the long term quality of life within the T2DM population.

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Appendices

Appendix 1

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE
THE UNIVERSITY OF DUBLIN

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

We are looking to recruit healthy men and women aged between 30 and 70 years who do not exercise regularly* and are currently overweight (BMI ≥ 25 kg/m^2)**.

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

- Free medical check.
- Body composition.
- Maximal aerobic capacity (VO_{2max}).
- Maximal leg strength and endurance.
- Leg circulation with ultrasound.
- Report of your results (subject to completion of the study).

Participants would attend the human performance laboratory in the Department of Physiology (Watts building) on 4 occasions (plus medical visit). For further information, please contact:

Norita Gildea gildean@tcd.ie

* Less than 1 bout of 60 min of moderate-intensity exercise per week for the past 6 months

** Body Mass Index (BMI) is calculated as body mass in kilograms divided by the square of height in metres, i.e. body mass (kg) / height (m)^2

Appendix 2

Please note that the work for this thesis was the first 'baseline' work of a larger exercise intervention study, as per the participant information leaflet below.

Participant Information Sheet

TRINITY COLLEGE DUBLIN
COLÁISTE NA TRÍONÓIDE
THE UNIVERSITY OF DUBLIN

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

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

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

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

Q: What is required of me if I decide to take part in this study?
A: All participants who are interested in entering the study will be invited to meet a researcher from the Department of Physiology at Trinity College Dublin. You will have the opportunity to go through this information sheet again and ask any questions you might have about the study. You will be given a consent form to take home and complete. This is so you can take time to decide whether or not you want to take part in the study.

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

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

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

Visit 1: Calf blood flow and maximal cycling capacity (VO_{2max}) test (session will last approximately 130 min)

You will have your height and weight recorded and you will be familiarised with the cycle ergometer. During this session you will perform moderate-intensity exercises on the calf ergometer (exercise machine). Each contraction will last 1 second, accompanied by a 2 second rest. You will do this 3 times for 5-6 minutes at a time, for a total of 100-120 contractions per bout, with a 10-minute rest between bouts. The calf test requires you to exercise your right calf muscle by pushing your toes against a plate (plantar-flexion). While performing these tests, the investigator will be monitoring your blood pressure, heart rate, and leg blood circulatory data. You will then complete an incremental cycling exercise test to failure in the upright position. During the exercise test you will be required to increase your level of effort in a stepwise manner until you reach, and cannot sustain a maximum effort. The level of effort will increase by approximately 15-25 watts every minute. Five minutes after the end of the incremental test you will perform a constant-load cycling exercise to volitional fatigue at 85% of the peak power achieved during the incremental test.

Visit 2: Calf blood flow and four submaximal cycling bouts (session will last approximately 125 min).

In this visit we will assess the ability of your popliteal artery to dilate and increase blood flow to the calf after an occlusion test as well as measuring several cardiorespiratory variables (i.e. oxygen uptake, heart rate, cardiac output - heart's blood pumping capacity and muscle's oxygen extraction capacity) during four short cycling bouts. For the first test we will inflate a cuff to a high pressure (i.e. 250mmHg) around your calf to cut off the circulation to this muscle. After a 5-minute period, the cuff will be released, and blood flow will be measured by observing changes in the diameter of the arteries and blood velocity (these measurements will be made using an ultrasound probe on the back of your leg). Following this, you will perform four 6-minute submaximal trials of constant-force cycling exercise, with a 15-minute rest between bouts. Three of these will be at a moderate intensity (80% of your ventilatory threshold) and one at a higher intensity (intensity corresponding to 50% between your ventilatory threshold and peak capacity). These intensities of exercise are individualised according to each person's physical fitness and determined from Visit 1.

Visit 3: Calf and quadriceps muscle deoxygenation and four submaximal cycling bouts (session will last approximately 115 min).

In this visit we will assess the ability of your calf and quadriceps muscles to extract oxygen during occlusion tests followed by four short cycling bouts. For the first two tests we will inflate a cuff to a high pressure (i.e. 250mmHg) around your lower/upper quadriceps to cut off the circulation to the calf/quadriceps muscles. The occlusion in each of these tests should take up to 7 minutes (this time is dependent on each person's individual response and therefore can vary but will not exceed 7 minutes) and will be conducted while a near-infrared lighting device measures the extraction of oxygen in the specified muscles. After this you will repeat the same cycling bouts as per visit 2 (i.e. four 6-minute submaximal trials of constant-force cycling exercise, with a 15-minute rest between bouts where three will be at a moderate intensity and one at a higher intensity). As in the previous visit there will be recordings of the rate of increase of oxygen uptake, cardiac output (heart's blood pumping capacity) and muscle oxygen extraction capacity (rate of muscle oxygenation-deoxygenation) during these exercise bouts.

Visit 4: Four submaximal cycling bouts (session will last up to 120 min).

In this visit you will perform four 6-minute submaximal trials of constant-force cycling exercise at a high intensity. There will be a period of 15 minutes rest between the first and second bout as well as between the third and fourth bout. A period of 45 minutes rest will also be completed between the second and third bout. As in the previous visits there will be recordings of the rate of increase of oxygen uptake, cardiac output (heart's blood pumping capacity) and muscle oxygen extraction capacity (rate of muscle oxygenation-deoxygenation) during these exercise bouts.

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

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

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

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

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

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

VO_{2max} and constant load cycling tests: The study involves both a VO_{2max} test (maximal aerobic capacity test) and a constant load cycling test to determine VO_{2max}, VO_2 kinetics, and cardiac output. Due to its nature, all participants will experience physical stress which may cause some discomfort such as temporary muscle fatigue and shortness of breath. These feelings go away very quickly once the exercise bout has finished. It is possible that you may also experience light headedness, chest discomfort, cramping in the legs and irregular heartbeats during this test. With a VO_{2max} test there is a very slight risk that participants may suffer a heart attack (one occurrence per 5000 tests in a population with a low prevalence of known coronary heart disease). For this reason, a qualified medical doctor will deem the participant suitable to complete an exercise test to exhaustion. All VO_{2max} tests will be supervised by a minimum of two exercise physiologists. Other potential risks, including fainting, nausea, muscle strain, and muscle soreness, will be minimised by a proper warm-up, familiarisation procedure, and a cool-down.

Q: How do I know if I am suitable to take part?
A: You will be considered suitable to take part in this study if you are aged between 18 and 70 years, have a BMI $\geq$ 25 kg/m^2, are untrained ($\leq$ 2 bouts of 60 min of moderate-intensity or "somewhat hard" exercise per week and have not participated in a continuous exercise program for the last 6 months) and have been approved for participation following the free full medical examination.

Please note that in order to be considered you must be free from any diagnosis of cardiovascular or otherwise serious medical ailments/comorbid conditions including autonomic insufficiency/dysfunction, symmetrical neuropathy, abnormal cardiac function or evidence of ischaemic heart disease, angina or other cardiac or pulmonary symptoms limiting exercise performance. Absence of comorbid conditions will be established and confirmed by history, physical

examination and laboratory testing. Controlled hypertensives (<160/90mmHg at rest) will be admitted to the study.

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

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

Q: What if I change my mind during the study?
A: As stated above, you are free to withdraw at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits that you had before entering the study. Although unlikely, please be aware that the investigators may also stop your participation in the study at any time without your consent.

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

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

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

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

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

Ms. Norita Gildea 01-8961770 gildean@tcd.ie

Dr Mikel Egaña 01-8961770 megana@tcd.ie

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

Appendix 3

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

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

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

If interested in receiving more information, please contact:

Norita Gildea: gildean@tcd.ie / 01-8961770

Appendix 4

ELM PARK, DUBLIN 4

Department of endocrinology and diabetes mellitus

PARTICIPANT INFORMATION AND CONSENT FORM

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

NAME OF PRINCIPAL INVESTIGATOR:

Prof Donal O'Shea

Dr. Ronan Canavan

Ms Norita Gildea

Dr Joel Rocha

Prof Simon Green

Dr Mikel Egaña

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

WHAT IS THE PURPOSE OF THIS STUDY?

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

WHY HAVE I BEEN CHOSEN?

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

WHAT WILL HAPPEN IF I VOLUNTEER?

Your participation is entirely voluntary. If you initially decide to take part you can subsequently change your mind without difficulty. This will not affect your future treatment in any way. Furthermore your doctor may decide to withdraw you from this study if he/she feels it is in your best interest.

If you agree to participate, before the commencement of the project you will be requested to provide 2 blood samples (approx. 2.5 tsp.) and you will undergo a screening visit to determine if your current health status meets the inclusion criteria for this study. Upon receiving medical clearance to participate in this, you will be randomly allocated into one of the EXERCISE (continuous or interval training) or CONTROL (non-exercise) groups. Participants in the EXERCISE groups will perform a 12-week supervised exercise training programme. Participants in the CONTROL

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

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

Visit before the start of the study

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

Visits during assessment weeks 0 and 12

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

Visit 2 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Fat tissue thickness by Ultrasound	Allows the measurement of the fat layer above your muscles in the leg.	You will need to rest whilst the fat layer in your leg is measured using an ultrasound machine.	10	175
Calf and quadriceps NIRS occlusion	Allows the assessment of the ability of the muscles of the back of your lower leg and front of the upper thigh to use oxygen.	You will need to be in a lying position whilst a blood pressure cuff is inflated to a high pressure of 250mmHg for up to 7 min for each muscle.	25	
Flow-mediated dilation (FMD)	Allows the assessment of the ability of the artery at the back of your knee to widen and increase the amount of blood that goes to the muscles of the lower leg.	You will need to be in a lying position whilst a blood pressure cuff is inflated around the lower leg to a high pressure of 250mmHg for 5 min.	20	
Calf exercise	Allows the measurement of the amount of blood that goes to the muscles of the lower leg during moderate-intensity exercise.	You will need to push your toes against a force plate that measures the strength of your lower leg. Each push will last 1 second, followed by a 2 second rest and this exercise will continue for 6min. This exercise will then need to be repeated two more times but you will have a 10-minute rest between each 6 min session.	80 (18 min of exercise)	
Constant-load cycling test at moderate-intensity	Allows the measurement of how much oxygen your muscles use during moderate-intensity cycling exercise.	You will need to cycle for 9 min (including 3min warm-up) in the seated position whilst the resistance felt on the pedals will be of a constant moderate-intensity for 6 min. This will be repeated once but you will have 12 min of rest in between.	40 (18 min of exercise)	

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

Visit 4 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Constant-load cycling test at moderate and high intensity	Allows the measurement of how much oxygen your muscles use during moderate and high intensity cycling exercise.	You will need to cycle for 15 min (including 3 min of warm-up) in the seated position whilst the resistance felt on the pedals will be of a moderate-intensity for the first 6 min and a high-intensity for the last 6 min. This will be repeated three more times but there will be a total rest period of 69 min	139 (60 min of exercise)	139

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

Visit 1 **(Location: Trinity College Dublin)**				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Pulse wave velocity (PWV)	Allows the measurement of how stiff your arteries are.	You will need to rest whilst the arteries in your neck and groin are monitored using an external pressure sensor.	20	169
Body measurements	Allows the measurement of your physical characteristics.	You will need to stand still and relaxed whilst measures of height, weight, neck, hip and waist circumferences and body fat are taken.	10	
Constant-load cycling test at moderate and high intensity	Allows the measurement of how much oxygen your muscles use during moderate and high intensity cycling exercise.	You will need to cycle for 15 min (including 3 min of warm-up) in the seated position whilst the resistance felt on the pedals will be of a moderate-intensity for the first 6 min and a high-intensity for the last 6 min. This will be repeated three more times but there will be a total rest period of 69 min	139 (60 min of exercise)	

Visit 2 (Location: Trinity College Dublin)				
Name of test	**What it does**	**What is required**	**Total test time (min)**	**Total visit time (min)**
Fat tissue thickness by Ultrasound	Allows the measurement of the fat layer above your muscles in the leg.	You will need to rest whilst the fat layer in your leg is measured using an ultrasound machine.	10	159
Flow-mediated dilation (FMD)	Allows the assessment of the ability of the artery at the back of your knee to widen and increase the amount of blood that goes to the muscles of the lower leg.	You will need to be in a lying position whilst a blood pressure cuff is inflated around the lower leg to a high pressure of 250mmHg for 5 min.	20	
Calf exercise	Allows the measurement of the amount of blood that goes to the muscles of the lower leg during moderate-intensity exercise.	You will need to push your toes against a force plate that measures the strength of your lower leg. Each push will last 1 second, followed by a 2 second rest and this exercise will continue for 6min. This exercise will then need to be repeated two more times but you will have a 10-minute rest between each 6 min session.	80 (18 min of exercise)	
Constant-load cycling test at moderate-intensity	Allows the measurement of how much oxygen your muscles use during moderate-intensity cycling exercise.	You will need to cycle for 9 min (including 3min warm-up) in the seated position whilst the resistance felt on the pedals will be of a constant moderate-intensity for 6 min.	19 (9 min of exercise)	
Maximal cycling capacity test	Allows the measurement of the maximum amount of oxygen you can use during a maximal cycling effort.	You will need to cycle in the seated position where the resistance felt on the pedals will go up until you cannot rotate them. During this test you will be required to increase your level of effort until you cannot cycle any further.	30 (approx. 12 min of exercise)	

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

Summary of all visits over the 3 month period			
Week	**Number of visits**	**Location of visits**	**Total time per visit (min)**
Screening	1	St. Columcille's Hospital	80
0	4	St. Columcille's Hospital (1) Trinity College Dublin (3)	70 175 115 139
3	2	Trinity College Dublin (2)	169 159
6	2	Trinity College Dublin (2)	169 159
9	2	Trinity College Dublin (2)	169 159
12	4	Trinity College Dublin (4)	70 175 115 139
		Total (min)	**2062**
		Total (hours)	**34.4**

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

- Supervised exercise training programme (Exercise groups only):

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

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

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

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

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

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

ARE THERE ANY BENEFITS FROM MY PARTICIPATION?

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

ARE THERE ANY RISKS INVOLVED IN PARTICIPATING?

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

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

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

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

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

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

WHAT HAPPENS IF I DO NOT AGREE TO PARTICIPATE?

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

CONFIDENTIALITY

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

COMPENSATION

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

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

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

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

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

HAS THIS STUDY BEEN REVIEWED BY AN ETHICS COMMITTEE?

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

CONTACT DETAILS

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

PLEASE TICK YOUR RESPONSE IN THE APPROPRIATE BOX

- I have read and understood the Participant Information. YES □ NO □

- I have had the opportunity to ask questions and discuss the study. YES □ NO □

- I have received satisfactory answers to all my questions. YES □ NO □

- I have received enough information about this study. YES □ NO □

- I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care. YES □ NO □

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

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

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

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

Participant's Signature: _____

Date: _____

Participant's Name in print: _____

Investigator's Signature: _____

Date: _____

Investigator's Name in print: _____

Appendix 5

Medical Questionnaire

DEPARTMENT OF PHYSIOLOGY, TRINITY COLLEGE, DUBLIN.

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

Supervising Researchers: Dr. Joel Rocha and Ms. Norita Gildea

Principal Investigator: Dr Mikel Egaña

Medical Personnel/Physician:_____

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

Subject Name:_____Date:_____

Height:_____Weight:_____

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

Contact Telephone Numbers and E-mail:_____

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

1. Are you a smoker? YES NO _____
2. Do you suffer from asthma? YES NO _____
3. Do you drink alcohol? YES NO _____
4. Do you drink tea/coffee? YES NO _____
5. Do you drink Coke/Pepsi etc? YES NO _____
6. Are you a diabetic? YES NO _____
7. Are you lactose intolerant? YES NO _____
8. Have you ever had any soft tissue injuries (ie: broken bones, ligament damage…)?
   YES NO _____
   _____
9. Does your family have a history of stroke and/or heart disease?
   YES NO _____
   _____
10. Do you have any allergies? YES NO _____

11. Do you have any other medical/health related complaints that should be made aware to the investigators?

    YES NO _____

    _____

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

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

    _____

    _____

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

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

    _____

    _____

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

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

    _____

    _____

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

    YES NO

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

    _____

    _____

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

Signature of Subject:_____Date:_____

Signature of Supervising Researcher:_____Date:_____

Signature of Principal Investigator:_____Date:_____

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

Signature of Physician:___Date:_____

General Examination (Doctors Use Only)

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

Medical Summary

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

Appendix 6

Low activity physical activity recall questionnaire (LOPAR)

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

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

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

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

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

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

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

Household or yard hours/week _____

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

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

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

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

CARD A PHYSICAL ACTIVITIES AT WORK

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

CARD B HOUSEHOLD CHORES AND YARD WORK

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

CARD C RECREATIONAL ACTIVITIES

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

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

Appendix 7

RATE OF PERCEIVED EXERTION

(BORG SCALE)

6	
7	**Very, very light**
8	
9	**Very light**
10	
11	**Fairly light**
12	
13	**Somewhat hard**
14	
15	**Hard**
16	
17	**Very hard**
18	
19	**Very, very hard**
20	**Maximum exertion**

Appendix 8

Experiment 1

Physical characteristics – ND and T2DM

Diabetic Participants	Sex		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	Waist-To-Hip Ratio	Age Predicted HR
2	M		41.2	1.68	92	32.60	5.90	1.09	1	8.2	120	80	1.08	178.8
4	M		52.1	1.83	116.5	34.79	5.40	1.1	1.2	8.9	152	80	1.00	167.9
9	M		52.3	1.80	100.5	31.02	4.00	1.2	1.1	9.4	140	80	1.07	167.7
12	M		48.3	1.82	108.8	32.85	5.00	1.2	1.1	9.4	132	74	1.02	171.7
13	M		40.0	1.80	116.3	35.90	6.90	0.99	0.96	9.5	124	66	1.15	180.0
15	M		41.4	1.74	115	37.98	5.80	0.97	0.99	12.7	138	88	1.07	178.6
21	F		51.4	1.58	76	30.44	9.90	1.1	1.2	8.1	114	76	0.96	168.6
24	F		36.9	1.70	116	40.14	X	1.08	1.1	x	114	66	1.03	183.1
25	M		42.0	1.85	106.4	31.09	3.60	1.23	0.93	8.0	127	90	1.07	178.0
28	M		54.1	1.74	94.7	31.28	7.30	1.1	1.2	7.8	118	78	1.04	165.9
29	M		44.9	1.73	83.2	27.80	3.40	1.05	1.03	7.3	118	72	0.93	175.1
30	M		52.7	1.88	94.1	26.62	6.10	0.98	0.95	9.7	122	88	0.97	167.3
32	M		48.2	1.77	116.7	37.25	5.90	1.1	1.2	x	143	84	1.16	171.8
38	F		60.2	1.65	74.5	27.36	6.10	1.1	1.1	10.5	140	80	0.95	159.8
39	M		42.2	1.70	72.5	25.09	5.90	1	1.2	6.2	110	62	0.96	177.8
42	F		58.7	1.55	87	36.21	X	0.87	0.92	X	124	72	0.89	161.3
43	F		43.8	1.58	59	23.63	7.40	x	x	6.1	102	70	0.89	176.2
Males	**12**	**Mean**	**47.7**	**1.73**	**95.8**	**31.9**	**5.9**	**1.1**	**1.1**	**8.7**	**125.76**	**76.82**	**1.02**	**172.3**
Females	**5**	**S.D.**	6.8	0.1	18.3	4.8	1.6	0.1	0.1	1.73	13.33	8.19	0.08	**6.8**
Total	**17**	**SEM**	1.1	0.0	2.9	0.8	0.3	0.0	0.0	0.55	4.22	2.59	0.03	0.03

Non-Diabetic Participants	Sex		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	WHR	Age Predicted HR
2	M		36.0	1.70	80.4	27.82	6.2	1.2	1.13	7.8	133	78	0.9	184.0
3	F		47.5	1.61	66	25.46	2.1	1.3	1.21	7.4	110	68	0.8	172.5
5	M		40.3	1.78	97.9	30.90	9.4	0.94	1.02	6.0	139	81	0.9	179.7
6	M		59.3	1.78	92.8	29.29	7.9	1.07	1.03	8.6	138	90	1.0	160.7
7	F		52.5	1.63	80.2	30.19	12.7	1.13	1.24	5.3	120	70	0.9	167.5
8	M		34.5	1.69	80.6	28.22	4.2	1.29	1.25	4.7	128	70	0.9	185.5
9	F		53.3	1.60	104.8	40.94	17.4	0.9	1	x	128	86	0.9	166.7
12	M		51.2	1.74	104.4	34.48	5.7	0.86	0.99	6.1	147	92	1.0	168.8
14	M		47.9	1.70	87.7	30.35	7.03	1.32	1.34	7.1	137	80	1.0	172.1
16	F		32.8	1.55	76.4	31.80	14.4	1.22	1.26	4.8	108	76	0.9	187.2
18	M		35.7	1.67	76	27.25	4.2	1.31	1.31	7.9	118	86	1.0	184.3
19	M		35.4	1.81	91.9	28.05	5.3	1.07	1.05	x	128	80	1.0	184.6
21	M		42.8	1.82	100	30.19	5.03	1.23	1.12	7.9	140	84	1.1	177.2
J&P(22)	F		44.0	1.63	85	31.99	X	x	x	x	x	x	x	176.0
J&P(23)	M		34.0	1.77	105	33.52	X	x	x	x	x	x	x	186.0
J&P(24)	M		50.0	1.93	120	32.22	X	x	x	x	x	x	x	170.0
J&P(25)	M		45.0	1.79	100	31.21	X	x	x	x	x	x	x	175.0
Males	**12**	**Mean**	**43.7**	**1.72**	**91.1**	**30.8**	**7.8**	**1.1**	**1.2**	**6.7**	**128.8**	**80.1**	**0.9**	**176.3**
Females	**5**	**S.D.**	8.1	0.1	13.8	3.5	4.5	**0.2**	**0.1**	**1.4**	**11.9**	**7.6**	**0.1**	**8.1**
Total	**17**	**SEM**	1.8	0.0	3.0	0.8	1.0	0.0	0.0	0.3	2.6	1.7	0.0	1.8

ND VO_2 Data

NDSP RAMP															
ID	Oxygen Uptake										Power Output				
	Peak (L.min)	Peak (mL.kg.min)	RCP (L.min)	RCP (mL.kg.min)	VT (L.min)	VT (mL/kg/min)	VO2 @ VT %	VO2 @ RCP %	Slope Pre-VT (mL.min.W)	Slope Post-VT (mL.min.W)	Peak (W)	RCP (W)	RCP (%)	VT (W)	VT (%)
2	2.15	26.72	1.98	24.61	1.46	18.11	67.8	92.1	8.39	8.52	195	162	83	108	55.4
3	1.72	26.02	1.45	21.97	1.16	17.59	67.6	84.4	9.86	9.52	154	116	75	105	68.2
5	3.21	32.83	2.86	29.19	1.75	17.83	54.3	88.9	8.84	11.69	260	228	88	123	47.3
6	1.93	20.77	1.78	19.16	1.14	12.30	59.2	92.3	5.80	8.22	160	137	86	58	36.3
7	2.41	30.07	2.19	27.24	1.88	23.44	77.9	90.6	10.64	10.56	182	157	86	129	70.9
8	3.12	38.70	2.33	28.85	2.33	28.95	74.8	74.5	10.48	10.39	273	190	70	180	65.9
9	2.06	19.64	1.83	17.48	1.71	16.35	83.3	89.0	10.17	7.99	137	105	77	92	67.2
12	2.72	26.05	2.45	23.47	1.62	15.54	59.6	90.1	8.86	10.91	196	174	89	87	44.4
14	2.06	23.48	1.73	19.69	1.40	15.92	67.8	83.9	7.55	8.72	166	104	63	73	44.0
16	1.81	23.70	1.60	20.97	1.05	13.78	58.1	88.5	8.17	11.13	129	100	78	54	41.9
18	2.83	37.29	2.68	35.28	1.84	24.16	64.8	94.6	10.12	10.74	233	213	91	130	55.8
19	3.12	33.93	2.66	28.91	2.16	23.51	69.3	85.2	9.03	8.84	274	221	81	156	56.9
21	2.88	28.77	2.35	23.47	1.65	16.49	57.3	81.6	10.07	10.28	238	181	76	113	47.5
ADG22	2.23	26.21	1.91	22.47	1.98	23.29	88.9	85.7	9.74	8.33	190	160	84	145	76.3
DDG23	3.14	29.87	2.67	25.43	2.25	21.47	71.9	85.1	9.85	14.11	272	210	77	180	66.2
DPG24	3.30	27.48	2.72	22.67	2.33	19.42	70.7	82.5	9.64	11.42	269	220	82	180	66.9
RDG25	3.50	35.04	3.15	31.50	2.47	24.70	70.5	89.9	9.70	9.69	311	270	87	205	65.9
MEAN	**2.60**	**28.62**	**2.25**	**24.84**	**1.78**	**19.58**	**68.46**	**87.00**	**9.23**	**10.06**	**214.06**	**173.40**	**80.66**	**124.59**	**57.46**
SD	**0.58**	**5.50**	**0.49**	**4.75**	**0.44**	**4.53**	**9.34**	**4.89**	**1.23**	**1.59**	**56.01**	**50.05**	**7.41**	**45.05**	**12.01**

ID	Peak Cardiac Output (L.min)	Rest SBP	Rest DBP	Rest Map	Peak HR	Time to failure	100%SBP	100% DBP	100% MAP	RPE	RER
2	x	133.0	78.0	96.3	182.0	585.0	x	x	x	20.0	1.3
3	x	110.0	68.0	82.0	187.0	616.0	x	x	x	19.0	1.2
5	x	139.0	81.0	100.3	X	624.0	x	x	x	19.0	1.3
6	x	138.0	90.0	106.0	153.0	640.0	x	x	x	18.0	1.2
7	x	120.0	70.0	86.7	160.0	728.0	x	x	x	15.0	1.1
8	16.0	128.0	70.0	89.3	175.0	819.0	122.0	75.0	91.6	19.0	1.2
9	x	128.0	86.0	100.0	141.0	548.0	198.9	108.2	148.3	19.0	1.1
12	15.6	147.0	92.0	110.3	149.0	588.0	143.5	95.1	111.6	17.0	1.1
14	13.8	137.0	80.0	99.0	174.0	664.0	174.4	105.5	131.0	20.0	1.1
16	11.6	108.0	76.0	86.7	182.0	516.0	174.6	133.6	146.2	18.0	1.1
18	-	118.0	86.0	96.7	185.0	699.0	x	x	x	20.0	1.2
19	17.2	128.0	80.0	96.0	167.0	657.6	x	x	x	15.0	1.2
21	18.0	140.0	84.0	102.7	179.0	714.0	x	x	x	17.0	1.1
ADG22	x	109.0	74.0	85.7	X	742.0	186.0	96.0	123.0	X	X
DDG23	x	115.0	79.0	91.0	X	764.0	183.0	114.0	139.0	X	X
DPG24	x	118.0	78.0	91.3	X	747.0	165.0	104.0	125.0	X	X
RDG25	x	115.0	80.0	95.0	X	939.0	186.0	96.0	123.0	X	X
MEAN	**15.37**	**125.35**	**79.53**	**95.00**	**169.50**	**681.80**	**170.37**	**103.05**	**126.53**	**18.15**	**1.16**
SD	**2.34**	**12.25**	**6.77**	**7.69**	**15.38**	**105.17**	**23.95**	**15.95**	**17.71**	**1.72**	**0.08**

ND [HHb+Mb] Data

NDSP NIRS HHb VL% v's PO(%) (Double-Linear)						
ID	Intercept	Slope 1	C	Breakpoint	Slope 2	r^2
2	-12.459	1.131	0.027	89.997	1.104	0.925
3	-3.366	1.153	1.010	86.863	0.143	0.984
5	23.322	0.602	-1.000	94.119	1.602	0.637
6	x	x	x	x	x	x
7	-7.783	1.040	0.251	92.377	0.789	0.921
8	-6.137	0.955	-0.273	69.861	1.228	0.996
9	x	x	x	x	x	x
12	3.086	1.062	1.495	85.346	-0.433	0.960
14	-5.921	1.298	1.315	80.004	-0.017	0.980
16	11.115	1.305	1.542	56.532	-0.238	0.914
18	-4.464	1.320	0.850	65.006	0.470	0.990
19	-4.730	1.217	0.906	80.891	0.311	0.995
21	4.032	1.095	1.259	87.133	-0.165	0.990
ADG22	x	x	x	x	x	x
DDG23	-13.996	1.429	2.268	78.417	-0.839	0.983
DPG24	-14.000	1.215	1.590	91.993	-0.375	0.929
RDG25	x	x	x	x	x	x
MEAN	**-2.41**	**1.14**	**0.86**	**81.43**	**0.28**	**0.94**
SD	**10.61**	**0.21**	**0.89**	**11.47**	**0.73**	**0.10**

ND [HHb+Mb] Data

NDSP NIRS HHb VL% v's PO (W)(Double-Linear)						
ID	Intercept	Slope 1	C	Breakpoint	Slope 2	r^2
2	-23.992	0.582	-0.497	165.000	1.079	0.959
3	-27.761	0.923	0.809	135.107	0.114	0.984
5	-105.771	0.551	-2.159	225.000	2.710	0.768
6	x	x	x	x	x	x
7	-128.821	1.271	0.089	169.295	1.182	0.920
8	-15.116	0.395	-0.265	240.370	0.660	0.997
9	x	x	x	x	x	x
12	-12.262	0.641	0.905	169.836	-0.264	0.953
14	-14.395	0.896	0.688	120.000	0.208	0.978
16	-29.849	1.463	1.721	76.750	-0.258	0.900
18	-13.995	0.581	0.424	180.000	0.157	0.989
19	-24.777	0.532	0.396	222.417	0.136	0.995
21	1.471	0.474	0.544	208.338	-0.070	0.989
ADG22	x	x	x	x	x	x
DDG23	-18.914	0.559	0.889	209.379	-0.330	0.976
DPG24	-21.801	0.483	0.632	242.990	-0.150	0.953
RDG25	x	x	x	x	x	x
MEAN	**-33.54**	**0.72**	**0.32**	**181.88**	**0.40**	**0.95**
SD	**38.33**	**0.33**	**0.93**	**49.81**	**0.85**	**0.06**

T2DM VO_2 Data

DSP RAMP															
ID	Oxygen Uptake										Power Output				
	Peak (L.min)	Peak (mL.kg.min)	RCP (L.min)	RCP (mL.kg.min)	VO2@RCP%	VT ml.kg.min	VT (L.min)	V02@ VT %Peak	Slope Pre-VT (mL.min.W)	Slope Post-VT (mL.min.W)	Peak (W)	RCP (W)	RCP (%)	VT (W)	VT (%)
2	1.86	20.24	1.59	17.32	85.6		1.27	68.05	8.08	9.35	139	108	78	70	50.4
4	3.18	27.30	2.99	25.63	93.9	19.02	2.22	69.66	10.42	11.54	231	207	90	133	57.6
9	2.39	23.74	2.35	23.34	98.3	20.29	2.04	85.46	7.36	7.00	203	175	86	140	68.8
12	2.43	22.30	2.18	20.06	90.0	12.81	1.39	57.46	9.73	9.44	200	168	84	87	43.5
13	2.58	22.18	2.01	17.28	77.9	12.24	1.42	55.19	8.24	12.12	185	143	77	84	45.4
15	3.22	28.02	2.95	25.66	91.6	22.24	2.56	79.39	9.51	13.03	245	190	78	150	61.2
21	1.66	21.82	1.40	18.36	84.0	15.66	1.19	71.69	9.24	10.57	120	83	70	65	53.8
24	2.07	17.84	2.00	17.25	96.7	15.76	1.83	88.35	7.57	8.19	155	140	90	93	59.7
25	2.63	24.67	2.37	22.23	90.3	19.35	2.06	78.44	9.99	9.54	221	179	81	152.5	69.0
28	1.79	18.85	1.71	18.08	95.6	15.06	1.43	79.66	9.64	7.28	186	128	69	89.6	48.2
29	2.22	26.72	1.88	22.55	84.6	18.58	1.55	69.55	9.70	8.96	208	160	77	91	43.8
30	2.45	25.98	2.28	24.26	93.3	16.97	1.60	65.32	9.21	10.88	213	191	90	124	58.2
32	3.12	26.69	x	x	x	x	x	x	x	x	246	x	x	x	x
38	1.51	20.24	1.46	19.53	96.8	14.20	1.06	70.16	4.95	4.01	97	93	96	48.8	50.3
39	1.16	15.93	1.08	14.87	93.5	13.71	0.99	86.06	6.51	-1.63	96	71.5	75.0	58.6	60.9
42	1.62	18.61	1.53	17.55	94.5	13.69	1.19	73.56	8.88	11.12	91	79.7	87.6	57	62.8
43	1.24	20.98	1.13	19.19	91.4	16.07	0.95	76.58	9.13	6.37	103	80	77.6	82	80.0
MEAN	**2.18**	**22.48**	**1.93**	**20.20**	**91.1**	**16.1**	**1.55**	**73.41**	**8.63**	**8.61**	**172.90**	**141.04**	**81.79**	**95.27**	**57.09**
SD	**0.65**	**3.66**	**0.57**	**3.32**	**5.6**	**3.0**	**0.47**	**9.53**	**1.45**	**3.59**	**55.07**	**45.07**	**8.04**	**34.11**	**10.19**

ID	Peak Cardiac Output (L.min)	Rest SBP	Rest DBP	Rest MAP	Peak HR	Time to Failure	100%SBP	100% DBP	100% MAP	RPE	RER
2	x	120.0	80.0	93.3	137.0	507.0	175.9	106.5	138.2	18.0	1.2
4	x	152.0	80.0	104.0	157.0	1004.0	219.8	120.1	162.8	19.0	1.2
9	12.6	140.0	80.0	100.0	171.0	699.0	189.3	96.6	134.5	20.0	1.1
12	13.2	132.0	74.0	93.3	158.0	690.0	179.5	115.3	142.9	17.0	1.2
13	14.5	124.0	66.0	85.3	163.0	645.0	x	x	x	20.0	1.1
15	14.5	138.0	88.0	104.7	174.0	825.0	x	x	x	20.0	1.2
21	x	114.0	76.0	88.7	169.0	780.0	189.3	96.6	134.5	17.0	1.1
24	12.5	114.0	66.0	82.0	178.0	700.0	x	x	x	17.0	1.1
25	x	127.0	90.0	102.3	165.0	626.4	200.3	127.8	158.1	17.0	1.1
28	x	118.0	78.0	91.3	167.0	648.0	216.7	145.1	180.9	12.0	1.1
29	11.7	118.0	72.0	87.3	176.0	714.0	185.2	98.4	129.3	20.0	1.1
30	11.3	122.0	88.0	99.3	144.0	729.0	154.7	92.0	119.6	19.0	1.1
32	x	143.0	84.0	103.7	181.0	828.0	x	x	x	17.0	1.1
38	x	130.0	75.0	93.3	178.0	642.0	x	x	x	17.0	1.1
39	x	110.0	62.0	78.0	143.0	465.0	159.1	48.9	85.1	18.0	1.1
42	8.8	118.0	78.0	91.3	135.0	606.0	185.1	95.8	129.2	18.0	1.4
43	9.6	102.0	70.0	80.7	155.0	492.0	185.2	98.4	129.3	20.0	1.3
MEAN	**12.08**	**124.82**	**76.88**	**92.86**	**161.82**	**682.38**	**186.68**	**103.46**	**137.04**	**18.00**	**1.16**
SD	**1.97**	**13.00**	**8.08**	**8.52**	**14.80**	**133.64**	**19.43**	**23.49**	**23.87**	**2.00**	**0.08**

T2DM [HHb+Mb] Data

DSP NIRS HHb VL% v's PO (%) (Double-Linear)						
ID	Intercept	Slope 1	C	Breakpoint	Slope 2	r^2
2	-16.341	1.593	1.709	71.000	-0.116	0.943
4	-2.519	1.194	1.411	84.426	-0.217	0.989
9	-14.604	1.376	2.050	82.516	-0.675	0.982
12	5.265	1.033	2.496	91.651	-1.463	0.978
13	0.501	1.144	0.848	81.140	0.296	0.992
15	-0.841	1.218	0.889	74.083	0.329	0.986
21	x	x	x	x	x	x
24	-30.999	2.252	2.003	56.458	0.249	0.909
25	-22.791	1.355	0.626	81.999	0.728	0.951
28	-4.400	1.540	1.559	64.900	-0.019	0.975
29	1.560	1.230	2.080	79.700	-0.850	0.970
30	-18.000	1.358	0.453	72.510	0.905	0.637
32	-3.780	1.067	2.822	94.347	-1.755	0.978
38	x	x	x	x	x	x
39	-49.057	1.730	1.242	81.580	0.488	0.876
42	x	x	x	x	x	x
43	-92.986	2.625	1.755	62.799	0.870	0.993
MEAN	**-17.79**	**1.48**	**1.57**	**77.08**	**-0.09**	**0.94**
SD	**26.28**	**0.46**	**0.70**	**10.76**	**0.83**	**0.09**

T2DM [HHb+Mb] Data

DSP NIRS HHb VL% v's PO (W)(Double-Linear)						
ID	Intercept	Slope 1	C	Breakpoint	Slope 2	r^2
2	-24.754	1.098	1.299	112.570	-0.201	0.964
4	-11.338	0.568	0.673	196.850	-0.105	0.989
9	-36.486	0.809	1.209	169.680	-0.400	0.979
12	-4.047	0.569	1.401	184.330	-0.832	0.972
13	-11.214	0.696	0.515	152.095	0.180	0.990
15	-8.957	0.538	0.393	184.085	0.146	0.984
21	x	x	x	x	x	x
24	-49.999	1.547	1.306	93.856	0.241	0.918
25	-28.277	0.626	0.420	194.998	0.207	0.964
28	-9.533	0.837	0.874	126.380	-0.037	0.978
29	-6.916	0.617	0.993	169.007	-0.376	0.973
30	-20.000	0.657	0.229	158.710	0.428	0.740
32	-10.175	0.497	1.044	214.990	-0.547	0.977
38	x	x	x	x	x	x
39	-46.925	1.335	1.247	110.000	0.087	0.960
42	x	x	x	x	x	x
43	-140.760	5.205	3.460	41.012	1.745	0.992
MEAN	**-29.24**	**1.11**	**1.08**	**150.61**	**0.04**	**0.96**
SD	**35.39**	**1.22**	**0.79**	**48.20**	**0.60**	**0.06**

ND and T2DM Haematological results

NDSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
2	3.80	4.70	2.77	1.35	1.50	1.10
3	4.00	4.40	3.24	1.76	1.10	1.20
5	x	X	X	X	X	X
6	x	X	X	X	X	X
7	3.90	X	4.82	3.37	1.20	0.60
8	3.90	5.08	2.72	1.27	1.20	0.60
9	4.90	X	3.37	1.35	1.50	1.10
12	x	X	X	X	X	X
14	4.00	5.10	4.56	2.54	1.20	1.80
16	3.90	4.90	3.13	1.58	1.00	1.10
18	5.90	5.20	4.48	2.75	1.10	1.50
19	3.70	X	2.67	1.63	0.70	0.70
21	4.10	5.30	4.63	2.62	1.40	1.90
ADG22	x	X	X	X	X	X
DDG23	x	X	X	X	X	X
DPG24	x	X	X	X	X	X
RDG25	x	X	X	X	X	X
Mean	4.21	4.95	3.64	2.02	1.19	1.16
SD	**0.68**	**0.31**	**0.88**	**0.73**	**0.24**	**0.46**

DSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
2	16.40	10.70	X	x	x	8.40
4	7.00	6.10	4.00	1.90	1.60	1.10
9	7.60	6.80	3.90	1.80	1.50	1.30
12	5.70	6.50	5.60	3.20	0.93	3.22
13	x	6.90	4.00	2.00	1.20	1.80
15	8.50	7.60	4.90	x	1.00	1.80
21	6.90	6.20	x	x	x	x
24	7.00	6.50	3.70	1.98	0.96	1.70
25	10.50	9.70	x	x	x	x
28	7.40	6.70	4.70	2.70	1.40	1.40
29	9.70	7.40	4.30	1.80	0.80	3.80
30	x	6.50	4.40	2.60	1.30	1.00
32	x	7.40	3.30	1.10	1.80	0.90
38	6.900	5.300	5.000	3.300	1.030	1.500
39	4.800	x	x	x	x	x
42	x	x	4.800	x	1.500	1.400
43	9.800	7.300	x	x	x	x
Mean	8.32	7.17	4.38	2.24	1.25	2.26
SD	**2.92**	**1.38**	**0.65**	**0.69**	**0.31**	**2.03**

ND and T2DM Accelerometry Data

NDSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
2	X	X	X	X
3	X	X	X	X
5	19.81	3.26	0.55	0.37
6	18.91	4.62	0.42	0.06
7	19.40	3.73	0.68	0.19
8	22.16	1.67	0.16	0.01
9	19.44	4.07	0.20	0.29
12	19.06	4.59	0.32	0.03
14	17.28	4.53	1.47	0.71
16	18.60	4.28	0.93	0.19
18	18.11	4.74	0.94	0.21
19	X	X	X	X
21	X	X	X	X
ADG22	18	5.437	0.440	0.053
DDG23	19.4567	3.853	0.620	0.070
DPG24	16.7267	5.860	1.123	0.290
RDG25	19.0033	4.093	0.820	0.083
Mean	18.925	4.210	0.667	0.197
SD	**1.326**	**1.028**	**0.381**	**0.192**

DSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
2	x	x	x	x
4	18.3833	3.793	1.583	0.240
9	17.81	5.97	0.16	0.06
12	x	x	x	x
13	x	x	x	x
15	17.04	6.13	0.80	0.03
21	18.74	5.07	0.18	0.01
24	16.82	6.60	0.51	0.07
25	x	x	x	x
28	x	x	x	x
29	x	x	x	x
30	19.2767	4.403	0.253	0.067
32	x	x	x	x
38	x	x	x	x
39	x	x	x	x
42	x	x	x	x
43	x	x	x	x
Mean	18.012	5.327	0.583	0.078
SD	**0.967**	**1.091**	**0.548**	**0.083**

ND and T2DM LOPAR Data

NDSP	LOPAR MET/hours Per Week
2	177.5
3	480.3
5	186.8
6	110.5
7	x
8	192.8
9	152.1
12	126.5
14	95.3
16	272.0
18	125.3
19	x
21	x
ADG22	x
DDG23	x
DPG24	x
RDG25	x
Mean	191.9
SD	**113.6**

DSP	LOPAR MET/hours Per Week
2	124
4	116.5
9	125.8
12	74.0
13	270.8
15	150.5
21	55.5
24	x
25	x
28	x
29	x
30	119.0
32	x
38	280
39	x
42	x
43	143.8
Mean	146.0
SD	**74.1**

Appendix 9

Experiment 2

Physical Characteristics – ND & T2DM

Non-Diabetic Participants	Sex		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	WHR
2	M		36.0	1.70	80.4	27.82	6.2	1.2	1.13	7.8	133	78	0.9
3	F		47.5	1.61	66	25.46	2.1	1.3	1.21	7.4	110	68	0.8
4	F		33.5	1.73	84.2	28.13	8.7	0.94	1.02	5.0	116	68	0.8
5	M		40.3	1.78	97.9	30.90	9.4	1.07	1.03	6.0	139	81	0.9
6	M		59.3	1.78	92.8	29.29	7.9	1.13	1.24	8.6	138	90	1.0
7	F		52.5	1.63	80.2	30.19	12.7	1.29	1.25	5.3	120	70	0.9
8	M		34.5	1.69	80.6	28.22	4.2	0.9	1	4.7	128	70	0.9
9	F		53.3	1.60	104.8	40.94	17.4	0.86	0.99		128	86	0.9
12	M		51.2	1.74	104.4	34.48	5.7	1.32	1.34	6.1	147	92	1.0
14	M		47.9	1.70	87.7	30.35	7.03	1.22	1.26	7.1	137	80	1.0
16	F		32.8	1.55	76.4	31.80	14.4	1.31	1.31	4.8	108	76	0.9
18	M		35.7	1.67	76	27.25	4.2	1.23	1.12	7.9	118	86	1.0
Males	**7**	**Mean**	**43.7**	**1.68**	**86.0**	**30.4**	**8.3**	**1.1**	**1.2**	**6.4**	**126.8**	**78.8**	**0.9**
Females	**5**	**S.D.**	9.3	0.1	12.0	4.1	4.5	**0.2**	**0.1**	**1.4**	**12.4**	**8.6**	**0.1**
Total	**12**	**SEM**	2.0	0.0	2.6	0.9	1.0	0.0	0.0	0.3	2.7	1.9	0.0

Diabetic Participants	Sex		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	Waist-To-Hip Ratio
2	M		41.2	1.68	92	32.60	5.90	1.09	1	8.2	120	80	1.08
3	M		55.2	1.74	94.5	31.21	7.80	1.14	1.35	12.6	146	76	1.06
5	M		55.7	1.70	75.5	26.12	4.90	1.14	1.27	7.1	110	60	0.93
9	M		52.3	1.80	100.5	31.02	4.00	1.2	1.1	9.4	140	80	1.07
13	M		40.0	1.80	116.3	35.90	6.90	0.99	0.96	9.5	124	66	1.15
15	M		41.4	1.74	115	37.98	5.80	0.97	0.99	12.7	138	88	1.07
18	F		42.8	1.58	65	26.04	7.50	1.2	1.2	9.4	118	70	0.87
21	F		51.4	1.58	76	30.44	9.90	1.1	1.2	8.1	114	76	0.96
24	F		36.9	1.70	116	40.14	X	1.08	1.1	x	114	66	1.03
38	F		60.2	1.65	74.5	27.36	6.10	1.07	1.05	9	130	75	x
39	M		42.2	1.70	72.5	25.09	5.90	1	1.2	6.2	110	62	0.96
40	F		57.4	1.58	102	40.86	X	0.95	1	x	x	x	x
Males	**7**	**Mean**	**48.0**	**1.69**	**91.7**	**32.1**	**6.5**	**1.1**	**1.1**	**9.2**	**124.00**	**72.64**	**1.02**
Females	**5**	**S.D.**	8.1	0.1	18.6	5.6	1.7	0.1	0.1	2.10	12.71	8.61	0.08

Ramp Data – ND & T2DM

NDSP Ramp										
NDSP ID	Oxygen Uptake				Power Output		Peak Cardiac Output (L.min)	Peak Hr	Time to failure	CO @ 80% VT (L.min)
	Peak (L.min)	Peak (mL.kg.min)	VO2@VT (L.min)	VO2 @ VT (mL.kg.min)	Peak (W)	VT (W)				
2	2.15	26.72	1.46	18.11	195	108	x	182.0	585.0	9.1
3	1.72	26.02	1.16	17.59	154	105	x	187.0	616.0	6.8
4	2.18	25.89	1.24	14.73	175	89	14.3	173.0	x	8.3
5	3.21	32.83	1.75	17.83	260	123	x	x	624.0	11.5
6	1.93	20.77	1.14	12.30	160	58	x	153.0	640.0	9.9
7	2.41	30.07	1.88	23.44	182	129	x	160.0	728.0	17.5
8	3.12	38.70	2.33	28.95	273	180	16.0	175.0	819.0	16.0
9	2.06	19.64	1.71	16.35	137	92	x	141.0	548.0	11.6
12	2.72	26.05	1.62	15.53	196	87	15.6	149.0	588.0	11.5
14	2.06	23.48	1.40	15.91	166	73	13.8	174.0	664.0	12.4
16	1.81	23.70	1.05	13.78	129	54	11.6	182.0	516.0	10.3
18	2.83	37.29	1.84	24.15	233	130	-	185.0	699.0	15.7
MEAN	**2.35**	**27.60**	**1.55**	**18.22**	**188.33**	**102.33**	**14.26**	**169.18**	**638.82**	**11.72**
SD	**0.51**	**6.04**	**0.38**	**4.87**	**45.98**	**35.24**	**1.74**	**15.86**	**86.16**	**3.25**

DSP Ramp										
DSP ID	Oxygen Uptake				Power Output		Peak Cardiac Output (L.min)	Peak Hr	Time to failure	CO @ 80% VT (L.min)
	Peak (L.min)	Peak (mL.kg.min)	VO2@VT (L.min)	VO2 @ VT (mL.kg.min)	Peak (W)	VT (W)				
2	1.86	20.24	1.27	13.77	139	70	x	137.0	507.0	12.9
3	1.79	18.95	1.33	14.08	139	81	x	149.0	636.0	10.3
5	2.22	29.46	1.54	20.37	184	97	x	176.0	816.0	13.0
9	2.39	23.74	2.04	20.29	203	140	12.6	171.0	699.0	13.0
13	2.58	22.18	1.42	12.24	185	84	14.5	163.0	645.0	x
15	3.22	28.02	2.56	22.24	245	150	14.5	174.0	825.0	13.2
18	1.41	21.62	1.11	17.06	107	70	10.0	160.0	508.0	8.7
21	1.66	21.82	1.19	15.66	120	65	x	169.0	780.0	x
24	2.07	17.84	1.83	15.76	155	93	14.7	178.0	700.0	12.5
38	1.51	20.24	1.06	14.20	97	48.77	x	178.0	642.0	5.6
39	1.16	15.93	0.99	13.71	96	58.58	x	143.0	465.0	x
40	1.74	17.09	1.40	13.76	141	79	x	x	644.0	
MEAN	**1.97**	**21.43**	**1.48**	**16.10**	**150.92**	**86.20**	**13.26**	**163.45**	**655.58**	**11.15**
SD	**0.57**	**4.10**	**0.46**	**3.22**	**45.75**	**30.66**	**2.01**	**14.56**	**118.62**	**2.76**

ND VO2 kinetics data

NDSP 80%VT Unprimed						
ID	**Baseline**	**Amp**	**TD1**	**Tau**	**End Amp**	**VO2 gain**
2	0.859	0.822	20.238	49.397	1.681	10.762
3	0.637	0.677	29.003	36.505	1.314	9.154
4	0.660	0.598	20.306	39.993	1.258	9.774
5	0.770	0.826	23.083	30.248	1.596	9.343
6	0.817	0.309	19.588	44.335	1.126	8.490
7	0.653	0.815	28.574	21.503	1.468	8.747
8	0.958	1.274	29.957	26.534	2.232	9.505
9	0.721	0.766	20.917	33.469	1.487	12.040
12	0.899	0.561	25.003	27.651	1.460	9.418
14	0.759	0.163	30.632	28.851	0.922	3.364
16	0.810	0.353	21.253	47.131	1.163	10.634
18	0.756	1.019	23.385	30.485	1.775	10.842
MEAN	**0.775**	**0.682**	**24.328**	**34.675**	**1.457**	**9.339**
SD	**0.100**	**0.311**	**4.163**	**8.834**	**0.346**	**2.138**

NDSP 80%VT Primed						
ID	**Baseline**	**Amp**	**TD1**	**Tau**	**End Amp**	**VO2 gain**
2	0.969	0.827	19.956	47.075	1.796	10.823
3	0.757	0.831	26.999	33.970	1.588	11.226
4	0.720	0.650	24.452	29.847	1.370	10.616
5	0.806	0.837	23.417	30.163	1.643	9.474
6	0.805	0.321	31.840	26.047	1.126	8.826
7	0.645	0.619	18.335	19.067	1.264	6.642
8	0.898	1.470	27.278	17.501	2.368	10.969
9	0.816	0.725	32.130	22.190	1.541	11.393
12	0.911	0.563	27.614	26.109	1.474	9.440
14	0.747	0.253	16.328	11.831	1.000	5.237
16	0.895	0.353	6.753	40.235	1.248	10.620
18	0.909	1.147	17.375	27.388	2.056	12.198
MEAN	**0.823**	**0.716**	**22.706**	**27.618**	**1.539**	**9.789**
SD	**0.096**	**0.348**	**7.320**	**9.763**	**0.392**	**2.046**

T2DM VO_2 kinetics data

DSP 80% VT Unprimed						
ID	**Baseline**	**Amp**	**TD1**	**Tau**	**End Amp**	**VO2 gain**
2	1.053	0.497	27.091	53.036	1.550	10.808
3	0.504	0.800	19.631	30.265	1.304	14.590
5	0.911	0.700	24.836	61.481	1.611	10.350
9	1.465	0.779	26.677	49.298	2.244	7.662
13	0.931	0.585	17.249	62.180	1.516	10.230
15	1.133	1.089	9.767	52.762	2.222	9.898
18	0.606	0.434	17.720	27.007	1.040	9.525
21	0.777	0.407	16.753	24.006	1.184	9.784
24	1.037	0.502	17.131	36.598	1.540	7.849
38	0.806	0.235	8.001	49.880	1.041	8.103
39	0.736	0.289	26.976	30.981	1.025	7.833
40	0.937	0.325	36.386	32.244	1.262	6.109
Mean	**0.908**	**0.554**	**20.685**	**42.478**	**1.461**	**9.395**
SD	**0.254**	**0.250**	**8.035**	**13.715**	**0.417**	**2.160**

DSP 80% VT Primed						
ID	**Baseline**	**Amp**	**TD1**	**Tau**	**End Amp**	**VO2 gain**
2	1.049	0.434	24.803	34.148	1.483	9.444
3	0.889	0.443	36.000	25.160	1.332	8.092
5	1.008	0.586	25.000	57.705	1.594	8.662
9	1.497	0.754	27.755	37.471	2.251	7.416
13	1.011	0.559	22.948	43.203	1.570	9.768
15	1.238	0.989	21.381	30.592	2.227	8.991
18	0.643	0.469	18.677	46.662	1.112	10.275
21	0.795	0.426	22.745	18.743	1.221	10.238
24	1.067	0.462	13.042	30.049	1.529	7.222
38	0.830	0.260	3.037	18.252	1.089	8.955
39	0.723	0.334	25.809	30.493	1.057	9.063
40	1.070	0.310	17.100	35.633	1.380	5.828
Mean	**0.985**	**0.502**	**21.525**	**34.009**	**1.487**	**8.663**
SD	**0.234**	**0.202**	**8.172**	**11.362**	**0.398**	**1.323**

ND [HHb+Mb] kinetics

NDSP 80%VT Unprimed						
ID	**Baseline**	**A1**	**TD1**	**Tau**	**TD1+TAU**	**a+b**
2	-29.096	28.500	15.000	7.000	22.000	-0.596
3	-128.232	94.408	10.266	18.033	28.298	-33.824
4	-58.999	33.440	13.591	9.027	22.618	-25.559
5	-143.000	39.608	11.039	16.937	27.975	-103.391
6	-72.905	59.479	16.836	12.466	29.301	-13.427
7	-44.252	16.901	12.703	8.763	21.467	-27.351
8	-106.713	114.413	8.666	30.808	39.474	7.700
9	-64.599	11.076	8.233	17.128	25.361	-53.523
12	-98.204	68.383	10.917	10.428	21.345	-29.821
14	-56.465	36.770	4.452	22.135	26.587	-19.695
16	-43.759	15.182	13.459	8.817	22.276	-28.577
18	20.862	170.647	10.496	10.450	20.945	191.508
MEAN	**-68.78**	**57.40**	**11.30**	**14.33**	**25.64**	**-11.38**
SD	**45.19**	**47.95**	**3.32**	**6.99**	**5.31**	**69.78**

NDSP 80%VT Primed						
ID	Baseline	A1	TD1	Tau	TD1+TAU	a+b
2	-26.900	21.970	11.968	8.997	20.965	-4.930
3	18.664	86.000	9.000	14.000	23.000	104.664
4	-46.100	38.915	10.013	15.045	25.058	-7.185
5	-46.000	36.053	13.091	24.248	37.339	-9.947
6	-27.333	76.374	12.001	31.898	43.899	49.041
7	-23.153	27.979	10.270	17.154	27.425	4.825
8	-222.800	101.967	11.992	18.018	30.010	-120.833
9	-23.760	10.499	10.240	10.136	20.376	-13.261
12	-86.800	85.431	10.158	15.000	25.158	-1.369
14	-6.767	31.689	13.667	30.459	44.126	24.922
16	-62.003	21.439	11.794	13.859	25.653	-40.564
18	36.261	154.448	8.547	21.035	29.582	190.709
MEAN	**-43.06**	**57.73**	**11.06**	**18.32**	**29.38**	**14.67**
SD	**65.57**	**43.11**	**1.59**	**7.32**	**8.20**	**76.51**

T2DM [HHb+Mb] kinetics

DSP 80%VT Unprimed						
ID	Baseline	A1	TD1	Tau	TD1+TAU	a+b
2	-11.808	138.443	7.679	27.482	35.161	126.6349
3	-44.532	68.074	10.436	12.077	22.513	23.54122
5	68.596	208.746	8.558	15.027	23.585	277.3426
9	-45.228	161.148	14.446	17.054	31.500	115.9196
13	-55.744	56.235	12.718	12.444	25.162	0.490667
15	-19.106	99.409	10.482	13.674	24.156	80.30346
18	-64.980	85.008	11.992	18.558	30.550	20.02824
21	-9.109	9.300	14.937	6.698	21.635	0.191
24	39.149	123.000	12.355	29.387	41.742	162.149
38	-30.208	10.291	20.901	8.870	29.771	-19.917
39	7.800	58.566	22.606	7.116	29.722	66.366
40	x	x	x	x	x	x
MEAN	**-15.02**	**92.57**	**13.37**	**15.31**	**28.68**	**77.55**
SD	**40.84**	**61.74**	**4.71**	**7.51**	**6.11**	**88.734**

DSP 80%VT Primed						
ID	Baseline	A1	TD1	Tau	TD1+TAU	a+b
2	22.655	117.701	14.725	7.448	22.173	140.3559
3	-13.922	83.374	9.406	13.492	22.898	69.45242
5	81.761	203.545	11.302	13.508	24.810	285.3059
9	-57.178	161.274	14.756	14.395	29.151	104.0957
13	-28.087	77.372	13.361	14.147	27.508	49.28541
15	134.356	169.150	12.037	15.804	27.841	303.5054
18	-33.813	137.886	13.199	20.709	33.908	104.0732
21	-10.500	15.114	15.404	11.026	26.430	4.61415
24	-12.823	8.000	14.000	4.500	18.500	-4.82287
38	-18.410	21.002	15.710	26.057	41.767	2.59237
39	-22.899	72.012	12.570	18.007	30.577	49.11236
40	x	x	x	x	x	x
MEAN	**3.74**	**96.95**	**13.32**	**14.46**	**27.78**	**100.69**
SD	**56.24**	**66.62**	**1.90**	**5.92**	**6.28**	**106.34**

ND & T2DM Δ [HHb+Mb]/ΔVO_2 Index

ND	Mean HHB.VO2 ratio (20-150s)	
	Unprimed	Primed
2	1.32	1.22
3	1.01	1.09
4	1.09	1.11
5	0.99	0.96
6	1.06	1.04
7	0.95	0.95
8	0.88	0.90
9	1.00	1.07
12	1.06	1.05
14	0.89	0.78
16	1.12	1.06
18	1.08	0.93
MEAN	**1.04**	**1.01**
SD	**0.12**	**0.12**

T2DM	Mean HHB.VO2 ratio (20-150s)	
	Unprimed	Primed
2	1.36	1.27
3	1.03	0.98
5	1.27	1.02
9	1.08	1.07
13	1.16	1.27
15	1.20	1.05
18	0.98	1.01
21	1.03	1.03
24	x	x
38	1.54	0.76
39	1.14	1.06
40	x	x
MEAN	**1.18**	**1.05**
SD	**0.17**	**0.15**

ND & T2DM ΔHR Responses

NDSP	80% VT Unprimed			80% VT (Primed)		
	Baseline	**End**	**Delta HR**	**Baseline**	**End**	**Delta HR**
2	82.0	137	55	96	142	46
3	93.0	148	55	111	160	49
4	85.0	115	30	94	129	35
5	94.0	115	21	101	121	20
6	82.0	100	18	90	104	14
7	88.0	124	36	99	133	34
8	93.0	141	48	103	151	48
9	83.0	116	33	94	126	32
12	87.0	106	19	93	114	21
14	99.0	120	21	116	141	25
16	116.0	158	42	126	149	23
18	96.0	143	47	125	162	37
Mean	**91.5**	**126.9**	**35.4**	**104.0**	**136.0**	**32.0**
SD	18.07	18.07	13.91	12.56	18.09	11.67

DSP	80% VT			80% VT (Primed)		
	Baseline	**End**	**Delta**	**Baseline**	**End**	**Delta**
2	120.0	133	13	127	143	16
3	102.0	122	20	112	128	16
5	95.0	117	22	98	117	19
9	114.0	144	30	130	160	30
13	100.0	124	24	122	147	25
15	112.0	149	37	129	158	29
18	110.0	134	24	110	143	33
21	103.0	126	23	109	134	25
24	128.0	150	22	137	155	18
38	107.0	129	22	125	137	12
39	114.0	127	13	119	132	13
40	x	x	x	x	x	
Mean	**109.5**	**132.3**	**22.7**	**119.8**	**141.3**	**21.5**
SD	9.51	11.05	6.77	11.48	13.34	7.26

ND & T2DM Haematological Results

NDSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
2	3.80	4.70	2.77	1.35	1.50	1.10
3	4.00	4.40	3.24	1.76	1.10	1.20
4	4.20	5.20	3.80	1.63	1.86	0.71
5	x	x	x	x	x	x
6	x	x	x	x	x	x
7	3.90	X	4.82	3.37	1.20	0.60
8	3.90	5.08	2.72	1.27	1.20	0.60
9	4.90	X	3.37	1.35	1.50	1.10
12	x	x	x	x	x	x
14	4.00	5.10	4.56	2.54	1.20	1.80
16	3.90	4.90	3.13	1.58	1.00	1.10
18	5.90	5.20	4.48	2.75	1.10	1.50
Mean	**4.28**	**4.94**	**3.65**	**1.95**	**1.30**	**1.08**
SD	**0.69**	**0.30**	**0.79**	**0.75**	**0.27**	**0.40**

DSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
2	16.4	10.7	7.9	x	x	8.4
3	12.8	9	4.4	2.3	1.2	x
5	5.3	5.4	5.1	3	1.2	x
9	7.6	6.8	3.9	1.8	1.5	1.3
13	x	6.9	4	2	1.2	1.8
15	8.5	7.6	4.9	x	1	1.8
18	9.8	7.3	x	x	x	x
21	6.9	6.2	x	x	x	x
24	7	6.5	3.7	1.98	0.96	x
38	6.9	5.3	5	3.3	1.03	1.4
39	4.8	x	x	x	x	3.8
40	x	6.7	x	3.9	x	1
Mean	**8.60**	**7.13**	**4.86**	**2.61**	**1.16**	**2.79**
SD	**3.57**	**1.56**	**1.34**	**0.80**	**0.18**	**2.64**

ND & T2DM Accelerometry

NDSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
2	x	x	x	x
3	x	x	x	x
4	x	x	x	x
5	19.81	3.260	0.553	0.373
6	18.91	4.617	0.417	0.057
7	19.40	3.73	0.68	0.19
8	22.16	1.67	0.16	0.01
9	19.44	4.07	0.20	0.29
12	19.06	4.59	0.32	0.03
14	17.28	4.53	1.47	0.71
16	18.60	4.28	0.93	0.19
18	18.11	4.74	0.94	0.21
Mean	**19.196**	**3.943**	**0.631**	**0.229**
SD	**1.351**	**0.981**	**0.426**	**0.217**

DSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
2	x	x	x	x
3	x	x	x	x
5	18.39	3.72	1.58	0.30
9	17.81	5.97	0.16	0.06
13	x	x	x	x
15	17.04	6.13	0.80	0.03
18	x	x	x	x
21	18.74	5.07	0.18	0.01
24	16.82	6.60	0.51	0.07
38	x	x	x	x
39	x	x	x	x
40	x	x	x	x
Mean	**17.761**	**5.498**	**0.648**	**0.093**
SD	**0.831**	**1.137**	**0.584**	**0.120**

ND & T2DM LOPAR

NDSP	LOPAR MET/hours Per Week
2	177.5
3	480.3
4	186.8
5	110.5
6	x
7	192.8
8	152.1
9	126.5
12	95.3
14	272.0
16	125.3
18	94.5
Mean	**183.0**
SD	**111.7**

DSP	LOPAR MET/hours Per Week
2	124
3	29
5	110.5
9	125.8
13	270.8
15	150.5
18	143.8
21	55.5
24	x
38	280.0
39	x
40	x
Mean	**138.6**
SD	**60.7**

Appendix 10

Experiment 3

Physical characteristics – ND & T2DM

Non-Diabetic Participants	Sex	Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	WHR
2	M	36.0	1.70	80.4	27.82	6.2	1.17	1.08	7.8	133	78	0.9
4	F	33.5	1.73	84.2	28.13	8.7	1.25	1.13	5.0	116	68	0.8
5	M	40.3	1.78	97.9	30.90	9.4	0.89	1	6.0	139	81	0.9
6	M	59.3	1.78	92.8	29.29	7.9	1	1.28	8.6	138	90	1.0
7	F	52.5	1.63	80.2	30.19	12.7	1.09	1.16	5.3	120	70	0.9
8	M	34.5	1.69	80.6	28.22	4.2	1.27	1.32	4.7	128	70	0.9
9	F	53.3	1.60	104.8	40.94	17.4	0.88	0.97		128	86	0.9
12	M	51.2	1.74	104.4	34.48	5.7	0.97	0.99	6.1	147	92	1.0
14	M	47.9	1.70	87.7	30.35	7.03	1.31	1.31	7.1	137	80	1.0
16	F	32.8	1.55	76.4	31.80	14.4	1.1	1.27	4.8	108	76	0.9
18	M	35.7	1.67	76	27.25	4.2	1.24	1.19	7.9	118	86	1.0
19	M	35.4	1.81	91.9	28.05	5.3	1.17	1.05		128	80	1.0
Males	**8**	**Mean**	**42.7**	**1.70**	**88.1**	**30.6**	**8.6**	**1.1**	**1.15**	**6.3**	**128.3**	**79.8**	**0.9**
Females	**4**	**S.D.**	9.5	0.1	10.2	3.8	4.2	**0.1**	**0.1**	**1.4**	**11.3**	**7.9**	**0.1**
Total	**12**	**SEM**	2.1	0.0	2.2	0.8	0.9	0.0	0.0	0.3	2.5	1.7	0.0

Diabetic Participants	Sex	Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	Waist-To-Hip Ratio
2	M	41.2	1.68	92	32.60	5.90	1.09	1	8.2	120	80	1.08
3	M	55.2	1.74	94.5	31.21	7.80	1.14	1.35	12.6	146	76	1.06
5	M	55.7	1.70	75.5	26.12	4.90	1.14	1.27	7.1	110	60	0.93
12	M	48.3	1.82	108.8	32.85	5.00	1.2	1.1	9.4	132	74	1.02
13	M	40.0	1.80	116.3	35.90	6.90	0.99	0.96	9.5	124	66	1.15
15	M	41.4	1.74	115	37.98	5.80	0.97	0.99	12.7	138	88	1.07
18	F	42.8	1.58	65	26.04	7.50	1.2	1.2	9.4	118	70	0.87
21	F	51.4	1.58	76	30.44	9.90	1.1	1.2	8.1	114	76	0.96
24	F	36.9	1.70	116	40.14	x	1.08	1.1		114	66	1.03
25	M	42.0	1.85	106.4	31.09	3.60	1.23	0.93	8	127	90	1.07
38	F	60.2	1.65	74.5	27.36	6.10	1.07	1.05	9	130	75	x
39	M	42.2	1.70	72.5	25.09	5.90	1	1.2	6.2	110	62	0.96
Males	**8**	**Mean**	**46.4**	**1.71**	**92.7**	**31.4**	**6.3**	**1.1**	**1.1**	**9.1**	**123.58**	**73.58**	**1.02**
Females	**4**	**S.D.**	7.5	0.1	19.4	4.8	1.7	0.1	0.1	2.02	11.41	9.43	0.08
Total	**12**	**SEM**	1.2	0.0	3.1	0.8	0.3	0.0	0.0	0.64	3.61	2.98	0.03

Ramp Physiological Responses – ND & T2DM

NDSP RAMP										
NDSP ID	Oxygen Uptake				Power Output		Peak Cardiac Output (L.min)	Peak Hr	RER	CO @ 50% delta (L.min)
	Peak (L.min)	Peak (mL.kg.min)	VO2@VT (L.min)	VO2 @ VT (mL.kg.min)	Peak (W)	VT (W)				
2	2.15	26.72	1.46	18.11	195	108	x	182.0	1.2	16.6
4	2.18	25.89	1.24	14.73	175	89	14.3	173.0	1.3	17.4
5	3.21	32.83	1.75	17.83	260	123	x	x	1.2	25.1
6	1.93	20.77	1.14	12.30	160	58	x	153.0	1.1	11.0
7	2.41	30.07	1.88	23.44	182	129	x	160.0	1.2	13.4
8	3.12	38.70	2.33	28.95	273	180	16.0	175.0	1.0	19.3
9	2.06	19.64	1.71	16.35	137	92	x	141.0	1.1	13.5
12	2.72	26.05	1.62	15.53	196	87	15.6	149.0	1.1	14.6
14	2.06	23.48	1.40	15.91	166	73	13.8	174.0	1.1	13.8
16	1.81	23.70	1.05	13.78	129	54	11.6	182.0	1.3	10.3
18	2.83	37.29	1.84	24.15	233	130	-	185.0	1.1	16.8
19	3.12	33.93	2.16	23.51	274	156	17.2	167.0		x
MEAN	**2.47**	**28.26**	**1.63**	**18.72**	**198.33**	**106.58**	**14.75**	**167.36**	**1.15**	**15.62**
SD	**0.51**	**6.28**	**0.39**	**5.10**	**50.65**	**38.51**	**1.97**	**14.72**	**0.08**	**4.15**

DSP RAMP										
DSP ID	Oxygen Uptake				Power Output		Peak Cardiac Output (L.min)	Peak Hr	RER	CO @ 50% delta (L.min)
	Peak (L.min)	Peak (mL.kg.min)	VO2@VT (L.min)	VO2 @ VT (mL.kg.min)	Peak (W)	VT (W)				
2	1.86	20.24	1.27	13.77	139	70	x	137.0	1.2	11.1
3	1.79	18.95	1.33	14.08	139	81	x	149.0	1.2	10.2
5	2.22	29.46	1.54	20.37	184	97	x	176.0	x	13.0
12	2.43	22.30	1.39	12.81	200	87	13.2	158.0	1.2	x
13	2.58	22.18	1.42	12.24	185	84	14.5	163.0	1.2	14.6
15	3.22	28.02	2.56	22.24	245	150	14.5	174.0	1.2	x
18	1.41	21.62	1.11	17.06	107	70	10.0	160.0	1.0	8.0
21	1.66	21.82	1.19	15.66	120	65	x	169.0	1.1	11.2
24	2.07	17.84	1.83	15.76	155	93	14.7	178.0	1.1	14.5
25	2.63	24.67	2.06	19.35	221	153	x	165.0	1.1	11.8
38	1.51	20.24	1.06	14.20	97	48.77	x	178.0	1.2	x
39	1.16	15.93	0.99	13.71	96	58.58	x	143.0	1.0	x
MEAN	**2.04**	**21.94**	**1.48**	**15.94**	**157.33**	**87.95**	**13.38**	**162.50**	**1.12**	**11.80**
SD	**0.60**	**3.92**	**0.46**	**3.19**	**49.58**	**32.74**	**1.98**	**13.76**	**0.07**	**2.22**

VO_2 kinetics – ND

NDSP 50% Delta Unprimed								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
2	0.861	1.155	14.971	29.269	0.231	133.770	38.525	2.02
4	0.691	1.120	13.341	34.722	0.193	144.492	100.845	1.81
5	0.786	1.781	18.854	29.735	0.466	136.440	112.423	2.57
6	0.858	0.769	19.677	30.173	0.222	135.726	102.002	1.63
7	0.728	0.786	14.593	28.590	0.236	123.094	70.600	1.51
8	1.011	2.172	17.158	33.728	0.397	139.602	91.802	3.18
9	0.776	1.073	13.894	30.094	0.183	139.002	98.045	1.85
12	0.918	1.196	7.619	35.239	0.428	124.210	324.916	2.11
14	0.915	0.804	18.580	43.230	0.388	138.392	107.173	1.72
16	0.747	0.802	13.516	32.444	0.221	124.088	101.174	1.55
18	0.889	1.270	13.781	20.165	0.687	62.271	104.716	2.16
19	1.125	1.515	15.015	29.720	0.679	94.934	89.703	2.64
MEAN	**0.86**	**1.20**	**15.08**	**31.43**	**0.36**	**124.67**	**111.83**	**2.06**
SD	**0.12**	**0.44**	**3.25**	**5.39**	**0.18**	**23.61**	**70.04**	**0.51**

NDSP 50% Delta Primed								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
2	0.857	1.157	21.362	27.950	0.325	132.807	88.977	2.01
4	0.691	1.078	14.057	31.185	0.276	139.683	88.757	1.77
5	0.859	1.978	21.410	28.278	0.419	212.568	78.840	2.84
6	0.953	0.847	15.577	28.179	0.211	134.881	77.382	1.80
7	0.812	1.416	14.166	23.467	0.497	83.420	124.718	2.23
8	1.077	1.861	10.299	24.071	0.587	110.379	79.257	2.94
9	0.747	0.985	14.424	24.090	0.227	100.497	45.093	1.73
12	0.997	1.278	14.960	37.914	0.138	126.426	95.793	2.27
14	1.189	0.754	27.321	40.086	0.235	172.547	90.148	1.94
16	0.827	0.871	13.112	39.596	0.230	200.644	82.043	1.70
18	1.019	1.465	17.533	22.920	0.278	86.712	29.649	2.48
19	1.168	1.631	17.287	22.182	0.806	93.247	129.219	2.80
MEAN	**0.933**	**1.277**	**16.792**	**29.160**	**0.352**	**132.818**	**84.156**	**2.21**
SD	**0.161**	**0.402**	**4.616**	**6.632**	**0.193**	**42.970**	**27.784**	**0.46**

VO_2 kinetics – T2DM

DSP 50% Delta Unprimed								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
2	0.988	0.517	21.228	16.549	0.235	90.193	17.418	1.51
3	0.860	0.677	16.843	34.307	0.211	107.739	42.823	1.54
5	0.761	1.332	2.870	56.808	0.237	196.230	125.287	2.09
12	1.026	1.233	16.321	32.072	0.572	156.817	199.729	2.26
13	0.960	1.031	14.415	43.434	0.435	115.900	187.476	1.99
15	1.110	1.618	8.568	44.008	0.309	170.837	36.173	2.73
18	0.601	0.750	15.383	33.088	0.142	156.989	137.338	1.35
21	0.812	0.830	15.000	46.653	0.099	221.496	26.281	1.64
24	0.875	0.732	16.525	32.911	0.120	131.966	13.250	1.61
25	0.802	1.490	7.798	35.605	0.098	78.065	49.999	2.29
38	0.849	0.354	18.001	27.092	0.180	111.723	76.644	1.20
39	0.646	0.581	14.575	42.665	0.441	205.674	78.961	1.23
MEAN	**0.86**	**0.93**	**13.96**	**37.10**	**0.26**	**145.30**	**82.61**	**1.79**
SD	**0.15**	**0.41**	**5.08**	**10.40**	**0.15**	**46.81**	**65.10**	**0.48**

DSP 50% Delta Primed								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
2	1.009	0.650	17.723	29.797	0.050	73.000	30.782	1.66
3	0.855	0.771	25.534	16.211	0.182	115.986	11.555	1.63
5	0.830	1.322	7.994	44.768	0.145	172.247	14.885	2.15
12	1.097	1.312	15.031	38.673	0.253	153.948	89.701	2.41
13	0.969	1.055	15.126	39.675	0.224	126.717	30.490	2.02
15	1.190	1.630	10.599	37.097	0.161	147.800	77.776	2.82
18	0.580	0.727	15.611	29.913	0.136	100.699	168.885	1.31
21	0.830	0.800	16.948	22.615	0.159	225.940	38.635	1.63
24	0.963	0.734	12.620	28.000	0.230	131.000	29.350	1.70
25	0.855	1.630	16.860	27.990	0.032	86.400	5.150	2.49
38	0.942	0.340	20.500	20.190	0.108	167.766	31.800	1.28
39	0.644	0.671	24.846	36.877	0.149	220.356	40.335	1.32
MEAN	**0.897**	**0.970**	**16.616**	**30.984**	**0.152**	**143.488**	**47.445**	**1.87**
SD	**0.172**	**0.415**	**5.187**	**8.644**	**0.067**	**48.151**	**45.583**	**0.51**

[HHb+Mb] kinetics – ND

NDSP 50%Delta Unprimed								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
2	-49.200	53.414	13.453	9.290	52.500	91.400	167.000	4.21
4	-51.071	60.060	12.791	7.022	11.500	102.200	63.000	8.99
5	-81.999	28.624	18.266	9.522	65.000	98.600	183.000	-53.37
6	4.694	72.000	16.723	11.143	58.000	113.000	200.000	76.69
7	-55.100	30.540	8.265	3.467	2.672	175.518	53.143	-24.56
8	-57.250	187.000	9.570	36.608	21.000	115.000	97.000	129.75
9	-92.000	11.172	13.819	6.289	1.817	25.421	1.567	-80.83
12	-70.949	90.019	11.208	14.210	22.235	92.000	103.000	19.07
14	-34.243	31.106	6.380	14.136	29.500	71.000	147.000	-3.14
16	-99.345	37.146	8.426	11.667	14.561	100.892	70.466	-62.20
18	-17.755	181.027	5.804	16.993	6.000	66.000	5.000	163.27
19	7.260	280.000	8.798	13.998	7.000	234.000	5.000	287.26
MEAN	**-49.75**	**88.51**	**11.13**	**12.86**	**24.32**	**107.09**	**91.26**	**38.76**
SD	**34.77**	**83.18**	**3.95**	**8.42**	**22.35**	**53.22**	**70.62**	**107.95**

NDSP 50%Delta Primed								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
2	-21.314	62.500	8.819	14.115	29.800	85.000	97.000	41.19
4	-20.528	64.511	11.198	11.990	11.450	102.200	25.000	43.98
5	-91.964	24.600	18.382	8.500	64.000	107.600	198.000	-67.36
6	-12.889	92.983	15.991	9.999	60.000	119.000	155.000	80.09
7	-39.746	31.004	8.797	4.005	9.000	206.000	68.000	-8.74
8	-82.841	61.992	20.818	30.011	41.000	107.000	65.000	-20.85
9	-47.404	9.501	19.496	9.986	1.917	38.980	3.988	-37.90
12	-27.641	74.987	11.198	12.796	23.000	99.200	121.000	47.35
14	-22.678	49.067	7.758	13.041	23.000	105.000	120.000	26.39
16	-67.269	44.000	7.599	16.900	8.700	91.000	23.000	-23.27
18	37.402	218.000	6.400	18.797	2.000	80.000	2.000	255.40
19	12.975	290.000	6.000	11.990				302.98
MEAN	**-31.99**	**85.26**	**11.87**	**13.51**	**24.90**	**103.73**	**79.82**	**53.27**
SD	**37.27**	**83.43**	**5.37**	**6.45**	**21.91**	**40.01**	**64.52**	**114.01**

[HHb+Mb] kinetics – T2DM

DSP 50%Delta Unprimed								
ID	**Baseline**	**Ap**	**TDp**	**Taup**	**As**	**TDs**	**Taus**	**Baseline + Ap**
2	12.161	156.000	14.540	17.000				168.16
3	-120.385	54.000	13.000	10.700	5.000	68.000	21.000	-66.39
5	27.893	255.500	11.440	10.000				283.39
12	-170.635	232.570	13.778	12.180				61.94
13	-109.486	84.275	13.537	9.509	8.909	146.065	52.451	-25.21
15	-5.758	122.000	10.453	13.699	18.000	208.000	63.000	116.24
18	-72.595	100.570	12.730	9.600	39.500	126.000	73.000	27.98
21	-66.550	41.500	13.960	8.780	7.800	159.000	21.000	-25.05
24	-71.449	117.574	9.099	7.317				46.13
25	-78.600	211.500	8.520	15.858				132.90
38	-43.040	33.008	15.967	22.682	26.045	85.940	94.782	-10.03
39	21.101	94.973	18.719	7.749	77.853	170.110	194.581	116.07
MEAN	**-56.45**	**125.29**	**12.98**	**12.09**	**26.16**	**137.59**	**74.26**	**68.84**
SD	**61.50**	**74.31**	**2.86**	**4.52**	**25.82**	**48.63**	**59.41**	**99.49**

DSP 50%Delta Primed								
ID	**Baseline**	**Ap**	**TDp**	**Taup**	**As**	**TDs**	**Taus**	**Baseline + Ap**
2	56.492	220.000	15.000	20.000				276.49
3	-46.632	69.001	11.305	12.699	6.475	73.987	5.004	22.37
5	64.023	245.000	12.500	8.000				309.02
12	-74.177	252.000	12.485	10.500				177.82
13	-72.782	96.590	13.050	8.990	8.000	203.000	18.000	23.81
15	55.900	182.000	6.011	15.017	9.000	297.000	63.000	237.90
18	-49.350	105.235	12.228	9.385	12.494	147.024	37.099	55.88
21	-36.950	54.055	12.994	10.448	20.000	132.988	75.160	17.11
24	-22.150	140.500	8.300	12.000				118.35
25	-30.450	261.000	8.004	14.986				230.55
38	47.600	21.940	14.549	19.782	1.308	107.744	12.098	69.54
39	-6.750	97.188	14.401	8.652	48.000	89.530	158.680	90.44
MEAN	**-9.602**	**145.38**	**11.736**	**12.538**	**15.040**	**150.182**	**52.720**	**135.8**
SD	**52.060**	**83.839**	**2.845**	**4.127**	**15.628**	**77.391**	**53.562**	**106.0**

VO_2 MRT – ND & T2DM

VO2 MRT	
NDSP 50% Delta Unprimed	
ID	MRT
2	50.98
4	55.99
5	53.72
6	50.16
7	56.58
8	55.72
9	54.08
12	52.00
14	71.22
16	60.00
18	44.15
19	51.71
Mean	54.69
SD	6.54

VO2 MRT	
DSP 50% Delta Unprimed	
ID	MRT
2	73.97
3	75.98
5	81.98
12	81.98
13	72.99
15	78.98
18	66.99
21	74.98
24	62.99
25	54.00
38	81.00
39	91.00
Mean	74.74
SD	9.81

NDSP 50% Delta Primed	
ID	MRT
2	43.71
4	46.99
5	43.99
6	46.17
7	43.46
8	48.06
9	45.06
12	49.06
14	60.93
16	58.07
18	42.90
19	52.90
Mean	48.44
SD	5.92

DSP 50% Delta Primed	
ID	MRT
2	43.56
3	40.20
5	58.19
12	62.19
13	66.19
15	55.98
18	42.99
21	45.40
24	53.99
25	37.97
38	72.70
39	81.00
Mean	55.03
SD	13.67

[HHb+Mb] MRT – ND & T2DM

NIRS MRT	
NDSP 50% Delta Unprimed	
ID	MRT
2	95.29
4	29.00
5	142.00
6	9.50
7	9.40
8	58.00
9	10.00
12	54.00
14	114.00
16	80.00
18	20.00
19	13.09
Mean	**52.86**
SD	**45.81**

NIRS MRT	
DSP 50% Delta Unprimed	
ID	MRT
2	17.00
3	13.98
5	10.03
12	12.11
13	43.63
15	21.00
18	74.59
21	37.75
24	7.35
25	15.79
38	45.05
39	92.24
Mean	**32.54**
SD	**27.30**

NDSP 50% Delta Primed	
ID	MRT
2	68.11
4	27.52
5	148.00
6	82.00
7	9.64
8	76.01
9	13.99
12	58.79
14	77.00
16	39.04
18	18.80
19	12.00
Mean	**52.57**
SD	**40.82**

DSP 50% Delta Primed	
ID	MRT
2	20.00
3	14.98
5	8.00
12	10.50
13	12.96
15	14.96
18	15.00
21	30.45
24	12.01
25	14.98
38	19.89
39	20.00
Mean	**16.14**
SD	**5.87**

Heart rate responses – ND & T2DM

NDSP	50% Delta Unprimed			50% Delta (Primed)		
	Baseline	End	Delta HR	Baseline	End	Delta HR
2	107	175	68	115	183	68
4	91	150	59	91	160	69
5	92	157	65	120	160	40
6	89	137	48	97	152	55
7	95	153	58	104	159	55
8	99	176	77	111	176	65
9	87	138	51	91	136	45
12	95	134	39	107	145	38
14	106	166	60	114	174	60
16	117	173	56	108	172	64
18	125	167	42	x	x	x
19	x	x	x	x	x	x
Mean	**100.3**	**156.9**	**56.6**	**105.8**	**161.7**	**55.9**
SD	**12.2**	**15.7**	**11.2**	**10.0**	**14.8**	**11.4**

DSP	50% Delta Unprimed			50% Delta Primed		
	Baseline HR	End HR	Delta HR	Baseline HR	End HR	Delta HR
2	112	151	39	128	152	24
3	109	155	46	113	142	29
5	98	150	52	102	152	50
12	98	155	57	110	165	55
13	108	157	49	123	160	37
15	117	171	54	130	176	46
18	126	169	43	127	169	42
21	92	138	46	100	148	48
24	131	177	46	138	182	44
25	x	x	x	x	x	x
38	x	x	x	x	x	x
39	x	x	x	x	x	x
Mean	**110.1**	**158.1**	**48.0**	**119.0**	**160.7**	**41.7**
SD	**13.1**	**12.2**	**5.6**	**13.3**	**13.4**	**10.0**

Haematological results – ND & T2DM

NDSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
2	3.80	4.70	2.77	1.35	1.50	1.10
4	4.20	5.20	3.80	1.63	1.86	0.71
5	X	X	X	X	X	X
6	X	X	X	X	X	X
7	3.90	X	4.82	3.37	1.20	0.60
8	3.90	5.08	2.72	1.27	1.20	0.60
9	4.90	X	3.37	1.35	1.50	1.10
12	X	X	X	X	X	X
14	4.00	5.10	4.56	2.54	1.20	1.80
16	3.90	4.90	3.13	1.58	1.00	1.10
18	5.90	5.20	4.48	2.75	1.10	1.50
19	3.70	X	2.67	1.63	0.70	0.70
Mean	**4.24**	**5.03**	**3.59**	**1.94**	**1.25**	**1.02**
SD	**0.71**	**0.20**	**0.85**	**0.75**	**0.34**	**0.42**

DSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
2	16.4	10.7	7.9	x	x	8.4
3	12.8	9	4.4	2.3	1.2	2
5	5.3	5.4	5.1	3	1.2	2
12	5.7	6.5	5.6	3.2	0.93	3.22
13	x	6.9	4	2	1.2	1.8
15	8.5	7.6	4.9	x	1	1.8
18	9.8	7.3	x	x	x	x
21	6.9	6.2	x	x	x	x
24	7	6.5	3.7	1.98	0.96	1.7
25	10.5	9.7	x	x	x	x
38	6.9	5.3	5	3.3	1.03	1.5
39	4.80	x	x	x	x	x
Mean	**8.60**	**7.37**	**5.08**	**2.63**	**1.07**	**2.80**
SD	**3.54**	**1.75**	**1.30**	**0.61**	**0.12**	**2.32**

Accelerometry data – ND & T2DM

NDSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
2	19.813	3.260	0.553	0.373
4	18.910	4.617	0.417	0.057
5	19.400	3.727	0.683	0.190
6	22.163	1.667	0.160	0.010
7	19.437	4.067	0.203	0.293
8	21.780	1.277	0.620	0.323
9	19.057	4.593	0.323	0.027
12	17.283	4.533	1.473	0.710
14	18.597	4.283	0.927	0.193
16	18.107	4.743	0.943	0.207
18	x	x	x	x
19	x	x	x	x
Mean	**19.49**	**3.59**	**0.66**	**0.25**
SD	**1.83**	**1.47**	**0.48**	**0.24**

DSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
2				
3	x	x	x	x
5	18.39	3.72	1.58	0.30
12	x	x	x	x
13	x	x	x	x
15	17.04	6.13	0.80	0.03
18	x	x	x	x
21	18.74	5.07	0.18	0.01
24	16.82	6.60	0.51	0.07
25	x	x	x	x
38	x	x	x	x
39	x	x	x	x
Mean	**17.75**	**5.38**	**0.77**	**0.10**
SD	**0.96**	**1.28**	**0.60**	**0.14**

LOPAR data – ND & T2DM

NDSP	LOPAR MET/hours Per Week
2	177.5
4	94.5
5	186.8
6	110.5
7	x
8	192.8
9	152.1
12	126.5
14	95.3
16	272.0
18	125.3
19	x
Mean	**153.3**
SD	**55.3**

DSP	LOPAR MET/hours Per Week
2	124
3	29
5	110.5
12	x
13	270.8
15	150.5
18	143.8
21	55.5
24	x
25	x
38	280.0
39	x
Mean	**138.6**
SD	**60.7**

Appendix 11

Experiment 4

Physical characteristics – ND & T2DM

Non-Diabetic Participants	Sex		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	WHR
7	F		52.5	1.63	80.2	30.19	12.7	1.09	1.16	5.3	120	70	0.9
8	M		34.5	1.69	80.6	28.22	4.2	1.27	1.32	4.7	128	70	0.9
9	F		53.3	1.60	104.8	40.94	17.4	0.88	0.97		128	86	0.9
13	F		30.1	1.70	86	29.76	14	1.1	1.1	6.1	130	76	0.8
14	M		47.9	1.70	87.7	30.35	7.03	1.31	1.31	7.1	137	80	1.0
16	F		32.8	1.55	76.4	31.80	14.4	1.1	1.27	4.8	108	76	0.9
18	M		35.7	1.67	76	27.25	4.2	1.24	1.19	7.9	118	86	1.0
Males	**3**	**Mean**	**41.0**	**1.65**	**84.5**	**31.2**	**10.6**	**1.1**	**1.19**	**6.0**	**124.1**	**77.7**	**0.9**
Females	**4**	**S.D.**	9.9	0.1	10.0	4.5	5.3	**0.1**	**0.1**	**1.3**	**9.5**	**6.7**	**0.1**
Total	**7**	**SEM**	2.2	0.0	2.2	1.0	1.2	0.0	0.0	0.3	2.1	1.5	0.0

Diabetic Participants	Sex		Age	Stature (m)	Body Mass (kg)	BMI (kg.m2)	Fat Layer VL (mm)	Pre Ex ABI	Post Ex ABI	PWV	Rest SBP	Rest DBP	Waist-To-Hip Ratio
13	M		40.0	1.80	116.3	35.90	6.9	0.99	0.96	9.5	124	66	1.2
18	F		42.8	1.58	65	26.04	7.5	1.2	1.2	9.4	118	70	0.9
21	F		51.4	1.58	76	30.44	9.9	1.1	1.2	8.1	114	76	1.0
24	F		36.9	1.70	116	40.14	X	1.08	1.1		114	66	1.0
29	M		44.9	1.73	83.2	27.80	3.4	1.05	1.03	7.3	118	72	0.9
38	F		60.2	1.65	74.5	27.36	6.1	1.07	1.05	9.0	130	75	
39	M		42.2	1.70	72.5	25.09	5.9	1	1.2	6.2	110	62	1.0
Males	**3**	**Mean**	**45.5**	**1.68**	**86.2**	**30.4**	**6.6**	**1.1**	**1.1**	**8.3**	**118.29**	**69.57**	**0.98**
Females	**4**	**S.D.**	7.9	0.1	21.1	5.6	2.1	0.1	0.1	1.31	6.78	5.16	0.10
Total	**7**	**SEM**	1.2	0.0	3.3	0.9	0.3	0.0	0.0	0.41	2.14	1.63	0.03

Ramp physiological responses – ND & T2DM

NDSP RAMP										
NDSP ID	Oxygen Uptake				Power Output		Peak Cardiac Output (L.min)	Peak Hr	RER	CO @ 50% delta (L.min)
	Peak (L.min)	Peak (mL.kg.min)	VO2@VT (L.min)	VO2 @ VT (mL.kg.min)	Peak (W)	VT (W)				
7	2.41	30.07	1.88	23.44	182	129	x	160.0	1.2	13.4
8	3.12	38.70	2.33	28.95	273	180	16.0	175.0	1.0	19.3
9	2.06	19.64	1.71	16.35	137	92	x	141.0	1.1	13.5
13	1.90	22.08	1.27	14.80	137	73	12.0	x	1.1	x
14	2.06	23.48	1.40	15.91	166	73	13.8	174.0	1.1	13.8
16	1.81	23.70	1.05	13.78	129	54	11.6	182.0	1.3	10.3
18	2.83	37.29	1.84	24.15	233	130	-	185.0	1.1	16.8
MEAN	**2.31**	**27.85**	**1.64**	**19.63**	**179.57**	**104.43**	**13.35**	**169.50**	**1.14**	**14.52**
SD	**0.50**	**7.62**	**0.43**	**5.83**	**54.70**	**44.05**	**2.01**	**16.43**	**0.07**	**3.12**

DSP RAMP										
DSP ID	Oxygen Uptake				Power Output		Peak Cardiac Output (L.min)	Peak Hr	RER	CO @ 50% delta (L.min)
	Peak (L.min)	Peak (mL.kg.min)	VO2@VT (L.min)	VO2 @ VT (mL.kg.min)	Peak (W)	VT (W)				
13	2.58	22.18	1.42	12.24	185	84	14.5	163.0	1.2	14.6
18	1.41	21.62	1.11	17.06	107	70	10.0	160.0	1.0	8.0
21	1.66	21.82	1.19	15.66	120	65	x	169.0	1.1	11.2
24	2.07	17.84	1.83	15.76	155	93	14.7	178.0	1.1	14.5
29	2.22	26.72	1.55	18.58	208	91	14.5	176.0	1.1	14.2
38	1.51	20.24	1.06	14.20	97	49	x	178.0	1.2	x
39	1.16	15.93	0.99	13.71	96	59	x	143.0	1.0	x
MEAN	**1.80**	**20.91**	**1.31**	**15.32**	**138.29**	**72.69**	**13.43**	**166.71**	**1.10**	**12.50**
SD	**0.50**	**3.45**	**0.30**	**2.13**	**44.96**	**16.85**	**2.29**	**12.70**	**0.08**	**2.88**

VO_2 kinetics – ND

NDSP 80% VT Unprimed Work to Work						
ID	Baseline	A	TD	Tau	Baseline + A	VO2 Gain
7	0.570	0.741	13.481	25.306	1.31	7.951
8	0.911	1.298	19.831	28.261	2.21	9.685
9	0.792	0.664	6.367	38.292	1.46	10.447
13	0.725	0.386	14.648	26.833	1.11	7.983
14	0.550	0.170	23.137	29.370	0.72	3.508
16	0.808	0.326	13.974	32.219	1.13	9.822
18	0.780	1.013	10.957	33.141	1.79	10.773
MEAN	**0.73**	**0.66**	**14.63**	**30.49**	**1.39**	**8.60**
SD	**0.13**	**0.40**	**5.52**	**4.42**	**0.49**	**2.50**

NDSP 80% VT Primed Work to Work						
ID	Baseline	A	TD	Tau	Baseline + A	VO2 Gain
7	0.639	0.622	20.168	21.512	1.26	6.678
8	0.832	1.570	16.938	21.576	2.40	11.718
9	0.743	0.738	10.560	35.650	1.48	11.606
13	0.819	0.357	15.684	25.707	1.18	7.374
14	0.680	0.227	17.261	12.215	0.91	4.695
16	0.903	0.330	13.040	23.359	1.23	9.936
18	0.930	1.099	15.768	24.162	2.03	11.688
MEAN	**0.792**	**0.706**	**15.631**	**23.455**	**1.50**	**9.10**
SD	**0.109**	**0.483**	**3.093**	**6.928**	**0.53**	**2.85**

NDSP 50% Delta Unprimed Work to Work								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
7	1.368	0.394	19.884	34.693	0.131	125.270	129.459	1.76
8	2.381	0.705	11.925	49.028	0.074	60.049	11.730	3.09
9	1.460	0.443	11.223	32.250	0.082	138.857	39.452	1.90
13	1.227	0.475	1.736	56.498	0.104	144.036	101.397	1.70
14	0.870	0.542	45.630	48.180	0.574	234.684	93.722	1.41
16	1.207	0.405	15.857	26.892	0.301	104.869	121.669	1.61
18	1.976	0.758	14.778	48.580	0.145	117.635	57.455	2.73
MEAN	**1.50**	**0.53**	**17.29**	**42.30**	**0.20**	**132.20**	**79.27**	**2.03**
SD	**0.51**	**0.15**	**13.70**	**10.93**	**0.18**	**53.07**	**44.04**	**0.63**

NDSP 50% Delta Primed Work to Work								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
7	1.274	0.433	16.748	34.788	0.048	151.000	9.318	1.71
8	2.506	0.540	14.828	27.049	0.101	39.000	16.480	3.05
9	1.502	0.448	13.090	34.379	0.037	118.522	32.246	1.95
13	1.300	0.437	14.216	36.625	0.088	172.460	21.515	1.74
14	0.853	0.513	1.008	42.091	0.325	219.037	15.221	1.37
16	1.287	0.629	3.831	53.166	0.202	122.141	199.999	1.92
18	2.189	0.883	9.773	56.705	0.039	134.114	15.969	3.07
MEAN	**1.559**	**0.555**	**10.499**	**40.686**	**0.120**	**136.611**	**44.393**	**2.11**
SD	**0.580**	**0.161**	**5.964**	**10.734**	**0.107**	**55.313**	**68.984**	**0.67**

VO_2 kinetics – T2DM

DSP 80% VT Unprimed Work to Work						
ID	Baseline	A	TD	Tau	Baseline + A	VO2 Gain
13	0.920	0.576	1.383	48.350	1.50	10.073
18	0.627	0.451	13.000	28.639	1.08	9.880
21	0.744	0.440	1.000	27.995	1.18	10.577
24	1.039	0.469	25.627	28.055	1.51	7.328
29	0.928	0.650	0.001	57.000	1.58	10.350
38	0.647	0.387	0.001	40.398	1.03	13.342
39	0.721	0.252	19.500	42.800	0.97	6.838
MEAN	**0.80**	**0.46**	**8.64**	**39.03**	**1.26**	**9.77**
SD	**0.16**	**0.13**	**10.69**	**11.37**	**0.26**	**2.18**

DSP 80% VT Primed Work to Work						
ID	Baseline	A	TD	Tau	Baseline + A	VO2 Gain
13	1.012	0.563	22.117	38.322	1.57	9.834
18	0.643	0.465	1.504	46.863	1.11	10.204
21	0.790	0.440	12.760	25.929	1.23	10.577
24	1.068	0.460	17.551	26.383	1.53	7.180
29	1.034	0.544	16.334	24.857	1.58	8.638
38	0.830	0.260	3.037	18.252	1.09	8.950
39	0.722	0.359	0.001	51.502	1.08	9.735
MEAN	**0.871**	**0.441**	**10.472**	**33.158**	**1.31**	**9.30**
SD	**0.167**	**0.105**	**8.857**	**12.518**	**0.24**	**1.15**

DSP 50% Delta Unprimed Work to Work								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
13	1.554	0.570	14.791	50.135	0.454	93.098	188.884	2.12
18	1.067	0.275	7.569	47.241	0.059	94.673	20.100	1.34
21	1.208	0.315	0.000	46.861	0.103	184.392	22.099	1.52
24	1.592	0.601	10.611	81.443	0.021	99.821	9.518	2.19
29	1.584	0.616	14.718	49.439	0.179	167.302	26.333	2.20
38	1.060	0.220	6.090	64.437	0.063	117.272	89.999	1.28
39	1.008	0.228	17.455	40.609	0.055	74.000	10.083	1.24
MEAN	**1.30**	**0.40**	**10.18**	**54.31**	**0.13**	**118.65**	**52.43**	**1.70**
SD	**0.27**	**0.18**	**6.08**	**13.98**	**0.15**	**41.37**	**66.22**	**0.45**

DSP 50% Delta Primed Work to Work								
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	Baseline + Ap
13	1.588	0.652	19.950	48.327	0.295	146.590	102.829	2.24
18	1.070	0.333	6.104	28.357	0.023	110.317	12.554	1.40
21	1.226	0.388	1.074	47.861	0.102	200.299	50.770	1.61
24	1.590	0.533	17.580	67.130	0.012	99.998	18.300	2.12
29	1.616	0.704	1.251	54.301	0.025	169.003	8.305	2.32
38	1.044	0.216	22.646	22.125	0.010	98.019	40.948	1.26
39	1.063	0.121	23.401	26.932	0.087	71.449	9.051	1.18
MEAN	**1.314**	**0.421**	**13.144**	**42.148**	**0.079**	**127.954**	**34.680**	**1.73**
SD	**0.272**	**0.219**	**9.985**	**16.660**	**0.102**	**45.548**	**34.285**	**0.48**

[HHb+Mb] kinetics – ND

NDSP 80% VT Unprimed						
ID	Baseline	A	TD	Tau	Baseline + A	TD+T
7	-38.832	15.190	18.74	2.60	-23.6	21.34
8	-146.437	86.035	9.35	42.97	-60.4	52.32
9	-40.302	6.478	14.45	4.65	-33.8	19.10
13	-95.950	22.623	14.20	22.74	-73.3	36.94
14	-23.247	11.106	15.54	18.82	-12.1	34.36
16	-42.587	11.207	16.89	8.89	-31.4	25.78
18	7.882	153.233	14.31	12.92	161.1	27.23
MEAN	**-54.21**	**43.70**	**14.78**	**16.23**	**-10.5**	**31.0**
SD	**51.03**	**55.60**	**2.91**	**13.84**	**78.6**	**11.4**

NDSP80% VT Primed						
ID	Baseline	A	TD	Tau	Baseline + A	TD+T
7	-21.342	27.251	2.88	27.94	5.9	30.82
8	-222.772	130.182	2.04	28.19	-92.6	30.24
9	-23.700	10.113	11.72	7.37	-13.6	19.09
13	-38.400	10.131	21.56	12.58	-28.3	34.14
14	-6.507	31.499	12.78	30.81	25.0	43.60
16	-62.400	23.173	10.99	17.05	-39.2	28.04
18	27.883	167.097	9.14	24.88	195.0	34.02
MEAN	**-49.61**	**57.06**	**10.159**	**21.261**	**7.5**	**31.42**
SD	**81.2**	**63.968**	**6.581**	**8.971**	**90.7**	**7.4**

NDSP 50%Delta Unprimed										
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	TDp + taup	Baseline + Ap	Absolute End Amp
7	-24.900	8.961	5.74	11.07	2.026	80.07	6.913	16.809	-15.9	-13.914
8	-48.668	40.433	0.75	19.30	1.500	63.00	5.000	20.054	-8.2	-6.735
9	-22.900	2.617	12.89	15.16	16.382	114.50	106.574	28.054	-20.3	-3.901
13	-88.093	5.806	7.76	6.92	0.470	49.23	6.687	14.683	-82.3	-81.817
14	-4.079	20.986	10.19	61.57	11.979	169.27	97.430	71.759	16.9	28.887
16	-32.712	14.300	9.83	48.10	3.200	176.20	59.000	57.930	-18.4	-15.212
18	219.800	20.500	5.53	9.12	1.800	129.00	33.000	14.653	240.3	242.100
MEAN	**-0.22**	**16.23**	**7.53**	**24.46**	**5.34**	**111.61**	**44.94**	**32.0**	**16.0**	**21.344**
SD	**100.56**	**12.76**	**3.96**	**21.49**	**6.23**	**50.09**	**43.59**	**23.25**	**103.3**	**102.825**

NDSP 50%Delta Primed										
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	TDp + taup	Baseline + Ap	Absolute End Amp
7	17.277	11.800	6.00	19.81	1.000	42.80	12.000	25.811	29.077	30.077
8	-96.800	49.874	3.16	21.75	19.000	53.60	24.000	24.901	-46.925	-27.925
9	-1.330	1.291	18.86	12.63	2.795	188.02	35.076	31.491	-0.039	2.756
13	-26.720	1.400	27.97	7.00	6.900	54.00	20.000	34.970	-25.320	-18.420
14	22.700	20.313	20.72	33.11	10.700	211.84	47.039	53.829	43.013	53.713
16	-40.600	12.300	8.89	15.16	2.000	176.00	21.000	24.049	-28.300	-26.300
18	246.200	26.899	5.62	9.04	X	X	X	14.654	273.099	273.099
MEAN	**17.25**	**17.70**	**13.031**	**16.927**	**7.066**	**121.044**	**26.519**	**30.0**	**34.9**	**41.0**
SD	**108.8**	**16.959**	**9.446**	**8.905**	**6.875**	**78.633**	**12.525**	**12.3**	**109.8**	**106.8**

[HHb+Mb] kinetics – T2DM

DSP 80% VT Unprimed						
ID	Baseline	A	TD	Tau	Baseline + A	TD+T
13	-10.150	66.985	13.05	16.22	56.8	29.27
18	-36.010	93.338	10.22	23.31	57.3	33.53
21	-53.500	15.973	18.04	17.61	-37.5	35.64
24	39.100	124.078	13.05	28.02	163.2	41.07
29	81.000	65.000	16.72	12.03	146.0	28.75
38	-30.208	10.291	20.90	8.87	-19.9	29.77
39	7.790	58.011	20.10	10.22	65.8	30.32
MEAN	**-0.282**	**61.95**	**16.012**	**16.611**	**61.7**	**32.6**
SD	**47.125**	**40.162**	**4.006**	**7.047**	**75.2**	**4.5**

DSP 80% VT Primed						
ID	Baseline	A	TD	Tau	Baseline + A	TD+T
13	-21.840	71.988	15.98	13.99	50.1	29.96
18	-7.990	103.536	12.23	18.00	95.5	30.23
21	-11.200	16.278	14.92	13.23	5.1	28.15
24	5.160	167.505	10.18	21.90	172.7	32.07
29	141.660	110.019	17.01	23.99	251.7	41.01
38	-18.410	21.002	15.71	26.06	2.6	41.77
39	-18.709	67.888	14.36	19.30	49.2	33.66
MEAN	**9.810**	**79.75**	**14.342**	**19.493**	**89.6**	**33.8**
SD	**58.844**	**53.015**	**2.375**	**4.847**	**92.3**	**5.4**

DSP 50%Delta Unprimed										
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	TDp + taup	Baseline + Ap	Absolute End Amp
13	57.120	36.993	5.51	14.04	6.8	107	71	19.5455	94.1	100.9131
18	56.600	18.500	7.00	20.00	9	76	64	27	75.1	84.09997
21	-46.600	14.500	13.00	14.00	9.000	169.000	64.000	27	-32.1	-23.1
24	165.850	50.994	0.03	18.99	2.78208	30.22144	9.5888	19.01223	216.8	219.6262
29	179.000	62.094	14.82	13.92	5.139	30.09	2.183	28.73839	241.1	246.2337
38	-58.390	13.967	22.58	49.77	6.993	153.58	26.941	72.35274	-44.4	-37.4295
39	78.980	43.012	14.01	31.11	22.000	60.25	24.162	45.11813	122.0	143.9917
MEAN	**61.794**	**34.29**	**10.992**	**23.118**	**8.816**	**89.449**	**37.411**	**34.1**	**96.1**	**104.9**
SD	**92.288**	**19.103**	**7.398**	**13.237**	**6.208**	**55.997**	**28.409**	**18.9**	**110.2**	**109.4**

DSP 50%Delta Primed										
ID	Baseline	Ap	TDp	Taup	As	TDs	Taus	TDp + taup	Baseline + Ap	Absolute End Amp
13	49.332	39.965	5.42	16.30	1.500	110.60	7.000	21.716	89.3	90.797
18	101.490	22.985	6.90	25.91	1.570	138.80	7.063	32.806	124.5	126.044
21	12.220	20.511	3.98	23.87	11.300	103.81	64.353	27.844	32.7	44.031
24	183.560	34.197	4.51	19.09	3.489	38.99	8.298	23.603	217.8	221.246
29	290.680	37.298	13.99	12.00	2.300	28.40	3.990	25.990	328.0	330.277
38	1.570	5.933	24.84	10.71	2.600	109.80	24.000	35.546	7.5	10.103
39	67.720	45.011	6.98	34.95	17.000	70.82	68.960	41.933	112.7	129.731
MEAN	**100.939**	**29.41**	**9.516**	**20.404**	**5.680**	**85.888**	**26.238**	**29.9**	**130.4**	**136.0**
SD	**103.554**	**13.611**	**7.535**	**8.536**	**6.053**	**40.894**	**28.395**	**7.2**	**110.6**	**109.2**

VO_2 MRT – ND & T2DM

VO2 MRT				
NDSP 50% Delta Unprimed Work to Work			DSP 50% Delta Unprimed Work to Work	
ID	MRT		ID	MRT
7	57.55		13	66.59
8	59.21		18	61.78
9	81.27		21	78.80
13	62.44		24	82.80
14	70.26		29	85.29
16	64.60		38	68.00
18	62.24		39	60.00
Mean	65.37		Mean	71.89
SD	8.11		SD	10.27
NDSP 50% Delta Primed Work to Work			DSP 50% Delta Primed Work to Work	
ID	MRT		ID	MRT
7	42.75		13	70.62
8	33.00		18	35.56
9	52.99		21	67.62
13	47.99		24	71.00
14	69.99		29	60.27
16	70.99		38	28.41
18	60.09		39	38.84
Mean	53.97		Mean	53.19
SD	14.06		SD	18.30

[HHb+Mb] MRT – ND & T2DM

NIRS MRT				
NDSP 50% Delta Unprimed Work to Work			DSP 50% Delta Unprimed Work to Work	
ID	MRT		ID	MRT
7	17.05		13	29.87
8	21.30		18	54.02
9	108.05		21	78.00
13	5.92		24	20.91
14	94.56		29	16.86
16	64.20		38	104.23
18	19.11		39	58.94
Mean	47.17		Mean	51.83
SD	41.45		SD	32.01
NDSP 50% Delta Primed Work to Work			DSP 50% Delta Primed Work to Work	
ID	MRT		ID	MRT
7	18.00		13	18.98
8	31.00		18	27.01
9	12.11		21	63.10
13	43.00		24	17.00
14	75.00		29	10.00
16	34.98		38	68.00
18	11.03		39	52.04
Mean	32.16		Mean	36.59
SD	22.40		SD	23.88

Δ[HHb+Mb]/ΔVO 2 Index – 80% VT Element

NDSP	Mean HHB.VO2 ratio (5s)	
	Unprimed	Primed
7	1.085	0.936
8	0.789	0.910
9	1.003	1.071
13	0.870	0.950
14	1.520	0.756
16	1.108	1.054
18	1.037	0.898
Mean	1.059	0.939
SD	0.234	0.105

DSP	Mean HHB.VO2 ratio (5s)	
	Unprimed	Primed
13	1.217	1.291
18	0.970	1.000
21	0.951	1.012
24	0.948	1.127
29	1.250	0.840
38	1.537	0.760
39	1.794	1.109
Mean	1.238	1.020
SD	0.326	0.179

Heart rate responses – ND & T2DM

	80% VT Unprimed W-W			80% VT Primed W-W			50% Delta Unprimed W-W			50% Delta Primed W-W		
ND	HR Baseline	HR End	HR Delta	HR Baseline	HR End	HR Delta	HR Baseline	HR End	HR Delta	HR Baseline	HR End	HR Delta
8	91	150	59	99	158	59	150	182	32	158	171	13
9	84	110	26	91	117	26	110	137	27	117	139	22
13	113	142	29	122	155	33	141	182	41	155	175	20
14	101	123	22	116	143	27	123	164	41	143	172	29
16	122	153	31	126	148	22	153	176	23	148	176	28
18	91	145	54	125	161	36	145	185	40	161	186	15
Mean	99	135	36	111	145	34	135	167	32	145	169	22
SD	14	16	14	14	16	12	16	19	8	16	15	7

	80% VT Unprimed W-W			80% VT Primed W-W			50% Delta Unprimed W-W			50% Delta Primed W-W		
T2DM	HR Baseline	HR End	HR Delta	HR Baseline	HR End	HR Delta	HR Baseline	HR End	HR Delta	HR Baseline	HR End	HR Delta
13	110	137	27	122	147	25	137	163	26	147	165	18
18	113	141	28	114	143	29	141	157	16	143	159	16
21	102	127	25	109	134	25	127	147	20	134	154	20
24	128	150	22	137	155	18	150	171	21	155	170	15
29	120	153	33	128	153	25	153	173	20	153	178	25
38	107	129	22	125	137	12	129	162	33	137	163	26
39	117	132	15	119	132	13	132	144	12	132	147	15
Mean	114	138	25	122	143	21	138	162	21	143	162	19
SD	9	10	6	9	9	7	10	10	7	9	10	5

Haematological results – ND & T2DM

NDSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
7	3.90	X	4.82	3.37	1.20	0.6
8	3.90	5.08	2.72	1.27	1.20	0.6
9	4.90	X	3.37	1.35	1.50	1.1
14	4.00	5.10	4.56	2.54	1.20	1.8
16	3.90	4.90	3.13	1.58	1.00	1.1
18	5.90	5.20	4.48	2.75	1.10	1.5
13	x	x	x	x	x	x
Mean	**4.42**	**5.07**	**3.85**	**2.14**	**1.20**	**1.12**
SD	**0.83**	**0.13**	**0.88**	**0.86**	**0.17**	**0.48**

DSP	FPG (mmol.L)	HbA1c (%)	TCL (mmol.L)	LDL (mmol.L)	HDL (mmol.L)	TRY (mmol.L)
13	x	6.9	4	2	1.2	1.8
18	9.8	7.3	x	x	x	x
21	6.9	6.2	x	x	x	
24	7	6.5	3.7	1.98	0.96	x
38	6.9	5.3	5	3.3	1.03	1.4
39	4.8	x	x	x	x	3.8
29	x	x	x	x	x	x
Mean	**7.08**	**6.44**	**4.23**	**2.43**	**1.06**	**2.33**
SD	**1.78**	**0.76**	**0.68**	**0.76**	**0.12**	**1.29**

Accelerometry data – ND & T2DM

NDSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
7	x	x	x	x
8	19.400	3.727	0.683	0.190
9	22.163	1.667	0.160	0.010
13	19.437	4.067	0.203	0.293
14	17.283	4.533	1.473	0.710
16	18.597	4.283	0.927	0.193
18	18.107	4.743	0.943	0.207
Mean	**18.36**	**4.41**	**0.89**	**0.35**
SD	**0.90**	**0.29**	**0.52**	**0.24**

DSP	Sedentary hr/day	Light hr/day	Moderate hr/day	Vig hr/day
13	x	x	x	x
18	x	x	x	x
21	18.74	5.07	0.18	0.01
24	16.82	6.60	0.51	0.07
38	x	x	x	x
39	x	x	x	x
29	x	x	x	x
Mean	**17.78**	**5.83**	**0.35**	**0.04**
SD	**1.36**	**1.08**	**0.23**	**0.04**

LOPAR data – ND & T2DM

NDSP	LOPAR MET/hours Per Week
7	192.75
8	152.125
9	179.0
13	95.3
14	272.0
16	125.3
18	x
Mean	**169.4**
SD	**61.5**

DSP	LOPAR MET/hours Per Week
13	270.8
18	143.8
21	55.5
24	x
38	x
39	280.0
29	x
Mean	**187.5**
SD	**107.7**