DYSFUNCTIONAL MUSCLE BLOOD FLOW REGULATION DURING EXERCISE IN TYPE 2 DIABETES

by
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ABSTRACT

There is some evidence to suggest that oxygen consumption (VO$_2$) and oxygen delivery to muscle are reduced at exercise onset and steady state in individuals with type 2 diabetes (T2D), although no studies have combined measurements of both muscle blood flow and VO$_2$ during exercise in this population. OBJECTIVES: 1) To determine whether a reduction in VO$_2$ during exercise would be accompanied by reduced leg blood flow (LBF). 2) To examine the dynamic response characteristics of LBF to determine whether feedforward and/or feedback control systems of blood flow regulation are impaired. METHODS: Four men with T2D and six healthy, activity matched controls (CON) performed supine, two-leg knee extension/flexion exercise tests involving progressive increase in exercise intensity to exhaustion and step increases to a low intensity equivalent to lifting 7.5 kg (LO$_{7.5kg}$), and a moderate intensity equivalent to 90% of ventilatory threshold (VT$_{90\%}$). MEASUREMENTS: LBF, VO$_2$, mean arterial pressure, heart rate, and stroke volume were measured continuously. RESULTS: Means ± SE, CON vs. T2D. 1) ΔVO$_2$ was not different between groups during the incremental test (P = 0.264), ΔLBF in T2D tended to be lower (P = 0.098). 2) ΔVO$_2$ was not different between groups at any time during LO$_{7.5kg}$ (P = 0.351). Individuals with T2D demonstrated a lower ΔLBF at time = 15 s (3435.6 ± 275.0 vs. 2120.4 ± 218.4 ml/min, P = 0.018). 3) Gains for baseline (G$_0$) and phase I (G$_1$) LBF adaptation to LO$_{7.5kg}$ were lower in T2D compared to CON (G$_0$: 959.8 ± 111.3 vs. 617.0 ± 22.1 ml/min, P = 0.044; G$_1$: 3662.1 ± 229.0 vs. 2128.1 ± 161.6 ml/min, P = 0.002). 4) The time required to achieve 63% of the total response magnitude tended to be slower in T2D (LO$_{7.5kg}$: 14.3 ± 1.7 vs. 23.1 ± 4.2 s; VT$_{90\%}$: 26.2 ± 3.5 vs. 40.0 ± 7.5 s; P = 0.095). CONCLUSIONS: 1) The initiatory rise in
LBF is significantly lower in individuals with T2D, likely due to impairments in feedforward control mechanisms of blood flow regulation, 2) Individuals with T2D do not demonstrate lower VO₂ responses to exercise despite an impaired LBF response.
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LIST OF ABBREVIATIONS

ADP – adenosine diphosphate
ATP – adenosine triphosphate
bpm – beats per minute
cm – centimeters
CO – cardiac output
CO₂ – carbon dioxide
CON – control group
HbA₁c – glycosylated hemoglobin
HR – heart rate
kg – kilograms
l/min – litres per minute
LBF – leg blood flow
LO₇.₅kg – workload equivalent to lifting 7.5kg on knee extension and 2.25kg flexion
MAP – mean arterial pressure
ml – millilitres
mmol/L – millimoles per litre
MRT – mean response time
MVC – maximal voluntary contraction
NO – nitric oxide
O₂ – oxygen
PG – prostaglandins
P₁ – inorganic phosphate
PmO₂ – microvascular partial pressure of oxygen
Qm – microvascular oxygen delivery
s – seconds
SE – standard error
SV – stroke volume
τ – time constant
t – time
T2D – type 2 diabetes
TD – time delay
VO₂ – pulmonary oxygen uptake
VT – ventilatory threshold
VT₉₀% – workload equivalent to 90% of the subject’s ventilatory threshold
CHAPTER 1: INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disease characterized by hyperglycemia resulting from insulin resistance and relative insulin deficiency (2). Approximately 90 to 95% of individuals with diabetes mellitus are diagnosed with this form of the disease (2; 19). The worldwide prevalence of diabetes (type 1 and 2) was roughly 171 million in the year 2000, with projections of a doubling in the incidence of cases to 366 million by 2030 (143). In Canada, there were approximately 1.4 million individuals diagnosed with diabetes in 2000, and this number is expected to increase to 2.4 million by 2016 (79). The estimated cost of diabetes care in the United States in 2007 was estimated to be $174 billion dollars, with the average medical expenditures for people with diabetes being 2.3 times higher than a comparable individual without diabetes (19).

The risk for developing T2D increases with age, obesity, and lack of physical activity (2). A family history of diabetes, gestational diabetes, and race/ethnicity also play a role in developing this disease (19). Symptoms include polyuria, polydipsia, weight loss and blurred vision (2). Individuals with long term diabetes are known to develop complications leading to retinopathy, nephropathy, and autonomic neuropathy. Additionally, these individuals are also at greater risk of cardiovascular, peripheral arterial, and cerebrovascular disease (2).

The World Health Organization (WHO) recommended in 2006 that the criteria for diagnosis of diabetes mellitus include either a fasting plasma glucose concentration of greater than or equal to 7.0 mmol/L (126 mg/dl), or a plasma glucose concentration of greater than or equal to 11.1 mmol/L (200 mg/dl) 2-hrs following ingestion of a 75 g glucose load (oral glucose tolerance test) (2; 152). Glycosylated hemoglobin (HbA1c) is a
measure of glycemic control, reflecting an individual’s average plasma glucose over the previous 2-3 months (152). However, there are inconsistencies in its measurement and analysis techniques are not readily available in many countries (152). Therefore it is currently not recommended as criteria for diagnosis of diabetes (152).

The increased prevalence of T2D has lead to an urgent demand for effective treatment and prevention strategies. Physical activity has been identified as an important component of these interventions (4; 68; 121). The benefits of exercise programs for individuals with T2D are numerous, including improvement in glycemic control, maintenance of a healthy weight, and reduction of cardiovascular risk factors (74; 121; 126). However, T2D is also associated with a decreased exercise capacity, demonstrated by a slower increase in oxygen uptake (VO\(_2\)) at the onset of exercise, as well as reduced maximal oxygen uptake (VO\(_{2\text{max}}\)) compared to healthy, age and physical activity matched controls (14; 92). The slower activation of oxidative metabolism necessitates an increased reliance on substrate level phosphorylation to meet ATP demand, resulting in a greater perturbation of cellular homeostasis and a quicker onset of fatigue. Individuals with T2D may find participation in regular physical activity more difficult and are therefore less likely to attain the full benefits of training.

The exact nature of the reduced exercise tolerance in T2D is unclear, but deficiencies in both central and peripheral determinants of muscle oxygen uptake are likely contributors. Impaired cardiac function in the form of slowed heart rate kinetics has been found in some studies (92) but not others (10). The rise in heart rate increases cardiac output, the inflow to the large arteries, at the onset of exercise; this helps to maintain mean arterial pressure despite increased outflow due to vasodilation in the
skeletal muscle. If the initial increase in heart rate, and hence cardiac output, is delayed, constriction of blood vessels in the peripheral circulation would be required to preserve arterial pressure. As a result, the normal increase in skeletal muscle blood flow and oxygen delivery would be compromised. Despite this initial delay, however, it appears cardiac output eventually reaches the same steady state values as in healthy, matched individuals (60). During exercise, cardiac output serves to maintain mean arterial pressure and hence the driving pressure for muscle blood flow. Interestingly, steady state leg blood flow in one study of exercise in T2D is lower with similar cardiac output (60), suggesting that central factors are not the only aspect of oxygen delivery which may be impaired in this population.

Peripheral deficiencies include reports of altered skeletal muscle energy metabolism, including dysfunctional mitochondria (54), impaired glucose (55) and free fatty acid (56) utilization, as well as accelerated phosphocreatine hydrolysis and rapid decreases in pH (110) with exercise. The latter two findings are also accompanied by greater muscle deoxygenation compared to healthy matched controls, with the rate of deoxygenation being almost 3.1-fold faster during the first 3 minutes of exercise. These findings suggest that increased oxygen extraction was required to compensate for reduced oxygen delivery at the onset of exercise, an impairment that critically affects cellular homeostasis and hence exercise tolerance in this population.

Impaired vascular function is also a hallmark characteristic of T2D. Dysfunctional endothelial dependent vasodilation has been demonstrated in a number of studies (58; 76; 125). However, the extent to which this affects skeletal muscle oxygen delivery is unknown. Estimates of microvascular blood flow kinetics obtained from skeletal muscle
deoxygenation and pulmonary VO2 data (via Fick principle) demonstrate a relationship between reduced oxygen delivery and slowed muscle VO2 onset kinetics (10).

To date, very few studies have examined limitations to exercise in T2D in terms of muscle blood flow (bulk oxygen delivery). Kingwell et al. (58) found that individuals with T2D had a significantly reduced leg blood flow response to 25-mins of cycling at 60% VO2peak. Similarly, a recent study showed a reduced steady state blood flow response to supine leg extension exercise (60). Unfortunately, no data were available regarding the time course and magnitude of changes in blood flow leading up to steady state. Investigation of this adaptation phase is extremely important as insufficient adjustment of bulk muscle blood flow, both in terms of rate of increase and magnitude, limits adjustments in oxygen delivery and therefore could contribute to the slower increase in VO2 in T2D at the onset of exercise (14; 92).

Since oxygen uptake and oxygen delivery are critically related, exercise studies examining each independently are limited in providing insight into cardiovascular support of muscle metabolism. In addition, the latter has only been measured during steady state exercise. To our knowledge, no study has combined direct measures of both muscle blood flow and oxygen uptake to examine the dynamic response of both in T2D. The purpose of this thesis work was to investigate the impact of T2D on the oxygen delivery and utilization responses to exercise across a range of exercise intensities.

**Specific Objectives**

1) To confirm previous findings of reduced a) submaximal oxygen uptake (VO2), and b) peak oxygen uptake (VO2peak) responses to non steady state, incremental exercise in individuals with T2D versus healthy controls.
2) To determine whether a lower blood flow response accompanies reductions in VO\(_2\) during incremental and steady state exercise in individuals with T2D versus healthy controls.

3) To determine whether the *dynamic characteristics* of the leg blood flow response to constant work exercise in individuals with T2D is slowed versus healthy controls.

**Hypotheses**

1) a. Subjects with T2D will demonstrate a reduced submaximal VO\(_2\) response during incremental knee extension/knee flexion exercise regardless of workload compared to healthy controls.

   b. At exhaustion, they will also demonstrate a reduced peak VO\(_2\) response compared to healthy controls.

2) Subjects with T2D will demonstrate a reduced blood flow response to incremental and constant-work knee extension/knee flexion exercise regardless of workload compared to healthy controls.

3) Subjects with T2D will demonstrate an impaired adjustment of leg blood flow to constant-work knee extension/knee flexion exercise compared to healthy controls. This will be characterized by a delayed onset of response, as well as a slower increase to steady state in both the light and moderate exercise intensity domains.
CHAPTER 2: LITERATURE REVIEW

Muscle Contractile Function: Impact of Muscle Oxygenation

Muscle contraction requires energy in the form of adenosine triphosphate (ATP). There are various pathways within the cell which are responsible for the production of ATP, processes which can be divided into two main categories: those which require oxygen (aerobic), and those which do not (anaerobic). In order to sustain exercise for more than a few minutes, the demand for ATP from the contracting muscle must be met by aerobic metabolic pathways of ATP production (132). However, during a step transition in exercise intensity, the rate of ATP supply from oxidative phosphorylation is slowed compared to the immediate increase in ATP demand (Fig. 1). According to the net drive hypothesis, the rate of mitochondrial respiration is dependent upon: 1) the intracellular concentrations of the metabolic substrates ADP, P_i and NADH, and 2) the partial pressure of oxygen within the cell, PcellO_2 (132). The ratio of the concentrations of these substrates and their products, [NADH] / [NAD^+] and [ATP] / [ADP][P_i] represent the redox (reduction/oxidation) state and phosphate energy state of the cell, respectively. Together with PcellO_2, a measure of muscle oxygenation, these three factors interact to determine the rate of increase in mitochondrial respiration and hence aerobic ATP production (Fig. 2).

At the onset of muscle contraction, the increased rate of ATP hydrolysis results in a rise in [ADP] and [P_i]. As changes in the phosphate energy state are directly linked to ATP demand, they act as regulators of oxidative phosphorylation (132). At a given phosphate energy state, ATP production depends on PcellO_2 and the redox state, both of
which can independently modulate the rate of ATP supply (132). PcellO\textsubscript{2} is determined by a combination of both convective and diffusive oxygen delivery (132). While muscle blood flow and arterial oxygen content determine the bulk transport of oxygen from the lungs to the skeletal muscle, oxygen delivery into the myocyte is dependent on the muscle’s diffusive conductance, and on the oxygen pressure gradient between the microvasculature and the cell (PmO\textsubscript{2} – PcellO\textsubscript{2})(132). As arterial oxygen content and muscle diffusive conductance are constant under normal conditions, muscle blood flow and PmO\textsubscript{2} are the main factors effecting muscle oxygenation.

So what limits the rate of increase in oxidative phosphorylation? At the onset of a step increase in exercise intensity, the change in the phosphate energy state due to ATP hydrolysis communicates to the mitochondria a new ATP demand (131; 132). As the ratio of [ATP] / [ADP][Pi] decreases, this modulates an increase in oxygen uptake (VO\textsubscript{2}), which reflects oxidative phosphorylation (131). According to the net drive hypothesis, the rate at which VO\textsubscript{2} increases depends on the intracellular levels of oxygen, PcellO\textsubscript{2} (132). At lower PcellO\textsubscript{2}, a greater decrease in phosphate energy state (or increase in substrates ADP and P\textsubscript{i}) needs to occur before a given VO\textsubscript{2} can be achieved (146; 148-150). Therefore, at a given phosphate energy state, a myocyte with a lower PcellO\textsubscript{2} will have a lower VO\textsubscript{2}, compared to one with a higher PcellO\textsubscript{2}. However, as the substrates ADP and Pi are potent stimulators of glycolysis (124), and have inhibitory effects on contractile proteins (132), the effect of low muscle oxygenation is not only a delayed increase in VO\textsubscript{2}, but a contributor to early muscle fatigue (132). In contrast, at high levels of oxygenation, there exists a point in which further increases in PcellO\textsubscript{2} can no longer modulate increases in VO\textsubscript{2} (see Fig. 2, plateau). At this PcellO\textsubscript{2}, oxygen levels are
considered “saturating”, and muscle oxygenation is no longer rate limiting to the increase in VO₂ (131). Instead, the rate of oxidative phosphorylation depends solely on the metabolic machinery, the rate at which the mitochondria can utilize oxygen (39). This is unlikely to be the case during exercise in normoxic conditions as PcellO₂ can drop from ~30 mmHg at rest to less than 5 mmHg (97; 98). Furthermore, it has been suggested that even at PcellO₂s of 30 mmHg, changes in phosphate energy state and redox are already required to achieve a given rate of oxidative phosphorylation (131; 150). Finally, an increase in metabolic enzymes, such as with a greater number of mitochondria, requires smaller changes in phosphate energy state and redox state to achieve a given VO₂ (24; 46; 132). Similarly, at a given PcellO₂ and metabolic state, a lower activation of enzymes will result in a lower VO₂. The availability of metabolic enzymes impacts the rate of VO₂ in much the same way as oxygen availability. In summary, the rate of ATP supply via oxidative phosphorylation depends on the net drive of the phosphate energy and redox states, PcellO₂, and the levels of enzyme activation. Muscle oxygenation is not only a critical determinant of the adjustment of VO₂, but also has implications for muscle fatigue, as a slowed rate of increase in oxidative phosphorylation is accompanied by a greater stimulation of anaerobic energy pathways.

Individuals with type 2 diabetes (T2D) have impaired exercise tolerance compared to individuals of similar age and physical activity levels. The majority of the evidence for this comes from reports of impaired VO₂ responses to exercise (8; 10; 14; 92; 95). The inability to produce ATP through aerobic pathways necessitates an increase reliance on anaerobic means of ATP supply, namely phosphocreatine breakdown and glycolysis. These processes result in an accelerated accumulation of metabolic
byproducts, such as $P_i$, ADP and lactate, that can impair muscular function (132). Therefore, the ability to sustain physical activity at a given work output may be reduced.

Peak oxygen uptake, $VO_2\text{peak}$, is the highest rate at which the body can consume oxygen during maximal exercise, and is considered one of the best measures of an individual’s cardiorespiratory fitness (47). Individuals with T2D demonstrate lower $VO_2\text{peak}$ compared to matched controls (8; 14; 60; 92; 95). However, findings of no difference have also been reported (57; 58; 70; 93). Maximal treadmill performance in a study by Regensteiner et al. (95) was 20% lower in individuals with T2D compared to healthy, activity matched controls, and was not predicted by weight, body mass index, years of disease, fasting insulin levels, fasting and post-exercise glucose levels, percentage of glycosylated hemoglobin ($HbA_{1c}$), or whole blood viscosity. In a comparison of sedentary, age and weight matched individuals (8), reduced VO$_2$ in individuals with T2D was linearly correlated with reduced peripheral oxygen extraction, rather than cardiac output, at maximal exercise. In this study, impairment in peripheral oxygen delivery may have limited the VO$_2$ response to maximal exercise.

In addition to possible reductions in maximal exercise performance, there have also been reports of abnormal VO$_2$ responses to submaximal workloads. During a graded exercise test in which either treadmill speed (2 mph) or grade (3.5%) was increased every 2-mins, individuals with T2D demonstrated lower VO$_2$ responses from 4 mph and 7% grade until exhaustion, compared to healthy, age, sex and physical activity matched controls (95). These differences existed even when values were normalized for weight, height or BMI. Similarly, during an incremental cycling test (8), absolute VO$_2$ was significantly lower at 75 and 125 W, and tended to be lower at 50, 100 and 150 W in
individuals with T2D versus controls. As the previous two studies measured VO\textsubscript{2} responses during incremental exercise, where workloads were maintained for 2-mins or less, data were non-steady state values. Interestingly, when exercise is maintained for longer periods of time, submaximal steady state values of VO\textsubscript{2} are not different from that of healthy controls. Regenstein er et al. (92) found that VO\textsubscript{2} responses after 7-mins of constant-load cycling at 20, 40 and 80 W were not significantly different between groups. Therefore, the differences between groups during incremental exercise result from an impairment in the adjustment of VO\textsubscript{2} to steady state.

Recently, the use of VO\textsubscript{2} on-kinetics as a measure of aerobic conditioning has gained popularity, especially in elderly and patient populations as the tests do not require exertion of maximal effort (39). The rate of adjustment in VO\textsubscript{2} during a step transition from rest to exercise provides valuable information about the integrated response of the pulmonary, cardiovascular and muscular systems (39). As activities of daily living involve countless changes in work output, for example walking up stairs or lifting objects, the examination of VO\textsubscript{2} on-kinetics may provide a better understanding of the challenges facing individuals who have trouble performing these tasks. As discussed above, if the onset of oxidative metabolism is slowed, as demonstrated by delayed on-kinetics of VO\textsubscript{2}, increased reliance on anaerobic means of energy production negatively impact on the ability to sustain muscular work. Consistent with the finding of impaired non-steady state VO\textsubscript{2} responses, VO\textsubscript{2} on-kinetics for work rates above and below lactate threshold have been shown to be significantly delayed in individuals with T2D compared to healthy controls (14; 92). These findings were observed during constant-load cycling at 20, 30 (14; 92) and 80 W (14), and were independent of obesity related influences, as
comparisons were made with overweight-matched and lean controls. In both of these studies, it is unknown whether the delayed rise in VO$_2$ is due to limitations in oxygen delivery and/or utilization. However, slowed heart rate kinetics were observed in addition to the slowed on-kinetics of VO$_2$ in (92), leading to the possibility of an impairment in convective oxygen delivery in these individuals. According to the net drive hypothesis, a reduction in oxygen delivery would lower PcellO$_2$, therefore requiring larger changes in phosphate energy state and/or redox state before a given rate of VO$_2$ can be achieved. Therefore, at a given $[\text{ATP}] / [\text{ADP}][\text{Pi}]$, individuals with T2D would have lower rates of oxidative phosphorylation compared to their healthy counterparts. This would have led to the delayed on-kinetics observed. Interestingly, steady state submaximal VO$_2$ did not differ between groups in this study (92). Therefore, while the initial on-kinetics are slowed, the eventual rate of oxidative phosphorylation was not different between healthy and T2D. This may have resulted from increased PcellO$_2$ due to feedback mechanisms correcting muscle oxygenation, or if PcellO$_2$ remained reduced, greater intracellular changes in phosphate energy state and redox state, compared to CON, that maintained the require rate of oxidative phosphorylation.

As regular physical activity is an important strategy in the treatment and prevention of T2D, it is important to gain an understanding of why individuals in this disease population have a lowered capacity to sustain exercise. Whether the delayed kinetic response of oxidative phosphorylation is related to central deficiencies in oxygen delivery or peripheral abnormalities in oxygen diffusion or utilization is yet to be determined. Based on the important role of PcellO$_2$ in modulating VO$_2$ and determining muscle fatigue, it is possible that impairments in oxygen delivery contribute to the
exercise intolerance found in this population. The following is a review of the evidence supporting deficient muscle oxygenation in individuals with T2D.

Fig. 1. Time course changes in ATP supply and demand at the onset of exercise (time = 0). Initiation of muscle contraction results in an immediate increase in ATP demand (dotted line). ATP supply via oxidative metabolism is delayed and follows an approximately exponential rate of increase. During this delayed rise in VO₂, the difference between ATP demand and supply via oxidative metabolism (shaded region) is made up by phosphocreatine breakdown and anaerobic glycolysis.
Fig. 2. Schematic representation of the net drive hypothesis. A constant rate of oxidative phosphorylation can be maintained despite reductions in PcellO₂ (circle → triangle) if adjustments in both the phosphate energy state ([ATP]/[ADP][Pi]) and redox state ([NADH]/[NAD⁺]) are made. The vertical dotted line represents the PcellO₂ at which compensations by the two metabolic states can no longer maintain the given rate of VO₂. Modified from (132).
Evidence for Impaired Muscle Oxygenation in T2D

Recent studies in both human and animal models with T2D suggest that the slowed oxygen uptake kinetics found in this population may be a result of impaired oxygen delivery (10; 58; 60; 83; 84; 151). Evidence exists for impairments at both the whole muscle level (10; 58; 60), as well as in the microvasculature within skeletal muscle (83; 84; 151). A blunted increase in oxygen delivery at the onset of exercise will result in a greater drop in the partial pressure of oxygen within the microvasculature (PmO2), reducing the driving pressure for blood-myocyte oxygen diffusion, and therefore possibly constraining the early increase in VO2 by lowering PcellO2.

To date, only a handful of studies have examined conduit artery blood flow and oxygen delivery during exercise in individuals with T2D. In 2003, Kingwell et al. (58) used thermodilution techniques to compare leg blood flow (LBF) responses in nine subjects with T2D and nine healthy age-, sex-, weight- and fitness- matched control subjects. LBF was measured at rest and at steady state during 25-mins of supine cycling exercise at 60% of VO2peak. At rest, LBF was similar between subject groups. However, steady state blood flow, from 10- to 25-mins, was significantly lower in the group with T2D compared to controls (2.78 ± 0.19 vs. 3.73 ± 0.38 l/min; means ± SE, T2D vs. controls). As arterial perfusion pressure was higher in T2D during exercise, the lower LBF response observed was attributed to a higher leg vascular resistance. The second study, published by Lalande et al. (60) in 2008, demonstrates a similar finding of reduced LBF during submaximal exercise in subjects with T2D compared to weight and physical activity matched controls. Magnetic resonance imaging (MRI) was used to measure femoral blood flow responses to supine, leg extension exercise in which subjects lifted a
1.5 kg weight attached at their ankles. Steady state measurements were taken when heart rate fluctuated less than 5 beats within a 30-s interval. LBF, indexed to thigh lean mass, was similar between groups at rest, but was significantly lower in the group with T2D during steady state exercise (6.7 ± 0.8 vs. 8.3 ± 0.7 ml/s/kg; T2D vs. controls). Assuming 7 kg of thigh lean mass in both groups (Table 1 in (60)), this corresponds to LBFs of ~2.81 l/min in T2D and 3.49 l/min in the controls. There was no difference in cardiac output between groups during the exercise, however given the low exercise work rate employed, central hemodynamics would not be expected to play a role. In contrast to the two previous investigations, a study examining splanchnic and muscle metabolism in non-obese T2D subjects and weight matched controls (70) found no difference in LBF at rest or steady state during 40-mins of upright cycling at 60% VO2peak (3.09 ± 0.25 vs. 3.07 ± 0.25 l/min; T2D vs. controls). The absence of impairment in steady state LBF may be related to differences in fitness in this T2D group compared to those in previous studies. Peak oxygen uptake in this group was 3.63 l/min, compared to 2.53 l/min in (58) and 2.31 l/min in (60). Finally, in a study examining skeletal muscle capillary recruitment during intermittent handgrip exercise (151), brachial artery blood flow measured by Doppler ultrasound techniques was found to be similar in patients with T2D (with and without microvascular complications) and healthy controls. Results were consistent at rest, and during exercise at both low and high intensity workloads (20 and 80% of maximal voluntary contraction respectively). While previous studies have examined responses in lower body, large muscle mass cycling exercise, the use of a forearm model represents a unique investigation as hemodynamic responses are not limited by central cardiovascular factors (107; 109; 118; 120; 134). It is possible then, that the lack of
significant difference between the groups is a result of the examination of a much smaller muscle mass.

Recent investigations in both human and rodent models of T2D have provided valuable information on oxygen delivery within the microvasculature of skeletal muscle. An examination of microvascular oxygen pressures in the spinotrapezius muscle of Goto-Kakizaki rats (GK), a model of spontaneous, non-obese T2D (38), has provided further support of impairments in the skeletal muscle of this population (84). The partial pressure of oxygen in the microvasculature (PmO$_2$) is a measure of the dynamic balance between microvascular oxygen delivery (Qm) and oxygen uptake (VO$_2$m). Therefore, changes in PmO$_2$ signify a mismatch between the two. At rest, GK rats demonstrate a significantly lower PmO$_2$ compared to healthy, control rats (29 ± 2 vs. 18 ± 2 Torr, controls vs. GK respectively). As PmO$_2$ represents the high energy component of the pressure gradient driving oxygen diffusion from the blood into the myocyte, this finding implies a significant impairment in oxygen transport at rest. At the onset of twitch muscle contractions, the pattern of fall in PmO$_2$ is significantly altered in GK rats compared to controls. This implies a major divergence in oxygen delivery responses between the two groups. In the control rats, PmO$_2$ fell exponentially to steady state following a brief (~10-s) time delay (Fig. 3A). In the GK rat, after a similar time delay, PmO$_2$ fell significantly faster (time to 63% of initial drop in PmO$_2$: 16 ± 4 vs. 6 ± 2 s, controls vs. GK) and displayed an undershoot before recovering to a level close to or slightly higher than baseline (Fig. 3B). Interestingly, PmO$_2$ at end contraction is not different between the T2D and control animals, meaning there was eventually no difference in the Qm/VO$_2$m ratio between groups.
Fig. 3. Reduced baseline and accelerated pattern of fall in the microvascular partial pressure of oxygen (PmO\textsubscript{2}) within the spinotrapezius muscle of the rat model of T2D. 
A: Healthy, Wistar rats. B: Goto-Kakizaki diabetic rats. The onset of twitch muscle contraction occurred at Time = 0. Steady state PmO\textsubscript{2} was not significantly different between groups (dotted line). Modified from (84).
The physiology behind the response profiles can be discussed using the conservation of mass model where $PmO_2$, in the compartment of interest – the microvasculature within skeletal muscle, is a function of the balance between $Qm$ and $VO_2m$, the inflow to and outflow from the compartment, respectively (Fig. 4). At rest, inflow matches outflow as $PmO_2$ is constant (though lower in T2D) (Fig. 4A). During the brief time delay, despite the start of twitch muscle contractions, $PmO_2$ remains unchanged. This implies that the early increases in both variables were in balance. The drop in $PmO_2$ occurs when outflow eventually starts to exceed the rate of inflow. As $PmO_2$ drops, so does the driving pressure for oxygen diffusion into the myocyte. Concurrently, $Qm$ begins to increase in response to the metabolic demand. The result of these two changes is a reduction in the mismatch between $VO_2m$ and $Qm$, and therefore slowing of the fall in $PmO_2$. At steady state, inflow of oxygen into the microvasculature matches the outflow of oxygen into the myocyte, but at a lower $PmO_2$. This is the response observed in healthy skeletal muscle. In the microvasculature of T2D however, this pattern is significantly altered. The initial fall in $PmO_2$ occurs much more rapidly in T2D skeletal muscle, suggesting a delayed increase in $Qm$ compared to controls as outflow greatly exceeds inflow (Fig. 4B). The slow rise in $PmO_2$ back to baseline levels occurs due to one of two possibilities: 1) inflow starts to increase and eventually exceed the rate of outflow, or 2) a slowing of outflow occurs concurrent with a constant or increasing inflow. The latter possibility is unlikely, as the twitch contractions remained constant throughout the protocol, representing a fixed metabolic requirement and eliciting a constant $VO_2m$ in the muscle. The second possibility is a delayed increase in $Qm$ that eventually exceeds the rate of $VO_2m$ and restores $PmO_2$ back to the exercising steady
state value. Slowed Qm on-kinetics have been reported, however these were based on estimates made from using the Fick equation (10). To date, there are no studies that have directly examined the dynamics of the Qm response to exercise. Finally, the balance between inflow and outflow does not differ between healthy and T2D muscle, demonstrating that at steady state, feedback controlled mechanisms of oxygen delivery and utilization did not differ between these groups.

Fig. 4. Changes in the partial pressure of oxygen within the microvasculature (PmO₂) as explained by the conservation of mass model. PmO₂ is a function of the dynamic balance between muscle blood flow (Qm) and oxygen uptake (VO₂m), the inflow and outflow from the compartment respectively. A: When PmO₂ is unchanging (i.e. rest or steady state), this represents a balance between Qm and VO₂m. B: If Qm is reduced, outflow will exceed inflow, resulting in a drop in PmO₂.
Recently, investigation of microvascular blood flow in the GK rat spinotrapezius muscle revealed significantly altered capillary hemodynamics at rest (83). Compared to healthy control rats, this model of T2D revealed a significant reduction in the lineal density of flowing capillaries, with the percentage of capillaries supporting red blood cell (RBC) flow ranging from 93 ± 3% in controls to 66 ± 5% in the GK rat. Furthermore, in the capillaries that did support continuous flow, RBC velocity was reduced by 65%, RBC flux by 66%, and capillary hematocrit by 30%. The reduced number of RBC available for oxygen diffusion at any given time would therefore be expected to slow the rate of oxygen transport into the muscle cell. An estimate of total oxygen delivery calculated from these findings indicated an overall reduction of up to 70% in the diabetic model. In line with the findings of reduced PmO₂ in the previously mentioned study (84), these data indicate a significantly altered hemodynamic environment supporting muscle oxygenation at rest. The accelerated pattern of fall in PmO₂ that has been observed in this diabetic model during muscle contraction (84) indicates further abnormalities in microvascular flow responses to exercise are likely.

Studies in human models also provide support for impaired microvascular oxygen delivery during exercise. The rate of skeletal muscle deoxygenation, as measured by near-infrared spectroscopy (NIRS), has been found to be 1) significantly larger in magnitude as demonstrated by an overshoot of the steady state response (10), and 2) significantly faster (~3.1 fold) (110), at the onset of exercise in individuals with T2D compared to healthy controls. The eventual time course of change in muscle deoxygenation, however, is not different between groups (110). The immediate increase in deoxygenated hemoglobin/myoglobin concentration ([HHb]) reflects a greater
dependence on oxygen extraction early on (10), and correlates well with the previously described PmO$_2$ responses found in rat skeletal muscle (84). A slower increase in Qm (inflow) compared to VO$_2$m (outflow) would cause the observed drop in PmO$_2$, resulting in a shift down the oxyhemoglobin dissociation curve and therefore increased offloading of oxygen from hemoglobin. Correspondingly, estimates of microvascular blood flow kinetics based on phase II pulmonary VO$_2$ and [HHb] responses (Fick equation) suggest slowed responses of Qm as demonstrated by a longer mean response time (35.8 ± 10.7 vs. 47.7 ± 14.3 s, controls vs. T2D; time to achieve 63% of total response magnitude) (10). These estimates are supported by findings of blunted capillary blood volume (CBV) responses to both low (20% MVC) and high (80% MVC) intensity forearm exercise in individuals with T2D and microvascular complications (151). This implicates abnormalities in capillary recruitment that affect the distribution of blood flow at the microvascular level despite normal conduit artery flow responses (see above). Interestingly, individuals with uncomplicated T2D showed preserved CBV responses compared to healthy controls, suggesting that the impaired capillary flow responses may be a function of disease progression.

Based on the evidence to date, an oxygen delivery limitation to muscle oxygenation appears quite likely. If impairments indeed exist at both the macro- and microvascular level, the result would be a significantly reduced pressure gradient for oxygen diffusion into the muscle. This, in combination with reports of altered skeletal muscle metabolism (54-56; 70; 99; 110), represents a major challenge to exercise tolerance in individuals with T2D. Future research is needed to integrate the findings of both animal and human models, to develop methods to examine both oxygen delivery and
utilization, and finally, to discover strategies that would relieve the burden of physical activity in this highly exercise intolerant population.

**Mechanisms Supporting Impaired Muscle Oxygenation in T2D**

Recent evidence suggests that control of oxygen delivery during exercise, both at the conduit artery level as well as in the microvasculature, is impaired in the skeletal muscle of T2D. However, the factors governing the impaired response are currently unclear. It is likely that both central and peripheral regulators of muscle blood flow are involved. The discussion of these factors will be presented in the following order. 1) central cardiovascular control mechanisms and their effects on arterial perfusion pressure, and 2) local vascular control mechanisms effecting vascular conductance. Perfusion pressure refers to the local arteriovenous pressure gradient driving flow into the vasculature, while vascular conductance refers to the degree to which flow is allowed (132), the balance between vasodilator and vasoconstrictor influences. Changes that occur in either of these variables as a result of the T2D state will impact on the regulation of oxygen delivery during exercise.

**Central Determinants of Oxygen Delivery**

Mean arterial pressure (MAP) is a tightly regulated variable during both rest and exercise. It also represents the driving pressure for muscle blood flow during exercise. At the onset of skeletal muscle contraction, vasodilation in the vascular beds of the active musculature causes an immediate shift in blood volume from the central arteries into the peripheral circulation. As a result, there is an abrupt fall in MAP. However, with the onset of exercise, both heart rate (HR) and stroke volume (SV) also increase, resulting in
an increase in cardiac output (CO = HR x SV). The increase in CO, the inflow into the large arteries, helps to restore MAP despite the increased outflow due to peripheral vasodilation. If, however, there is a delay in the increase in CO (as a result of an impaired HR and/or SV response), a baroreflex mediated increase in sympathetic vasoconstriction will occur in order to preserve MAP. As a result, the normal increase in skeletal muscle blood flow and oxygen delivery at the onset of exercise may be compromised.

Very few studies have examined cardiac responses during exercise in individuals with T2D. Heart rate kinetics were found to be slowed in one study (92) but normal in another (10). While it is unclear whether or not there is a delay in the central hemodynamic response at the onset of exercise, heart rate and/or cardiac output appear to be comparable to that of healthy controls during steady state submaximal exercise (60) and peak exercise (8; 14; 92; 93; 95). Therefore, impairments in oxygen delivery that exist following the onset of exercise may be a result of peripheral, rather than central, deficiencies.

In a study examining cardiac function during incremental exercise (93), women with recently diagnosed T2D demonstrated significantly greater increases in pulmonary capillary wedge pressure (PCWP) at submaximal workloads, as well as at peak exercise, compared to healthy controls. Myocardial perfusion index, when normalized by myocardial mass (g), BMI (kg/m²), and peak exercise double index (mmHg x HR), was also significantly lower in the group with T2D. Lastly, there was an inverse correlation between PCWP and myocardial perfusion at peak exercise, suggesting that the abnormal cardiac response during exercise was related to reduced perfusion of the myocardium (93). It should be noted that these findings were present despite the absence of left
ventricular (LV) systolic and diastolic abnormalities at rest. Despite these abnormal findings, however, HR, SV, CO and absolute VO\textsubscript{2} were not significantly different between groups at peak exercise. Therefore, while these findings may have clinical significance regarding the future development of cardiac dysfunction, they as of yet do not impair exercise tolerance. The similar peak exercise measures in this study may be related to the recruitment of relatively symptom free individuals, as these subjects were chosen based on their recent diagnoses of T2D and were vigorously screened for systolic and diastolic dysfunction at rest. LV diastolic function has been found to limit maximal exercise performance in patients with T2D, compared to patients with normal LV function (89). While some studies have shown impaired diastolic function at rest in individuals with T2D (9; 88), much more research is needed to investigate the impact of this dysfunction on submaximal exercise performance as well as the effect of impairments on measures of stroke volume and cardiac output (15).

**Peripheral Determinants of Oxygen Delivery**

While the existence of central impairments in oxygen delivery requires further investigation, the evidence for impairments in the peripheral circulation is much more robust. Vascular dysfunction is a well known consequence of T2D, with reports of impairments in both endothelial-dependent vasodilation (43; 58; 69; 76; 141; 144), and endothelial-independent vasodilation (76; 141; 144). The latter, however, is preserved in some T2D subject populations (43; 69). The degree of impairment in endothelium dependent vasodilation has not been found to correlate with fasting glucose levels or levels of glycosylated hemoglobin (HbA\textsubscript{1c}) (69; 76; 141; 144). However, as the cause of vascular dysfunction in T2D is presently unclear, it may be possible that multiple
abnormalities associated with the disease act to determine the impairments that are observed.

Plasma levels of endothelin-1 (ET-1), a factor that is produced by endothelial cells and acts on smooth muscle to cause vasoconstriction, are elevated in individuals with T2D and appear to contribute to endothelial dysfunction (72; 128). Blockade of ET-1 receptors reveals a significant influence of ET-1-mediated vasoconstriction on reductions in basal flow, as well as flows induced by metacholine infusion, a test of endothelial-dependent vasodilation (72).

Elevated levels of C-reactive protein (CRP), a marker of inflammation, have been found in individuals with T2D (33; 129; 151), and is associated with impaired endothelial function (21; 32; 129). The inactivation of endothelium derived nitric oxide (NO) by oxygen-derived free radicals is also thought to contribute to the dysfunction (130). Indeed, treatments aimed at reducing systemic inflammation have been found to improve endothelium dependent vasodilation (26; 129; 130). CRP has also been found in-vitro to reduce the expression and bioactivity of endothelial nitric oxide synthase (137), the enzyme that catalyzes the formation of NO from L-arginine. Therefore, it appears as though the vascular dysfunction associated with elevated levels of CRP result from reductions in both the production and bioavailability of NO.

The effect of advanced glycosylation end products on NO function has been examined in models of experimental diabetes both in vitro and in vivo. These products form at an accelerated rate in the presence of hyperglycemia (111), and accumulate in the subendothelial layer of collagen that separates the endothelial cells from the vascular
smooth muscle (16). In vitro, NO activity is inactivated in the presence of these end products (16). In rat models of diabetes, responses to both acetylcholine and nitroglycerin, endothelium dependent and independent factors respectively, were increasingly impaired with duration of hyperglycemia (16). Administration of aminoguanidine, a treatment that prevents the accumulation of advanced glycosylation end products, resulted in a sparing of the decline in vascular function (16).

Elevated blood viscosity (95) is associated with microvascular complications (151) and has been found to correlate positively with glucose levels (20; 151), and negatively with insulin sensitivity (44). Reduced RBC deformability and increased aggregation due to plasma protein changes have been implicated in blood flow abnormalities seen in diabetes (7; 62; 75). As RBC must distort in order to pass through the comparatively smaller diameters of capillaries (20), increased RBC rigidity would limit the flow of RBC travelling through the microvasculature (83) and hence the number of RBCs available for oxygen diffusion.

Recently, the human RBC, in addition to its role as an oxygen carrier, has been found to be a regulator of skeletal muscle perfusion (27; 28; 123). Along with the offloading of oxygen, the RBC has been shown to release ATP in response to a low oxygen environment (i.e. low PO₂) (27). This ATP then binds to purinergic receptors on the vascular endothelium, triggering a vasodilatory response (123). The signal for ATP release is stimulated by the heterotrimeric G protein, G₁ (80; 81). A fall in hemoglobin oxygen content activates G₁, triggering a signal transduction pathway that eventually results in the release of ATP from the RBC (123). Recently, it was shown that the expression of G₁₂ in RBCs is reduced in humans with T2D, a finding that was associated
with a decreased release of ATP (122). Furthermore, the reduced ATP release was inversely correlated with levels of glycosylated hemoglobin, a measure of glycemic control (122). Therefore, a reduced ability for RBCs to stimulate increases in vascular conductance when oxygen delivery is impaired may be another contributor to the perfusion abnormalities observed.

The above mentioned factors are all possible mediators of the altered hemodynamic environment that has been reported in the skeletal muscle of both human and rat models of T2D. A decrease in vascular conductance lessens the amount of oxygen delivered to the vasculature, thereby reducing PmO\(_2\), the high energy component of the pressure gradient driving oxygen diffusion into the muscle.

**Control of muscle blood flow during exercise**

Exercising muscle blood flow rises in proportion to exercise intensity (105), and follows a very distinct pattern of increase. At the onset of contraction, there is an immediate and substantial increase that plateaus within 5-7 seconds (phase I) (51; 108; 119). A second, slower increase initiates at around 15 to 20 seconds and brings blood flow to its steady state value (phase II) (108; 119). At very high intensities of exercise, a third increase has been observed, and can start anywhere from 1.5 to 2 minutes (phase III) (108). The different time of onset and time course of each phase suggest distinct mechanisms or sets of mechanisms are involved for each (108).

Muscle blood flow is a function of the arteriovenous pressure gradient across the vascular bed, as well as vascular conductance. Vascular conductance is a measure of the degree to which flow is allowed, describing the amount of vasodilation in the resistance
vasculature. The mechanisms described below elevate blood flow and oxygen delivery by inducing vasodilation.

**Phase I: Feedforward Control Mechanisms**

At the onset of muscle contraction, blood flow increases immediately, reaching a plateau within 5-7 seconds (108; 134). Mechanisms in this stage reflect the cardiovascular control system’s attempt to minimize disturbances in muscle oxygenation by immediately increasing blood flow and hence, the supply of oxygen. Correspondingly, the rate of adjustment in this feedforward response appears to be coupled to muscle activation (119). This type of control system does not receive information about the state of its regulated variable (i.e. changes in muscle oxygenation), therefore the accuracy of its response cannot be re-assessed (132).

Locally, there are two feedforward mechanisms that have been theorized to help achieve the rapid adjustment in blood flow characteristically seen at the onset of exercise. One is the **muscle pump**. The mechanical effects of contraction cause venous emptying due to the presence of one-way valves that propel blood towards the heart (134). This results in zero venous pressure upon relaxation, and therefore a larger pressure gradient for arterial inflow into the muscle. The magnitude of this effect depends on how quickly venous volume is restored, as the enhanced pressure gradient is lost with venous refilling (78; 134; 135). The muscle pump is also maximized under conditions of high starting venous pressure, such as when the exercising limbs are below heart level (hydrostatic column effect) (132). Despite the blood flow enhancement effect of the muscle pump, it is also well known that contraction impedes arterial inflow by mechanical compression of the vessels within the vasculature (109; 134). Therefore, the muscle pump may only be
effective at low intensities of muscle contraction, when enhancement of flow overcomes impedance of flow (64). Indeed, it has recently been shown that muscle pump effectiveness is maximized at low contraction intensities (109; 133). As the magnitude of immediate hyperemia rises with increasing contraction intensity (105; 134), this provides evidence that the muscle pump mechanism is not the sole determinant of the phase I increase in exercise blood flow.

The alternate hypothesis for immediate hyperemia is that of rapid vasodilation. This mechanism increases blood flow by enhancing vascular conductance. Experiments controlling for muscle pump effects have shown that increases in vascular conductance can increase flow immediately in both the rest-to-exercise, as well as the exercise-to-exercise transition (100; 109; 133). Potential vasodilator substances can be released from the skeletal muscle and/or vascular endothelium upon initiation of muscle contraction, and include potassium, adenosine, acetylcholine, nitric oxide, adenosine triphosphate, and prostanoids (22; 153). Potassium and acetylcholine, in particular, have been considered ideal candidates as their presence coincides with muscle activation. Acetylcholine is released by motor neurons into the neuromuscular junction as a neurotransmitter to stimulate muscle contraction (132). However, its role in rapid vasodilation has been refuted by studies in which its action is blocked (118), or when motor nerves are stimulated but muscle contraction is prevented (78). In the latter, despite the release of acetylcholine, the absence of contraction abolished any increases in blood flow. Potassium, on the other hand, is released into the interstitial space during depolarization of the muscle fibre membrane potential (132). It is thought that the presence of potassium causes hyperpolarization in the vascular smooth muscle cells.
resulting in vasodilation (5). Still, as the mechanisms behind this are unclear (132), and as its effects appear to be transient in nature (5), the contribution of this factor to rapid vasodilation is currently still under investigation. The exact nature of the vasodilatory system(s) involved is unknown, as in vitro studies applying potential vasodilators on isolated arterioles demonstrate delayed increases in vessel diameter (78; 153) that are incompatible with the immediate blood flow seen in intact muscle. Blockades of certain vasodilators in the human forearm have also revealed little influence of individual vasodilators on elevated blood flow (22; 107; 118; 120). However, this may demonstrate that multiple, redundant systems are potentially responsible for the observed phenomenon (22).

Recently, mechanical compression-induced increases in vasodilation have been observed in isolated rat feed arteries (23), as well as in the intact human forearm (59). These effects appear to be greatest immediately after contraction (1-2 cardiac cycles), and for low to moderate intensity brief, single contractions (59). This mechanism appears to exert the majority of its influence early on, after which other vasodilatory systems take over. Therefore, deformation of the vasculature as a result of muscle contraction may represent another mechanism by which vasodilatory responses are stimulated at the onset of exercise.

In summary, the rapid hyperemic response observed at the onset of exercise is a function of the skeletal muscle pump and/or rapid vasodilation. It is likely that the reduction in venous pressure, the release of vasodilators associated with muscle activation, and mechanical compression of the vasculature act as feedforward mechanisms initiating the blood flow response to exercise (132). However, the magnitude
of this initial response is often, though not always, insufficient to meet metabolic demand and therefore further adjustments based on feedback control mechanisms are required.

**Phase II: Feedback Control Mechanisms**

Phase II feedback control mechanisms adjust blood flow and oxygen delivery to match metabolic demand (49; 108). In this type of control system, changes to the regulated variable, muscle oxygenation, are continuously monitored and adjustments are made based on the magnitude of the error that is detected (132). Currently, the nature of the error signal involved is unclear, though it is thought to be related to factors produced and/or released as a result of the mismatch between oxygen delivery and metabolic demand.

In the metabolic hypothesis of steady state blood flow regulation, a mismatch between metabolite production (metabolism) and removal (washout) results in an accumulation of byproducts in the interstitial space (25). The signals for the activation of this feedback system are the metabolic byproducts, which include carbon dioxide, lactate, hydrogen ions, adenosine, ATP, inorganic phosphate, heat and others (25; 49). As these substances build up, they will trigger vasodilation at the resistance vessels, which will subsequently increase blood flow in the area. The magnitude of the error signal will depend on the degree of mismatch between oxygen delivery and metabolic demand, and is directly related to metabolic rate (49). It is currently unknown which metabolite (or combination) triggers vasodilation leading to increased blood flow. It is doubtful that any one factor acts alone to control the feedback blood flow response observed (25). More probable is a synergistic effort of multiple vasodilators dependent on factors such as exercise intensity and duration.
The vascular endothelium may play a role in the hemodynamic response to exercise. The single layer of cells lining the inner surface of vessels is capable of responding to chemical stimuli and shear stress by releasing substances that adjust vascular tone (25). Three vasodilators that are known to be released by the endothelium are prostaglandins (PG), endothelial derived hyperpolarizing factor (EDHF), and nitric oxide (NO) (25). The flow-mediated dilation hypothesis of blood flow regulation predicts that an increase in shear stress along vessel walls due to increased blood velocity stimulates the release of vasodilators from the endothelial cells. The shear stress stimulus results from initial downstream vasodilation, and the corresponding rise in flow, that occurs in downstream vessels. The contribution of this mechanism to exercise hyperemia is currently unclear. While reductions in blood flow of ~10-20% occur during blockades of NO and/or PG, the hyperemic response to exercise is not abolished (107; 112; 118; 120). In some studies, the magnitudes of reduction are similar both at rest and in response to exercise (i.e. the absolute change in blood flow from rest to exercise remains the same) (107; 120). Recently, it was determined that blockade of NO consistently reduced flow by ~20% during steady state exercise, while blockade of PG only led to a transient reduction in flow (112). This indicates that while NO provides a consistent contribution to the magnitude of hyperemia during steady state exercise, the presence of redundant feedback systems are able to compensate for any loss in the effects of PG (112). Therefore while substances released by the endothelium, especially NO, contribute to the magnitude of skeletal muscle blood flow during exercise, their presence is additive to other control mechanisms (25).
It has been proposed that propagation of vasodilation helps to coordinate blood flow distribution by communicating demand to upstream vessels that are remote from the sites of metabolism. There are two mechanisms that have been proposed to explain how this ascending vasodilation occurs (117). The first is flow-mediated dilation as mentioned above, where an increase in vascular conductance in the microvasculature increases the flow through the upstream arterioles and feed arteries, triggering a shear stress mediated response. The second is conducted vasodilation, where a hyperpolarizing signal is conducted through gap junctions from cell to cell along the endothelium (116). The latter is supported by the finding in isolated feed arteries that the hyperemic response to muscle contraction was reduced by 47% when conducted vasodilation was blocked by endothelial cell damage (115). Of interest in that study, despite three fold increases in shear rate in the area upstream of the damage, no flow mediated dilation was observed (114). As resistances in the vascular tree are set up in series, dilation of larger feed arteries and arterioles concurrently with smaller downstream vessels is necessary to achieve sufficient increases in skeletal muscle perfusion (113), especially when metabolic demand is high. Therefore, conducted vasodilation provides a mechanism by which downstream metabolic demand can be communicated to the upstream resistance arteries.

Lastly, the red blood cell (RBC), in addition to its role as an oxygen carrier, is being considered as a possible oxygen sensor. The error signal generated by this feedback system is considered a direct measure of muscle oxygenation. When RBCs are exposed to regions of low PO₂, the offloading of oxygen stimulates the release of ATP (28). This ATP then binds to purinergic receptors on the endothelium, triggering conducted vasodilation (28). The result is an increase in vascular conductance, which improves
muscle blood flow and with it, muscle oxygenation. Therefore, this feedback system is thought to “sense” the imbalance between metabolic demand and oxygen supply, and subsequently correct the disparity by generating further increases in blood flow.

Feedback control systems of skeletal muscle perfusion rely on error signals that communicate the mismatch between metabolic rate and blood flow (108). While evidence shows the presence of multiple, and most likely redundant mechanisms, they all share the commonality of stimulating increases in blood flow and oxygen delivery to meet the metabolic demands of the contracting muscle.

**Summary**

Physical activity is an important aspect in the treatment and prevention of T2D (4; 71; 126). In order to sustain muscular work for more than a few minutes, the demand of ATP must be met through aerobic metabolic pathways of ATP production. Muscle oxygenation during exercise plays a critical role in supporting these pathways. Recently, it has become apparent that individuals with T2D demonstrate abnormal responses to exercise when compared to their healthy counterparts. The most important finding, and the one with the most implications to performance of physical activity, is the observation by some of reduced magnitudes and rates of increase in VO$_2$ during exercise. Evidence from both human and animals models of T2D suggest that impaired muscle oxygenation may contribute to this dysfunction. While the contribution and/or existence of central impairments in oxygen delivery remain to be determined, evidence for peripheral abnormalities in convective and diffusive oxygen transport is abundant. As endothelial dysfunction is a characteristic trademark of this disease, it is possible that abnormalities
in PG or NO-mediated vasodilation account for the observed reductions in blood flow and oxygen delivery. Recent reports of impairments in RBC-mediated vasodilation may also contribute.

The rise in muscle blood flow during low to moderate exercise occurs over two distinct phases. These phases are characterized based on time of onset and rate of increase, and are controlled by both feedforward and feedback regulatory systems. There have been reports of reduced steady state leg blood flows in individuals with T2D, however little is known about the initiatory mechanisms leading up to the blunted response. As muscle oxygenation plays a critical role in determining the rise in VO₂ during step increases in exercise workload, it is possible that a blunted rise in flow, both in terms of rate and magnitude, contributes to this observed response. Further, it is also unknown whether blood flow characteristics vary across different exercise intensities in this population. Therefore, the present thesis work was undertaken to examine the dynamic muscle blood flow response to both low and moderate intensity exercise. It is believed that the findings will contribute to the state of knowledge by providing insight into which blood flow regulatory mechanisms may be impaired and how they may contribute to the final steady state response observed. These results can then be used to guide the design of interventions to ease the stress of physical activity for individuals with T2D who display intolerance towards exercise.
Subject Medications

In any studies examining physiological responses in a disease, the issue of whether or not to withdraw medications is faced. The rationale for withdrawing medications is to isolate effects of the disease, rather than maintaining the possible effects of medication per se. In this thesis work, the decision was made to perform exercise testing on study participants without withdrawing their medication, and there are a number of reasons for making this decision. Firstly, activities of daily living are performed while individuals are on their medications. Exercise responses observed while testing subjects during a drug free period (i.e. withdrawal of medications 24 hours prior to testing in some studies) may not be representative of their day-to-day physiological responses. Secondly, while it would be ideal to recruit non-medicated subjects, the reality is that many individuals in the T2D population are on medications, either for treatment of their T2D or other related co-morbidities. Testing subjects as they are allows for conclusions to be made that would be representative of the functional characteristics of patients in their everyday life. Finally, while most exercise studies performed in the T2D population have subjects on various medications, there is no clear indication as to whether or not the subjects’ medications should be altered for testing. Some studies in the literature provide a list of exclusionary medications, but no detail on the medications that were included and/or what the status of these drugs was during the experiment. More detailed study protocols have subjects refrain from medications for a period of time prior to testing, with intervals ranging from 4 hours to 4 weeks. A review of relevant studies in the T2D population and their subject medication profiles is found in Table 1.
Table 1. Subject Medication Status in Studies Examining Exercise and/or Cardiovascular Responses in T2D

<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
<th>Medication Profile During Testing</th>
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<tbody>
<tr>
<td>Baldi, J.C. et al., 2003</td>
<td>Reduced exercise arteriovenous O₂ difference in Type 2 diabetes</td>
<td>8 of 11 on Metformin-Glipizide, 2 on angiotensin converting enzyme inhibitors</td>
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<td>(8)</td>
<td></td>
<td>Status during testing – unreported</td>
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<td>Skeletal muscle deoxygenation after the onset of moderate exercise</td>
<td>Unreported</td>
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<tr>
<td>(10)</td>
<td>suggests slowed microvascular blood flow kinetics in type 2 diabetes</td>
<td></td>
</tr>
<tr>
<td>Brandenburg, S.L. et al.,</td>
<td>Effects of exercise training on oxygen uptake kinetic responses in</td>
<td>Treated by diet or oral agents (not insulin), no other medications</td>
</tr>
<tr>
<td>1999 (14)</td>
<td>women with type 2 diabetes</td>
<td>Status during testing – unreported</td>
</tr>
<tr>
<td>Estacio, R.O., 1998</td>
<td>The association between diabetic complications and exercise capacity</td>
<td>Beta blockers and digoxin excluded</td>
</tr>
<tr>
<td>(29)</td>
<td>in NIDDM patients</td>
<td>Type of medications included – unreported</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Status during testing – unreported</td>
</tr>
<tr>
<td>Kelley, D.E. et al., 1994</td>
<td>Impaired free fatty acid utilization by skeletal muscle in non-insulin-</td>
<td>9 out of 11 treated with Sulfonylureas; Withdrawn 2 weeks prior to study.</td>
</tr>
<tr>
<td>(56)</td>
<td>dependent diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>Kelley, D.E. et al., 1996</td>
<td>The effect of non-insulin-dependent diabetes mellitus and obesity on</td>
<td>5 of 8 subjects treated with Sulfonylureas; Withdrawn 2 weeks prior to study.</td>
</tr>
<tr>
<td>(55)</td>
<td>glucose transport and phosphorylation in skeletal muscle</td>
<td></td>
</tr>
<tr>
<td>Kelley, D.E. et al., 2002</td>
<td>Dysfunction of mitochondria in human skeletal muscle in type 2</td>
<td>Treated by diet and exercise alone, a sulfonylurea or metformin; Withdrawn at least 4 weeks prior to the study.</td>
</tr>
<tr>
<td>Study</td>
<td>Summary</td>
<td>Details</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Kingwell, B.A. et al., 2002 (57)</td>
<td>Nitric oxide synthase inhibition reduces glucose uptake during exercise in individuals with type 2 diabetes more than in control subjects</td>
<td>2 of 9 on metformin, 1 of the 2 also on gliclazide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No medication the night before and morning of the experiment = 24 hour drug free period</td>
</tr>
<tr>
<td>Kingwell, B.A. et al., 2003 (58)</td>
<td>Type 2 diabetic individuals have impaired leg blood flow responses to exercise: role of endothelium-dependent vasodilation</td>
<td>2 of 9 on metformin, 1 of the 2 also on gliclazide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No medication the night before and morning of the experiment = 24 hour drug free period.</td>
</tr>
<tr>
<td>Lalande, S. et al., 2008 (60)</td>
<td>Reduced leg blood flow during submaximal exercise in type 2 diabetes</td>
<td>Excluded those on cardiovascular medications such as beta blockers and angiotensin-converting enzyme inhibitors.</td>
</tr>
<tr>
<td>Maiorana, A. et al., 2001 (67)</td>
<td>The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes</td>
<td>5 of 16 on angiotensin-converting enzyme inhibitors, 2 of 16 on lipid lowering therapy, 2 of 16 on aspirin, all but one on oral hypoglycemic drug (3- metformin, 3- gliclazide, 1- glipizide, 8- combined therapy); Medications not altered during the course of the trial. Vascular function examined 4 hours after medication use.</td>
</tr>
<tr>
<td>Martin, I.K. et al., 1995 (70)</td>
<td>Splanchnic and muscle metabolism during exercise in NIDDM patients</td>
<td>3 of 8 on glibenclamide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Withheld for 48 hours prior to testing.</td>
</tr>
<tr>
<td>McVeigh, G.E. et al., 1992 (76)</td>
<td>Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus</td>
<td>Diet alone, diet + sulfonylurea or biguanide or both. Status during testing - unreported</td>
</tr>
<tr>
<td>Regensteiner, J.G. et al., 1995 (95)</td>
<td>Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise</td>
<td>9 of 10 on oral agents (no insulin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Status during testing – unreported</td>
</tr>
<tr>
<td>Reference</td>
<td>Summary</td>
<td>Details</td>
</tr>
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<td>-----------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
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<tr>
<td>Regensteiner, J.G. et al., 1998 (92)</td>
<td>Abnormal oxygen uptake kinetic responses in women with type II diabetes mellitus</td>
<td>6 of 10 on glyburide, 2 of 10 on glipizide Status during testing - unreported</td>
</tr>
<tr>
<td>Regensteiner, J.G. et al., 2003 (94)</td>
<td>Oral L-arginine and vitamins E and C improve endothelial function in women with type 2 diabetes</td>
<td>Lipid lowering drugs, thiazolidinedione, metformin, antihypertensive agents excluded. Treated by diet or oral agents (no insulin). Status during testing – unreported</td>
</tr>
<tr>
<td>Regensteiner, J.G. et al., 2009 (93)</td>
<td>Cardiac Dysfunction during Exercise in Uncomplicated Type 2 Diabetes</td>
<td>Subjects treated by diet or sulfonylureas. Status during testing – unreported</td>
</tr>
<tr>
<td>Sanya, E.O. et al., 2003 (106)</td>
<td>Impaired Cardiovagal and Vasomotor Responses to Baroreceptor Stimulation in Type II Diabetes Mellitus</td>
<td>Not on any medications known to affect cardiovascular or autonomic function.</td>
</tr>
<tr>
<td>Scheuermann-Freestone, M. et al., 2003 (110)</td>
<td>Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes</td>
<td>6 of 21 on either sulfonylurea or metformin, 4 of 21 on both Status during testing- unreported</td>
</tr>
<tr>
<td>Williams, S.B. et al, 1996 (144)</td>
<td>Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus</td>
<td>18 of 21 on sulfonylurea and diet, 1 of 21 on diet and insulin No medications were taken within 12 hours of the study.</td>
</tr>
<tr>
<td>Womack, L. et al., 2009 (151)</td>
<td>Abnormal Skeletal Muscle Capillary Recruitment During Exercise in Patients with Type 2 Diabetes Mellitus and Microvascular Complications</td>
<td>Use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and metformin Medication use discontinued 3 days prior to testing.</td>
</tr>
</tbody>
</table>
CHAPTER 3: METHODS

Subjects

Four men with type 2 diabetes (T2D) and six healthy, control (CON) men of similar physical activity levels (see Table 2) were studied. Absence of overt cardiovascular disease and related comorbidities was confirmed through consultation with family physicians. One subject in the T2D group had an incidental finding of atherosclerosis in his abdominal artery. As he had no symptoms of claudication and normal pedal pulses, the individual was not excluded. Another subject, also in the T2D group, had quadruple coronary artery bypass graft surgery in 2001. An exercise electrocardiogram revealed no abnormalities in cardiac function during exercise. Diagnosis of T2D was confirmed by review of medical history and measurement of fasting blood glucose. One of the four subjects in the group with T2D was categorized as pre-diabetic, with fasting blood glucose measurements ranging from 5.8 to 8.3 mmol/L over the past 8 years. Two were on metformin, an oral hypoglycemic drug, while the other two had their diabetes controlled by diet and exercise. A list of other medications is found in Appendix B. Subjects in the CON group were not on any medications.

Screening/Familiarization

Recruitment for the study occurred over six months from January to June 2009. Poster advertisements and information brochures were placed in the waiting rooms of doctor’s offices, hospital hallways, local diabetes education centres, and various other medical clinics. Advertisements were placed in print and online in community newsletters, newspapers and forums. All recruitment materials contained a brief summary of the exercise involved, as well contact information for the Human Vascular Control
laboratory. See Appendix A for examples of recruitment materials used. Two potential participants with T2D were excluded from participation due to their medications. One healthy, control group volunteer was also turned away as quality data signals in this individual were difficult to obtain (see below for more details).

Initial screening consisted of a telephone interview (Appendix B) in which potential participants answered a series of questions designed to determine eligibility as well as their readiness to participate in physical activity. Information obtained included age, health status (healthy or type 2 diabetes), current medications, general physical activity level, smoking status, history of back pain, and frequency of alcohol consumption.

Individuals who passed the initial phone screening were then scheduled to come into the Human Vascular Control Laboratory in the School of Kinesiology and Health Studies at Queen’s University for a full experimental screening and familiarization session. The purpose of this visit was to provide subjects with a full understanding of the requirements of the experimental protocol, as well as provide the researchers with a chance to further screen and gather information regarding the eligibility of the potential participants.

The experiment was approved by the Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board. At the screening/familiarization session, subjects were given a tour of the laboratory and a thorough description of the experimental protocol prior to giving written, informed consent to participate in the
exercise study (Appendix B). Subjects also consented to providing access to their medical information as part of the screening process (see Medical History below).

**Anthropometric Measurements**

The subject’s height (cm), weight (kg), and waist circumference (cm) were recorded. Waist circumference was measured at the superior borders of the iliac crest (101). Subjects were asked to come to the screening/familiarization visit following an overnight (minimum 12 hours) fast. A small drop of blood was obtained from the tip of the index or middle finger with a contact activated lancet (BD Microtainer, Poland) and measured for glucose levels using a blood glucose monitoring system (Accu-Check Compact Plus, Roche Diagnostics, Germany). A 2 mL blood sample was also collected from the back of the hand using a 23-gauge Safety-Lok blood collection set (BD Vacutainer, Franklin Lakes, NJ), and analyzed for glycosylated hemoglobin (HbA1c) using high pressure liquid chromatography. This analysis was performed by the CORE laboratory in the Division of Clinical Laboratory Services at Kingston General Hospital.

**Medical History**

At the screening/familiarization visit, subjects were asked to fill out a detailed medical history questionnaire (Appendix B). This form served to confirm the absence of overt cardiovascular disease (hypertension excepted) and any other comorbid conditions that would contraindicate participation in exercise testing. This form consisted of two sections; the first was filled out by the potential participant, and the second by their family physician. A description of the experimental protocol, as well as a list of contraindications for exercise testing, were included to provide physicians with a full understanding of what would be required of their patient. Medical approval for
participation was required on two levels for this study. First, the subject had to be cleared by their family physician, the individual with the most detailed understanding of the subject’s health status. The patient’s medical questionnaire was then reviewed by the physician in charge of patient care during the study, Robert W. Hudson, M.D., Ph.D., of the Queen’s University Division of Endocrinology and Metabolism. This two step process served to ensure participants could engage in the exercise testing without risk to their present health. Finally, if participants did not regularly engage in physical activity, they were asked to undergo an exercise electrocardiogram (stress test) to ensure the testing could be performed safely. This procedure was arranged through their family physician. As this requirement was implemented later in the recruitment process, only one subject, in the T2D group, underwent the test. Results forwarded from the participant’s family physician indicated no abnormalities.

**Physical Activity Levels**

Physical activity levels were quantified using the Seven Day Physical Activity Recall (7-PAR) (104). This physical activity questionnaire has well documented reliability and validity (1), and has been previously used by other research groups performing exercise investigations in persons with T2D (14; 92; 95).

The interviewer-lead questionnaire requires subjects to recall the amounts of time spent sleeping, and engaging in moderate, hard, and/or very hard intensity physical activity over the previous seven days. Activities considered for the recall include any occupational, household, recreational, and/or sport activity. A moderate intensity activity is defined as one that feels similar to walking at a normal pace, while a very hard intensity activity feels similar to running. The hard intensity category is consequently
defined as any activity that feels easier than running, but more strenuous than walking. A fifth category, light intensity activity, is created post-interview by subtracting the time spent in sleep and each of the three previous categories from 24 hours. Definitions and examples of activities were printed on flash cards and provided as reference materials for the subjects.

The 7-PAR is scored based on the subject’s estimate of total time spent sleeping and in each of the four intensity categories of activity (light, moderate, hard, very hard) per week. Each intensity category is assigned a metabolic equivalent (MET) value (Appendix B), a number which is defined as the ratio of work metabolic rate to the standard resting metabolic rate of 4.184kJ/kg/hr (4.184kJ = 1 kcal) (3). For example, a moderate intensity activity is assigned a MET value of 4.0, meaning this activity requires four times the energy expenditure at rest. The total time in each category is multiplied by its associated MET value and summed to provide a total weekly energy expenditure score in kcal/kg/wk. Sample calculations are found in Appendix B.

Familiarization

Participants were familiarized with the mode of leg exercise to be performed (see Study Design). Individuals underwent an incremental exercise test to exhaustion consisting of small step increases in exercise intensity every minute to ensure they could cope with exercising at high workloads, and to become familiar with the discomfort associated with intense exercise. Subjects were reassured that both the “burning” feeling in their legs and the onset of heavy breathing were normal responses, and that these uncomfortable feelings would subside once the exercise was completed. This test was identical to the incremental test they would perform during the actual experimental
testing. The results gave researchers an idea of each individual’s maximal work capacities, and, following the second trial, allowed for an evaluation of the repeatability of this exercise test. Finally, the session ensured subjects were comfortable with having measurements taken as they exercised, as anxiety and/or unfamiliarity with the protocol can affect the exercise response. For example, the measurement of oxygen uptake during exercise required subjects to breathe into an air collection tube with their mouth tightly sealed around a rubber mouthpiece, and to wear a nose clip that prevented breathing through the nostril. Initially, this can be quite a constricting feeling, with some subjects taking longer, deeper breaths then they typically would. The familiarization visit allowed the subjects to practice breathing through the mouthpiece at rest and during exercise, so that ventilation rates during the actual experiment would reflect (as closely as possible) the individual’s normal response.

**Ultrasound Screening**

Doppler ultrasound measurements were performed at the subject’s femoral artery during the pre-testing exercise bout to ensure acceptable quality of measurement of femoral blood flow. Subjects were repeatedly reminded to keep their hips still during the exercise, as large movements at the site of measurement can result in loss of the ultrasound signal. One potential participant, in the healthy CON category, had to be turned away as the nature of the exercise, combined with a larger waist circumference, made valid measurements of femoral artery blood flow impossible.
**Study Design**

Exercise testing took place over two separate visits. Subjects were instructed to fast for 3 hours prior to the experiment, as well as to refrain from alcohol, caffeine and strenuous physical activity for 48 hours.

Subjects performed alternating, two-leg knee extension and flexion exercise on an electrically braked ergometer (Fig. 5). Subjects lay in a supine position at a hip angle of 160° during the exercise. The resistance on knee flexion was set to 30% of the weight on extension, as hamstring strength is characteristically less than quadriceps strength (82; 154). Pilot testing showed that a higher resistance on flexion resulted in the hamstring muscle group reaching maximal effort before the quadriceps, leading to a dramatically reduced time to exhaustion. The range of motion was ~ 90°, with the lower leg travelling from a position perpendicular to the horizontal to just before full extension (Fig. 5). A metronome provided an auditory indication of the required contraction frequency, approximately 30 extension/flexion cycles per minute per leg. To ensure participants achieved the desired cadence and range of motion on each extension/flexion duty cycle, one research assistant was assigned the job of providing feedback and motivation to the participant on each contraction.

The study consisted of two different exercise protocols. An examination of the relationship between exercise intensity and cardiovascular and respiratory response was achieved through an incremental exercise test to exhaustion. The workload was changed in small step increases every minute until the participant could no longer maintain the desired cadence. The dynamic cardiovascular and respiratory response to submaximal, steady state exercise was assessed through 5-min exercise bouts that transitioned from
rest to both low and moderate intensity workloads. Each workload was performed twice, and averaged to obtain a single response per workload per subject.

Fig. 5. Experimental setup. Subjects performed alternating knee extension and flexion exercise while in a supine position. The resistance on flexion was set to 30% of the weight on extension. The range of motion was ~ 90°.
Visit 1: Step Transition from Rest to Low Intensity Constant Work and Incremental Exercise Test to Exhaustion

Subjects arrived at the laboratory and were allowed to lay supine for a period of at least 30 minutes while being instrumented by the researchers. During this visit, the participant performed two 5-min low intensity exercise bouts at an absolute workload equivalent to lifting ~7.5kg on extension and ~2.25kg on flexion (LO7.5kg). This was a standardized low intensity for all subjects (~50% VO2peak).

The testing began with 2-mins of quiet, baseline rest, followed by 5-mins of exercise at LO7.5kg. The metronome was started prior to the start of baseline and remained on until the end of exercise. This allowed the individual to mentally settle into the rhythm of the contractions prior to the start. With 15-s left in the baseline period, subjects were given a verbal indication of the time remaining. A countdown of “3… 2… 1… start” (in time with the beeps of the metronome) was given to indicate the start of exercise. Subjects were not given any time feedback during the exercise, only being told when they were finished. The subject was given at least 15 minutes to recover and allow measurement variables to return to baseline before the start of the second exercise bout. Individuals were asked to remain supine during this time. Recovery duration varied among subjects and was dependent on the amount of time it took for femoral blood velocity to return to baseline values. This value was defined as a blood velocity within 2 cm/s of that recorded during the previous baseline.

Immediately following the second 5-min bout at LO7.5kg, the resistance on the kicking ergometer was increased by 1 kg every minute, and the subject continued to exercise until they reached exhaustion (Fig. 6A). Subjects were not given an indication of
the time that had elapsed or when the work would be increased, as it was important to avoid anticipatory responses. Exhaustion was defined as the workload at which subjects could no longer maintain the required contraction frequency and/or range of motion despite encouragement and motivation from the research assistant.

A rating of perceived exertion scale was used during this portion of the exercise test to provide researchers with an idea of the subject’s progression to exhaustion. During the last 15-s of each workload, subjects were asked to indicate with their hand on a scale of 1 to 10 how they were feeling; with 1 being no exertion at all, and 10 being a maximal effort that could only be maintained for another ten to twenty seconds. This tool was introduced during the initial screening session, so subjects were familiar with using the scale, and had practice gauging their own levels of fatigue.

Time to exhaustion ranged from 8 to 17 minutes. Of the ten participants, seven reached their pre-screening maximal workloads or higher, indicating consistent peak effort. Results from the maximal exercise test were initially analyzed to establish peak aerobic capacity for this mode of exercise, as well as provide an estimate of each individual’s ventilatory threshold (VT).

During an incremental exercise test, minute ventilation ($V_E$) increases linearly with respect to increasing workload and $VO_2$ (48). However, there exists a point in which $V_E$ will suddenly begin to increase out of proportion to increases in $VO_2$. This marks the onset/development of metabolic acidosis and reflects an excessive increase in $CO_2$ production (48). In subjects with normal ventilatory control responses, the increase in $V_E$ is thought to parallel the increase in $VCO_2$ (140). The ventilatory threshold is an important parameter to quantify as physiological responses to exercise differ above the
threshold as compared to below it (140). Specifically, the perception of effort is markedly increased (48), exercise duration is shortened, and ventilatory drive continually rises disproportional to metabolic work (140). Most importantly, however, is the finding that the dynamic rise in VO$_2$ is altered so that time to steady state, if achieved, may be delayed (140). An addition increase in VO$_2$ (phase III, slow component) may also manifest beyond the first 3-mins of exercise (140).

In order to ensure dynamic response characteristics were examined below this threshold and therefore could be compared between all subject groups, the workload for the moderate intensity exercise bout was chosen to represent 90% of the workload achieved at each individual’s ventilatory threshold (VT$_{90%}$). This was defined as being within the moderate intensity domain.

Visit 2: Step Transition from Rest to Moderate Intensity Constant Work

Similar to the first visit, subjects arrived at the laboratory and were allowed to lay supine for a period of at least 30 minutes while being instrumented by the researchers. During this second visit, the participant performed two 5-min moderate intensity exercise bouts at VT$_{90%}$ (Fig. 6B). The relative work intensity varied among subjects, ranging (on extension) from ~12 to 17 kg in the CON group (mean 14.4 ± 0.9 kg), and ~11 to 14 kg in the group with T2D (mean 12.6 ± 0.6 kg).

The testing began with 2-mins of quiet, baseline rest, followed by 5-mins of exercise at VT$_{90%}$. The subject was then given at least 15 minutes to recover and allow measurement variables to return to baseline before the start of the second exercise bout. Again, recovery duration varied among subjects and was dependent on the amount of
time it took for femoral blood velocity to return to baseline values (within 2 cm/s). For some subjects, one bout of either LO\textsubscript{7.5kg} or VT\textsubscript{90\%} had to be repeated if it was found that the data collected during the earlier trials was not of usable quality. Two subjects from both the CON and T2D groups repeated one bout of LO\textsubscript{7.5kg} exercise, while 2 subjects in the CON and 1 subject in the T2D group repeated one bout of VT\textsubscript{90\%}. If necessary, the LO\textsubscript{7.5kg} bout was always performed prior to the moderate intensity VT\textsubscript{90\%} exercise. As subjects were given unlimited rest between exercise bouts, and as measurement variables were monitored closely during recovery, the addition of an extra 5-min bout of exercise is not believed to have affected the integrity of the responses. This is supported by literature investigating the effects of prior exercise on the dynamic response characteristics of moderate intensity exercise. Performing a moderate or high intensity “warm-up” prior to a moderate exercise bout has not been found to alter the kinetic response profile of pulmonary VO\textsubscript{2} (17; 31; 35).
Fig. 6. A: Schematic representation of the exercise protocol performed during the first experimental visit. Subjects exercised for 5-min at LO7.5kg, and were then allowed to recover. After at least 15-mins of rest, a second 5-min bout at LO7.5kg was performed, followed by ~1 kg per minute increases in workload until exhaustion. B: Schematic representation of the exercise protocol performed during the second experimental visit. Subjects exercised for 5-min at a workload equivalent to 90% of the workload achieved at ventilatory threshold (VT90%). A period of at least 15-mins of rest was provided before a second 5-min bout at the same intensity.
Measurements

All variables were measured continuously during baseline rest, and throughout exercise.

*Mean Arterial Pressure, Heart Rate & Stroke Volume (MAP, HR and SV)*

MAP, HR and SV were measured beat by beat using a pneumatic finger cuff (Finometer MIDI, Finapres Medical Systems) placed on the middle finger of the right hand. The hand rested on the subject’s chest at heart level.

MAP was measured through the volume clamp method, in which the diameter of an artery in the finger is kept constant (despite changes in arterial pressure) using a finger cuff with an inflatable bladder (13). The target is an “unloaded” artery at its unstressed diameter, that corresponding to a transmural pressure of zero across the arterial wall (13). Departures from the unstressed diameter, monitored by photo-plethysmography, are counteracted by changes in finger cuff pressure. For example, when an increase in diameter is detected (i.e. during systole), the finger cuff is immediately inflated to counteract the change (13). As the cuff closely monitors and mirrors arterial volume changes, the pressure in the finger cuff reflects intra-arterial pressure (13).

SV was estimated by use of the Modelflow method. Intra-arterial pressure measured at the finger is used to compute an aortic flow waveform which is then integrated to provide an estimate of beat by beat left ventricular SV (13). The method utilizes a non-linear, three element model to simulate aortic flow. The cross sectional area of the vessel is computed with an arctangent equation based on data derived from human aortas (13; 61). The model requires age, sex, weight and height inputs to simulate individual aortic area-pressure relationships (61). The three elements used in the flow model are aortic characteristic impedance, arterial compliance, and peripheral vascular
resistance (13; 142). Aortic characteristic impedance represents the resistance in the aorta to the inflow from the left ventricle (13). When the left ventricle contracts, outflow from the aorta is opposed by the existing pressure in the aorta (ventricular afterload). However, as flow increases, aortic pressure will rise, dependent also on the cross sectional area of the aorta and its compliance (13). This element therefore describes the relationship between instantaneous flow and pressure measured at the entrance of the aorta (13). Arterial compliance is described as the change in aortic pressure for a given volume of blood, and is represented as the change in volume per change in unit pressure (13). Finally, peripheral vascular resistance, the ratio of mean pressure to mean flow, measures how easily blood flows from the aortic region to the vascular beds in the periphery (13).

Using these characteristics of arterial input impedance and subject-dependent parameters, the Modelflow method is able to simulate aortic flow and therefore provide an estimate of left ventricular SV. Cardiac output (CO) is calculated by multiplying the computed SV by the corresponding heart rate.

Oxygen Uptake and Minute Ventilation ($VO_2$, $V_E$)

Gas exchange and ventilation were measured with a MOXUS metabolic cart (AEI Technologies). Data were stored breath by breath during the exercise test and then averaged at 5-s intervals for analysis. Inspired and expired volumes were measured with a turbine volume transducer (Interface Associates, Laguna Niguel, CA) which was calibrated with a 3L calibration syringe (Hans Rudolph Inc., Kansas City, MO). Calibration of the hardware gas analyzers was performed before each test, with both
room air (20.93% O₂, 0.03% CO₂) and a certified gas calibration tank of low O₂ (4.65%) and high CO₂ (5.85%) gas concentrations.

*Leg blood flow (LBF)*

Femoral artery diameter was imaged by echo ultrasound (Vingmed System 5, GE Medical Systems) using a linear 10-MHz probe on the left leg at a site proximal to the femoral artery bifurcation. Video images of the artery were recorded continuously and stored on a computer based software system for offline analysis. Due to the large amount of movement at the site of measurement during exercise, clear images of the artery were often intermittent. Therefore, diameter measurements were taken whenever a clear image of the vessel was present. All measures were made by the same operator. Average response values were obtained by fitting the diameter data with either a linear or exponential curve. Slight variations in diameter due to measurement error or biological variability are inevitable, therefore a line of best fit was used to represent the best estimate of diameter over time.

Femoral artery blood velocity was obtained on the right leg with pulsed Doppler ultrasound (Multigon 500B, Transcranial Doppler, Multigon Industries) using a flat, 4-MHz probe. Data were recorded continuously at 200-Hz on a computer based system (Powerlab, ADInstruments). The angle of insonation was determined by obtaining an echo ultrasound image of the artery at the site of velocity measurement. See Fig. 7 for details on the calculation. The ultrasound gate was set to insonate the entire width of the artery lumen.
Fig. 7. Determination of angle of insonation. A: When the velocity probe is held on the surface of the skin parallel to the femoral artery, this results in a 57° angle of insonation. B: In this instance, tilting of the velocity probe resulted in a reduced angle of insonation, 57° - θ. C: In this instance, tilting of the velocity probe resulted in a greater angle of insonation, 57° + θ. An image of the femoral artery was obtained at the site of velocity measurement using echo ultrasound. θ was manually determined on a printout of the image with the use of a protractor.
Data Analysis

Ventilatory Threshold

VT was defined as the workload corresponding to a sudden break in $V_E$ (12; 48; 140). The $V_E$ versus time data plot was divided into two sections and linear regression was performed on each. Initial division of the data was determined visually and then fit with two individual lines. Data points close to the intersection point of the two lines were then moved between plots until the largest possible coefficient of determination ($r^2$) for each line was obtained. The 60-s time interval, and corresponding workload, containing the intersection point of the two lines of best fit was considered the workload at VT (Fig. 8). $VT_{90\%}$ was determined by multiplying the workload at VT by 0.9.

Fig. 8. Ventilation data from one representative subject during the incremental exercise test to exhaustion. Workload was increased by ~1 kg every min. Calculation of ventilatory threshold was performed by splitting data into two regions and performing linear regression on each section. The 60-s time interval (grey bar) containing the point of intersection was used to identify the workload at ventilatory threshold.
Hemodynamics

Beat by beat values for MAP, HR, SV and CO were time aligned to the onset of exercise and linearly interpolated to give values at 1-s intervals. The data were then averaged across the two bouts of LO7.5kg to produce a single data set for each subject, for each variable. VT90% was performed to analyze dynamic response characteristics at a moderate exercise intensity, therefore only HR, CO and LBF were analyzed for this workload.

Total LBF (both legs) was calculated using the following formula:

\[ LBF = \left[ \text{velocity} \times 60\text{s/min} \times \pi \times \left( \frac{\text{diameter}}{2} \right)^2 \right] \times 2 \]

The 200-Hz flow data were time aligned to the onset of exercise and ensemble averaged across bouts to obtain a single data set per subject, per workload. As LBF for each 5-min bout of exercise was not individually analyzed prior to averaging for each workload, the possibility exists for an improved kinetic response in the second bout as a result of the prior exercise performed in the first bout.

The data was then filtered in the frequency domain using a low-pass filter (lowpass.xfm, SigmaPlot 2001). A cut-off frequency of 0.2 Hz was used, as it has been found to eliminate high frequency noise, including that related to heart rate and muscle contraction, while still preserving the data components required to quantify the underlying kinetics response (30). The filtered data was then resampled at 10-Hz for regression analysis. Resampling of data has not been found to interfere with parameter estimates (30). The continuous 10-Hz data set was then fit with the appropriate
exponential model (see Kinetics Analysis) to obtain parameters defining the dynamic response characteristics.

Baseline Values

Baseline Values for MAP, HR, SV, and CO were taken as a 10-s average between time \( t = -35 \) and \( t = -25 \) of baseline, where exercise started at \( t = 0 \) and values are in seconds. Baseline values for LBF and VO\(_2\) were averaged over the last minute prior to exercise.

Incremental Exercise Test to Exhaustion

Three workloads from the incremental exercise test were chosen for comparison, a low intensity workload (7.5 kg), a moderate intensity workload (10 kg), and a high intensity workload (13 kg). Average values for MAP, HR, SV, CO, VO\(_2\), and LBF were taken during the last 10-s of each stage. For the 7.5 kg workload, average values were taken from \( t = 50 \) to 60 seconds of the 5-min LO\(_{7.5kg}\) exercise bout. All averaged data therefore represent non-steady state values averaged 50-s after the start of the workload. Peak values from the incremental exercise test for MAP, HR, SV, CO and LBF were taken as an average of the last 10 seconds prior to exhaustion. Peak VO\(_2\) was quantified as an average of the last 60-s prior to exhaustion. Comparisons between groups were made based on the absolute magnitude of change (\( \Delta \)) in each variable from baseline.

Step Transition from Rest to LO\(_{7.5kg}\) Constant Work

Comparisons between groups for MAP, HR, SV, CO, VO\(_2\) and LBF were made in terms of the absolute magnitude of change (\( \Delta \)) from baseline. Data were averaged at \( t = -30 \), and 0 (10-s time bins), at 15, 30, 45, and 60 (5-s time bins), and at 90, 120, 180, 240,
and 300 (10-s time bins), where time is in seconds and the start of exercise occurred at t = 0.

For HR, the value plotted at t = 15 represents an average of the largest increase in magnitude within the first 0 to 25-s and 1 data point before and after this value (i.e. if the largest change occurred at t = 17, data were averaged for t = 16, 17 and 18). This procedure was performed in order to quantitatively characterize the overshoot in heart rate found in all subjects at the onset of exercise.

For MAP, the value plotted at t = 15 represents a 3-s average of the largest decrease in magnitude within the first 0 to 25-s. This averaging was performed in order to quantitatively characterize the immediate drop in pressure seen at the onset of exercise.

**Kinetics Analysis**

The time course plots for HR, CO and LBF were fit with one, two or three component exponential models, using a least squares procedure, for analysis of dynamic response characteristics. The number of components in the model was chosen based on the response profile observed, and resulted in the most even distribution of residuals along the length of the fit. See Appendix C for an example of the regression performed.

The models have a baseline component (G0), and one or more amplitude terms (G1, G2 and/or G3), time constants (τ1, τ2, and/or τ3), and time delays (TD1, TD2, and/or TD3) consistent with the number of phases of the response (see Fig. 9). TD in seconds gives a measure of how quickly vascular control systems can begin to respond following muscle contraction. τ in seconds represents the time it takes to achieve 63% of the response magnitude, and describes the rate at which blood flow increases. Finally, G
represents the magnitude of the response. The model is described by the following equation:

\[ Y(t) = G_0 + G_1[1 - e^{-\alpha(t-TD_1)/\tau_1}] \cdot \mu_1 + G_2[1 - e^{-\alpha(t-TD_2)/\tau_2}] \cdot \mu_2 + G_3[1 - e^{-\alpha(t-TD_3)/\tau_3}] \cdot \mu_3 \]

where

\[ \mu_1 = 0 \text{ for } t < TD_1 \quad \text{and} \quad \mu_1 = 1 \text{ for } t \geq TD_1 \]
\[ \mu_2 = 0 \text{ for } t < TD_2 \quad \text{and} \quad \mu_2 = 1 \text{ for } t \geq TD_2 \]
\[ \mu_3 = 0 \text{ for } t < TD_3 \quad \text{and} \quad \mu_3 = 1 \text{ for } t \geq TD_3 \]

where \( t \) is time in seconds and \( Y(t) \) is the time dependent variation in HR, CO or LBF.

The mean response time (MRT) quantifies the total time taken to reach 63% of the total response magnitude, the difference between baseline and steady state, and is a weighted mean of the sum of the TD and \( \tau \) for each response phase (65). The MRT is calculated by the formula:

\[ \text{MRT} = \frac{G_1}{(G_1 + G_2 + G_3)} \times (TD_1 + \tau_1) + \frac{G_2}{(G_1 + G_2 + G_3)} \times (TD_2 + \tau_2) + \frac{G_3}{(G_1 + G_2 + G_3)} \times (TD_3 + \tau_3) \]
Fig. 9. Schematic representation of the exponential model used to investigate the dynamic heart rate, cardiac output, and leg blood flow responses to constant work exercise. G, gain or amplitude; TD, time delay; \( \tau \), time constant; 1, 2, 3, phase of the response. Modified from (108).
Statistical Analysis

Subject characteristics, baseline and peak exercise values were compared between groups using two tailed, unpaired t-tests. Significance was set at P < 0.05 for all statistical analyses. All values are presented as means ± standard error (SE). For the analyses described below, multiple comparison testing (if necessary) was performed using a Holms-Sidak post hoc test.

Incremental Exercise Test – 7.5, 10 and 13 kg workloads

A two-way repeated measures analysis of variance (2 way RM ANOVA) was used to compare differences between groups at each workload for MAP, HR, SV, CO, VO2 and LBF. This statistical analysis test was performed to test the following null hypotheses:

A) \( H_0 \): There is no main effect of T2D in response to progressive increases in exercise intensity on LBF, VO2, MAP, HR, SV and CO.

B) \( H_0 \): There is no interaction effect of group (T2D versus CON) and exercise intensity on MAP, HR, SV, CO, VO2 and LBF.

Step Transition from Rest to \( LO_{7.5kg} \) Constant Work

A 2 way RM ANOVA was used to compare differences between groups at t = 15, 30, 45, 60, 90, 120, 180, 240 and 300 seconds into exercise. The variables tested were MAP, HR, SV, CO, VO2 and LBF. This statistical analysis test was performed to test the following null hypotheses:
A) $H_0$: There is no main effect of T2D on changes in MAP, HR, SV, CO, VO$_2$ and LBF in response to a step transition from rest to LO7.5kg.

B) $H_0$: There is no interaction effect of group (T2D versus CON) and time ($t = 15, 30, 45, 60, 90, 120, 180, 240$ and $300$ seconds) on MAP, HR, SV, CO, VO$_2$ and LBF.

*Dynamic Response Characteristics*

A 2-way RM ANOVA was used to compare the dynamic response characteristics of HR, CO and LBF. The variables tested were the kinetic parameters time delay (TD) and time constant ($\tau$) for each response phase, and the overall mean response time (MRT). Values were compared between groups, and at both the light (LO7.5kg) and moderate (VT$_{90\%}$) intensity workloads. This statistical analysis test was performed to test the following null hypotheses:

A) $H_0$: There is no main effect of T2D on the TD and $\tau$ of each response phase, and on the MRT in response to a step transition from rest to exercise.

B) $H_0$: There is no interaction effect of group (T2D versus CON) and workload (LO7.5kg or VT$_{90\%}$) on the TD and $\tau$ of each response phase, and on the overall MRT.

A one-way analysis of variance (1-way ANOVA) was used to compare the magnitude of LBF achieved during each phase of response in LO7.5kg. Specifically, the parameters tested were $G_0$ (baseline amplitude), $G_1$ (phase 1 amplitude), and $G_2$ (phase 2 amplitude). This statistical analysis test was performed to test the following null hypothesis:

$H_0$: There is no effect of T2D on the gain achieved during each response phase.
CHAPTER 4: RESULTS

Subject characteristics are displayed in Table 2. As expected, subjects with T2D had higher levels of fasting plasma glucose and glycosylated hemoglobin (HbA$_{1c}$) compared to CON. Means for age, height, weight and waist circumference were not significantly different between groups, however the group with T2D tended to be older, and more abdominally obese ($P = 0.057$ for both measures). Body mass index (BMI) was also significantly greater in this group. Habitual physical activity levels, as evaluated by the 7-PAR, were not different between groups. For the incremental exercise test, peak workload at exhaustion varied from 15.5 to 23.4 kg (mean 18.7 ± 1.4 kg) in the CON group, and from 14.6 to 19.0 (mean 16.4 ± 1.0 kg) in the group with T2D. In contrast to previous studies, the peak oxygen uptake ($VO_2^{peak}$) achieved was not different between subject groups (1852.0 ± 162.0 vs. 1711.1 ± 170.8 ml/min; CON vs. T2D respectively).

Baseline values for $VO_2$, MAP, HR, SV, CO and LBF are shown in Table 3. At rest, subjects with T2D had significantly lower LBF and MAP, and higher HR. There was also a trend towards higher CO ($P = 0.063$) in this group. Resting SV and $VO_2$ were not significantly different between groups.
Table 2. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CON subjects</th>
<th>T2D subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>44.3 ± 2.7</td>
<td>54.8 ± 4.1</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>178.4 ± 3.0</td>
<td>170.8 ± 3.1</td>
<td>0.124</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>86.4 ± 3.1</td>
<td>98.8 ± 7.4</td>
<td>0.114</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>27.2 ± 1.2</td>
<td>33.9 ± 2.4*</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>Waist Circumference, cm</strong></td>
<td>96.3 ± 3.3</td>
<td>111.3 ± 6.7</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>Fasting plasma glucose, mmol/L</strong></td>
<td>5.6 ± 0.3</td>
<td>8.2 ± 1.1*</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td>5.3 ± 0.1</td>
<td>7.4 ± 0.9*</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>Disease duration, years</strong></td>
<td>---</td>
<td>5.8 ± 2.6</td>
<td>---</td>
</tr>
<tr>
<td><strong>Habitual Physical Activity, kcal/kg/wk</strong></td>
<td>249 ± 8</td>
<td>248 ± 10</td>
<td>0.914</td>
</tr>
<tr>
<td><strong>VO_{2peak}, ml/min</strong></td>
<td>1852.0 ± 162.0</td>
<td>1711.1 ± 170.8</td>
<td>0.579</td>
</tr>
<tr>
<td><strong>WL_{peak}, kg</strong></td>
<td>18.7 ± 1.4</td>
<td>16.4 ± 1.0</td>
<td>0.247</td>
</tr>
<tr>
<td><strong>CO_{peak}, l/min</strong></td>
<td>11.5 ± 1.4</td>
<td>14.2 ± 1.9</td>
<td>0.277</td>
</tr>
<tr>
<td><strong>HR_{peak}, bpm</strong></td>
<td>126.4 ± 4.8</td>
<td>116.8 ± 8.0</td>
<td>0.307</td>
</tr>
<tr>
<td><strong>SV_{peak}, ml</strong></td>
<td>92.2 ± 12.6</td>
<td>120.7 ± 11.6</td>
<td>0.155</td>
</tr>
</tbody>
</table>

Values are means ± SE. CON, control; T2D, type 2 diabetes; BMI, body mass index; HbA1c, glycosylated hemoglobin; VO_{2peak}, peak oxygen uptake; WL_{peak}, peak workload; HR_{peak}, peak heart rate; SV_{peak}, peak stroke volume; CO_{peak}, peak cardiac output. All peak values were obtained from the incremental exercise test and are averages of the last 10-s prior to exhaustion. *P < 0.05 for a significant difference between T2D and CON groups.
Table 3. Baseline Values for Cardiorespiratory Variables

<table>
<thead>
<tr>
<th></th>
<th>CON subjects</th>
<th>T2D subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂, ml/min</td>
<td>310.8 ± 11.8</td>
<td>309.2 ± 31.7</td>
<td>0.958</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>102.4 ± 3.9</td>
<td>85.0 ± 0.9*</td>
<td>0.008</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>60.1 ± 2.3</td>
<td>72.0 ± 4.9*</td>
<td>0.038</td>
</tr>
<tr>
<td>SV, ml</td>
<td>102.3 ± 7.8</td>
<td>117.0 ± 7.7</td>
<td>0.237</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>6.1 ± 0.6</td>
<td>8.5 ± 1.1</td>
<td>0.063</td>
</tr>
<tr>
<td>LBF, ml/min</td>
<td>959.8 ± 111.3</td>
<td>617.0 ± 22.1*</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Values are means ± SE. All variables were measured in the supine position. CON, control (n = 6); T2D, type 2 diabetes (n = 4); VO₂, oxygen uptake; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; LBF, leg blood flow; For LBF, n = 5 for CON subjects. *P < 0.05 for a significant difference between T2D and CON groups.
Incremental Exercise Test – 7.5, 10 and 13 kg workloads

Mean values for the 7.5, 10 and 13 kg workloads, as well as values at peak workload, are shown in Fig. 10 to 15. Peak values are shown with horizontal error bars to indicate variability in peak workload achieved. Individual subject data were also plotted to show the variability in responses. To aid in the comparison of exercise responses in subjects with different baselines, all values are expressed as the absolute change (Δ) in magnitude from rest.

Subjects did not differ in their ΔVO₂, and ΔHR responses to the step changes in exercise intensity (Fig. 10 and 12). Both variables increased linearly with exercise. Subjects with T2D demonstrated a trend towards larger increases in ΔMAP compared to CON at the 10 and 13 kg workloads but not at 7.5 kg (P = 0.094 for interaction) (Fig. 11). ΔMAP was significantly greater at 10 and 13 kg compared to 7.5 kg (P = 0.025, 0.017 respectively) in T2D, but did not change significantly in CON. ΔSV remained unchanged from baseline in the CON group, while the group with T2D demonstrated an initial increase from baseline at 7.5 kg and no further increases with subsequent workloads (Fig. 13). At peak exercise, SV fell to or below baseline values in all but two subjects (1 CON, 1 T2D) (Fig. 13). ΔSV tended to be higher in the group with T2D at all three workloads (P = 0.051, 0.106, 0.069 for 7.5, 10 and 13 kg respectively). The ΔCO response was not significantly different between groups (P = 0.212) (Fig. 14). While the differences between groups for ΔSV and ΔCO appear large, the lack of statistical difference between groups may be a result of both the small sample size in each group, and the variability in responses between subjects (see individual subject plots). Finally, there was a trend towards a greater ΔLBF in the CON group compared to the group with T2D (P = 0.098)
(Fig. 15A). As baseline flow was lower in T2D, the absolute value of LBF was significantly reduced during the 10 and 13 kg workloads ($P = 0.035, 0.007$ respectively) (Fig. 15B). The difference in baseline LBF, however, is difficult to interpret as the distribution of flow during rest differs from that during exercise, where the majority of LBF is directed to the active skeletal muscle.

LBF for one subject in the CON group had to be excluded as the Doppler ultrasound signal for measurement of blood velocity was not consistent throughout the first experimental visit. Use of this data would have resulted in an underestimation of LBF, therefore group means for LBF, in both the step transition to LO7.5kg and the incremental exercise test, were derived from $n = 5$ CON subjects. For all subjects, increased leg movement during contraction at higher workloads compromised the quality of the ultrasound data collected and therefore did not permit for the quantification of peak LBF.
Fig. 10. Individual subject and group oxygen uptake (VO₂) values at 7.5, 10 and 13 kg workloads, and peak workload during the incremental exercise test to exhaustion. Data for the 7.5 kg workload were averaged during the last 10-s of the first minute of LO₇.₅kg. Remaining data points are averages of the last 10-s of each workload, and the last 10-s prior to exhaustion (peak values). Symbols represent group means ± SE. Horizontal error bars for peak values were used to indicate variation in peak workload achieved. Values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4).
Fig. 11. Individual subject and group mean arterial pressure (MAP) values at 7.5, 10 and 13 kg workloads, and peak workload during the incremental exercise test to exhaustion. Data for the 7.5 kg workload were averaged during the last 10-s of the first minute of LO_{7.5kg}. Remaining data points are averages of the last 10-s of each workload, and the last 10-s prior to exhaustion (peak values). Symbols represent group means ± SE. Horizontal error bars for peak values were used to indicate variation in peak workload achieved. Values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4). There was a trend towards an interaction between group and workload (P = 0.094).
Fig. 12. Individual subject and group heart rate (HR) values at 7.5, 10 and 13 kg workloads, and peak workload during the incremental exercise test to exhaustion. Data for the 7.5 kg workload were averaged during the last 10-s of the first minute of LO₇.₅kg. Remaining data points are averages of the last 10-s of each workload, and the last 10-s prior to exhaustion (peak values). Symbols represent group means ± SE. Horizontal error bars for peak values were used to indicate variation in peak workload achieved. Values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4).
Fig. 13. Individual subject and group stroke volume (SV) values at 7.5, 10 and 13 kg workloads, and peak workload during the incremental exercise test to exhaustion. Data for the 7.5 kg workload were averaged during the last 10-s of the first minute of LO7.5kg. Remaining data points are averages of the last 10-s of each workload, and the last 10-s prior to exhaustion (peak values). Symbols represent group means ± SE. Horizontal error bars for peak values were used to indicate variation in peak workload achieved. Values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4). There was a main effect of subject group on the SV response (P = 0.041).
Fig. 14. Individual subject and group cardiac output (CO) values at 7.5, 10 and 13 kg workloads, and peak workload during the incremental exercise test to exhaustion. Data for the 7.5 kg workload were averaged during the last 10-s of the first minute of LO7.5kg. Remaining data points are averages of the last 10-s of each workload, and the last 10-s prior to exhaustion (peak values). Symbols represent group means ± SE. Horizontal error bars for peak values were used to indicate variation in peak workload achieved. Values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4). The responses are not significantly different (P = 0.212) between groups.
Fig. 15. A: Individual subject and group leg blood flow (LBF) values at 7.5, 10 and 13 kg workloads during the incremental exercise test to exhaustion. Data for the 7.5 kg workload were averaged during the last 10-s of the first minute of LO7.5kg. Remaining data points are averages of the last 10-s of each workload. Values are expressed as the absolute change in magnitude from baseline. There was a trend towards a greater Δ in the CON group (P = 0.098). B: As baseline LBF was lower in the group with T2D (See Table 3), absolute LBF was significantly lower in this group at the 10 and 13 kg workloads. ●, CON subjects (n = 5); ▲, subjects with T2D (n = 4). *P < 0.05 for a significant difference between T2D and CON.
Step Transition from Rest to LO7.5kg Constant Work

Average group responses over time are shown in Fig. 16 to 21. ΔVO₂, ΔMAP, and ΔHR were not significantly different between groups over time (Fig. 16, 17 and 18 respectively). As expected, MAP fell at the onset of exercise due to the shift in blood volume from the central arteries into the skeletal muscle vasculature. As heart rate and cardiac output rose, this increased the inflow into the large arteries, restoring MAP to baseline values by t = 30. MAP continued to rise until steady state at approximately t = 90, signifying achievement of a new equilibrium between inflow (cardiac output) and outflow (leg blood flow). Most subjects demonstrated an overshoot of HR at the onset of exercise (see Appendix C for individual responses). This response was characterized by a rapid peak and fall in heart rate within in the first 25-s of exercise, followed by a slow, gradual increase to steady state. The magnitude of the overshoot, calculated as the difference between the highest and lowest heart rate achieved, did not differ significantly between groups (7 ± 1 vs. 6 ± 2 beats; CON vs. T2D respectively, P = 0.617). Similar to the response observed in the incremental exercise test, SV increased from baseline and remained elevated during exercise in T2D, while values for CON did not change significantly (Fig. 19). Compared to CON, subjects with T2D had a significantly greater ΔSV at t = 30, 45, 60, 240 and 300, and a trend towards a greater increase at t = 90, 120 and 180 (P = 0.088, 0.063, 0.109 respectively). Due to a similar ΔHR and a larger ΔSV, ΔCO in subjects with T2D tended to be larger than in the CON group (P = 0.109) (Fig. 20). Examination of individual subject ΔLBF (Fig. 21A, dotted and dashed lines) reveals the presence of an outlier in the CON group with a dramatically greater exercise ΔLBF. Further analysis shows that the assumptions of the 2-way RM ANOVA are violated as
the two groups display unequal variances for this variable. The average group response with this subject removed (n = 4 in both CON and T2D) is shown in Fig. 21B. The 2-way RM ANOVA run on this reduced pool of subjects passes the test for equal variances and an interaction between group and time is revealed (P = 0.006). At the onset of exercise at t = 15, the group with T2D demonstrated a significantly smaller ΔLBF compared to the CON group (3435.6 ± 275.0 vs. 2120.4 ± 218.4 ml/min, CON vs. T2D; P = 0.018).

**Dynamic Response of LBF**

The kinetic parameters describing the dynamic LBF response to both LO\(_{7.5kg}\) and VT\(_{90\%}\) are shown in Table 4A. The gain parameters describing the absolute magnitudes of LBF achieved during LO\(_{7.5kg}\) are shown in Table 4B. Gains for VT\(_{90\%}\) were not reported as subjects worked at different absolute workloads, therefore eliciting different magnitudes of LBF (see below for alternate analysis). Group averaged LBF responses for LO\(_{7.5kg}\) and VT\(_{90\%}\) are shown in Fig. 22A and 23A respectively. To ease the comparison of dynamic response characteristics at VT\(_{90\%}\), data for this relative workload were plotted as a percentage of the total change observed (0% at baseline, 100% at steady state). The exponential model representations of the mean group responses, derived from the parameters in Table 4A, are shown in Fig. 22B for LO\(_{7.5kg}\) and Fig. 23B for VT\(_{90\%}\). The response to the transition from rest to LO\(_{7.5kg}\) elicited two phases of LBF increase in all subjects. The transition from rest to VT\(_{90\%}\) was biphasic in all but two subjects, as one individual in each of the T2D and CON groups demonstrated a third phase of increase (see Appendix C, subject codes CN05 and DM03). The phase III kinetic parameters for the two subjects were not included in Table 4A; however, these values were included in
the calculation of both individuals’ MRT. Visual analysis of the VO₂ responses during this workload revealed one of these two subjects did not achieve steady state, which may explain the additional increase in LBF after ~ 2-mins of exercise. Two additional subjects in the CON group did not achieve a steady state VO₂, however, their LBF responses consisted of only two response phases.

**Phase I response.** The start of exercise in both the LO₇.₅kg and VT₉₀% workloads resulted in an immediate increase in LBF. The time to the onset of this rapid response (TD₁) did not differ between groups, and was not affected by the magnitude of the workload increase (Fig. 24A). The rate of increase (τ₁) in LBF was not different between groups at LO₇.₅kg, but was significantly faster in the subjects with T2D at VT₉₀% (Fig. 24B).

**Phase II response.** The onset of the second, slower phase (TD₂) of LBF was significantly earlier in the T2D group during LO₇.₅kg (Fig. 25A). There was also a trend towards a smaller TD₂ during VT₉₀% compared to the CON group (P = 0.063) (Fig. 25A). The rate of increase (τ₂) in LBF, however, was not different between groups (Fig. 25B). On the other hand, τ₂ tended to be faster in both groups when the step transition from rest was to a low intensity (LO₇.₅kg), compared to a moderate intensity (VT₉₀%) workload (P = 0.069).

**Mean response time.** There was a trend for a slower rate of increase in LBF in the group with T2D (P = 0.095), as revealed by slightly larger MRTs for both LO₇.₅kg and VT₉₀% (Fig. 26). Additionally, the MRTs of the LBF response for the moderate intensity
workload (VT90%) were significantly longer than that of the low intensity workload (LO7.5kg) for both T2D and CON (P = 0.006, 0.038 respectively).

Magnitude of the LBF response. LBF gains for LO7.5kg are shown in Table 4B and Fig. 27. Baseline LBF (G₀) was significantly lower in T2D compared to CON (~ 64% of CON G₀). At the onset of exercise, LBF increased immediately in both groups. At the end of the phase I adaptation, the gain (G₁) achieved in the T2D group was significantly smaller, approximately 58% of the gain in the CON group. The gain for the slower, phase II response appears larger in the subjects with T2D compared to CON, however, this difference was not statistically significant (P = 0.366). The total gain (TG) of the response, G₁ + G₂, was not different between groups. While there is a roughly 900 mL difference in means for TG, this difference appears to be largely influenced by a single subject in the CON group (Fig. 27). Absolute LBF gains for VT90% were not compared in the same manner as subjects worked at different absolute workloads, therefore exhibiting different metabolic requirements. However, in relative terms, the contribution of G₁ as a percentage of TG tended to be lower in the group with T2D (61.3 ± 7.0 vs. 45.0 ± 2.0 %, CON vs. T2D; P = 0.104) (Fig. 23).

Dynamic Response of HR

The kinetic parameters describing the dynamic HR response to both LO7.5kg and VT90% are shown in Table 5. For LO7.5kg, two CON subjects and one with T2D demonstrated a mono-exponential increase in heart rate (see Appendix C; subject codes CN01, CN05, DM01). Therefore, the kinetic parameters for phase II are means of n = 4 CON subjects and n = 3 subjects with T2D. One subject in the T2D group also
demonstrated a third phase of response (Appendix C; DM02). Parameters describing this phase were not included in Table 5, however the values were used in the calculation of this individual’s MRT. For VT90%, all subjects demonstrated a biphasic increase in heart rate, except for one CON subject who displayed a third phase of response (Appendix C; CN06).

There was no significant difference between groups for any of the parameters describing the kinetic HR response to both LO7.5kg and VT90%. However, the time to 63% of the total increase in HR (MRT) was significantly faster in LO7.5kg compared to VT90% in the CON group (P = 0.002). There was also a trend towards a faster increase in the group with T2D (P = 0.068). Individual subject values and group means for MRT are shown in Fig. 28.

**Dynamic Response of CO**

The kinetic parameters describing the dynamic CO response to both LO7.5kg and VT90% are shown in Table 6. The CO response to LO7.5kg was biphasic in all but four subjects, who demonstrated a single exponential increase to steady state (Appendix C; CN04, CN06, DM01, DM04). A biphasic increase in CO was observed during VT90% in all but three subjects, who demonstrated a single exponential increase (Appendix C; CN02, CN06, DM01).

There was no significant difference between the T2D and CON groups for any of the parameters describing the kinetic CO response to both LO7.5kg and VT90%. There was also no effect of workload on any of the parameters. Individual subject values and group means for MRT are shown in Fig. 29.
Fig. 16. Time course of change in oxygen uptake ($\Delta VO_2$) during 5-mins of constant work exercise at LO$_{7.5kg}$. Values are group means ± SE, expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4).
Fig. 17. Time course of change in mean arterial pressure (ΔMAP) during 5-mins of constant work exercise at LO7.5kg. Values are group means ± SE, expressed as the absolute change in magnitude from baseline. Data plotted at t = 15 represent three second averages of the largest drop in MAP within the first 25-s. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4).
Fig. 18. Time course of change in heart rate (ΔHR) during 5-mins of constant work exercise at LO7.5kg. Values are group means ± SE, expressed as the absolute change in magnitude from baseline. Data plotted at t = 15 represent three second averages of the largest increase in HR within the first 25-s. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4).
Fig. 19. Time course of change in stroke volume (ΔSV) during 5-mins of constant work exercise at LO_{7.5kg}. Individual subject data is shown in the dotted (…, CON) and dashed (---, T2D) lines. Symbols represent group means ± SE. All values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4). *P < 0.05 for a significant difference between T2D and CON.
Fig. 20. Time course of change in cardiac output (ΔCO) during 5-mins of constant work exercise at LO_{7.5kg}. Individual subject data is shown in the dotted (…, CON) and dashed (---, T2D) lines. Symbols represent group means ± SE. All values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 6); ▲, subjects with T2D (n = 4). There was a trend towards a larger CO response in the group with T2D compared to CON (P = 0.109).
Fig. 21. A: Time course of change in leg blood flow ($\Delta$LBF) during 5-mins of constant work exercise at LO_{7.5kg}. Individual subject data is shown in the dotted (CON) and dashed (T2D) lines. Symbols represent group means ± SE. All values are expressed as the absolute change in magnitude from baseline. ●, CON subjects (n = 5); ▲, subjects with T2D (n = 4). The presence of an outlier in the CON group (arrow) results in unequal variances between groups, violating the assumptions of the 2-way RM ANOVA. B: Time course change in $\Delta$LBF with data from one control subject removed (A, arrow). Mean values are of 4 subjects in each group. *P < 0.05 for a significant difference between T2D and CON.
Table 4A. Dynamic response characteristics of LBF to 5-mins of constant work exercise at LO\textsubscript{7.5kg} and VT\textsubscript{90\%}.

<table>
<thead>
<tr>
<th></th>
<th>TD\textsubscript{1}, s</th>
<th>τ\textsubscript{1}, s</th>
<th>TD\textsubscript{2}, s</th>
<th>τ\textsubscript{2}, s</th>
<th>MRT, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>LO\textsubscript{7.5kg}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.5 ± 0.4</td>
<td>3.4 ± 0.4</td>
<td>29.2 ± 2.7</td>
<td>18.1 ± 4.1</td>
<td>14.3 ± 1.9</td>
</tr>
<tr>
<td>T2D</td>
<td>1.4 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>18.2 ± 1.0*</td>
<td>26.7 ± 7.5</td>
<td>23.1 ± 4.2</td>
</tr>
<tr>
<td>VT\textsubscript{90%}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.0 ± 0.5</td>
<td>3.2 ± 0.3</td>
<td>24.1 ± 2.3</td>
<td>36.9 ± 8.0</td>
<td>26.2 ± 3.5</td>
</tr>
<tr>
<td>T2D</td>
<td>1.7 ± 0.5</td>
<td>1.7 ± 0.3*</td>
<td>17.3 ± 1.5</td>
<td>44.8 ± 14.0</td>
<td>40.0 ± 7.5</td>
</tr>
</tbody>
</table>

P 0.680 0.027 0.029 0.493 0.095

Values are means ± SE. CON, control (n = 5 for LO\textsubscript{7.5kg}, n = 6 for VT\textsubscript{90\%}); T2D, type 2 diabetes (n = 4); TD\textsubscript{n}, time delay; τ\textsubscript{n}, time constant; where n is the phase of response. MRT, mean response time. P values are for the main effect of group, T2D or CON. Post hoc analyses were performed with the Holms Sidak test. *P < 0.05 for a significant difference between T2D and CON groups.

Table 4B. LBF gains during 5-mins of constant work exercise at LO\textsubscript{7.5kg}.

<table>
<thead>
<tr>
<th></th>
<th>G\textsubscript{0}, ml/min</th>
<th>G\textsubscript{1}, ml/min</th>
<th>G\textsubscript{2}, ml/min</th>
<th>TG, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>959.8 ± 111.3</td>
<td>3662.1 ± 229.0</td>
<td>1246.2 ± 490.2</td>
<td>4908.4 ± 603.2</td>
</tr>
<tr>
<td>T2D</td>
<td>617.0 ± 22.1*</td>
<td>2128.1 ± 161.6*</td>
<td>1956.1 ± 466.5</td>
<td>4084.2 ± 307.2</td>
</tr>
</tbody>
</table>

P 0.044 0.002 0.366 0.335

Values are means ± SE. CON, control (n = 5); T2D, type 2 diabetes (n = 4); G, gain or amplitude of response; Subscripts, phase of response; TG, total gain. *P < 0.05 for a significant difference between T2D and CON groups.
Fig. 22. A: Average LBF response during rest and 5-mins of exercise at LO\textsubscript{7.5kg}. The start of exercise occurred at time = 0. Data are group averages shown as absolute change in LBF from baseline. B: Exponential model representation of the LBF response in both groups. The parameters for each curve were obtained by taking the average value across subjects in each experimental group (Table 4A). Baseline gain was plotted as 0 ml/min for ease of comparison. Solid line, CON subjects (n = 5); Dotted line, subjects with T2D (n = 4).
Fig. 23. A: Average LBF response during rest and 5-mins of exercise at VT$_{90\%}$. The start of exercise occurred at time $= 0$. As subjects worked at different absolute workloads and had different magnitudes of LBF, data are shown as a percentage of the total change observed from rest to steady state exercise. B: Exponential model representation of the LBF response in both groups. The parameters for each curve were obtained by taking the average value across subjects in each experimental group (Table 4A). Solid line, CON subjects ($n = 6$); Dotted line, subjects with T2D ($n = 4$).
Fig. 24. Mean values ± SE of the calculated A: time delay (TD), and B: time constant (τ) for Phase I of the dynamic LBF response to low (LO_{7.5kg}) and moderate (VT_{90%}) work intensities. The TD parameter represents the time from the onset of exercise to the onset the response, while the τ parameter represents the time required to achieve 63% of the response magnitude. Individual data is shown in the open circles (CON, n = 5 for LO_{7.5kg}, n = 6 for VT_{90%}) and triangles (T2D, n = 4). *P < 0.05 for a significant difference between groups.
Fig. 25. Mean values ± SE of the calculated A: time delay (TD), and B: time constant (τ) for Phase II of the dynamic LBF response to low (LO7.5kg) and moderate (VT90%) work intensities. The TD parameter represents the time from the onset of exercise to the onset the response, while the τ parameter represents the time required to achieve 63% of the response magnitude. Individual data is shown in the open circles (CON, n = 5 for LO7.5kg, n = 6 for VT90%) and triangles (T2D, n = 4). *P < 0.05 for a significant difference between groups.
Fig. 26. Mean values ± SE of the calculated mean response time (MRT) of the dynamic LBF response to constant work exercise at low (LO7.5kg) and moderate (VT90%) work intensities. The MRT parameter represents the time required to achieve 63% of the response magnitude at steady state. Individual data is shown in the open circles (CON subjects, n = 5 for LO7.5kg, n = 6 for VT90%) and triangles (subjects with T2D, n = 4). MRT tended to be slower in the group with T2D compared to CON (P = 0.095).
Fig. 27. Mean values ± SE of the LBF gains (G) during 5-mins of constant work exercise at LO_{7.5kg}. The gain parameter represents the magnitude of the LBF increase for each phase. TG, total gain of the response at steady state. Subscripts, phase of response. Individual data is shown in the open circles (CON subjects, n = 5) and triangles (subjects with T2D, n = 4). *P < 0.05 for a significant difference between CON and T2D.
Table 5. Dynamic response characteristics of HR to 5-mins of constant work exercise at LO\(_{7.5kg}\) and VT\(_{90\%}\).

<table>
<thead>
<tr>
<th></th>
<th>TD(_1), s</th>
<th>(\tau_1), s</th>
<th>TD(_2), s</th>
<th>(\tau_2), s</th>
<th>MRT, s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LO(_{7.5kg})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.5 ± 0.1</td>
<td>5.8 ± 1.7</td>
<td>110.9 ± 16.5</td>
<td>45.8 ± 10.5</td>
<td>17.6 ± 5.3</td>
</tr>
<tr>
<td>T2D</td>
<td>1.5 ± 0.5</td>
<td>3.3 ± 0.8</td>
<td>55.5 ± 21.2</td>
<td>23.3 ± 10.0</td>
<td>24.0 ± 7.8</td>
</tr>
<tr>
<td><strong>VT(_{90%})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>2.2 ± 0.7</td>
<td>3.1 ± 0.5</td>
<td>64.2 ± 19.0</td>
<td>86.1 ± 22.5</td>
<td>65.1 ± 10.5</td>
</tr>
<tr>
<td>T2D</td>
<td>2.2 ± 0.8</td>
<td>3.1 ± 0.6</td>
<td>43.7 ± 8.1</td>
<td>78.4 ± 15.1</td>
<td>52.2 ± 6.0</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>1.000</td>
<td>0.291</td>
<td>0.151</td>
<td>0.476</td>
<td>0.693</td>
</tr>
</tbody>
</table>

Values are means ± SE. CON, control (n = 6); T2D, type 2 diabetes (n = 4); TD\(_n\), time delay; \(\tau_n\), time constant; where n is the phase of response. MRT, mean response time. For TD\(_2\) and \(\tau_2\) in LO\(_{7.5kg}\), n =4 for CON and n = 3 for T2D, as not all subjects displayed a second phase of response. P values are for the main effect of group, T2D or CON.

Table 6. Dynamic response characteristics of CO to 5-mins of constant work exercise at LO\(_{7.5kg}\) and VT\(_{90\%}\).

<table>
<thead>
<tr>
<th></th>
<th>TD(_1), s</th>
<th>(\tau_1), s</th>
<th>TD(_2), s</th>
<th>(\tau_2), s</th>
<th>MRT, s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LO(_{7.5kg})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>1.3 ± 0.4</td>
<td>10.4 ± 2.6</td>
<td>112.7 ± 17.7</td>
<td>66.5 ± 12.0</td>
<td>49.6 ± 20.6</td>
</tr>
<tr>
<td>T2D</td>
<td>0.8 ± 0.3</td>
<td>13.5 ± 3.0</td>
<td>106.0 ± 7.8</td>
<td>87.7 ± 3.9</td>
<td>32.8 ± 13.9</td>
</tr>
<tr>
<td><strong>VT(_{90%})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.5 ± 0.5</td>
<td>16.9 ± 6.4</td>
<td>126.1 ± 24.1</td>
<td>73.0 ± 16.2</td>
<td>51.3 ± 16.0</td>
</tr>
<tr>
<td>T2D</td>
<td>2.3 ± 1.3</td>
<td>15.0 ± 3.7</td>
<td>87.6 ± 13.3</td>
<td>40.5 ± 15.1</td>
<td>37.8 ± 9.9</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.359</td>
<td>0.923</td>
<td>0.452</td>
<td>0.451</td>
<td>0.441</td>
</tr>
</tbody>
</table>

Values are means ± SE. CON, control (n = 6); T2D, type 2 diabetes (n = 4); TD\(_n\), time delay; \(\tau_n\), time constant; where n is the phase of response. MRT, mean response time. For TD\(_2\) and \(\tau_2\) in LO\(_{7.5kg}\), n =4 for CON and n = 2 for T2D; in VT\(_{90\%}\), n =4 for CON and n =3 for T2D, as not all subjects displayed a second phase of response. P values are for the main effect of group, T2D or CON.
Fig. 28. Mean values ± SE of the calculated mean response time (MRT) of the dynamic HR response to constant work exercise at low (LO$_{7.5kg}$) and moderate (VT$_{90\%}$) work intensities. The MRT parameter represents the time required to achieve 63% of the response magnitude at steady state. Individual data is shown in the open circles (CON subjects, n = 6) and triangles (subjects with T2D, n = 4).

Fig. 29. Mean values ± SE of the calculated mean response time (MRT) of the dynamic CO response to constant work exercise at low (LO$_{7.5kg}$) and moderate (VT$_{90\%}$) work intensities. The MRT parameter represents the time required to achieve 63% of the response magnitude at steady state. Individual data is shown in the open circles (CON subjects, n = 6) and triangles (subjects with T2D, n = 4).
CHAPTER 5: DISCUSSION

This study was designed to confirm previous observations of impaired oxygen uptake responses to exercise in individuals with T2D, as well as determine whether dysfunctional muscle blood flow regulation during exercise may contribute to these reports. The main findings of the present investigation are:

1) In contrast to previous studies, subjects with T2D did NOT demonstrate impaired VO₂ responses to exercise compared to healthy, physical activity matched controls. This finding was consistent throughout a range of workloads, from low intensity to peak exercise.

2) Subjects with T2D demonstrated a significantly reduced LBF at rest, as well as a trend towards a smaller LBF response to incremental exercise compared to healthy, physical activity matched controls.

3) Subjects with T2D demonstrated an impaired adjustment of LBF to low and moderate intensity constant work exercise compared to healthy, physical activity matched controls. This was characterized by a smaller LBF gain at the onset of exercise (LO₇.₅kg), and a trend towards a slower rate of increase to steady state. Therefore, in the group with T2D, oxygen delivery in the form of LBF does not appear to be a limiting factor to the increase and maintenance of VO₂, as compared to healthy controls.

It is acknowledged that the extremely limited sample size of subjects recruited has resulted in an underpowered study. Group means were described as “tended to be lower” or “showed a trend towards” if differences appeared to be sufficiently large. In all of these cases, P-values were reported (P = 0.05 to 0.10) and individual subject data were
presented in order to demonstrate the variability of responses. Results will be discussed based on these trends towards significance, however, in the end, the reader should be aware that only statistically significant differences (P < 0.05) should be recognized.

**VO₂ and LBF Interactions**

The finding of similar VO₂ responses despite a smaller initial increase in LBF during LO₇.₅kg is in line with studies that report the greater reliance of T2D skeletal muscle on oxygen extraction during the onset of contractions (10; 110). The results of the present study are also consistent with exaggerated reductions in microvascular oxygen pressure (PmO₂) at the onset of exercise as observed in rat models of T2D (84), and previously discussed in the Chapter 2 literature review.

At rest, the absolute value of LBF was significantly less in the group with T2D. Resting VO₂, representing full body oxygen consumption, was not different between groups. The observation of impaired resting blood flow has not been previously reported, however, this finding concurs with reports in rat skeletal muscle of a lowered PmO₂ at rest (84).

During exercise, abnormal changes in PmO₂ indicate an altered relationship between muscle blood flow (Qm) and muscle oxygen uptake (VO₂m). At the onset of twitch contractions in the rat model of T2D, PmO₂ drops significantly faster compared to control, and demonstrates an undershoot of the eventual steady state value (Fig. 3, Chapter 2). Consistent with these findings are observations from the present study that individuals with T2D demonstrate a reduced hyperemic response at the onset of low intensity exercise (t = 15 in Fig. 21B, G₁ in Table 4B). As PmO₂ represents the dynamic
balance between Qm and VO2m, it is possible that this blunted increase in LBF contributes to a rapid fall in muscle oxygenation.

According to the Fick equation,

\[ \text{VO}_2 = \text{Qm} \times [\text{CaO}_2 - \text{CvO}_2] \]

where Qm is muscle blood flow, CaO2 and CvO2 represent arterial and venous oxygen content respectively, and the term CaO2 – CvO2 represents the amount of oxygen that is extracted as blood flows across the capillaries through the site of metabolic demand. The same VO2 can be achieved at different levels of Qm and CaO2 – CvO2. This appears to be the case in the present study. The reduced phase I LBF gain in LO7.5kg would have necessitated an increase in extraction in order to maintain sufficient delivery of oxygen to support the given metabolic demand. In line with this hypothesis, initial skeletal muscle deoxygenation (as measured by near infrared spectroscopy) has been shown to demonstrate an overshoot of the eventual steady state level at the onset of moderate cycling exercise in individuals with T2D (10). Therefore, it is possible that an early increase in oxygen extraction compensated for the blunted initial increase in LBF found in the group with T2D.

As mentioned previously, the rise in VO2 at the onset of exercise is likely due to the integrated response of both delivery and utilization of oxygen within the myocyte. The blunted rise in LBF observed in the group with T2D during LO7.5kg likely resulted in a large drop, compared to CON, in PmO2 within the skeletal muscle. As PmO2 represents the high energy component of the pressure gradient driving diffusive transport of oxygen into the cell, this may have resulted in a drop in the oxygenation within the cell (PcellO2),
and thus the oxygen available to support the given metabolic rate. The magnitude of this reduction in the group with T2D is unknown, however, given the similarity of their VO₂ responses to the CON group, it is possible that alterations in the metabolic state of the cell compensated for the reductions in PcellO₂. A given metabolic rate can be maintained, despite reductions in PcellO₂, provided adjustments in phosphate energy state (ratio of [ATP] to [ADP][Pi]) and redox state (ratio of [NADH] to [NAD⁺]) occur (132) (Fig. 2, Chapter 2). According to the net drive hypothesis, the rate of mitochondrial respiration is determined by the combined effects of the phosphate energy state, the redox state, and PcellO₂ (132). Changes in one of these variables can be offset by opposite changes in the others in order to maintain the same rate of ATP production (132). If PcellO₂ was reduced in this study, increases in both [ADP][Pi], and [NADH] would have occurred to maintain ATP production (132; 145; 147-149), and therefore, the observed VO₂. While a critical PcellO₂ exists, below which compensations by the two energy states can no longer maintain the given ATP production (dotted line, Fig. 2, Chapter 2), the similar VO₂s observed in this study show that this was not a concern. Finally, the similarity in VO₂, despite lower LBF, may also be attributed to a greater efficiency of mitochondrial respiration in the group with T2D. At a given metabolic rate, smaller changes in the phosphate energy state and redox state are required if there is a greater number of mitochondria present (24; 45; 132). This is unlikely to be the case however, as many studies report reduced, rather than enhanced, mitochondria function in the skeletal muscle of T2D (54; 99).

The eventual rise in LBF to steady state did not differ in magnitude between groups during LO7.5kg (Fig. 21 and Table 4B), which points to a greater compensatory
response in the subjects with T2D over time. Again, this delayed ΔLBF concurs with the PmO₂ response reported in the rat model of T2D (84). After the significantly faster drop and undershoot compared to healthy controls, PmO₂ in the diabetic rats rises and eventually reaches a steady state value not different from baseline. As discussed in the literature review, there are two possible explanations for this observed response. Either a reduction in VO₂m or an increase in Qm would be required in order for steady state (Qm = VO₂m) to be attained. The results of this study are evidence that the pattern in PmO₂ recovery is most likely due to increases in blood flow that eventually restore muscle oxygenation to a state not different from controls. Steady state ΔLBF did not differ between groups during LO7.5kg, consistent with the finding in the rat models that the balance between Qm and VO₂m (and hence PmO₂) was similar in both groups.

For the incremental exercise test, ΔLBF at the 7.5, 10 and 13kg workloads are non-steady state values, obtained during the last 10-s of each 1-min interval. Consistent with observation of blunted LBF responses at the onset of exercise, ΔLBF tended to be lower in the group with T2D, most likely due to an abnormal adjustment response. The differences between groups in ΔLBF values were ~ 770, 1184, and 1721 mL for the 7.5, 10 and 13 kg workloads respectively. Individual subject plots in Fig. 15 reveal that only one subject with T2D had ΔLBF responses comparable to those of the CON subjects. Therefore, it is predicted that with a larger sample size, these difference in ΔLBF would be statistically significant. Finally, VO₂ did not differ significantly between groups at any of the three workloads, pointing to the possibility that modulations in both phosphate energy state and redox state compensated for any reductions in oxygen transport that may
have occurred. Therefore, it does not appear as though blunted ΔLBF responses, if present, compromised muscle oxygenation support for mitochondrial respiration.

**Dynamic Response Characteristics of LBF**

We sought to measure the dynamic increase in LBF in individuals with T2D to determine whether dysfunctional muscle blood flow regulation was present in this population. Recent reports of slowed on-kinetics in VO$_2$ (14; 92) and microvascular blood flow (10), along with reduced steady state blood flows (58; 60) during exercise have led to the prediction that an impairment in oxygen delivery may be partly responsible for the abnormal exercise responses. Previous measurements of LBF have been limited to steady state exercise (58; 60; 151), therefore it was unknown which aspects of the blood flow control systems were responsible for the observed impairment. As discussed in Chapter 2, the dynamic increase in LBF is regulated by two different regulatory systems. The combined response of both feedforward and feedback controllers elevate flow and oxygen delivery to match metabolic demand. Deficiencies in either control system, in terms of magnitude and/or rate of response, would result in a lower LBF response.

*Phase I: Feedforward Vascular Control*

The significantly smaller contribution of $G_1$ to total ΔLBF in absolute magnitude (LO$_{7.5kg}$) and the trend towards a smaller $G_1$ in relative terms (VT$_{90\%}$) provide evidence that initiatory, feedforward mechanisms of blood flow regulation are impaired in individuals with T2D. The onset and rate of increase in this response, however, was not significantly slower in the group with T2D compared to CON. In fact, the rate of increase
(τ₁) was significantly faster during VT₉₀% in the group with T2D. Therefore, while it appears as though the activation of the feedforward control systems is preserved with T2D, the ability to elevate blood flow is significantly impaired.

There are currently two proposed mechanisms that regulate the immediate increase in LBF with muscle activation: contraction-induced rapid vasodilation, and the skeletal muscle pump (134). Subjects in this study performed knee extension/flexion exercise in a supine position. Therefore, it is unlikely that the emptying of venous volume with contraction resulted in a significant widening of the arteriovenous pressure gradient, as the hydrostatic column effect on venous pressure was very small. Consequently, impairments in contraction-induced rapid vasodilation may be responsible for the blunted LBF response observed. While the mechanisms controlling this increase in vascular conductance are currently unclear, available evidence points towards multiple contributors including vasodilatory factors released during skeletal muscle activation (22; 134), and contraction induced distortion of resistance vessels (23; 59). As endothelial dysfunction is characteristic of individuals with T2D (43; 58; 69; 76; 141; 144), it is plausible that the reduced production and/or bioavailability of nitric oxide (NO) (16; 129; 130) or prostaglandins (PG) contributed to the blunted LBF response found in this study. However, studies in healthy humans have shown that neither NO (118), PG (120), or the combination of the two (107) account for rapid vasodilation at the onset of exercise. Blockades of either or both of these substances at the onset of muscle contraction have failed to cause reductions in the normal magnitude and rate of increase in muscle blood flow. Further, the application of sodium nitroprusside, an NO donor, on isolated skeletal muscle arterioles revealed a 4-s delay to onset of vasodilation (153). Therefore, it is
unlikely that impaired release of these two vasodilators from the endothelium caused the blunted rapid vasodilatory response found in this study.

Potassium is a potential vasodilator that has been the subject of recent investigations. It is released from muscle fibres during membrane depolarization, therefore its increase in the interstitial space coincides with muscle activation. However, the mechanism underlying its effect (132), as well as the magnitude of its contribution to overall hyperemia during different types and duration of contraction (59), is currently unclear. Therefore, it is not possible to make predictions of changes in this factor with disease.

Recently, it was found that mechanical compression of isolated rat feed arteries produced a time course of vasodilation that was similar to that observed during muscle contraction (23). Furthermore, removal of the endothelium blunted, but did not eliminate the increase in vessel diameter (23). The mechanism behind this response is currently unknown, but it has been hypothesized that a reduction in transmural pressure across the vessel wall, due to increases in extravascular pressure, stimulates a myogenic mechanism that induces vasodilation (77). The endothelial factors NO, PG and endothelium derived hyperpolarizing factor are possible vasodilators that mediate the endothelium dependent component of this response, however there have not been any studies that have specifically examined their contribution to mechanical compression induced vasodilation. Also, as mentioned above, the presence of NO and PG have not been found to be essential contributors to immediate hyperemia (107; 118; 120). Nonetheless, as the mechanisms regulating rapid vasodilation at the onset of muscle contraction have yet to be fully explored, the role of substances released from endothelium should not be
overlooked. This is especially important as disease populations, such as those with T2D, demonstrate reduced hyperemic responses to exercise, a finding that has been associated with endothelial dysfunction (58).

**Phase II: Feedback Vascular Control**

The onset of the phase II increase in LBF (TD2) was significantly faster in the group with T2D during LO7.5kg, and tended to be faster during VT90%. This is consistent with the characteristics of a feedback control system, whereby an error signal relating a mismatch between oxygen delivery and metabolic demand initiates a response to correct the error (132). In the group with T2D, the significantly lower G1 presumably caused a large mismatch between oxygen supply and demand. As the magnitude of this error signal would be greater than that of the CON group, feedback control systems would be predicted to initiate earlier. As the total gain of the LBF response was not different between groups, one would have expected the phase II gain (G2) to be higher in the group with T2D. This would also be consistent with the feedback control system characteristic that the magnitude of the error determines the magnitude of the response (132). While the means for G2 were not significantly different between groups, visual analysis of the individual subject plots for this variable (Fig. 27) showed that the CON group mean was significantly influenced by one outlier, who had a G2 ~2000 mL higher than his counterparts. Therefore, despite the lack of statistical significance, it is predicted that the similar TGs observed in both groups resulted from a greater G2 in the group with T2D.

The rate of increase of this feedback response (τ2) was not different between groups for either LO7.5kg or VT90%. This finding, along with the shorter TD2 in the group
with T2D, and similar TG between groups at steady state, are evidence that feedback control mechanisms of blood flow regulation are preserved in individuals with T2D.

Recently, it was shown that a reduced expression of heterotrimeric G protein, G_{i2}, in humans with T2D was associated with a decreased release of ATP from red blood cells (RBC) (122). As RBC-ATP stimulated vasodilation has been considered an important feedback mechanism of blood flow regulation (27; 28), this impairment would have negative consequences for the maintenance of muscle oxygenation during exercise. While we are unable to quantify the contribution of this mechanism in the current study, the observation of preserved phase II LBF responses demonstrate that the RBC-O_{2} sensor hypothesis of feedback control is not impaired in this group with T2D. This conclusion is supported by the fact that our subjects with T2D demonstrated good control of blood glucose levels (mean HbA_{1c} of 7.4%), as impairment in ATP release is inversely associated with glycemic control (122).

**Mean Response Time**

The mean response time (MRT) tended to be slower in the group with T2D in both LO_{7.5kg} and VT_{90%}. MRT represents the time needed to achieve 63% of the total response magnitude, and is a weighted mean of the TD and τ for each response phase (65). While τ_{1} and TD_{2} were faster in the group with T2D, and τ_{2} not significantly different between groups, the smaller contribution of G_{1} to TG in the group with T2D may implicated in the trend towards the longer MRT in the subjects with T2D. In other words, while the speed of the kinetic response was not slower in T2D compared to CON, the rise in LBF to 63% of the total response may delayed as a result of the blunted increase in LBF at the onset of low intensity exercise. Therefore, the rate limiting step in
LBF regulation during exercise in these individuals with T2D lies in the inability of feedforward mechanisms to elevate LBF to a level comparable to CONs.

**Central Hemodynamics**

*Dynamic Response Characteristics of HR and CO*

The possibility of a central impairment in oxygen delivery in T2D has been hypothesized as a result of the findings of slowed heart rate and VO₂ on-kinetics (92) during submaximal exercise, and altered cardiac hemodynamics to graded increases in work rate (93). Consistent with the finding of preserved LBF dynamic response characteristics (TD, τ), there were no significant differences between any of the parameters describing the dynamic response of HR and CO to both LO₇.₅kg and VT₉₀%. Despite the blunted increase in ΔLBF at the start of muscle contraction, the initial onset and increase in HR and CO was virtually identical between groups (TD₁ and τ₁). Therefore, a central limitation to oxygen delivery is not expected to have contributed to the impaired response. After the initial increase, the kinetic responses of both heart rate and cardiac output varied between subjects regardless of group, with individuals displaying anywhere from one to three phases of response. Therefore, the use of MRT, similar to that calculated for LBF, allowed for comparisons between groups regardless of the pattern of response. As demonstrated in Fig. 28 and 29 for HR and CO respectively, there is significant overlap of the values for MRT between both groups. Hence, central hemodynamic responses do not appear to play a limiting role in the present study.

During exercise in the upright position, 63% of the increase in cardiac output is attributed to an increase in heart rate, and the remainder due to increases in SV (52). However, in the supine position SV is elevated quite significantly compared to rest (52).
Consequently, large increases in HR are not required to achieve elevated values of CO. It is possible, then, that measuring cardiac responses during exercise in the supine position do not allow limitations in central hemodynamics to be readily detected. Further research into central limitations to exercise in T2D is required, specifically in upright, large muscle mass exercise where increases in HR and CO are expected to play a significant role.

*Arterial perfusion pressure*

MAP was significantly lower at rest in the individuals with T2D, and may have contributed to the lower LBF responses observed. The reason for this finding is unclear, although one of the subjects in this group was on anti-hypertensive medication. As the magnitude of increase in MAP (ΔMAP) did not differ between groups during exercise at LO7.5kg, the lower perfusion pressure persisted throughout this 5-min workload. The reduced perfusion pressure may have contributed to the blunted initial increase in ΔLBF at t = 15, but its effect appeared to minimize over time as ΔLBF did not differ between groups after this time. This would mean that an increase in vascular conductance occurred in the group with T2D. These findings are consistent with a previous study that examined LBF responses under manipulations of perfusion pressure (65). During leg exercise in the supine position, the magnitude of the initial LBF gain (G1) was significantly less than that of G1 in the upright position, where MAP is ~ 20 mmHg higher. However, it is acknowledged that the comparisons in the latter study were performed in the same individuals, whereas the difference in perfusion pressure in this study was between subject groups, where differences in the initial change in vascular conductance may also play a role.
During non steady state exercise at 10 and 13 kg, $\Delta$MAP was significantly higher compared to 7.5 kg in the group with T2D. If vasodilation was blunted in the skeletal muscle of the group with T2D, as is characteristic of this population (16; 43; 58; 69; 76; 144), the rise in $\Delta$MAP would have been a compensatory response to increase LBF. This response, however, was limited in its effectiveness as $\Delta$LBF for the group with T2D did not reach the same levels as the CON group.

*Stroke volume*

The baseline values for SV are characteristic of those measured in the supine position (40; 53; 63). This is due to the increase in central blood volume and ventricular diastolic volume that result from being in a supine position (52). Peak SV fell at exhaustion in both groups (Fig. 13). In untrained individuals, such as those found in the present study, the SV response at maximal exercise is varied, as reviews on the topic have found reports of a small increase, plateau or decline (136; 138). Much of the debate surrounding the response of SV at maximal exercise involves differences between trained and untrained individuals, timing of measurements, as well as limitations in measurement techniques (37; 138).

The initial increase in SV in subjects with T2D, compared to no change in the CON group, may be a result of lower MAP, representing a decreased ventricular afterload, compared to CON. At rest, SV was not different between groups, despite a ~17 mmHg greater MAP in the CON group. This could reflect a greater preload capacity (enhanced ventricular filling) or increased myocardial contractility (52). At the onset of exercise, the difference in MAP persisted, therefore assuming similar changes in preload
and contractility between groups, it is possible that the lower impedance to aortic flow contributed to the increased SV observed in the group with T2D.

SV did not change significantly from rest to exercise in the CON group. In the group with T2D, after the initial increase, values also remained constant. These findings are similar to previous studies that show minor changes in SV during supine, graded (52; 53; 103) and steady state cycling exercise (50; 63). SV at rest in the supine position has been reported to be only 20% less than maximum SV (52). In endurance trained subjects, exercise in the supine position leads to substantially reduced increases in SV compared to the upright position (139). Enhanced ventricular filling is thought to allow athletes to be able to continuously increase SV during upright incremental exercise, compared to untrained individuals who demonstrate a plateau around 40% VO₂max (36). Therefore, given that our subjects are untrained individuals, the already elevated resting SV, and tendency for stroke volume to plateau at low intensities of exercise (~40% VO₂max, (6; 36)), the pattern of SV response in our subjects is not unexpected.

Cardiac Output

The measurement of changes in CO (ΔCO) did not correlate with measures of ΔLBF. Normally with low to moderate dynamic exercise, the increase in CO contributes entirely to the increase in LBF (52). However, in the CON group during LO7.5kg, ΔCO was ~ 2 l/min, whereas ΔLBF was ~ 5 l/min. The change observed in the group with T2D was closer to expected, with ~ 4 l/min increases in both. Hence, there appears to be a discrepancy between the change in magnitude of blood volume being pumped out by the heart into the central circulation, and the amount being measured in the lower limbs. Fig. 30 plots ΔLBF as a function of ΔCO for the 7.5, 10 and 13 kg workloads. The line of
identity denotes the point where ΔLBF equals ΔCO, and points to the left of the line are subjects where ΔLBF exceeds ΔCO. From this plot it appears that some subjects, regardless of condition, display this pattern. The following is a discussion of the possibilities by which this may have occurred.

Fig. 30. Changes in LBF (ΔLBF) as a function of the change in CO (ΔCO) during non-steady state knee extension/flexion exercise at 7.5, 10 and 13 kg workloads in CON subjects (circles) and subjects with T2D (triangles). The line of identity denotes the point in which ΔCO matches ΔLBF, a finding that is expected during dynamic exercise at low to moderate intensities (52). Data points to the left of the line of identity are subjects who displayed ΔLBFs that exceeded ΔCO.
Sympathetic vasoconstriction of inactive tissues

Based on the law of conservation of mass, in order for $\Delta LBF$ to exceed $\Delta CO$, there must be a diversion of blood volume from other vascular beds (for example, skin, splanchnic, inactive muscle beds) to the active muscle. Sympathetic vasoconstriction in inactive tissues occurs as part of the baroreflex-mediated regulation of arterial blood pressure (132). When the rise in muscle blood flow exceeds the rate at which cardiac output can increase, such as during progressively heavier intensities of exercise, an increase in sympathetic outflow results in vasoconstriction at inactive tissue beds. This results in a shift in blood volume from the inactive tissues to the central circulation, where it is then delivered to active muscle (102; 132). Increasing inflow into the vasculature of active muscle by decreasing outflow to inactive vascular beds prevents a drop in central blood volume and allows for the maintenance of arterial blood pressure. While this sympathetically mediated response would explain the observations in the present study, it is unlikely to have been the case. The rise in sympathetic nerve activity in humans during dynamic exercise only occurs once withdrawal of parasympathetic activity is completed around a heart rate of ~ 100 bpm (102). The absolute heart rates achieved were less than 100 bpm in both the 7.5 and 10 kg workloads, and just over 100 bpm at the 13 kg workload. Therefore, it is unlikely that increased sympathetic activity during these workloads accounted for the discrepancy between $\Delta LBF$ and $\Delta CO$.

Underestimation of CO

The absolute values measured for SV during rest and exercise are comparable to those reported in the literature for exercise in a supine position (40; 50; 53; 63). The values are also consistent with the report that SV increases to approximately 20% of
maximal SV in the transition from upright to supine (52), assuming an untrained maximal SV of ~ 120 ml/beat (36). However, while values for group means are comparable to that reported in the literature, examination of individual subject SV responses reveals a large range of values in the CON group. At rest and exercise (values did not change significantly in this group), values range from ~ 80 ml/beat (typical of values in the upright position, (11; 40; 63)) to 130 ml/beat. The CON subjects who had the three lowest supine SVs (83 to 89 ml/beat) all demonstrated ΔCO < ΔLBF. As the estimation of SV by the Modelflow method is based on average age and gender characteristics of aortic input impedance (see Chapter 3, METHODS), values calculated may not always reflect a given individual’s actual SV. The Modelflow method has been found to underestimate absolute values for CO in older adults (96), though its reliability in measuring relative changes in young adults has been described (127). Therefore, it is possible that an error in the estimation of SV by the Modelflow method resulted in a reduced CO response.

It is unlikely that lower than normal exercise HR responses in the present study contributed to the reduced ΔCO. During the incremental exercise test to exhaustion, the peak HR achieved was ~ 70% of the average age-predicted maximal heart rate. In a similar study performing two leg knee extension in an upright position, peak heart rate achieved during an incremental exercise test was 166 beats per minute (bpm), ~ 88% of average age predicted heart rate (41). The higher HR achieved in this study can be attributed to the upright position. An incremental exercise test performed with supine, single knee extension elicited maximal heart rate responses of 120 bpm in young subjects and 90 bpm in older subjects (changes of < 40 bpm from rest) (86). Short bouts of
bilateral, supine knee extension exercise at ~70-75% of peak power elicited heart rate responses as low as 100 to 110 bpm (34). Therefore, as the heart rate responses observed in this study are consistent with values reported in similar studies, it is unlikely that a low HR contributed to a lower than normal CO response.

*Overestimation of LBF*

Absolute values for the LBFS observed in the present study range from approximately 1 l/min at rest to 6 l/min during steady state exercise at LO7.5kg (ΔLBF ~5L/min). These values are higher in comparison to a study by Macdonald et al. (65), who examined LBF kinetics with an identical experimental protocol (Doppler ultrasound measurement during supine, knee extension and flexion exercise at a low to moderate work rate). Heart rate and dynamic response characteristics of LBF are comparable, but the mean age of the subjects is much lower (27 ± 5 years). Despite the finding that aging results in an attenuation of exercise muscle blood flow (86; 87; 90), LBFS reported in these younger subjects was lower, ~ 0.7 l/min at rest, and 4 l/min during steady state exercise. Similar protocols of single or double knee extension exercise also demonstrate values of ~ 1 l/min at rest and 3-4 l/min during low to moderate intensity exercise (30; 41). This however, is in contrast to values obtained by Kingwell et al. (58), who measured LBF responses with thermodilution techniques during upright moderate intensity cycling at 60% VO2peak. This group reported values of ~ 0.7 l/min at rest and ~ 4.5 l/min in T2D and 7 l/min in CON during exercise. Similarly, Martin et al. (70), who utilized the same cycling protocol, reported ~ 0.8 l/min at rest and 6 l/min during exercise in both groups. The latter two studies have the advantage of measuring LBF responses in subjects of similar weight, age and health profile to the subjects in this study, though the
mode of exercise and pattern of muscle recruitment is different. Nevertheless, based on data available from similar exercise protocols, the possibility exists that an overestimation of LBF occurred.

The measurement of muscle blood flow by Doppler ultrasound is commonly used in exercise studies (30; 41; 42; 65; 66; 85; 86; 100; 108; 109; 133), and has been shown to provide valid measurements during dynamic knee extension exercise (91). One of its limitations, however, is the sensitivity of the measurement to motion artifact, such as with muscle contraction. While increased movement during exercise at higher workloads can result in poor acquisition of Doppler signals, this would only act to reduce the magnitude of velocity measured, not enhance it. Therefore, it is unlikely that errors in measurement due to inconsistent data signals accounted for an overestimation of LBF.

A correction factor was applied to blood velocity measurements during offline analysis to account for differences in the angle of insonation of the Doppler ultrasound probe (see Chapter 3, Fig. 7). As most of the subjects studied demonstrated some degree of abdominal obesity (Chapter 4, Table 2), adjustments in the angle of the probe relative to the surface of the skin was necessary in order to access the vessel and obtain a clear signal. The adjusted angle of insonation was estimated by obtaining an Echo ultrasound image of the vessel at the site of velocity measurement. While every effort was taken to replicate the degree of adjustment made by the operator during testing, it is possible that the calculated angle of insonation differed from the one applied during testing. This process is further confounded by the fact that large movements at the site of measurement, due to muscle contraction, require the operator to make slight adjustments during exercise to maintain clear data signals. Therefore, it is possible that an
overestimation of LBF response occurred during this stage of data analysis. It should be noted, however, that corrections made only affect the absolute magnitudes of blood velocity, and do not have any influence on dynamic response characteristics.

In summary, it is possible that errors in the measurement of outcome variables (underestimation of CO, overestimation of LBF) resulted in the abnormal ΔCO : ΔLBF ratios observed. It is unlikely that these changes are due to physiological differences between CON and T2D individuals, as the workloads performed were of low to moderate intensity and did not interfere with neural regulation of arterial blood pressure.

Advantages and Limitations

The major advantage of this study over previous investigations is the simultaneous measurement of both LBF and VO2. To our knowledge, this is the first study to combine measures of both oxygen delivery and utilization to examine limitations to exercise performance in the T2D population. While separate reports of lower LBF and slowed VO2 responses to exercise have been suggested as evidence for a muscle oxygenation limitation to oxidative phosphorylation, these studies were limited by the fact that only one or the other were measured.

The subjects in this study had identical levels of habitual physical activity. This was confirmed with observations of similar VO2peak in both groups. Therefore, study findings were not influenced by underlying abnormalities associated with differences in cardiorespiratory fitness. Finally, supine, knee extension and flexion exercise is a non-weight bearing form of activity. Therefore, there were no differences in work performed, despite the fact that the group with T2D was on average 10 kg heavier than the CON group.
The major limitation of the present study is the small sample size of subjects in each group (n = 6 and 4 for CON and T2D respectively). Difficulties in subject recruitment, complicated by strict inclusion/exclusion criteria (see Appendix B), were the main factors behind the limitation. Given the low statistical power associated with the small sample sizes (see Appendix D), certain results of the present study should be interpreted cautiously. Plots of individual subject data were included in result figures if mean values appeared to be significantly different. For example, the mean values for non steady state $\Delta$LBF during the 7.5, 10 and 13 kg workloads show a trend towards being significantly lower in the group with T2D. This trend is supported by the finding of a blunted initial increase in LBF during LO7.5kg. Examination of individual means in Fig. 15 reveal that only one subject with T2D had $\Delta$LBF values in the range of the CONs. Therefore, with additional subjects, it is possible that a significant difference would be found. For instance, a mean difference of 1700 ml/min with a standard deviation of 800 ml/min (values for 13 kg workload), requires a sample size of 5 subjects per group for a power of 0.8 and alpha of 0.05.

Measurement of VO$_2$ with the MOXUS metabolic cart did not provide sufficient time resolution for quantification of VO$_2$ on-kinetics. Therefore, while the averaged values reported in this study indicate no differences in oxidative phosphorylation within the first 1 to 2-mins of exercise, it is possible that differences, undetectable by our measurements, were present in the rate of increase in VO$_2$ between the two subjects groups. However, group means from the first minute of exercise in Fig. 16 seem to indicate that individuals with T2D demonstrate slightly higher values compared to CON.
Nevertheless, future investigations of both VO$_2$ and LBF on-kinetics will help to clarify these findings.

There were no estimates of leg muscle mass in this study. However, the lower flow is unlikely to be attributed to a smaller leg mass, as the group with T2D weighed on average 10 kg more than the CON group (Chapter 4, Table 2). The average age of the group with T2D is approximately 10 years older than the CON group. It is possible that observed differences in early LBF responses are a result of an aging effect. Indeed, a blunting of rapid vasodilation has been reported in older adults compared to young adults (18), though the age difference in those groups was ~ 35 years. Metformin is known to improve endothelial function in individuals with T2D (73). As two of the individuals in our group with T2D were on metformin (50% of the group), it is possible that improved vascular function in these individuals minimized differences that may have emerged in the absence of the drug. However, despite the inclusion of the medicated individuals, significant differences in phase I LBF gain (LO$_{7.5kg}$) were still detected.

The absence of a requirement for a medical examination prior to participation in the study may have led to recruitment of subjects with underlying comorbidities. While completion of a medical history form by both the subject and their family physician were required, the information provided by these individuals was not always very detailed. Further, the inclusion of subjects with various comorbidities (see Appendix B) mean the findings of the present study cannot be solely attributed to the T2D state. On the other hand, this allows the results of the study to be more applicable to the general disease population, where the presence of comorbidities is more often than not, observed in individuals with T2D.
**Perspectives**

The workloads employed in this study were of low to moderate intensity for all subjects. These are reflective of the metabolic requirements encountered while performing activities of daily living such as walking up stairs or lifting and carrying objects. The findings of this study indicate that while muscle oxygenation may be transiently impaired in individuals with T2D, it does not ultimately affect their ability to sustain work at a given intensity. While these results can only be generalized to the individuals in the present study, and for this mode of exercise, it emphasizes the importance of measuring both oxygen delivery and utilization in studies of exercise tolerance in the T2D population.
CHAPTER 6: SUMMARY AND CONCLUSIONS

While the observation of a reduced leg blood flow response to exercise in T2D has been previously reported (58; 60), the measurements were limited to steady state exercise. Further, these findings were limited to a single work rate. To our knowledge, this is the first study to systematically characterize the dynamic response of LBF to both low and moderate intensity exercise in individuals with T2D. Additionally, the simultaneous measurement of both VO₂ and LBF allowed for differences in both to be compared, and associations, if any, to be made. The main findings of the present study are as follows: 1) The initiatory rise in LBF during LO7.5kg was significantly lower in individuals with T2D compared to healthy controls, a finding attributed to impairments in feedforward control mechanisms of blood flow regulation. In contrast, the magnitude of the total change in LBF was not different between groups, indicating that feedback regulatory systems are preserved. 2) Despite a blunted LBF response at the onset of low intensity exercise, oxygen delivery to the skeletal muscle was not compromised to the point that oxygen uptake was reduced. It is possible that adjustments in the metabolic state within the cell compensated for any reductions in muscle oxygenation that may have occurred. Therefore, in the present study, oxygen delivery in the form of LBF does not diminish the ability of individuals with T2D to perform exercise at the given work intensities.
REFERENCE LIST


120. **Shoemaker JK, Naylor HL, Pozeg ZI and Hughson RL.** Failure of prostaglandins to modulate the time course of blood flow during dynamic forearm exercise in humans. *J Appl Physiol* 81: 1516-1521, 1996.


APPENDIX A: Subject Recruitment Materials
Examples of advertisements placed in local community newsletters and newspapers.

**Research Opportunity for MEN**

The Human Vascular Control Laboratory at Queen’s is looking for males to participate in studies investigating the functioning of muscle blood vessels during exercise.

**WHO?**

30-55 year old non-smoking males who: 1) are HEALTHY, or 2) have TYPE 2 DIABETES.

**WHAT/WHERE/WHEN?**

Three 1.5-2 hour visits per study are required. Compensation is provided and times are flexible. We are located in the Physical Education Centre at Queen’s University.

**WHY?**

Your participation will help us to understand why people with Type II Diabetes have a hard time performing the physical activity that is so essential for their health.

*For more information, please contact Veronica or Melissa at (613) 533-6000, ext. 78425 or vascular.lab@queensu.ca.*

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**CLASSIFIED ADS**

**Nurses wanted for study**

Looking for nurses aged 28-60 who do not perform repetitive movements at the wrist or hand to participate in a study at Queen’s University on the prevention of carpal tunnel syndrome. Non-invasive procedure, takes approximately two hours. Details: Rob Trachter at 613-533-6000 ext. 77850 or robert.trachter@queensu.ca.

**Free barn cats**

Owner moving to city, needs to relocate mother cat and three older kittens, one female tabby and set of twins (male & female, black & white). Cats are very well socialized with humans and animals alike. Excellent addition to any facility requiring feline assistance. Details: Tanya Henry at 613-544-3400 ext. 2005 or tanya@kadhim.net.

**Research opportunity for men**

The Human Vascular Control Laboratory at Queen’s University is looking for men aged 30-55 years to participate in studies examining blood vessel responses to exercise. Compensation is provided. Details: Melissa or Veronica at vascular.lab@queensu.ca or 613-533-6000 ext. 78425.

**ATV for sale**

1991 Yamaha MOTO4 250 ATV in phenomenal shape. Well maintained, 5-speed, high/low range and reverse, shaft driven, 2-wheel drive, rear cargo carrier with seat, front and rear storage racks, winch and windshield included. Great, all-around ATV. Asking $2,000. Details: Ted at 613-634-9168. Can be viewed on Kijiji.

**Motorcycle for sale**

2004 Kawasaki KLR650 motorcycle, great bike in phenomenal shape. Well maintained, 5-speed, chain driven, 46,000 kms. (mostly highway), heated grips. Great, all-around bike for the trails and the open road. Asking $4,000. Details: Ted at 613-634-9168. Can be viewed on Kijiji.

**SUBMISSION GUIDELINES**

- Ads must be received in writing
- Ads must be no longer than about 50 words
- Ads are subject to editing
- Ads promoting for-profit ventures are not accepted
- Ads benefiting non-profit organizations are welcome
- Publication is subject to time and space requirements

*E-mail: spectrum@kijiji.net Fax: 613-548-1354*  
*in person: Public Affairs, Nickie 2*  
*Deadline for April 15 issue is April 6*
Example of poster advertisements displayed on bulletin boards at Queen’s University, private medical clinics, and local diabetes education centres.

MALE PARTICIPANTS NEEDED!

WHO?

30-65 year old non-smoking males with TYPE 2 DIABETES

WHAT/WHEN/WHERE?

Three 1.5-2 hour visits per study are required. Compensation is provided and times are flexible. We are located in the Physical Education Centre at Queen’s.

WHY?

Your participation will help us to understand why people with Type 2 Diabetes have a hard time performing physical activity, and to find interventions that will help make exercise easier.

Please contact Veronica or Melissa at vascular.lab@queensu.ca OR (613) 533-6000 ext. 78425 if you have any questions or would like to participate!

STUDY PARTICIPANTS NEEDED!

Are you a healthy, non-smoking, inactive MALE, between the ages of 30-55? OR, Are you an inactive MALE with Type II Diabetes?

If so, a few hours of your time could contribute to important research relating to exercise in health and disease!

*Compensation for your time is Provided*

Please contact Veronica or Melissa at vascular.lab@queensu.ca OR (613) 533-6000 ext. 78425 if you have any questions or would like to participate!
Sample letter of information to health care practitioners.

Human Vascular Control Laboratory
School of Kinesiology and Health Studies
69 Union Street
Kingston, ON K7L 3N6

Dear Health Care Practitioner,

Thank you for allowing us to use your office as a site of recruitment for our research studies. We are currently looking for males, both with and without Type 2 Diabetes, to participate in studies examining the functioning of muscle blood vessels during exercise. We have included a recruitment poster, as well as a few brochures, to provide you and your clients with additional information.

Should you have any questions, please feel free to contact us at vascular.lab@queensu.ca, or (613) 533-6000, ext. 78425.

Once again, thank you for your assistance with our project.

Sincerely,

Michael E. Tschakovsky, PhD
Principle Investigator

Melissa Pak  Veronica Poitras
M.Sc. Candidates, School of Kinesiology and Health Studies
Detailed information brochure for interested participants.

DO I NEED TO DO ANYTHING BEFORE I COME IN TO THE LAB?

Participants Cannot:
- Consume food within 3 hours of the study.
- Exercise or consume alcohol within 12 hours of the study.
- Take aspirin or other anti-inflammatories within 48 hours of the study.

Also, since this study involves caffeine, it is very important that participants do not consume caffeine within 48 hours of the study. The following is a list of common items that contain caffeine:
- Coffee
- Tea
- Chocolate
- Energy drinks (e.g. Red Bull)
- Soft drinks (e.g. Coke)
- Cocoa
- Some over-the-counter drugs (e.g. Excedrin)

"If you are unsure whether an item contains caffeine, please err on the side of caution and do not eat/drink it."

First Visit to the Research Facility:
- The above instructions do not apply for your first visit to the lab.
- Arrive at the lab having fasted for 12 hours (no food or beverages other than water).
- Breakfast will be provided at the screening visit.

RESEARCH PURPOSE

- Being physically active is an essential part of treating Type II Diabetes.
- When we are physically active, muscles need lots of oxygen.
- The flow of blood to muscles delivers this oxygen.
- Recent research suggests that this flow of blood may be reduced in persons with Type II Diabetes.
- This may be a major reason why physical activity feels so much harder for people with this disease.

WE WANT TO KNOW:
1. Is the flow of blood to muscle reduced in persons with Type II Diabetes?
2. If so, is this because of problems with muscle blood vessels?
3. Does caffeine help improve the flow of blood to muscle?

BENEFITS OF THIS RESEARCH:
- Identify what is wrong with the flow of blood to muscle in Type II Diabetes and how to correct it.
- Make physical activity easier so that people will "stick with it" and enjoy the benefits to their health and well-being.

WHO CAN PARTICIPATE?
- 30-65 yr old MALES with or without Type II Diabetes.

Directions to the Human Vascular Control Lab
- Located at 69 Union St, Room 303A of the Physical Education Centre (PEC) at Queen’s University.
- On the north side of Union St., between Division St. and University Ave.
- Union St. is accessible from both Sir John A MacDonald Blvd. to the west and Division St. to the east, both of which are accessible from the 401.
- Parking is located 500m away in the Queen’s underground parking lot on Stuart St.

STUDY PARTICIPANTS WANTED!

WHAT WILL I DO IF I PARTICIPATE?

ARM STUDY
You will:
- Do hand gripping exercise using your forearm muscles until you are too tired to continue (usually ~15 minutes) on two separate days.
- Consume either a caffeine pill or a sugar pill on those days.

We will:
- Measure blood pressure, heart rate and the flow of blood to your forearm muscles.
- Take some blood samples from a vein at your elbow to measure how much oxygen your forearm muscles are using during exercise.

WHAT IS THE TIME COMMITMENT?
The study requires three 1.5-2 hour visits to the lab over a 2-3 week period (including a screening visit).

LEG STUDY
You will do:
- Increasing intensities of leg kicking exercise (bending and straightening your leg) until you can no longer continue (~15 minutes) on one day.
- Two repeats of a moderately difficult intensity of leg kicking for 5 minutes on another day.

We will measure:
- Blood pressure and heart rate, and the flow of blood to your leg muscles.
- How much oxygen you use by measuring your breathing through a mouthpiece and air tube.

WHAT IS THE TIME COMMITMENT?
The study requires three 1.5-2 hour visits to the lab over a 3 week period (including a screening visit).

ANYTHING ELSE?
You will be compensated a minimum of $75 for your time upon completion of the study. Parking is available at the Queen’s underground parking lot, located on Stuart Street (see map on back). We will cover your parking expenses.

If you have any questions or would like to book an appointment please contact Veronica Poitras or Melissa Pak at vascular.lab@queensu.ca or (613) 533-6000 ext. 78425.
Recommendations for future subject recruitment

• Attend community meetings/symposiums held by local diabetes education centres.
• Post information on internet forums and public websites.
• Large sized advertisements in local newspapers.
• No cost to publish in KGH Spectrum (hospital newsletter, proved to be quite effective in attracting interest).
• Local newspapers: Wednesday and Saturday classifieds receive most readership.
• Place poster advertisements with removable information tabs EVERYWHERE.
• The less information included in the ad, the greater the likelihood a potential participant will call and ask for more details. Face to face contact improves chances of recruitment.
• Medical clinics and hospitals are good areas to post information.
• Use of the words “inactive”, “sedentary” etc. may deter individuals from participating as these terms are perceived negatively.
• Only publish general inclusion criteria (ie. age, sex requirements) so individuals will call to obtain more information before excluding themselves based on criteria that may be too strict (ie. medications, physical activity levels)
APPENDIX B: Subject Information Forms
Subject Inclusion/Exclusion Criteria

- Male
- Ages 30-65
- Non-smokers
- Absence of overt cardiovascular disease (hypertension excepted)
- Absence of other serious disease
- Absence of condition for which exercise is contraindicated (e.g. severe arthritis)

Drugs that *exclude* participation:
- Insulin
- Insulin secretagogues
- Verapamil
- Diltiazem
- Sulphonylureas
- β-blockers

Drugs that are ok to *include*:
- Metformin
- Angiotensin converting enzyme (ACE) inhibitors
- Diuretics
- Calcium channel blockers (except for Verapamil and Diltiazem)
- Angiotensin II receptor blockers
- α-blockers
- Lipid-lowering drugs
- Rosiglitazone, pioglitazone

May need to undergo a stress test if at higher risk for cardiac event
Table B1. Subject Medications

<table>
<thead>
<tr>
<th>Subject Code</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN01-6</td>
<td>None</td>
</tr>
<tr>
<td>DM01</td>
<td>Crestor, Ciprofloxacin (in preparation for transurethral resection of the prostate)</td>
</tr>
<tr>
<td>DM02</td>
<td>Aspirin</td>
</tr>
<tr>
<td>DM03</td>
<td>Lipitor, Metformin, Novo-hydrazide, Ramipril</td>
</tr>
<tr>
<td>DM04</td>
<td>Aspirin, Cilazapril, Cyanocobalamin, Lipitor, Metformin</td>
</tr>
</tbody>
</table>
Pre-Screening Questions and Information

Name: _____________________ Phone #: ___________________ Date: __________________
E-mail: ___________________

Thank you for your interest in the study. Do you have one of the information brochures about the study?
YES → Excellent, so you know the gist of what is involved then.
NO → Ok, well let me tell you a bit about the study then… Does this sound like something you’d be interested in participating in? Do you have an e-mail account where I could send you a copy of our information brochure?

Next we need to ask you a few questions to determine whether you meet the criteria for the studies. All of your answers will be kept confidential.

How old are you? (30-55 years)
Do you have Type II diabetes?
Are you currently taking any medications?

Have you performed fewer than three 20-minute sessions per week of moderate-intensity physical activity over the past 3 months? (Moderate is similar to how you feel when walking at a normal pace).

Are you comfortable with giving blood samples?

Are you a smoker?

Do you currently experience back pain, or have you experienced back pain in the past?

On average, do you consume more than 2 alcoholic beverages per day?

PAR-Q Questions

1. Y / N Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

2. Y / N Do you feel pain in your chest when you do physical activity?

3. Y / N In the past month, have you had chest pain when you were not doing physical activity?

4. Y / N Do you lose your balance because of dizziness or do you ever lose consciousness?

5. Y / N Do you have a bone or joint problem (e.g. back, knee or hip) that could be made worse by a change in your physical activity?

6. Y / N Is your doctor currently prescribing drugs (e.g. water pills) for your blood pressure or heart condition?

7. Y / N Do you know of any other reason you should not do physical activity?
Thanks for answering our questions. The next step is for you to come in to the lab for a screening visit. The purpose of the screening visit is to see if we can get clear measurements of the variables we need to measure during the exercise trials, to fill out a few more information forms, and to do a complete run-through of the exercise protocol so that you are comfortable with it for the days where we will actually be collecting data. Do you have any questions so far?

The screening visit takes approximately 1.5-2 hours. For this visit…

1. It is important that you arrive at the lab without having consumed food or beverages other than water since the night before, since we’ll be taking a measurement of your fasting blood sugar levels. We’ll be doing this using a home glucose meter kit, which you are likely familiar with. It involves pricking the finger and then touching the drop of blood to a test strip.
2. After we have taken this blood sample, we will provide you with a modest breakfast. You are welcome to bring a snack of your own if you would prefer.
3. (V only) Also, since this study involves caffeine, it is very important that you don’t consume caffeine within 48 hours of the study. If you’re unsure whether an item contains caffeine, please err on the side of caution and do not eat/drink it.
4. (M only) Please bring a pair of loose fitting shorts

When would be a good time for you to come in for the screening?

Scheduled Date and Time: ______________________________________________________________

Do you need Directions to the Human Vascular Control Lab?
We are located at 69 Union St., in room 303A of the Physical Education Centre (PEC) at Queen’s University. The PEC is located on the north side of Union St., between Division St. and University Ave. Union St. is accessible from both Sir John A MacDonald Blvd to the west and Division St. to the east, both of which are accessible from the 401. Parking is located 500m away in the Queen’s underground parking lot on Stuart St.

Do you have any questions?
If you think of any questions or have any concerns at a later time, or if you need to change your appointment for any reason, please contact us here at the lab. The # is 533-6000 x78425. Alternatively, you can e-mail us at vascular.lab@queensu.ca.
CONSENT FORM
FOR RESEARCH PROJECT ENTITLED:
Dysfunction of Exercising Muscle Oxygen Delivery and Utilization
in Type II Diabetes

BACKGROUND INFORMATION:
You are being invited to participate in a research study directed by Dr. Michael E. Tschakovsky, PhD and Co-Investigators Dr. Robert Hudson, MD, PhD, FRCPC and Dr. Katherine Kovacs, MD, MSc, FRCPC designed to improve our understanding of potential problems with the delivery and use of oxygen in exercising muscle in persons with Type II Diabetes. Designated research personnel in the Human Vascular Control Laboratory of Dr. Tschakovsky will read through this consent form with you and describe procedures in detail and answer any questions you may have. This study is being sponsored by the Natural Sciences and Engineering Research Council of Canada. This study has been reviewed for ethical compliance by the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.
DETAILS OF THE STUDY:

There are three (3) different study protocols under this consent form. You can indicate which of these you are consenting to participate in at the end of the consent form.

**Purpose:** The purpose is to answer the following specific questions:

Study 1 - Does blood flow to muscle increase more slowly and to a lower level when exercise begins in persons with Type II Diabetes?

Study 2 - Does caffeine improve this response in persons with Type II Diabetes, and does this improve tolerance to exercise?

Study 3 - Do sympathetic nerves cause a greater narrowing of exercising muscle blood vessels in persons with Type II Diabetes?

You will be considered as a healthy control subject for a study if you are currently free of any cardiovascular, liver or kidney conditions listed on the accompanying medical screening form.

You will be considered as a person with Type II Diabetes subject for a study if your medical history relevant to the current study is deemed acceptable for participation by the study Co-Investigators responsible for patient care, Dr. Robert Hudson, Chair, Division of Endocrinology and Metabolism, Department of Medicine, and Dr. Katherine Kovacs, General Internal Medicine. *This requires that you consent to your family physician providing this information to Dr. Hudson and Dr. Kovacs.*

We must also confirm for all subjects that we will be able to properly measure blood vessels and blood flow in your forearm and/or legs.

**Description of Study(s):**
What will happen?

For all studies, an initial visit (Visit 1) will occur to obtain informed consent, conduct the screening test to determine if we can measure your blood vessels and blood flow, and for you to become familiar with the exercise that you would be performing in the study.

Depending on which study you consent to participate in, there will be 1-2 subsequent visits to the laboratory that can last between 1.5 to ~3 hours each.

Study 1:
Visit 2 - You will be asked to perform leg exercise, where the exercise intensity progressively increases until you are unable to continue. We will measure your heart rate, blood pressure, blood flow to your leg, blood vessel size, and the amount of oxygen your exercising muscles use.

Visit 3 - You will be asked to perform leg exercise again, but this time the exercise intensity will immediately be at a level that is of moderate intensity, and you will maintain that exercise for 5 minutes. This will be repeated 3 times, with 20 minutes of rest between exercise sessions. The same measurements as in Visit 2 will be performed.

Study 2:
Visit 2 and 3 - You will be asked to perform handgrip squeezing exercise using your forearm muscles until you are too tired to continue (no more than 20 minutes), on two separate days. You will consume either a caffeine pill or a sugar pill on those days. We will measure your heart rate, blood pressure, blood flow to your forearm, blood vessel size, and the amount of oxygen consumed and lactate produced by your exercising forearm.

Study 3:
Visit 2 - You will be asked to perform handgrip squeezing exercise, where the exercise intensity is immediately at either 20% (mild exercise) or 40% (moderate to heavy exercise) of your maximum grip strength for 10 minutes. You will perform one of each. Additionally, you will perform another bout of forearm exercise at each intensity, but this time at 5 minutes of forearm exercise, a cuff around your right leg just above the knee will be inflated to temporarily stop blood flow to your leg, and you will add calf muscle contractions to the remaining 5 minutes of forearm exercise. There are therefore a total of 4 exercise bouts, with 20 minutes of rest between each of them. We will measure
your heart rate, blood pressure, blood flow to your forearm, blood vessel size, the amount of oxygen consumed and lactate produced by your exercising forearm, and the amount of adrenalin and noradrenalin in your blood.

The following list describes details of the techniques we use to make our measurements and the nature of the exercise that you will be performing. They also provide an indication of any risks associated with each specific technique. Based on which of the above studies you are willing to participate in, the check box beside the appropriate techniques and exercises will be marked. Read only those, and initial at the checked box.

- **HEART RATE MEASUREMENTS:** Heart rate is continuously monitored by an electrocardiogram (EKG) through 3 spot electrodes on the skin surface. The electrodes are normally placed in the lower portion of the chest and they can detect the electrical activity that makes your heart beat.
  **RISKS:** *This procedure is non-invasive. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.*

- **BLOOD PRESSURE MEASUREMENTS:** A small cuff is fit around your finger. This cuff inflates to pressures that match the blood pressure in your finger, so you feel the cuff pulsing with your heart beat. It shines infrared light through your finger to measure changes in the size of your finger with each heart beat.
  **RISKS:** *This technique is non-invasive and there are no known/associated risks.*

- **LIMB BLOOD FLOW AND BLOOD VESSEL DIAMETER MEASUREMENTS:** The blood flowing through your brachial (above the elbow) or femoral (above the groin) artery can be detected and your artery diameter measured using Doppler and imaging ultrasound. A probe will be placed on the skin over your artery and adjustments in its position controlled by hand by the investigator. Measurement of femoral artery flow takes place on the lower abdomen just above the groin. Shorts will be tied up at the site of measurement to expose the skin in this region. High frequency sound (ultrasound) will penetrate your skin. The returning sound provides information on blood vessel size and blood flow.
  **RISKS:** *This technique is non-invasive and poses no risk.*
**BLOOD OXYGEN CONTENT:** A plastic clip is placed over your index finger. This clip aims light through your finger, and the absorption of that light by the blood provides information on how much oxygen the blood contains.

*RISKS: This technique is non-invasive and there are no known/associated risks.*

**GAS EXCHANGE:** This measures your breathing and the changes in oxygen and carbon dioxide as a result of your body utilizing oxygen and producing carbon dioxide. It involves breathing through a mouthpiece attached to a one way valve system, and wearing nose clips.

*RISKS: This procedure is entirely safe. There are no known/associated risks.*

**VENOUS BLOOD SAMPLING:** Blood samples from veins are used to measure the amount of lactic acid, oxygen, adrenalin and noradrenalin in your blood. We need to take a blood sample from a vein on the back of your hand, after we have increased blood flow to that hand by having you hold it in tolerably hot water until blood flow is maximized. For this, a researcher trained and certified in venipuncture (needle or catheter placement into a vein) will use sterile technique to draw a blood sample of ~1-3 ml into a syringe or a vacutainer tube. We also need to take multiple 1-3 ml samples of blood from a vein at the elbow, at various times during forearm exercise (no samples are taken during leg exercise). In this instance, the researcher will place a teflon catheter into your vein using sterile technique. The catheter will be secured to your skin with tape and a self-sealing access attached to allow for drawing blood from the vein. We will take a volume of blood much less than the volume of blood taken when you donate blood (370-400 ml). For Study 1 - 16 ml, Study 2 - 92 ml, Study3 - 144 ml. Periodically, the researcher may, after drawing some blood, inject (flush) sterile saline through the catheter into your vein. When the study is over, we will remove the catheter and secure sterile gauze over the puncture site.

*RISKS: The most common complications of inserting a small catheter in the arm is a small bruise and pain at the site of catheter insertion. This might last several days after removal of the catheter. When the catheter is removed pressure must be applied to the vein to prevent internal bleeding. If adequate pressure is not applied a bruise and some discomfort might result for a short period of time. The puncture site should be kept clean and covered with a sterile gauze pad while stopping the bleeding after catheter removal to prevent infection. There is very little risk of infection or injury to the vein. The amount of blood taken can result in at most a 2% reduction in the hemoglobin content in your blood (hemoglobin carries oxygen in your blood), in comparison to ~7.5% reductions experienced when you donate blood. Nevertheless, this 2% does constitute a very mild
anemia, and in the case of a person with chronic hemoglobin disorders it could increase the risk of adverse health consequences.

- **BLOOD GLUCOSE MEASUREMENT:** Blood samples from capillaries are used to measure the amount of glucose in your blood. We need to take a blood sample from the capillaries in your finger. To do this, a researcher will prick your fingertip using a lancing device (a sterile, single-use instrument), and draw a drop of blood onto a test strip in a glucose monitoring system. The puncture site will be smaller than the size of a small paper cut. 
  
  **RISKS:** You may experience some minor discomfort when your finger is pricked with the lancing device. There is a very small risk of infection at the puncture site.

- **FOREARM VOLUME:** The volume of your forearm will be measured by having you lower it into a tube of water, displacing the volume of water that equals your forearm volume into a measuring cylinder. 
  
  **RISKS:** There are no known/associated risks with this technique.

- **WAIST AND HIP CIRCUMFERENCE:** A tape measure will be used to measure the circumference of your waist and hip. 
  
  **RISKS:** There are no known/associated risks with this technique.

- **HANDGRIP EXERCISE:** You will be asked to perform handgrip squeezing exercise at a moderate or a heavy intensity for 8-12 minutes depending on the Study. Repeats of exercise will be separated by ~20 minutes rest. For the heavy intensity exercise in Study 2 you will be encouraged to continue exercising until you can no longer maintain the exercise, even though the last minute may be uncomfortable. You have the right to declare that you are unable to continue without any consequence to you. 
  
  **RISKS:** When forearm muscle contractions continue at a heavy intensity, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no known/associated risk to your muscles in performing this exercise. You may experience muscle soreness in the muscles of your forearm for 24-72 hours after performing the handgrip exercise, much as you would if you had been lifting weights.

- **LEG EXERCISE:** You will be asked to contract your leg muscles, either
continuously or intermittently. The duration of this exercise can vary from a few minutes to 10-20 minutes, and at an intensity that can range from very mild to maximal contraction force.

RISKS: When leg muscle contractions continue at a heavy intensity, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no known/associated risk to your muscles in performing this exercise. You may experience muscle soreness in the muscles of your leg for 24-72 hours after performing the leg exercise, much as you would if you had been lifting weights.

TALKING AND MOVEMENTS:

Talking or moving during the times that we are taking measurements will cause variations in the measurements we are making. If you have any discomfort, please let us know prior to the period where we begin taking measurements so that any adjustments can be made to restore comfort. However, if everything is comfortable, please maintain a very quiet posture. Even very slight movements interfere with our experiments.

RISK OF CARDIAC EVENT:

Participation in exercise has an inherent risk of a serious cardiac event during or shortly after strenuous exertion, regardless of health status. This risk is present in the case of the leg exercise study. However, this risk is very small for healthy persons or persons that have passed an exercise stress test with no complications.

SPECIAL INSTRUCTIONS:

You are asked to not drink alcohol during the 12 hrs or caffeine during the 48 hrs prior to the study. Also, we require that you are overnight fasted, to assess fasting blood glucose. You should empty your bladder immediately prior to starting the test. When the study is finished, we will have you sit in the laboratory for a period of time (possibly up to an hour) to allow you to readjust to the upright posture and to ensure that there are no other complications after exercise. These precautions should be enough to prevent any sensations of dizziness. Please be aware that sensations of dizziness are not normal and you should also let us know if you experience any new pain in your chest, jaw or arms following the exercise.
Following participation in the arm study, we recommend that you abstain from caffeine consumption for an additional 12 hours. The amount of caffeine consumed as part of the study is equivalent to ~2-4 cups of coffee, however consuming additional caffeine could lead to adverse effects (e.g. sensation of racing heart, acute sleep disturbance).

Benefits: There are no immediate benefits to you for participation in this study.

Exclusions: Participation in this study requires that you complete a medical information sheet (control subjects and diabetic subjects) and that you consent to medical history information relevant to this study being provided by your family physician to Co-Investigators in charge of patient care, Dr. Robert Hudson and Dr. Katherine Kovacs. The former asks some simple questions about your health. This information is used to guide us with your entry into the study. Other current health problems may exclude you from this study. This information is stored in your own file in a locked filing cabinet. Additionally, the inability for us to properly measure your blood vessels and blood flow as assessed during the initial screening test will require exclusion from the study.

SAFETY PRECAUTIONS:

Safety precautions for the study will include the following:

All control subjects who enter the study will be healthy men.

All persons with Type II diabetes will have their health history assessed by Dr. Hudson and/or Dr. Kovacs to ensure there are no contra-indications to the exercise required for this study. If deemed necessary by the co-investigators Dr. Hudson and Dr. Kovacs in charge of patient care, an exercise stress test will be carried out at either the Kingston Heart Clinic, Hotel Dieu Hospital or Kingston General Hospital. In these cases, passing this exercise stress test will be required for a subject to participate in the study.

Before entering the study you will be screened using a medical screening form. You will not be able to enter the study if anything is found which indicates that it is dangerous for you to participate.

We will continuously monitor your heart rate and blood pressure, and you will be laying on your back, or be seated. These precautions allow us to quickly identify
if you are becoming faint and simply stopping the experimental manipulation will allow you to quickly recover. Fainting can occur due to your tolerance for having a catheter placed in your vein or the site of blood. Once the experiment is over and you sit up, you may experience dizziness as a consequence of the effect of gravity. You will remain seated for a few minutes and in conversation with research personnel prior to standing up. **You may also be required to stay in the laboratory for ~1 hour to ensure no other post exercise issues arise.**

**CONFIDENTIALITY:**

All information obtained during the course of the study is strictly confidential and will not be released in a form traceable to you, except to you and your personal physician. Additionally, your personal health information, which has been obtained by Dr. Hudson from your family physician in order to assess qualification for participation in the study, will be safeguarded as follows: Your data will be kept in locked files and electronic data in password protected file folders on our analysis computers, which are available only to the investigators and research assistants who will perform statistical analysis of the data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular government functions. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Kinesiology and Health Studies at Queen’s University.

**STUDY COMPENSATION:** You will receive $10 per hour of your time in the laboratory for expenses and imposition on your time incurred by your participation in this study.

**FREEDOM TO WITHDRAW FROM THE STUDY**

Your participation in this study is voluntary. You may refuse to participate or you may discontinue participation at any time during the duration of the study without penalty and without affecting your future medical care.

**PRINCIPAL INVESTIGATOR INITIATED WITHDRAWAL FROM THE STUDY:**

The principal investigator, Dr. Michael Tschakovsky and the graduate student co-investigators, can at any time decide to terminate the experiment and have you withdraw from the study, based on problems with adequate quality of data, or signs of unusual risk to you.

**LIABILITY:**
In the event that you are injured as a result of taking part in this study, medical care will be provided to you until resolution of the medical problem. By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

**Subject Statement and Signature Section**

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I will contact:

- **Michael E. Tschakovsky, Ph.D.**
  (Principal Investigator)
  Room 303, Physical Education Centre
  Queen’s University, Kingston, ON, K7L 3N6
  Tel: (613) 533-6000, ext, 74697
  Cell: 613-328-9632
  E-mail: mt29@queensu.ca

- **Veronica Poitras or Melissa Pak or Terry Hong, MSc Candidates**
  (Co-investigators)
  Room 303, Physical Education Centre
  Queen’s University, Kingston, ON, K7L 3N6
  Tel: (613) 533-6000, ext 78425

- **Patrick Costigan, Ph.D.**
  (Acting Department Head)
  Room 225, Physical Education Centre
  Queen’s University, Kingston, ON, K7L 3N6
  Tel: (613) 533-6288
  E-mail: pat.costigan@queensu.ca

If I have any questions concerning research subject’s rights, I will contact:

- **Dr. Albert F. Clark, Chair, Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board**
  Office of Research Services, Fleming Hall, Jemmett Wing 301
  Queen’s University, Kingston, ON, K7L 3N6, Tel: 613-533-6081
By signing this consent form, I am indicating that I agree to participate in the study(s) checked below.

- [ ] Study 1
- [ ] Study 2
- [ ] Study 3

______________________   _________________________
Subject Signature     Signature of Witness

______________________   _________________________
Subject Name (please print)   Name of Witness (please print)

______________________   _________________________
Date (day/month/year)    Date (day/month/year)

STATEMENT OF INVESTIGATOR:

I, or one of my graduate student co-investigators, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

______________________
Date (day/month/year)
MEDICAL QUESTIONNAIRE FOR RESEARCH STUDY

Investigation of the Vascular Response to Acute Exercise in Men with and without Type II Diabetes.

Principal Investigator:
Michael E. Tschakovsky, PhD, School of Kinesiology and Health Studies

Co-Investigators:
Robert W. Hudson, M.D., PhD, FRCPC, Chair, Division of Endocrinology and Metabolism
Katherine A. Kovacs, M.D., M.Sc., FRCPC, General Internal Medicine
Melissa Pak, M.Sc. Candidate
Veronica J. Poitras, M.Sc. Candidate

To the study participant: Please answer all questions in sections 1 and 2 of this form.

To the physician: Please fill out section 3 of this form (pages 3-4). Completing this form may not require a medical re-evaluation of your patient. If the results of recent tests are readily available that might prove useful to study personnel while dealing with the participant, please include that information in this questionnaire.

PLEASE FAX COMPLETED QUESTIONNAIRE TO:
Robert W. Hudson, M.D., PhD, FRCPC, Chair, Division of Endocrinology and Metabolism
Fax Number: 613-533-6574

Please note that we will cover all costs for completing this questionnaire.
Please direct all invoices to:
Michael E. Tschakovsky, PhD
School of Kinesiology and Health Studies
Queen’s University
Kingston, ON K7L 3N6.
**SECTION 1: PERSONAL DATA (please print)**

Name: ____________________________________________

Date of Birth: ______________________________________

Family Physician Name: ______________________________

Family Physician Phone Number: _______________________

**SECTION 2: MEDICAL HISTORY**

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Has your doctor ever said you have heart trouble?</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>B. Do you get pains, pressure or tightness in your chest?</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>C. Do you often feel faint or experience dizziness?</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>D. Has your doctor ever told you that you have high blood pressure?</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>E. Do you know if you have any complications from your diabetes?</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>F. Is there a good reason, not mentioned above, why you should avoid exercise?</td>
<td>___</td>
<td>___</td>
</tr>
</tbody>
</table>

G. Do you have, or have you ever had, problems with any of the following?

<table>
<thead>
<tr>
<th>Problem</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Heart or blood vessels</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>ii. Nerves or brain</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>iii. Breathing or lungs</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>iv. Hormones, thyroid, or diabetes</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>v. Muscles, joints, or bones</td>
<td>___</td>
<td>___</td>
</tr>
<tr>
<td>vi. Other (please list)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
H. Please list any serious injuries suffered, or surgeries you have had. If you have had surgery, was any metal (e.g., pins or screws) left in your body?

______________________________________________________________________________
______________________________________________________________________________

I. Have you ever undergone an exercise stress test?

______________________________________________________________________________
______________________________________________________________________________

J. Are you presently taking any medications? If yes, please list.

______________________________________________________________________________
______________________________________________________________________________

K. Are you presently under the care of any other health care professional (i.e., Physiotherapist etc.)? If yes, please list.

______________________________________________________________________________
______________________________________________________________________________

L. Do you have any allergies to medications, adhesive tape, latex, etc.?

______________________________________________________________________________
______________________________________________________________________________

I acknowledge that the study investigators completed this form according to my specifications; this information is true to the best of my knowledge.

______________________________  ______________________________
Participant Name     Participant Signature

Date (dd/mm/yyyy) :  ______________
SECTION 3: MEDICAL REFERRAL

Physician: The applicant is considering participation in a research study that is investigating the cardiovascular response to acute upper or lower body exercise. As a participant in this study, your patient would undergo short bouts of exercise during which heart rate, blood pressure, cardiac output and muscle blood flow are measured non-invasively.

Should you have any questions regarding the participation of your patient in this project, please contact Michael E. Tschakovsky, PhD., School of Kinesiology and Health Studies, Queen’s University (613-533-6000, ext 74697).

Name of Patient: _______________________________________________________________

I. Review of Systems - please include diagnoses.

a) Cardiovascular ______________________________________________________

b) Respiratory ______________________________________________________

c) Neurological ______________________________________________________

d) Gastrointestinal ______________________________________________________

e) Genitourinary ______________________________________________________

f) Endocrine ______________________________________________________

g) Musculoskeletal ______________________________________________________

h) Skin ______________________________________________________

II. Additional abnormalities of which you are aware

____________________________________________________________________________

____________________________________________________________________________

____________________________________________________________________________

III. Current medications and doses

____________________________________________________________________________

____________________________________________________________________________
IV. Diabetes History (if applicable)

Duration of disease (years): __________

Fasting plasma glucose (mmol/l): __________  HbA1c (%): __________

Current treatment strategy (diet, exercise, medication etc.):
______________________________________________________________________________
______________________________________________________________________________

Please list known diabetic complications:
______________________________________________________________________________

V. Exercise Stress Test (if applicable)

Date performed (dd/mm/yyyy): __________________________________

Results: _______________________________________________________________________
______________________________________________________________________________

VI. On the basis of your knowledge and medical evaluation of the applicant, you would recommend (mark the appropriate answer):

_____ Participation in an exercise study supervised by a kinesiology graduate, or
_____ Participation in an exercise study is not recommended

Note: An explanation of the study protocol, as well as the absolute and relative contraindications to exercise testing, is provided on page 5 and 6 of this form.

Physician’s Name: ________________________________

Physician’s Signature: ________________________________

Date: ________________________________

Phone Number: ________________________________

Thank you very much for your help. Upon completion, please mail/fax pages 3 and 4 to:

Robert W. Hudson, M.D., PhD, FRCPC, Chair, Division of Endocrinology and Metabolism
Room 1036, Etherington Hall, Queen's University, Kingston, ON K7L 3N6.
Phone: 613-533-2973, Fax: 613-533-6574
American College of Sports Medicine Contraindications to Exercise Testing

**Absolute Contraindications**
- A recent change in the resting ECG suggesting infarction or other acute cardiac events
- Recent complicated myocardial infarction
- Unstable angina
- Uncontrolled ventricular dysrhythmia
- Uncontrolled atrial dysrhythmia that compromises cardiac function
- Third-degree A-V block
- Acute congestive heart failure
- Severe aortic stenosis
- Suspected or know dissecting aneurysm
- Active or suspected myocarditis or pericarditis
- Thrombophlebitis or intracardiac thrombi
- Recent systemic or pulmonary embolus
- Acute infection
- Significant emotion distress (psychosis)

**Relative Contraindications**
- Resting diastolic blood pressure >120 mm Hg or systolic blood pressure >200 mm Hg.
- Moderate valvular heart disease
- Known electrolyte abnormalities (hypokalemia, hypomagnesemia)
- Fixed-rate pacemaker (rarely used)
- Frequent of complex ventricular ectopy
- Ventricular aneurysm
- Cardiomyopathy, including hypertrophic cardiomyopathy
- Uncontrolled metabolic disease (e.g., diabetes, thyrotoxicosis, or myxoedema)
- Chronic infectious disease (e.g., mononucleosis, hepatitis, AIDS)
- Neuromuscular, musculoskeletal, or rheumatoid disorders that are exacerbated by exercise
- Advanced or complicated pregnancy
STUDY DETAILS

Background

The increased prevalence of Type II Diabetes in recent years has lead to an urgent demand for effective treatment and prevention strategies. Physical activity has been identified as an important component of these interventions (1). However, Type II Diabetes is also associated with a decreased exercise capacity (2, 3). Individuals find participation in regular physical activity more difficult and are unable to attain the full benefits of training.

Purpose

During exercise, our bodies need oxygen to produce energy for movement. Our heart and blood vessels help to deliver oxygen from our lungs to the working muscles. Recently, it has been found that this delivery system is impaired in persons with Type II Diabetes. This may be a major reason why exercise feels harder in people with this disease. Therefore, the aims of this research are to answer these questions:

Is the flow of blood to muscle reduced in persons with Type II Diabetes?
If so, is this because of problems with muscle blood vessels?
Does caffeine help improve the flow of blood to muscle?

We are trying to understand the nature of the dysfunction in order to guide the design of strategies that can enable persons with Type II Diabetes to exercise more comfortably.

Subjects

We are looking for 30-65 yr old MALES with or without Type II Diabetes. Certain medications are allowed. These will be assessed if you are interested in participating in the study.

Protocol

Arm Study  This study requires that individuals lie on their back and squeeze a handgrip device every 2 seconds until they can no longer continue. This should take about 15 minutes. Participants will complete this task on two occasions, after having consumed either a caffeine or sugar capsule. During this exercise, we will measure blood pressure, stroke volume, and heart rate, and use ultrasound to examine blood flow through the brachial artery. We will also take some blood samples from a vein at the elbow in order to measure muscle oxygenation.

Leg Study  This study requires individuals to lie in a supine position and perform different intensities of knee extension/flexion “kicking” exercise. During this task, we will measure blood pressure, stroke volume, and heart rate, and use ultrasound to
examine blood flow through the femoral artery. Participants will also be asked to breathe into a mouthpiece, which will measure their oxygen consumption during the exercise.

Note: All measurements (except for the blood samples) are non-invasive.


Dear Melissa Pak and Veronica Poitras,

RE: Review of Medical Questionnaire

I have reviewed the medical information form for _______________________________, and

APPROVE / DO NOT APPROVE

his participation in your exercise studies.

Sincerely,

Robert W. Hudson, MD., PhD, FRCPC, Chair, Division of Endocrinology and Metabolism

Please mail/fax completed form to the Department of Kinesiology and Health Studies at (613) 533 – 2009.
Dear Dr. [Name],

A patient of yours is interested in participating in an exercise study in the Human Vascular Control Laboratory at Queen’s University. The exercise consists of repeatedly bending and straightening the legs at gradually increasing intensities until exhaustion. As this is considered a maximal exercise test, we require our participants to undergo an exercise stress test to ensure our protocols can be performed safely. As our laboratory does not have the resources to perform this procedure, we kindly ask for your assistance in booking [Name] for a stress test.

We request that the results for the stress test be forwarded as soon as possible to:

Robert W. Hudson, M.D., PhD, FRCPC, Chair, Division of Endocrinology and Metabolism

Room 1036, Etherington Hall, Queen's University, Kingston, ON K7L 3N6.
Phone: 613-533-2973, Fax: 613-533-6574

Thank you for your assistance in this matter. Any costs incurred in this process will be reimbursed to you. Please direct all invoices to: Michael Tschakovsky, PhD, School of Kinesiology and Health Studies, Queen’s University, Kingston, ON K7L 3N6.

Should you have any questions, please feel free to contact us at (613) 533-6000, ext. 78425 or vascular.lab@queensu.ca.

Regards,

Melissa Pak
M.Sc. Candidate, School of Kinesiology and Health Studies
Physical Activity Recall

1. Were you employed in the last seven days (paid or volunteer)?  ☐ YES  ☐ NO  

2. How many days of the last seven did you work?   ☐ (round to nearest day)

3. How many total hours did you work in the last seven days?   ☐ ☐ hours

4. What days of the week do you consider to be your weekend or non-work days? For most people, this would be Saturday and Sunday, but it may be different for you.

☐ Sunday  ☐ Monday  ☐ Tuesday  ☐ Wednesday  ☐ Thursday  ☐ Friday  ☐ Saturday

**************************************************
Explain Moderate, Hard, and Very Hard Intensity levels **************************************************

At the end of the interview:

5. Compared to your physical activity over the past three months, was last week’s physical activity more, less or about the same?

☐ More
☐ Less
☐ About the same
<table>
<thead>
<tr>
<th></th>
<th>Yesterday</th>
<th></th>
<th></th>
<th>One Week Ago</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Moderate</th>
<th></th>
<th></th>
<th>Very Hard</th>
<th></th>
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<tr>
<td></td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very Hard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Moderate</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Very Hard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Rounding:**
- 10-22 mins = .25 hrs
- 23-37 mins = .50 hrs
- 38-52 mins = .75 hrs
- 53-1:07 mins = 1.0 hrs
- 1:08-1:22 = 1.25 hrs

ID# _______________  Interviewer Initials: ____________
7-Day Physical Activity Recall (PAR): Interviewer Evaluation Form

Date (dd/mm/yyyy): _________________  Subject Code: _________________  Interviewer: _________________  Interviewer Assessment:

Were there any problems with the 7-day PAR interview?  (circle one)  a) Yes  b) No

Explain:
________________________________________________________________________________________________________________________________________________________________________________________________________________________
________________________________________________________________________________________________________________________________________________________________________________________________________________________

Do you think this was a valid 7-day PAR interview?  a) Yes  b) Maybe  c) No

Please list below any activities reported by the participant that you don’t know how to classify.
________________________________________________________________________________________________________________________________________________________________________________________________________________________

Other Comments/Concerns:
________________________________________________________________________________________________________________________________________________________________________________________________________________________
________________________________________________________________________________________________________________________________________________________________________________________________________________________
Table B2. Scoring of the 7-PAR. Levels of physical activity and their corresponding MET values.

<table>
<thead>
<tr>
<th>Category</th>
<th>MET Range</th>
<th>MET value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Light</td>
<td>1.1 to 2.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Moderate</td>
<td>3.0 to 4.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Hard</td>
<td>5.0 to 6.9</td>
<td>6.0</td>
</tr>
<tr>
<td>Very Hard</td>
<td>&gt;7.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Table B3. Scoring of the 7-PAR. Excel spreadsheet of data from one representative subject.

<table>
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<th>day 2</th>
<th>day 3</th>
<th>day 4</th>
<th>day 5</th>
<th>day 6</th>
<th>day 7</th>
<th>Totals</th>
<th>Mets</th>
</tr>
</thead>
<tbody>
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<td>sleep</td>
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<td>mm</td>
<td>hh</td>
<td>mm</td>
<td>hh</td>
<td>mm</td>
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<td>6</td>
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<td>30</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
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<td>15.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>hard</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>very hard</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
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<td>moderate</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>15.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>30.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
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<tr>
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<tr>
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<td>0.00</td>
<td>0.00</td>
<td>15.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
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<tr>
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<td>light</td>
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<tr>
<td>Summary:</td>
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<tr>
<td>Totals</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EE (kcal/kg/wk)</td>
<td>234.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EE (kcal/kg/d)</td>
<td>33.55</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
APPENDIX C: Individual Subject Data
Sample plots from a representative subject of the exponential curve fitting process used to describe the dynamic response characteristics of LBF. A: Initially, a two-component exponential model was used to fit the LBF data for this subject. The resulting pattern of residuals around $y=0$ is shown in the dotted line (Inset). B: When a three-component exponential model is used to fit the data, the result is a more even distribution of residuals around $y=0$ (Inset).
Individual Subject LBF Responses to LO7.5kg
Individual Subject LBF Responses to VT_{90\%}
Individual Subject HR Responses to LO-7.5kg

CN01

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 55 60 65 70 75 80 85

CN02

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 55 60 65 70 75 80 85 90

CN03

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 50 55 60 65 70 75 80 85 90

CN04

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 40 50 60 70 80 90 100

CN05

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 60 65 70 75 80 85 90 95 100

CN06

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 40 50 60 70 80 90

DM01

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 55 60 65 70 75 80 85 90

DM02

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 70 75 80 85 90 95 100 105 110

DM03

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 65 70 75 80 85 90 95 100 105

DM04

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 75 80 85 90 95 100 105

DM05

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 70 75 80 85 90 95 100 105 110

DM06

Time (sec) -60 -30 0 30 60 90 120 150 180 210 240 270 300
HR (bpm) 65 70 75 80 85 90 95 100 105 110
Individual Subject HR Responses to VT_{90\%}
Individual Subject CO Responses to LO_7.5kg
Individual Subject CO Responses to VT$_{90\%}$
APPENDIX D: Sample Statistical Analyses
C – CON
D – T2D

One Way Analysis of Variance

Data source: LO7.5kg GAINS

Normality Test: Passed (P > 0.050)

Equal Variance Test: Failed (P = 0.029)

<table>
<thead>
<tr>
<th>Group Name</th>
<th>N</th>
<th>Missing</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG0</td>
<td>5</td>
<td>0</td>
<td>959.802</td>
<td>272.626</td>
<td>121.922</td>
</tr>
<tr>
<td>DG0</td>
<td>4</td>
<td>0</td>
<td>616.962</td>
<td>44.172</td>
<td>22.086</td>
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</table>

Source of Variation

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1</td>
<td>261197.606</td>
<td>261197.606</td>
<td>6.031</td>
<td>0.044</td>
</tr>
<tr>
<td>Residual</td>
<td>7</td>
<td>303152.862</td>
<td>43307.552</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>564350.468</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.044).

Power of performed test with alpha = 0.05: 0.485

The power of the performed test (0.485) is below the desired power of 0.800. You should interpret the negative findings cautiously.

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparison for factor:

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG0 vs. DG0</td>
<td>342.840</td>
<td>2.456</td>
<td>0.044</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
One Way Analysis of Variance

Data source: LO7.5kg GAINS

Normality Test: Passed (P > 0.050)

Equal Variance Test: Passed (P = 0.066)

<table>
<thead>
<tr>
<th>Group Name</th>
<th>N</th>
<th>Missing</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG1</td>
<td>5</td>
<td>0</td>
<td>3662.140</td>
<td>560.930</td>
<td>250.856</td>
</tr>
<tr>
<td>DG1</td>
<td>4</td>
<td>0</td>
<td>2128.100</td>
<td>323.250</td>
<td>161.625</td>
</tr>
</tbody>
</table>

Source of Variation

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5229508.270</td>
<td>23.286</td>
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<tr>
<td>Residual</td>
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<td>1572040.692</td>
<td>224577.242</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>6801548.962</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002).

Power of performed test with alpha = 0.050: 0.985

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG1 vs. DG1</td>
<td>1534.040</td>
<td>4.826</td>
<td>0.002</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Condition – CON or T2D
Intensity – LO7.5kg or VT90%
MRT – mean response time

**Two Way Repeated Measures ANOVA (One Factor Repetition)**

Data source: MRT for LBF Kinetic Parameters

General Linear Model

Dependent Variable: MRT

Normality Test (Shapiro-Wilk) Passed (P = 0.715)

Equal Variance Test: Passed (P = 0.960)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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</thead>
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<td>474.561</td>
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<td>Intensity</td>
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<td>20.906</td>
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<tr>
<td>Condition x Intensity</td>
<td>1</td>
<td>53.093</td>
<td>53.093</td>
<td>1.392</td>
<td>0.277</td>
</tr>
<tr>
<td>Residual</td>
<td>7</td>
<td>267.006</td>
<td>38.144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>2827.552</td>
<td>157.086</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The difference in the mean values among the different levels of Condition is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Intensity. There is not a statistically significant difference (P = 0.095).

The difference in the mean values among the different levels of Intensity is greater than would be expected by chance after allowing for effects of differences in Condition. There is a statistically significant difference (P = 0.003). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Condition does not depend on what level of Intensity is present. There is not a statistically significant interaction between Condition and Intensity. (P = 0.277)

Power of performed test with alpha = 0.0500: for Condition : 0.284
Power of performed test with alpha = 0.0500: for Intensity : 0.973
Power of performed test with alpha = 0.0500: for Condition x Intensity : 0.0817

Expected Mean Squares:
Approximate DF Residual for Condition = 8.057

Expected MS(Condition) = var(res) + 1.875 var(Subject(Condition)) + var(Condition)
Expected MS(Subject(Condition)) = var(res) + 1.852 var(Subject(Condition))
Expected MS(Intensity) = var(res) + var(Intensity)
Expected MS(Condition x Intensity) = var(res) + var(Condition x Intensity)
Expected MS(Residual) = var(res)

Least square means for Condition:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>21.234</td>
<td>3.657</td>
</tr>
</tbody>
</table>
Least square means for Intensity:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>LO</td>
<td>19.703</td>
</tr>
<tr>
<td>HI</td>
<td>33.098</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 3.110

Least square means for Condition x Intensity:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C x LO</td>
<td>16.264</td>
<td>4.146</td>
</tr>
<tr>
<td>C x HI</td>
<td>26.203</td>
<td>4.146</td>
</tr>
<tr>
<td>D x LO</td>
<td>23.141</td>
<td>4.636</td>
</tr>
<tr>
<td>D x HI</td>
<td>39.993</td>
<td>4.636</td>
</tr>
</tbody>
</table>

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: **Intensity**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI vs. LO</td>
<td>13.395</td>
<td>4.572</td>
<td>0.003</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Intensity within C**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI vs. LO</td>
<td>9.939</td>
<td>2.544</td>
<td>0.038</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: **Intensity within D**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI vs. LO</td>
<td>16.851</td>
<td>3.859</td>
<td>0.006</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Condition – CON or T2D  
Workload – 7.5, 10 or 13 kg

**Two Way Repeated Measures ANOVA (One Factor Repetition)**

Data source: VO2 in 7.5kg, 10 and 13 kg Workloads

General Linear Model

Dependent Variable: VO2

Normality Test (Shapiro-Wilk) Passed (P = 0.682)

Equal Variance Test: Failed (P < 0.050)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>1</td>
<td>86609.251</td>
<td>86609.251</td>
<td>1.446</td>
<td>0.264</td>
</tr>
<tr>
<td>Subject(Condition)</td>
<td>8</td>
<td>479159.036</td>
<td>59894.880</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workload</td>
<td>2</td>
<td>1790694.809</td>
<td>895347.405</td>
<td>98.731</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Condition x Workload</td>
<td>2</td>
<td>6100.359</td>
<td>3050.180</td>
<td>0.336</td>
<td>0.719</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td>145096.753</td>
<td>9068.547</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>2540673.078</td>
<td>87609.416</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The difference in the mean values among the different levels of Condition is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Workload. There is not a statistically significant difference (P = 0.264).

The difference in the mean values among the different levels of Workload is greater than would be expected by chance after allowing for effects of differences in Condition. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Condition does not depend on what level of Workload is present. There is not a statistically significant interaction between Condition and Workload. (P = 0.719)

Power of performed test with alpha = 0.0500: for Condition : 0.0873  
Power of performed test with alpha = 0.0500: for Workload : 1.000  
Power of performed test with alpha = 0.0500: for Condition x Workload : 0.0500

Expected Mean Squares:
Approximate DF Residual for Condition = 8.000

Expected MS(Condition) = var(res) + 3.000 var(Subject(Condition)) + var(Condition)  
Expected MS(Subject(Condition)) = var(res) + 3.000 var(Subject(Condition))  
Expected MS(Workload) = var(res) + var(Workload)  
Expected MS(Condition x Workload) = var(res) + var(Condition x Workload)  
Expected MS(Residual) = var(res)

Least square means for Condition:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>693.215</td>
<td>57.684</td>
</tr>
<tr>
<td>D</td>
<td>802.892</td>
<td>70.649</td>
</tr>
</tbody>
</table>
Least square means for Workload:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>432.411</td>
</tr>
<tr>
<td>10</td>
<td>769.703</td>
</tr>
<tr>
<td>13</td>
<td>1042.047</td>
</tr>
</tbody>
</table>

Std Err of LS Mean = 52.052

Least square means for Condition x Workload:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C x 7.5</td>
<td>392.743</td>
<td>65.842</td>
</tr>
<tr>
<td>C x 10</td>
<td>719.326</td>
<td>65.842</td>
</tr>
<tr>
<td>C x 13</td>
<td>967.576</td>
<td>65.842</td>
</tr>
<tr>
<td>D x 7.5</td>
<td>472.080</td>
<td>80.639</td>
</tr>
<tr>
<td>D x 10</td>
<td>820.080</td>
<td>80.639</td>
</tr>
<tr>
<td>D x 13</td>
<td>1116.517</td>
<td>80.639</td>
</tr>
</tbody>
</table>

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: Workload

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 vs. 7.5</td>
<td>609.635</td>
<td>14.026</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>10 vs. 7.5</td>
<td>337.292</td>
<td>7.760</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>13 vs. 10</td>
<td>272.344</td>
<td>6.266</td>
<td>&lt;0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: Workload within C

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 vs. 7.5</td>
<td>574.833</td>
<td>10.455</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>10 vs. 7.5</td>
<td>326.583</td>
<td>5.940</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>13 vs. 10</td>
<td>248.250</td>
<td>4.515</td>
<td>&lt;0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comparisons for factor: Workload within D

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 vs. 7.5</td>
<td>644.437</td>
<td>9.570</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>10 vs. 7.5</td>
<td>348.000</td>
<td>5.168</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>Yes</td>
</tr>
<tr>
<td>13 vs. 10</td>
<td>296.437</td>
<td>4.402</td>
<td>&lt;0.001</td>
<td>0.050</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Two Way Repeated Measures ANOVA (One Factor Repetition) Wednesday, August 05, 2009, 11:34:12 AM

Data source: 7.5kg, 10 and 13 kg Workloads

General Linear Model

Dependent Variable: LBF

Normality Test (Shapiro-Wilk)  Passed  (P = 0.739)

Equal Variance Test:  Passed  (P = 0.486)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>1</td>
<td>10008565.326</td>
<td>10008565.326</td>
<td>3.634</td>
<td>0.098</td>
</tr>
<tr>
<td>Subject(Condition)</td>
<td>7</td>
<td>19277794.842</td>
<td>2753970.692</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workload</td>
<td>2</td>
<td>6111132.915</td>
<td>305566.458</td>
<td>8.225</td>
<td>0.004</td>
</tr>
<tr>
<td>Condition x Workload</td>
<td>2</td>
<td>1010226.909</td>
<td>505113.455</td>
<td>1.360</td>
<td>0.289</td>
</tr>
<tr>
<td>Residual</td>
<td>14</td>
<td>5201063.002</td>
<td>371504.500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>42256444.554</td>
<td>1625247.867</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The difference in the mean values among the different levels of Condition is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Workload. There is not a statistically significant difference (P = 0.098).

The difference in the mean values among the different levels of Workload is greater than would be expected by chance after allowing for effects of differences in Condition. There is a statistically significant difference (P = 0.004). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Condition does not depend on what level of Workload is present. There is not a statistically significant interaction between Condition and Workload. (P = 0.289)

Power of performed test with alpha = 0.0500:  for Condition : 0.281
Power of performed test with alpha = 0.0500:  for Workload : 0.879
Power of performed test with alpha = 0.0500:  for Condition x Workload : 0.0943

Expected Mean Squares:
Approximate DF Residual for Condition = 7.000

Expected MS(Condition) = var(res) + 3.000 var(Subject(Condition)) + var(Condition)
Expected MS(Subject(Condition)) = var(res) + 3.000 var(Subject(Condition))
Expected MS(Workload) = var(res) + var(Workload)
Expected MS(Condition x Workload) = var(res) + var(Condition x Workload)
Expected MS(Residual) = var(res)

Least square means for Condition:
<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5260.589</td>
<td>428.483</td>
</tr>
<tr>
<td>D</td>
<td>4035.320</td>
<td>479.059</td>
</tr>
</tbody>
</table>

Least square means for Workload:
<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
</tr>
</thead>
</table>

188
7.5  4103.320
10  4572.017
13  5268.526

Std Err of LS Mean = 362.128

Least square means for Condition x Workload:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C x 7.5</td>
<td>4488.430</td>
<td>482.837</td>
</tr>
<tr>
<td>C x 10</td>
<td>5164.251</td>
<td>482.837</td>
</tr>
<tr>
<td>C x 13</td>
<td>6129.086</td>
<td>482.837</td>
</tr>
<tr>
<td>D x 7.5</td>
<td>3718.210</td>
<td>539.829</td>
</tr>
<tr>
<td>D x 10</td>
<td>3979.783</td>
<td>539.829</td>
</tr>
<tr>
<td>D x 13</td>
<td>4407.966</td>
<td>539.829</td>
</tr>
</tbody>
</table>

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor: Workload

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 vs. 7.5</td>
<td>1165.206</td>
<td>4.030</td>
<td>0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>13 vs. 10</td>
<td>696.509</td>
<td>2.409</td>
<td>0.030</td>
<td>0.025</td>
<td>No</td>
</tr>
<tr>
<td>10 vs. 7.5</td>
<td>468.697</td>
<td>1.621</td>
<td>0.127</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: Workload within C

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 vs. 7.5</td>
<td>1640.656</td>
<td>4.256</td>
<td>&lt;0.001</td>
<td>0.017</td>
<td>Yes</td>
</tr>
<tr>
<td>13 vs. 10</td>
<td>964.835</td>
<td>2.503</td>
<td>0.025</td>
<td>0.025</td>
<td>No</td>
</tr>
<tr>
<td>10 vs. 7.5</td>
<td>675.820</td>
<td>1.753</td>
<td>0.101</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: Workload within D

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 vs. 7.5</td>
<td>689.756</td>
<td>1.600</td>
<td>0.132</td>
<td>0.017</td>
<td>No</td>
</tr>
<tr>
<td>13 vs. 10</td>
<td>428.183</td>
<td>0.993</td>
<td>0.337</td>
<td>0.025</td>
<td>No</td>
</tr>
<tr>
<td>10 vs. 7.5</td>
<td>261.573</td>
<td>0.607</td>
<td>0.554</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
Two Way Repeated Measures ANOVA (One Factor Repetition)

Data source: LO7.5kg vs Time

General Linear Model

Dependent Variable: VO2

Normality Test (Shapiro-Wilk)  Passed  \( P = 0.099 \)

Equal Variance Test:  Passed  \( P = 0.749 \)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>1</td>
<td>56616.630</td>
<td>56616.630</td>
<td>0.982</td>
<td>0.351</td>
</tr>
<tr>
<td>Subject(Condition)</td>
<td>8</td>
<td>461044.109</td>
<td>57630.514</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>8</td>
<td>2028670.826</td>
<td>253583.853</td>
<td>51.908</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Condition x Time</td>
<td>8</td>
<td>48865.373</td>
<td>6108.172</td>
<td>1.250</td>
<td>0.285</td>
</tr>
<tr>
<td>Residual</td>
<td>64</td>
<td>312654.480</td>
<td>4885.226</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>3075057.737</td>
<td>34551.211</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The difference in the mean values among the different levels of Condition is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Time. There is not a statistically significant difference \( P = 0.351 \).

The difference in the mean values among the different levels of Time is greater than would be expected by chance after allowing for effects of differences in Condition. There is a statistically significant difference \( P = <0.001 \). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Condition does not depend on what level of Time is present. There is not a statistically significant interaction between Condition and Time. \( P = 0.285 \)

Power of performed test with alpha = 0.0500:  for Condition : 0.0500
Power of performed test with alpha = 0.0500:  for Time : 1.000
Power of performed test with alpha = 0.0500:  for Condition x Time : 0.120

Expected Mean Squares:
Approximate DF Residual for Condition = 8.000

Expected MS(Condition) = var(res) + 9.000 var(Subject(Condition)) + var(Condition)
Expected MS(Subject(Condition)) = var(res) + 9.000 var(Subject(Condition))
Expected MS(Time) = var(res) + var(Time)
Expected MS(Condition x Time) = var(res) + var(Condition x Time)
Expected MS(Residual) = var(res)

Least square means for Condition :

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>453.713</td>
<td>32.669</td>
</tr>
<tr>
<td>D</td>
<td>504.910</td>
<td>40.011</td>
</tr>
</tbody>
</table>

Least square means for Time :

<table>
<thead>
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<th>Mean</th>
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<tbody>
<tr>
<td>15</td>
<td>217.443</td>
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<tr>
<td>30</td>
<td>246.401</td>
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<tr>
<td>Time</td>
<td>Value</td>
</tr>
<tr>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>45</td>
<td>400.214</td>
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<tr>
<td>60</td>
<td>435.193</td>
</tr>
<tr>
<td>90</td>
<td>567.891</td>
</tr>
<tr>
<td>120</td>
<td>570.651</td>
</tr>
<tr>
<td>180</td>
<td>616.516</td>
</tr>
<tr>
<td>240</td>
<td>626.766</td>
</tr>
<tr>
<td>300</td>
<td>632.731</td>
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Std Err of LS Mean = 33.457

Least square means for Condition x Time:

<table>
<thead>
<tr>
<th>Group</th>
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<th>SEM</th>
</tr>
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<tbody>
<tr>
<td>C x 15</td>
<td>147.118</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 30</td>
<td>197.160</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 45</td>
<td>411.243</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 60</td>
<td>398.785</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 90</td>
<td>532.951</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 120</td>
<td>571.972</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 180</td>
<td>593.799</td>
<td>42.320</td>
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<tr>
<td>C x 240</td>
<td>610.201</td>
<td>42.320</td>
</tr>
<tr>
<td>C x 300</td>
<td>620.188</td>
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<tr>
<td>D x 15</td>
<td>287.767</td>
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</tr>
<tr>
<td>D x 30</td>
<td>295.642</td>
<td>51.831</td>
</tr>
<tr>
<td>D x 45</td>
<td>389.184</td>
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<td>D x 60</td>
<td>471.601</td>
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<td>D x 90</td>
<td>602.830</td>
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<td>D x 120</td>
<td>569.330</td>
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<td>639.233</td>
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<td>D x 240</td>
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<tr>
<td>D x 300</td>
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</table>
Two Way Repeated Measures ANOVA (One Factor Repetition)

Data source: LO7.5kg vs Time

Balanced Design

Dependent Variable: LBF with CN05 removed

Normality Test (Shapiro-Wilk)  Passed  (P = 0.341)

Equal Variance Test:  Passed  (P = 0.385)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<tbody>
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<td>3452833.754</td>
<td>3452833.754</td>
<td>1.170</td>
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<td>Subject(Condition)</td>
<td>6</td>
<td>17708302.020</td>
<td>2951383.670</td>
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<tr>
<td>Time</td>
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<td>13958786.054</td>
<td>1744848.257</td>
<td>13.768</td>
<td>&lt;0.001</td>
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<td>3199580.445</td>
<td>399947.556</td>
<td>3.156</td>
<td>0.006</td>
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<tr>
<td>Residual</td>
<td>48</td>
<td>6083132.043</td>
<td>126731.918</td>
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<tr>
<td>Total</td>
<td>71</td>
<td>44402634.316</td>
<td>625389.216</td>
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</table>

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Condition depends on what level of Time is present. There is a statistically significant interaction between Condition and Time.  (P = 0.006)

Power of performed test with alpha = 0.0500:  for Condition : 0.0627
Power of performed test with alpha = 0.0500:  for Time : 1.000
Power of performed test with alpha = 0.0500:  for Condition x Time : 0.793

Least square means for Condition :

<table>
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<th>Mean</th>
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<tr>
<td>C</td>
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</tr>
<tr>
<td>D</td>
<td>3586.033</td>
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</table>

Std Err of LS Mean = 286.327

Least square means for Time :

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<tbody>
<tr>
<td>15</td>
<td>2778.005</td>
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<td>30</td>
<td>3443.820</td>
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<tr>
<td>45</td>
<td>3661.114</td>
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<tr>
<td>60</td>
<td>3838.611</td>
</tr>
<tr>
<td>90</td>
<td>4051.261</td>
</tr>
<tr>
<td>120</td>
<td>4145.105</td>
</tr>
<tr>
<td>180</td>
<td>4242.239</td>
</tr>
<tr>
<td>240</td>
<td>4212.109</td>
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<td>300</td>
<td>3872.934</td>
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Std Err of LS Mean = 234.676

Least square means for Condition x Time :

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<tbody>
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<td>3435.628</td>
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<tr>
<td>C x 30</td>
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<tr>
<td>C x 45</td>
<td>3886.177</td>
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<tr>
<td>C x 60</td>
<td>4045.494</td>
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<tr>
<td>Comparison</td>
<td>Diff of Means</td>
</tr>
<tr>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>90 vs. 15</td>
<td>927.529</td>
</tr>
<tr>
<td>240 vs. 15</td>
<td>817.948</td>
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<tr>
<td>120 vs. 15</td>
<td>804.452</td>
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<td>180 vs. 15</td>
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<tr>
<td>45 vs. 15</td>
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<tr>
<td>30 vs. 15</td>
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<td>240 vs. 30</td>
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<tr>
<td>240 vs. 45</td>
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<td>353.903</td>
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<tr>
<td>90 vs. 300</td>
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<tr>
<td>90 vs. 60</td>
<td>317.662</td>
</tr>
<tr>
<td>180 vs. 30</td>
<td>278.582</td>
</tr>
<tr>
<td>180 vs. 45</td>
<td>241.178</td>
</tr>
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<td>90 vs. 180</td>
<td>235.802</td>
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<td>240 vs. 60</td>
<td>208.082</td>
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<tr>
<td>60 vs. 30</td>
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<td>90 vs. 120</td>
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<tr>
<td>120 vs. 180</td>
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<tr>
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<td>37.405</td>
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### Comparisons for factor: **Time within D**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 vs. 15</td>
<td>2236.740</td>
<td>8.886</td>
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<tr>
<td>240 vs. 15</td>
<td>2050.259</td>
<td>8.145</td>
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<tr>
<td>120 vs. 15</td>
<td>1929.749</td>
<td>7.666</td>
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<td>90 vs. 15</td>
<td>1618.982</td>
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<td>300 vs. 15</td>
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<td>60 vs. 15</td>
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<td>0.002</td>
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<td>180 vs. 30</td>
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<td>0.002</td>
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<td>120 vs. 30</td>
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<td>180 vs. 45</td>
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<td>30 vs. 15</td>
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<tr>
<td>180 vs. 60</td>
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<tr>
<td>90 vs. 30</td>
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<tr>
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<td>120 vs. 45</td>
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<td>0.003</td>
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<td>60 vs. 30</td>
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<td>240 vs. 300</td>
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<tr>
<td>240 vs. 90</td>
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<td>180 vs. 120</td>
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<td>300 vs. 45</td>
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<td>186.481</td>
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<td>240 vs. 120</td>
<td>120.511</td>
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<td>300 vs. 60</td>
<td>98.281</td>
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<td>90 vs. 300</td>
<td>9.356</td>
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### Comparisons for factor: **Condition within 15**

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<th>Unadjusted P</th>
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<tbody>
<tr>
<td>C vs. D</td>
<td>1315.245</td>
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### Comparisons for factor: **Condition within 30**

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<tr>
<td>C vs. D</td>
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### Comparisons for factor: **Condition within 45**

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<th>Unadjusted P</th>
<th>Critical Level</th>
<th>Significant?</th>
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<tbody>
<tr>
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<td>1.261</td>
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</tr>
<tr>
<td>Comparison</td>
<td>Diff of Means</td>
<td>t</td>
<td>Unadjusted P</td>
<td>Critical Level</td>
<td>Significant?</td>
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<td>--------------</td>
<td>-----</td>
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<td>----------------</td>
<td>--------------</td>
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<tr>
<td>C vs. D</td>
<td>413.766</td>
<td>0.882</td>
<td>0.397</td>
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<td>No</td>
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<tr>
<td>C vs. D</td>
<td>623.792</td>
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<td>0.212</td>
<td>0.050</td>
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<tr>
<td>C vs. D</td>
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<tr>
<td>D vs. C</td>
<td>229.768</td>
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<td>C vs. D</td>
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<tr>
<td>C vs. D</td>
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<td>0.609</td>
<td>0.555</td>
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