INFLUENCE OF CAFFEINE ON EXERCISING MUSCLE BLOOD FLOW AND EXERCISE TOLERANCE IN TYPE II DIABETES

A Pilot Project

by

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ABSTRACT

Background: Exercise is a critical treatment modality in persons with Type II Diabetes Mellitus (T2DM), however people with this disease experience chronic fatigue and a decreased exercise capacity, which affects their ability or willingness to participate in physical activity. Studies suggest that this exercise intolerance may be partly due to a reduced exercising muscle blood flow (MBF), and in particular to a reduced ability of red blood cells (RBCs) to evoke ATP-mediated vasodilation and an increase in MBF as they traverse areas of high O$_2$ demand. Additional evidence suggests that caffeine may attenuate this impairment by enhancing the release of ATP from RBCs.

Hypothesis: Persons with T2DM would have reduced Forearm Blood Flow (FBF), oxygen consumption (VO$_2$), and exercise tolerance responses to exercise compared to control (CON) subjects, and caffeine would attenuate these impairments.

Methods: T2DM ($n = 4$) and CON ($n = 4$) participants performed rhythmic forearm handgrip exercise at an intensity equivalent to 17.5 kg until “task failure” or 20 minutes of exercise was reached, after having consumed either a caffeine (5mg/kg; Caff) or placebo (Pl) capsule. FBF (Doppler and Echo ultrasound of the brachial artery), VO$_2$ and lactate efflux (deep venous blood sampling), forearm vascular conductance (FVK), mean arterial pressure (MAP) and heart rate (HR) were quantified for each minute of exercise.

Results: Steady state FBF was similar across groups and treatment conditions (mean ± SE ml/min; CONCaff 553.80 ± 82.35, CONPl 583.42 ± 112.62, T2DMCaff 523.33 ± 105.39, T2DMPl 569.08 ± 134.20, NS), and this was due to similar MAP and FVK
(across groups and treatment conditions, NS). VO2 and Time to Task Failure (TTF) were not different between groups and treatment conditions (NS), although TTF tended to be improved with caffeine versus placebo (10.00 ± 2.02 vs 8.24 ± 1.79 min, P=0.295). There was a strong positive relationship between FBF and TTF ($r^2=0.763$; $P=0.005$).

**Conclusions:** In the exercise model utilized, persons with T2DM do not have impaired cardiovascular responsiveness or reduced exercise tolerance, and caffeine does not provide any benefit. Differences in exercising MBF may be an underlying mechanism regarding differences in exercise tolerance.
ACKNOWLEDGMENTS

First, I would like to thank the participants, who so willingly donated their time (and gave up caffeine for 48 hours in advance of each visit to the lab). Without you this study would not have been possible.

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Venous Blood Constituents
Serum Caffeine Concentration
HbA1c
C\textsubscript{o2}, Hb, La
Forearm Oxygen Consumption (VO\textsubscript{2})
Lactate Efflux
Time to Task Failure
Across-Subject Averaging
Statistical Analysis

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<th>Description</th>
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<tr>
<td>2,3-DPG</td>
<td>2,3-diphosphoglycerate</td>
</tr>
<tr>
<td>AC</td>
<td>Adenylyl Cyclase</td>
</tr>
<tr>
<td>ANG II</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
</tr>
<tr>
<td>C\textsubscript{a}O\textsubscript{2}</td>
<td>Arterial Oxygen Content</td>
</tr>
<tr>
<td>CNP</td>
<td>Cyclic Nucleotide Phosphodiesterase</td>
</tr>
<tr>
<td>CO</td>
<td>Cardiac Output; Carbon Monoxide</td>
</tr>
<tr>
<td>CON</td>
<td>Control</td>
</tr>
<tr>
<td>C\textsubscript{v}O\textsubscript{2}</td>
<td>Venous Oxygen Content</td>
</tr>
<tr>
<td>DK\textsubscript{muscle}</td>
<td>Diffusive Conductance of Muscle</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>Epi</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>FBF</td>
<td>Forearm Blood Flow</td>
</tr>
<tr>
<td>FVK</td>
<td>Forearm Vascular Conductance</td>
</tr>
<tr>
<td>G\textsubscript{i}</td>
<td>Heterotrimeric G Protein</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated Hemoglobin</td>
</tr>
<tr>
<td>HHb</td>
<td>Deoxygenated hemoglobin/myoglobin</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>KGH</td>
<td>Kingston General Hospital</td>
</tr>
<tr>
<td>La\textsuperscript{-}</td>
<td>Lactate</td>
</tr>
<tr>
<td>MBF</td>
<td>Muscle Blood Flow</td>
</tr>
<tr>
<td>MBV</td>
<td>Mean Blood Velocity</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NIRS</td>
<td>Near Infrared Spectroscopy</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>NS</td>
<td>Not Significant</td>
</tr>
<tr>
<td>O\textsubscript{2}</td>
<td>Oxygen</td>
</tr>
<tr>
<td>P\textsubscript{1}</td>
<td>Inorganic Phosphate</td>
</tr>
<tr>
<td>PcellO\textsubscript{2}</td>
<td>Intracellular Partial Pressure of Oxygen</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PmvO\textsubscript{2}</td>
<td>Microvascular Partial Pressure of Oxygen</td>
</tr>
<tr>
<td>Qm</td>
<td>Microvascular Blood Flow</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of Perceived Exertion</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic Nervous System</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke Volume</td>
</tr>
<tr>
<td>TTF</td>
<td>Time to Task Failure</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type II Diabetes Mellitus</td>
</tr>
<tr>
<td>TVK</td>
<td>Total Vascular Conductance</td>
</tr>
<tr>
<td>VK</td>
<td>Vascular Conductance</td>
</tr>
<tr>
<td>VO\textsubscript{2}</td>
<td>Oxygen Consumption</td>
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Type II Diabetes Mellitus (T2DM) is a disease of epidemic proportions; it is projected to affect more than 377 million people worldwide by the year 2030 (177). Although it is by definition a metabolic disorder, characterized by poor glycemic control with hyperglycemia, hyperinsulinemia, insulin resistance, and elevated plasma free fatty acids (143), the primary cause of morbidity and mortality in this population is cardiovascular disease (114; 172); cardiovascular disease accounts for up to 80% of deaths in persons with T2DM (153). In this regard, exercise is a major therapeutic modality; not only does exercise exert beneficial effects on the management of blood glucose levels, but it confers a protective effect on cardiovascular health (4; 160). Unfortunately however, persons with T2DM experience a reduced exercise tolerance, making it difficult for individuals in this population to participate in regular physical activity.

Recent evidence suggests that this exercise intolerance may be due, at least in part, to impaired muscle blood flow (MBF) and concomitant impaired oxygen (O\textsubscript{2}) delivery (76; 81), factors which are known to hasten the development of fatigue (62; 165). Furthermore, evidence from \textit{in vitro} studies implicates a defective functioning of the Red Blood Cell (RBC)-O\textsubscript{2} Sensor feedback mechanism of blood flow regulation in the attenuated MBF response in this population (61; 155). An impairment in this mechanism could conceivably explain the blunted blood flow response to exercise (76; 81) that is observed in individuals with T2DM, and could therefore represent a target for intervention.
In brief, the RBC-O₂ Sensor mechanism is purported to function as follows. RBCs contain millimolar amounts of adenosine 5’-triphosphate (ATP), and they release this ATP in response to a reduction in the oxygen content of hemoglobin (Hb), the O₂-transport protein of RBCs (157). In other words, when the offloading of O₂ from RBCs increases to meet increasing O₂ demands, as occurs in active skeletal muscle, the release of ATP from RBCs also increases. This ATP can diffuse in the plasma and bind to purinergic (P₂y) receptors on the endothelium, causing it to release vasodilators such as nitric oxide, which then evoke an increase in vascular conductance and thus an increase in blood flow (34; 157). The amount of ATP released is in proportion to the degree of saturation of Hb; thus the greater the oxygen demand in the muscle, the greater the desaturation of RBCs, the greater the release of ATP, and the greater the degree of vasodilation elicited. In other words, as RBCs traverse areas of high O₂ demand, they release ATP that mediates a local and conducted vasodilation and consequent increase in blood flow. In this way, the demand for O₂ in the muscle is effectively communicated to the blood vessels that supply it, such that O₂ supply increases to match the new, greater O₂ demand.

A signal transduction pathway for the release of ATP from RBCs has been proposed, and while the pathway has not yet been completely characterized, several components have been identified (Figure 1A). For instance, this pathway contains the heterotrimeric G protein Gᵢ (115; 116) which activates adenylyl cyclase (AC), a transmembrane protein that catalyzes the conversion of ATP to cyclic AMP (cAMP), a common intracellular second messenger (155); accumulation of intraerythrocyte cAMP leads to an increase in ATP efflux downstream (156). Importantly, a reduced expression
Figure 1. (A) Proposed pathway for ATP release from RBCs. (B) Mechanism by which caffeine is proposed to augment the RBC’s ability to function as an O₂ sensor. Caffeine may enhance the release of ATP from RBCs by both inhibiting the breakdown of intraerythrocyte cAMP (by inhibiting CNPs, the enzymes responsible for hydrolyzing cAMP), and/or by stimulating its production (secondary to increased epinephrine release). AC = Adenylyl Cyclase; ATP = Adenosine Triphosphate; cAMP = cyclic Adenosine Monophosphate; CNP = cyclic nucleotide phosphodiesterase; Gᵢ = heterotrimeric G protein; Hb = hemoglobin; ? = unidentified mechanism; + = stimulates; dashed arrow = multiple steps. Adapted from Sprague et al., 2007 (157).
of $G_i$ has been observed in both animal models of diabetes (45; 101) and in the RBCs of humans with T2DM (156). Specifically, it has been shown that the expression of the $G_{i2}$ subtype is reduced in humans with T2DM, and that this reduction is associated with an impaired release of ATP in response to either direct activation of $G_i$ with a drug (156), or in response to exposure to low oxygen tension (61). These findings suggest that the decreased release of ATP from RBCs in response to Hb desaturation is a mechanism underlying the observed impairment in exercising MBF in individuals with T2DM.

Anecdotal evidence suggests that caffeine improves exercise tolerance in individuals with T2DM, and additional in vitro research has linked this improvement to an attenuation of the impairment in the RBC-O$_2$ Sensor mechanism of vasoregulation (38). Caffeine, a methylxanthine, inhibits some forms of cyclic nucleotide phosphodiesterases (CNPs), enzymes that hydrolyze cAMP (38). Since the signal transduction pathway for the release of ATP from RBCs requires increases in intracellular cAMP (formed via $G_i$ activation of AC) (155; 157), inhibiting its breakdown should theoretically result in an elevation in ATP release (Figure 1B). Ellsworth and colleagues (2007) tested this hypothesis in an in vitro investigation in which they exposed rabbit RBCs to a level of caffeine which would be expected in an individual who has consumed about 1-2 cups of coffee (38). They found that this amount of caffeine increased the release of ATP from RBCs by ~70%, and that intracellular cAMP levels also significantly increased by 22%, supporting the proposed mechanism of caffeine’s enhancement of ATP release.

Furthermore, in vivo, caffeine is known to increase circulating levels of epinephrine (Epi) (70; 171), and Epi is a stimulator of AC (155). Accordingly, Epi could
potentially increase intraerythrocyte cAMP levels and result in increased ATP efflux from RBCs.

Thus there is evidence to suggest that caffeine may enhance the ability of the RBC to act as an O$_2$ sensor, both by attenuating the degradation of intraerythrocyte cAMP, and by stimulating its production, secondary to Epi. Therefore, if caffeine does in fact augment ATP release from RBCs in humans in vivo, and if this results in greater vasodilation and increased blood flow and oxygen delivery, then caffeine administration may represent a practical therapeutic intervention to aid T2DM patients in tolerating the exercise that is of such importance to their health.

With this as a foundation, the objectives of this study were twofold: 1) to confirm and characterize the nature of the impairment in steady state muscle blood flow and oxygen delivery during small muscle mass (forearm) exercise in individuals with T2DM, and 2) to ascertain the effects of caffeine on exercise tolerance and exercising MBF in individuals with T2DM versus healthy controls.

In order to attain these objectives, this study comprised a characterization of the MBF, vascular conductance, oxygen consumption, exercise tolerance, and other relevant responses (mean arterial pressure, lactate concentration and efflux, heart rate, stroke volume, cardiac output) in exercising human forearm muscle under normal conditions, and following oral consumption of caffeine. This characterization was done in individuals with Type II Diabetes, and in control subjects who were of similar age and physical activity level.

The forearm exercise model was utilized for a few key reasons. First, there is evidence of both central (cardiac) and peripheral (vascular) contributions to the abnormal
exercise response in persons with T2DM (127), and it is difficult to isolate these contributions using a large muscle mass model.

To elaborate, mean arterial pressure (MAP) is a key regulated variable during exercise; it is determined by the volume of blood in the arteries, which is determined by the balance between the inflow (Cardiac Output; CO) and outflow (Total Vascular Conductance; TVK) of blood. During exercise, the blood vessels of the exercising muscles dilate, or increase their conductance, resulting in the needed increase in blood flow and oxygen delivery. If this dilation were the only change, then MAP would fall; to maintain MAP either an increase in CO, or a reduction in the conductance of another vascular bed(s), or both would be required. If there is inadequate cardiac function such that CO is reduced at a given work rate, then a compensatory sympathetic vasoconstriction – that is, a reduction in TVK – must occur in order to maintain an appropriate MAP. In large muscle mass exercise this sympathetic restraint of muscle blood flow occurs even in healthy individuals who experience a normal rise in CO with exercise (135); it is necessary to maintain an appropriate MAP.

Thus in studying large muscle mass exercise in a diseased population it is difficult to determine whether an abnormally small vascular conductance is the result of an inadequate CO response and a resultant compensatory vasoconstriction, or is simply due to an impaired vasodilatory response. In small muscle mass exercise however, the increase in vascular conductance relative to TVK is small enough that it is unlikely to threaten MAP regulation. Therefore, small muscle mass exercise is unlikely to be limited by inadequate cardiac function, and as such the forearm allows for a better isolation of local peripheral contributions to the dysfunction during exercise.
Furthermore, the forearm was an attractive exercise model for practical reasons (129); the anticubital fossa allows for relative ease of catheter placement for measurement of venous oxygen content and calculation of oxygen uptake, and the brachial artery is easily accessible with ultrasound to measure blood flow. Thus the forearm allowed access to measures that are critical in understanding the nature of the dysfunction.

The forearm exercise consisted of squeezing a handgrip dynamometer (2 seconds of contraction to 2 seconds of relaxation) with oscilloscope output (i.e. live computer feedback displaying force output) at an intensity equivalent to 17.5 kg until “task failure” or 20 minutes was reached. “Task failure” was defined as an inability to maintain the appropriate cadence or contraction intensity for three consecutive contractions, as visualized on the oscilloscope output, and time to task failure was used to assess “exercise tolerance”. It was hypothesized that persons with T2DM would have reduced exercising MBF compared to controls, and that caffeine would increase MBF and result in greater time to task failure, or improved exercise tolerance.

**Specific Objectives:**

1. To confirm and characterize the nature of the impairment in muscle blood flow and O$_2$ consumption during steady state forearm exercise in individuals with T2DM.

2. To ascertain the effects of caffeine on exercising muscle blood flow, O$_2$ consumption, and exercise tolerance in individuals with T2DM versus healthy controls of similar age and physical activity level.
Specific Hypotheses:

1. T2DM patients would have reduced forearm blood flow and O₂ consumption versus controls at the same absolute work rate, and that this would be due to an impaired vasodilatory response.

2. T2DM patients would have a lower exercise tolerance than controls, as evidenced by demonstrating a shorter time to task failure at the same absolute work rate.

3. Caffeine would attenuate the impairment in forearm blood flow and O₂ consumption in individuals with T2DM versus the placebo, and this would be related to an improvement in the vasodilatory response.

4. Caffeine would increase the time to task failure in both T2DM patients and controls, although the percent improvement would be greater in the T2DM patients assuming a basal impairment.
Chapter 2: LITERATURE REVIEW

Exercise Intolerance in Type 2 Diabetes: The Role of the Cardiovascular System and Rationale for Caffeine as an Intervention

Type II Diabetes (T2DM) is one of the most common chronic diseases. As of 2005 the total prevalence of T2DM was estimated to be greater than 170 million individuals worldwide (9; 161), and the growth in prevalence and incidence continues to increase at unprecedented rates with no signs of slowing down (4). It is projected that the global prevalence of T2DM will be 377 million by the year 2030 – just 20 short years from now (11; 177).

A disease with both genetic and environmental components (4; 161), T2DM is a metabolic disorder that is identified by the World Health Organization’s diagnostic classification: a fasting plasma glucose level of \( \geq 7.0 \) mmol/L, or a 2-hr plasma glucose level of \( \geq 11.1 \) mmol/L (i.e. 2-hr post-ingestion of a 75g oral glucose load) (73). The pathophysiology of T2DM involves defects in both insulin action (i.e. insulin resistance – a reduced physiological response to a given amount of insulin (109)) and insulin secretion (4). T2DM is characterized by poor glycemic control with hyperglycemia, hyperinsulinemia, insulin resistance and elevated plasma free fatty acids (143), as well as symptoms of polyuria (excessive passage of urine), polydipsia (excessive thirst), and polyphagia (excessive hunger) (73).

The health consequences and complications of T2DM are substantive. For instance, 25% of those with diabetes have “severe visual impairment”, a proportion approximately double that in persons without the disease (139), and diabetic retinopathy leads to \( \sim 12,000-24,000 \) new cases of blindness in the US alone every year (1).
Peripheral neuropathy and peripheral arterial disease result in elevated rates of lower-extremity amputations (40), and persons with T2DM have an approximately doubled risk for both physical (138) and cognitive disability (59). These are only some of the cluster of complications associated with T2DM, yet they serve to illustrate the seriousness of the disease. Perhaps most significantly, T2DM increases the risk of cardiovascular disease by 200-400% (159), and cardiovascular disease accounts for up to 80% of deaths in persons with this disease (153). In other words, while T2DM is a metabolic disorder by definition, people with this disease die primarily from cardiovascular disease (153), and the age-adjusted mortality among adults with T2DM is about twice that of people who do not have diabetes (46).

Given the prevalence of this disease and the severity of its acute and chronic complications, it is clear that effective treatment and prevention strategies are urgently needed. The goal of T2DM treatment is to achieve and maintain near-normal blood glucose levels and optimal lipid levels in order to prevent or delay complications (4). To this end, exercise has long been considered the cornerstone of diabetes management (4; 147; 160). Not only does exercise have beneficial effects on glycemia and insulin sensitivity, but it has a protective influence on cardiovascular health (160; 172). The problem is that while exercise is extremely important, persons with T2DM suffer from exercise intolerance (127). Persons with T2DM report experiencing chronic fatigue, weakness, and a decreased exercise capacity (32), which affects their ability or willingness to participate in physical activity (9).

Evidence is accumulating to suggest that the cardiovascular system plays an important role in establishing the exercise intolerance experienced by this population.
Namely, in persons with T2DM there is evidence for impairments in the convective delivery of oxygen (O$_2$) from the lungs to the skeletal muscle capillaries (76; 81) and the diffusive capacity for the movement of O$_2$ from the capillaries into the skeletal muscle cells (myocytes) (35; 118) – both of which would impact muscle oxygenation (PcellO$_2$) and contribute to the onset of fatigue processes – as well as factors affecting the myocytes’ ability to use the O$_2$ once it has been taken up (122). Each of these impairments would be expected to contribute to the exercise intolerance experienced by this population.

In particular, a few recent investigations have demonstrated that impaired blood flow to exercising muscle, and concomitant impaired O$_2$ delivery, is a probable contributor to the development of muscular fatigue and reduced exercise tolerance in persons with T2DM (76; 81). Evidence from in vitro studies suggests that this impairment is probably due, at least in part, to a diminished ability of red blood cells (RBC) to elicit a widening of the blood vessels and an increase in blood flow in response to a low-oxygen environment (61; 154; 155). A defect in this mechanism, referred to as the “RBC-O$_2$ Sensor”, could conceivably explain the blunted blood flow response to exercise that is observed in individuals with T2DM.

Furthermore, evidence from an in vitro study (38) suggests that caffeine, arguably the most commonly-consumed drug worldwide (54), may act to attenuate this impairment in the RBC-O$_2$ Sensor mechanism of vasoregulation. If in fact caffeine does improve the RBC’s ability to evoke increases in muscle blood flow (MBF) and oxygen delivery during exercise, then caffeine administration may be a practical intervention to help
enable individuals with T2DM to engage in the exercise that is of such importance to their health.

In summary, T2DM is a highly prevalent disease with considerable consequences to health. Exercise is an important treatment modality, however individuals with T2DM experience exercise intolerance; it is becoming increasingly apparent that the cardiovascular system has a key contribution to this intolerance. Specifically, impairment in the RBC-O\textsubscript{2} Sensor mechanism of MBF-metabolism matching could partly explain observations of reduced exercising MBF and oxygen delivery in this population, and caffeine has the potential to be used as an intervention to improve blood flow during exercise and thereby make exercise easier. With this as a foundation, the purposes of this review are six-fold: 1) to provide a brief overview of how muscle oxygenation is established by the cardiovascular system and how PcellO\textsubscript{2} influences muscle metabolism and processes related to fatigue, 2) to describe the nature of the exercise intolerance in persons with T2DM, with specific reference to factors affecting convective O\textsubscript{2} delivery, diffusive O\textsubscript{2} flux, and metabolic O\textsubscript{2} utilization during exercise, 3) to elaborate on how the RBC-O\textsubscript{2} Sensor mechanism is purported to function in terms of matching O\textsubscript{2} delivery to metabolic demand, and to identify how this mechanism is impaired in T2DM, 4) to provide a rationale for testing caffeine’s utility as an intervention in improving the RBC-O\textsubscript{2} Sensor mechanism, 5) to identify other relevant characteristics and effects of caffeine, and 6) to highlight some important considerations in studying cardiovascular responses to exercise in a disease population.
(1) Muscle Oxygenation – Determinants and Impact of PcellO₂

A primary role of the cardiovascular system during exercise is to provide the muscles with adequate O₂ to support aerobic metabolism. Limitations to exercise tolerance that are due to the cardiovascular system are thus due to the muscle oxygenation environment (PcellO₂) that is established by the convective delivery of O₂ from the lungs to the skeletal muscle capillaries, and the diffusive capacity for the movement of O₂ from the capillaries to the inside of the myocytes (167).

To elaborate, the convective delivery of O₂ is the amount of O₂ “offered” to the muscle, and it occurs according to the Fick Principle (66); the convective delivery of O₂ is a function of the muscle blood flow (MBF) and arterial O₂ content (CaO₂) (Figure 2A). Once the O₂ has been convectively delivered to the muscle, it must diffuse from the blood into the myocytes (175). Diffusive flux occurs according to Fick’s law (66); it is a function of the diffusive conductance of the muscle for oxygen (DKmuscle), and the oxygen pressure gradient from the capillary to the inside of the cell (PcapO₂-PcellO₂) (Figure 2B). Convective O₂ delivery and diffusive O₂ flux interact to determine the PcellO₂ at a given O₂ consumption (VO₂).

Briefly, PcellO₂ is determined by the balance between the “inflow” and “outflow” of O₂ to and from the cytosol. With respect to the cell, “inflow” is represented by the diffusion of O₂ from the capillary space into the muscle cell space, which is dependent both on the diffusive conductance and the gradient of the partial pressure of O₂ from the capillary (the “high” part of the gradient, which is established by the convective delivery of O₂) to the cell (the “low” part of the gradient). “Outflow” is represented by the
Figure 2. (A) The Fick Principle and (B) Fick’s Law interact to determine oxygen transport from the lungs to the inside of the cells. 

CaO\textsubscript{2} = Arterial O\textsubscript{2} content; CO = cardiac output; DK\textsubscript{muscle} = diffusive conductance of the muscle; HbO\textsubscript{2} = oxyhemoglobin; HR = heart rate; MBF = Muscle Blood Flow; Pa = arterial pressure; PaO\textsubscript{2} = partial pressure of O\textsubscript{2} in the arteries; PcapO\textsubscript{2} = partial pressure of O\textsubscript{2} in the capillaries; PcellO\textsubscript{2} = partial pressure of O\textsubscript{2} in the capillaries; Pv = venous pressure; SV = stroke volume; TVK = total vascular conductance; VK\textsubscript{muscle} = muscle vascular conductance
utilization of O\textsubscript{2} by the mitochondria in the cell during oxidative phosphorylation (i.e. O\textsubscript{2} consumption, or VO\textsubscript{2}). Thus for PcellO\textsubscript{2} to be altered, “inflow” and “outflow” must change relative to one another. At steady state, inflow equals outflow (i.e. the diffusive flux of O\textsubscript{2} into the cell is equal to the VO\textsubscript{2}) and PcellO\textsubscript{2} is stable.

Importantly, there can be different partial pressures of O\textsubscript{2} within the cell at the same rates of entry and consumption, or there can be the same partial pressure of O\textsubscript{2} within the cell for different rates of entry and consumption. For example, relevant to persons with T2DM, at a fixed work rate (i.e. fixed O\textsubscript{2} consumption or “outflow” of O\textsubscript{2} from the cell), and a fixed diffusive conductance, if the convective delivery of O\textsubscript{2} is reduced, this will result in a reduced PcellO\textsubscript{2}. Sequentially, at the moment the convective O\textsubscript{2} delivery is reduced, the pressure gradient for the movement of O\textsubscript{2} into the cell is also reduced. This means that the “inflow” of O\textsubscript{2} into the cell is reduced relative to the outflow of O\textsubscript{2} (i.e. VO\textsubscript{2} is unchanged). Since inflow is less than outflow, the PcellO\textsubscript{2} will decrease. As PcellO\textsubscript{2} decreases, the pressure gradient between the capillary and the cell increases, affecting an increase in inflow. Eventually inflow will again equal outflow, but at a new and lower PcellO\textsubscript{2}. Thus, all other things being equal, any factor altering the convective O\textsubscript{2} delivery or the diffusive capacity of the muscle for O\textsubscript{2} can ultimately impact PcellO\textsubscript{2}.

PcellO\textsubscript{2} is a critical determinant of one’s ability to perform or tolerate physical activity, as explained by the “Net Drive” concept (167). The concept of “Net Drive” is that the rate of mitochondrial respiration is determined by the combined “drive” from the phosphate energy state of the cell ([ADP][Pi]/[ATP]), redox energy state ([NADH]/[NAD+]), and the partial pressure of O\textsubscript{2} in the cell and therefore immediately
available to the mitochondria (PcellO₂), such that the effect on mitochondrial ATP production by changes in any one of these variables can be compensated for by changes in any one or all of the others (167; 182). Thus a lower PcellO₂ requires compensations by the phosphate energy state (i.e. ↑[ADP][Pi]/[ATP]) and redox energy state (i.e. ↑[NADH]/[NAD⁺]) in order to maintain ATP production (181). These compensations represent a metabolic cost to maintaining the required force output, or level of ATP production, and contribute to fatigue processes (5). In other words, for a lower PcellO₂ a greater metabolic perturbation is required to generate the necessary ATP. A discussion on how these metabolic adjustments contribute to fatigue is beyond the scope of this review, however it is important to emphasize that minimizing disruptions to the intracellular milieu is vital to being able to sustain a given work rate over time.

In this way, the cardiovascular system determines PcellO₂, and PcellO₂ influences one’s ability or willingness to sustain a given level of physical activity.

(2) Exercise Intolerance in T2DM: Impairments in Convective O₂ Delivery, O₂ Diffusive Capacity, and Metabolic O₂ Consumption

In persons with T2DM, there is evidence for impairments in the convective delivery of O₂ from the lungs to the skeletal muscle capillaries (76; 81) and the diffusive capacity for the movement of O₂ from the skeletal muscle capillaries into the myocytes (35; 118) – both of which would impact PcellO₂ – as well as factors affecting the myocytes’ ability to use the O₂ once it has been taken up (122). Each of these
impairments would be expected to contribute to the exercise intolerance experienced by this population.

**Convective O₂ Delivery**

*Convective O₂ Delivery - Nature of the Impairment*

As mentioned, convective O₂ delivery is a function of both MBF and CaO₂ (Figure 2); since CaO₂ does not change during submaximal exercise, MBF determines O₂ delivery (128). In the transition from rest to exercise, MBF increases in a biphasic manner and reaches a plateau that is in proportion to metabolic demand, such that steady state blood flow is linearly related to exercise intensity (49; 123; 128). Evidence indicates that the rapid initial increase in MBF is due to feedforward control mechanisms (i.e., mechanisms that “anticipate” changes in regulated variables and act to minimize these changes), whereas feedback control helps to determine what level MBF gets to and how fast it gets there (i.e. mechanisms that are initiated in response to an error signal that provides information about the magnitude of the change in the regulated variable and act to keep it within the appropriate range) (31; 68; 167). There is evidence for impairment in MBF (i.e. convective O₂ delivery) both at the onset of exercise (9; 11; 117), and during the steady state (76; 81; 184) in persons with T2DM.

*Slowed Adjustment of MBF at the Onset of Exercise*

Indication of impairment in MBF at the onset of exercise in persons with T2DM is somewhat indirect, and comes from three primary sources of evidence: O₂ uptake kinetic responses and corresponding measures of skeletal muscle energy metabolism, estimated
microvascular blood flow (Qm) kinetics, and characterization of the microvascular partial pressure of $O_2$ (PmvO$_2$) response.

The rate of increase in oxygen uptake (i.e. VO$_2$ kinetics) at the onset of exercise is influenced by a combination of cardiovascular and peripheral factors (18). At the onset of exercise, there is an immediate increase in demand for Adenosine Triphosphate (ATP), the energy currency of the cell. At moderate levels of muscular activity, oxidative phosphorylation (i.e. aerobic metabolism) supplies most of the ATP used for muscle contraction, however while ATP demand increases in an instantaneous step-wise fashion with an increase in work rate, the adjustment of aerobic metabolism is delayed and follows an approximately exponential time course (165). The delay in the “turning on” of aerobic metabolism relative to ATP demand constitutes what is termed an “O$_2$ deficit” (the classical representation of this concept is shown in Figure 3) (90). As is evident in Figure 3, the magnitude of the O$_2$ deficit is sensitive to the time course (kinetics) of the O$_2$ uptake response, such that a slower adjustment of muscle O$_2$ uptake results in a greater O$_2$ deficit. This is significant because a lower VO$_2$ at any given time point, for a constant work rate, necessitates a greater reliance on substrate level phosphorylation to meet the energy demands of the cell, which results in a greater accumulation of metabolic by-products that can contribute to fatigue (27). A slowed VO$_2$ kinetic response has been observed in persons with T2DM (9; 18; 126), and it is likely that this is due at least in part to impairments in skeletal MBF (9).

First, Regensteiner and colleagues (1998) measured VO$_2$ kinetics during constant load submaximal cycling exercise at three different intensities (20, 30 and 80 W) in women with uncomplicated T2DM and lean or overweight healthy controls (matched for
Figure 3. (A) O$_2$ deficit at the onset of exercise. (B) Magnitude of O$_2$ deficit is determined by O$_2$ kinetics; the slower the kinetics, the larger the O$_2$ deficit. Dashed arrows indicate a lower VO$_2$ at time “x” for the dashed curve versus the solid curve. This is significant because it indicates a greater reliance on substrate-level phosphorylation to meet the identical ATP demand. Solid line = Control, Dashed line = T2DM. Adapted from Lukin and Ralston (1962) (90), and Silverthorn (2004) (148).
It was found that VO\textsubscript{2} kinetics were significantly slowed in the T2DM group compared to the control groups at work rates below the lactate threshold, and tended to be slowed at the work rate above the lactate threshold. The same research group replicated these findings in a training study, with significantly slowed VO\textsubscript{2} kinetics being observed in the T2DM participants at all three work rates (18). Likewise, Bauer and colleagues (2007), again part of the same research group, observed slowed VO\textsubscript{2} kinetics in men and women with T2DM compared to healthy controls, during exercise transitions from unloaded to moderate cycling exercise (at \(\sim\) 85\% of each individual’s pre-determined lactate threshold) (9). Together, these studies demonstrate the accruement of a greater O\textsubscript{2} deficit at the onset of exercise in persons with T2DM.

Corresponding with these observations of slowed VO\textsubscript{2} kinetics are measures of skeletal muscle energy metabolism. For instance, Scheuermann-Freestone and colleagues (2003) measured skeletal muscle high-energy phosphate metabolites and pH (\(^{31}\text{P}\) nuclear magnetic resonance spectroscopy; MRS) and oxygenation (near infrared spectroscopy; NIRS) during plantar flexion exercise against progressively increasing intensities to fatigue (143). They found that skeletal muscle pH, concentrations of PCr, free ADP and P\text{r}, and oxygen saturation were all the same at rest in T2DM participants and controls (matched for age, sex, and body mass index; BMI). During exercise however, PCr hydrolysis was 2-fold faster and the pH decrease 3-fold faster in the T2DM participants. Additionally, the rate of muscle deoxygenation was 3.1-fold faster during the first 3 minutes of exercise in the T2DM group, indicating that the greater metabolic disturbance was accompanied by a decreased PcellO\textsubscript{2}. In accordance with these observations, the T2DM participants exhibited a functional exercise impairment, with the average exercise
times being ~32% shorter for the T2DM group versus the controls (~7 vs. 11 min). Importantly, although the T2DM participants were able to exercise for significantly less time than the controls, there were no differences in the muscle cellular environment at the end of exercise once fatigue was reached. This illustrates how a greater disturbance in the metabolic environment per unit time, as a result of a greater reliance on substrate-level phosphorylation and a reduced PcellO₂, leads to a faster progression of fatigue (27). Thus slowed VO₂ kinetics and the accompanying metabolic disturbances influence the ability to sustain physical activity over time. In other words, the slowed VO₂ kinetic response and associated greater metabolic disturbances are both indicators of a decreased exercise tolerance; that a slower rate of adjustment in MBF contributes to these responses is supported by estimates of Qm kinetics and characterization of the PmvO₂ response profile.

In the study by Bauer and colleagues (2007) mentioned earlier, in addition to quantifying pulmonary VO₂, measurements of deoxygenated hemoglobin/myoglobin [HHb] were made using NIRS (9). [HHb] reflects the local balance of O₂ delivery and O₂ uptake within the muscle, and as such provides an estimate of muscle oxygen extraction (i.e., [HHb]~(a – v O₂ difference)). Importantly, utilizing the [HHb] and VO₂ measurements together allowed for calculation of the Qm response via rearrangement of the Fick principle (i.e., Qm = \( \frac{\text{VO}_2}{[\text{HHb}]} \)). It was found that although the phase I kinetics of estimated Qm were similar between the groups, the phase 2 time constant and mean response time of Qm were significantly slower in the T2DM participants. The authors concluded that a slowed increase in microvascular blood flow at the onset of exercise
contributes to the observations of slowed VO$_2$ kinetics, and hence the reduced exercise tolerance in individuals with T2DM.

Similarly, that there is impairment in the rate of adjustment of MBF with exercise is supported by the characterization of the PmvO$_2$ response. PmvO$_2$ is representative of the MBF/VO$_2$ ratio (117); it can be viewed as the pool of O$_2$ offered to the cell, which is determined by the balance between the inflow of O$_2$ into the capillaries (MBF) and outflow of O$_2$ from the capillaries (diffusion into the cell and consumption; VO$_2$). In other words, for a given VO$_2$, if MBF increases PmvO$_2$ will increase, or for a given MBF if VO$_2$ increases PmvO$_2$ will decrease; any change in MBF or VO$_2$ relative to the other will therefore alter the PmvO$_2$.

When healthy rat muscle is electrically stimulated as a model of exercise, PmvO$_2$ shows a short (~10-12 sec) delay in which it remains at resting level, followed by a monoexponential decline to a new and lower steady state (9; 11; 117). This decline is representative of the time period that is required for MBF to increase such that O$_2$ delivery is again in balance with the new and higher VO$_2$. In other words, at the onset of exercise there is an immediate increase in VO$_2$ (i.e. outflow of O$_2$ from the capillary space), but a relative lag in the MBF response (i.e. inflow of O$_2$ to the capillary space), causing a lowering of PmvO$_2$. Eventually, MBF increases to again match the VO$_2$ such that the inflow of O$_2$ equals its outflow (i.e. MBF/VO$_2$ ratio), and PmvO$_2$ stabilizes at a new and lower steady state.

In contrast, in animal models of diabetes (11; 117), resting PmvO$_2$ is reduced and there is a markedly different response of PmvO$_2$ to electrical stimulation. In T2DM,
PmvO\textsubscript{2} declines more quickly and to a level that is below where it eventually stabilizes at steady state (9; 11; 117). This faster kinetic response, and “undershoot” of the steady state level, are consistent with an impairment in the rate of adjustment in MBF with exercise (i.e. a reduction in PmvO\textsubscript{2} due to a reduction in the MBF/VO\textsubscript{2} ratio), and indicate a greater fractional O\textsubscript{2} extraction. In addition to providing support for a slowed adjustment of MBF at the onset of exercise, this is significant as the PmvO\textsubscript{2} represents the driving pressure for the diffusion of O\textsubscript{2} into the cells; a lower PmvO\textsubscript{2} signifies a lower pressure gradient for the movement of O\textsubscript{2}, and can therefore contribute to a lowered PcellO\textsubscript{2}. PmvO\textsubscript{2} eventually stabilizes at the same steady state level in both normal and T2DM individuals, which indicates that after the initial “undershoot” there is a period of time for which MBF is greater relative to VO\textsubscript{2}. Given that this “recovery” of PmvO\textsubscript{2} occurs while VO\textsubscript{2} is still increasing to steady state (i.e. at a slowed rate versus controls) (127), this suggests that convective O\textsubscript{2} delivery is not the only factor limiting the adjustment of VO\textsubscript{2} at the onset of exercise. It is possible that a diffusion limitation for O\textsubscript{2} and/or metabolic impairment in the ability to consume O\textsubscript{2} are also involved in the reduced exercise tolerance in persons with T2DM, as discussed later. The characteristic VO\textsubscript{2}, PmvO\textsubscript{2}, and MBF responses are depicted schematically in Figure 4.

The PmvO\textsubscript{2} response profiles in healthy and T2DM rats are qualitatively similar but directionally opposite to the [HHb] responses mentioned earlier in the study by Bauer and colleagues (9). That is, a reduction in PmvO\textsubscript{2} and an increase in [HHb] are both indicative of increased O\textsubscript{2} extraction, and the PmvO\textsubscript{2} responses observed in the animal models (11; 117) mirror the [HHb] response measured in humans (9) (i.e. the “undershoot” in PmvO\textsubscript{2} has a corresponding “overshoot” in [HHb]). This is important as
Figure 4. Schematic of characteristic exercise responses in persons with T2DM (dashed lines) vs. healthy controls (solid lines).

(A) Slowed VO₂ kinetics in T2DM (9; 127) are probably partly due to a reduced adjustment of MBF at the onset of exercise.

(B) Faster PmvO₂ kinetics in T2DM are suggestive of a greater MBF/VO₂ mismatch at the onset of exercise. Quantified in electrically stimulated animal muscle (11; 117), this response is qualitatively similar though directionally opposite to the [HHb] response observed in humans in vivo during voluntary exercise (9).

(C) MBF. Onset of exercise is circled with question marks since impairment at the onset of exercise has yet to be directly demonstrated, although this can be inferred from estimated microvascular blood flow kinetics (9) and the data in panel (B). Steady state MBF has been shown to be reduced during low- (81) and moderate-intensity (76) leg exercise.
it suggests that these findings from stimulated animal muscle are representative of the conditions inside human muscle in vivo during voluntary exercise.

Also noteworthy, it has been proposed that different fibre types mandate different magnitudes of contraction-induced hyperemia, such that slow-twitch (Type I) fibres exhibit an augmented MBF-to-VO₂ relationship (103). In agreement with this, electrically stimulated Type I muscle has a PmvO₂ response much like that of the “control” rats as just described, whereas the Type II muscle response typifies that of the T2DM rats (11; 12; 103; 117). Interestingly, some (44; 95) although not all (63; 170) studies show a reduced proportion of Type I and increased proportion of Type II fibres in persons with T2DM. Thus it is possible that a change in muscle morphology characteristic of the population could explain the differential PmvO₂ response in persons with T2DM.

Thus exercise intolerance in persons with T2DM may be partly explained by a slowing of VO₂ kinetics and a greater disturbance in the metabolic environment inside the cell at the onset of exercise, and these responses may in turn be partly explained by impairment in the adjustment of MBF. This slowed adjustment of MBF at the onset of exercise is indicated by observations of slowed Qm kinetics and the altered PmvO₂ (or [HHb]) response in persons with T2DM versus controls. To reiterate, impairment in MBF at the onset of exercise is significant because it results in a greater reliance on anaerobic means of energy generation and disruption of the intracellular milieu, and also because it results in a lowering of PcellO₂ as identified in Section 1. Thus impairment in MBF at the onset of exercise has a negative impact not only at the start of exercise but
also during the steady state via the intracellular environment that it establishes, and can thereby contribute to premature muscular fatigue.

**Reduced Steady State MBF**

Regardless of the kinetics of the response, the same steady state VO$_2$ is eventually reached in both persons with T2DM and healthy controls (18; 127), which is expected given the established linear relationship between work rate and VO$_2$ (60). However the few direct measurements of MBF performed so far suggest that convective O$_2$ delivery is reduced at steady state in persons with T2DM versus controls (76; 81). Since at steady state the diffusive flux of O$_2$ into the cell is equal to the VO$_2$ (i.e. inflow = outflow; PcellO$_2$ is stable at steady state), and since the MBF establishes the high part of the pressure gradient for the movement of O$_2$ into the cell (i.e. PcapO$_2$), at a given diffusive conductance a reduced MBF necessitates a lower PcellO$_2$ for the same VO$_2$ to be achieved. In other words, a reduced steady state MBF indicates that the lowered PcellO$_2$ environment that is established at the onset of exercise remains at steady state and thereby contributes to the perceived difficulty of exercise.

Only five published studies to date have examined steady state MBF in persons with T2DM, and all examined MBF during leg exercise. First, Kingwell and colleagues (2003) measured the leg blood flow responses to moderate-intensity exercise (25-min supine cycling at 60% VO$_2$ peak) in subjects with T2DM and controls matched for age, VO$_2$ peak and body weight. They found that the steady state leg blood flow was significantly lower in the T2DM participants versus the controls (76). Similarly, Lalande and colleagues (2008) used Magnetic Resonance Imaging (MRI) to measure femoral
arterial blood flow during low-intensity leg extensor exercise (contralateral leg extensions with 1.5-kg ankle weights at a rate of 60±5rpm for ~15min). They demonstrated that steady state femoral blood flow, indexed to lean thigh mass, was significantly lower in the T2DM group versus healthy controls matched for a variety of characteristics including age, BMI, and fat-free mass (81). Likewise, early analysis of recent work in our lab demonstrated a reduced steady state MBF during low-intensity leg kicking exercise, as well as a reduced MBF during a progressive exercise test (unpublished observations).

Additionally, some rudimentary work by Menon and colleagues (1992) demonstrated that MBF (measured by $^{133}$Xe clearance) immediately following plantar and dorsiflexion exercise (i.e. post-exercise MBF) was significantly reduced in persons with diabetes versus controls (107), and Young and colleagues (1991) found that the MBF response to 5 minutes of cycle ergometer exercise at 75W was significantly lower in persons with diabetes (184). These two studies are somewhat limited in their generalizability, however, as both persons with Type I Diabetes Mellitus (T1DM) and T2DM were included, and some participants were smokers; it has been demonstrated that hemodynamic responses may differ between persons with T1DM and T2DM (41), and smoking affects blood vessel responses (23). Nonetheless, the findings from these studies are consistent with the more recent results of Kingwell et al. (76) and Lalande et al. (81). Together, these studies demonstrate a reduced steady state MBF in persons with T2DM at both low and moderate exercise intensities.

In contrast, Martin and colleagues (1995) found no difference in exercising leg blood flow between T2DM and control subjects (matched for age, BMI and VO$_2$max)
during 40 minutes of bicycle exercise at 60% of maximal oxygen uptake (98). It has been suggested that this discrepancy may be due to the recruitment of a fitter cohort (peak VO$_2$ achieved by participants was on average ~1L/min greater than that of the participants in the study by Kingwell and colleagues) (76). In general, most studies show a reduced steady state MBF in persons with T2DM versus controls at a variety of work rates.

It is worth mentioning here that two studies have been cited as showing a reduced VO$_2$ at constant sub-maximal work rates in T2DM (7; 126). In both of these studies, however, participants were completing graded exercise tests where the work rate was increased every one (7) or two minutes (126). Given that there are consistent observations of slowed VO$_2$ kinetics in persons with T2DM (9; 18; 127), it is likely that these participants simply had not yet reached steady state at the time of measurements. It can be speculated that given more time to adjust, VO$_2$ would reach the same values between T2DM and control participants in these studies. This is substantiated by the fact that the VO$_2$ was only statistically significantly different at some work rates but not others (7; 126).

Thus it is apparent that there are defects in convective O$_2$ delivery that are present both at the onset of exercise and during the steady state. It will be important to understand the mechanisms of these impairments so that appropriate interventions can be developed.

**Convective O$_2$ Delivery - Potential Mechanisms of Impairment**

The precise mechanisms of reduced MBF during exercise in persons with T2DM have not yet been elucidated, although there are a number of probable contributors.
MBF is a function of the pressure gradient from the arteries to the veins and the conductance of the blood vessels (i.e. vascular conductance; VK) (Figure 2). Since the pressure in the veins is minimal, mean arterial pressure (MAP) is often taken to directly represent the magnitude of this gradient, and MAP is itself a function of cardiac output (CO) and total systemic vascular conductance (TVK). VK is the inverse of resistance and it represents the degree to which flow is allowed. VK is described by the inverse of Poiseuille’s Law for resistance to flow through a tube: \( VK = \frac{\pi r^4}{8L\eta} \), where \( L \) is the length of the blood vessels (relatively constant in an individual), \( r \) is the radius of the blood vessels (determined by the interaction of vasodilator and vasoconstrictor influences and the primary determinant of VK in the systemic circulation), and \( \eta \) (“eta”) is the viscosity of the blood (determined by the hematocrit, the protein content of the plasma and the mechanical properties of the RBCs) (148). That is, conductance for flow increases as the length of the blood vessels or viscosity of the blood decrease, and as the radius of the blood vessels increases. Thus any factor affecting MAP or VK has the potential to alter MBF, from the volume of blood the heart pumps out per beat to the degree of dilation of the blood vessels.

In T2DM, it is probable that there are many contributing factors to the impaired MBF, including: 1) a central (cardiac) contribution, 2) a peripheral (vascular) contribution, and 3) altered hemorheological properties.

**Impaired MBF - Central (Cardiac) Contribution**

Impaired cardiac function, manifest as a reduced CO (due to either a reduced heart rate (HR), stroke volume (SV), or both), would affect MBF via its influence on
MAP, a key regulated variable of the cardiovascular system. CO represents the “inflow” to the large arteries, while the “outflow” is determined by the degree of dilation of the peripheral vasculature. A reduced CO, either transiently at the onset of exercise or during the steady state, would therefore necessitate a compensatory vasoconstriction in order to maintain MAP. Thus a reduced MBF could result from vasoconstriction, secondary to impaired cardiac function. Consistent with this notion, Ferraro and colleagues (1993) demonstrated that patients with T2DM had reduced CO and increased systemic vascular resistance responses compared with control subjects at rest (vascular resistance was calculated as: \( VR = \frac{MAP}{CO \cdot 80} \)) (41).

Indication of a central contribution to the impairment in MBF during exercise in persons with T2DM, however, has not been consistent. For instance, Regensteiner et al. (1998) demonstrated slowed HR kinetics at the onset of exercise in persons with T2DM (127), while Bauer et al. (2007) showed no difference in HR kinetics between persons with T2DM and healthy, matched individuals (9). Similarly, one study revealed that T2DM participants had significantly lower HR values than healthy subjects at the same relative submaximal exercise intensities (7), while another showed that HR was actually higher in T2DM participants than controls and was able to compensate for a reduced SV such that CO was not different between groups (81). Thus the role of a cardiac contribution to the reduced exercising MBF in T2DM is unclear.

Importantly, a lower exercising leg blood flow has been observed in patients with T2DM in the absence of a difference in CO compared to matched controls (81). This indicates that while central factors may play a role in some situations, they are not the only determinants of the impairment in MBF; peripheral factors must also play a role.
Impaired MBF – Peripheral (Vascular) Contribution

There are several possible vascular contributors to the reduced exercising MBF in persons with T2DM, including: 1) endothelial dysfunction, 2) heightened sympathetic nervous system-mediated vasoconstriction, and 3) dysfunction of the RBC-O₂ Sensor mechanism of feedback regulation of MBF.

Endothelial Dysfunction

The most common mechanism cited for reducing exercising MBF in persons with T2DM is an impaired endothelial function (9; 76; 81). The endothelium, the innermost cellular lining of the blood vessels, plays an important role in vasoregulation both at rest and during exercise. Endothelial cells respond to both mechanical forces (e.g. shear stress) and pharmacological agents (e.g. acetylcholine; ACh) by releasing factors into the interstitium [namely nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF)], that act on the smooth muscle in the vessel walls to cause relaxation (and therefore blood vessel dilation) (30). Thus specifically with respect to vasoregulation, “endothelial dysfunction” may be defined as “a state in which there is a reduction in nitric oxide (NO) bioactivity and increase in activity of mediators that favour vasoconstriction” (89). The bioavailability of NO is determined by the balance between the inflow and outflow of NO to and from the interstitial space, according to the conservation of mass model. The “inflow” of NO is represented by the endothelial production of NO, which is determined both by the availability of L-arginine (the precursor molecule to NO), and the activity of endothelial nitric oxide synthase (eNOS), the endothelial enzyme responsible for the synthesis of NO. The “outflow” of NO is
represented by the rate of NO degradation, which occurs as a function of its half-life and via its reaction with reactive oxygen species (ROS), such that the greater the concentration of ROS, the faster the breakdown of NO (Figure 5) (58; 176). Thus a reduced NO bioavailability may be the result of either a reduced NO production, an increased NO degradation, or both. Impaired endothelium-dependent dilation has been demonstrated in various vascular beds in humans with T2DM (39; 76; 125; 179), and it is possible that this dysfunction contributes to the attenuated exercise hyperemia in this population (76).

**Figure 5.** Influence of T2DM on pathways of endothelial nitric oxide (NO) generation and degradation. Proposed mechanisms of endothelial dysfunction in T2DM include: 1) increased production of reactive oxygen species (ROS) and resultant increase in NO degradation, 2) increased presence of advanced glycosylation end products, which also serve to degrade NO, and 3) activation of protein kinase C, which leads to both increased production of vasoconstrictor prostanoids and phosphorylates muscarinic receptors on the endothelium (180). Ach = acetylcholine; eNOS = endothelial NO synthase; L-Arg = L-arginine; M = muscarinic receptor; NO = nitric oxide; ROS = reactive oxygen species. Illustration adapted from Walther et al., 2004 (176).
Endothelial function is typically assessed by measuring the magnitude of increase in flow or arterial diameter in response to stimulation of NO release, either via stepwise infusions of endothelium-dependent vasodilator substances (such as ACh) or by a reactive hyperemia protocol (i.e. flow-mediated dilation, or FMD, stimulated by temporary arterial occlusion) (89). Control tests of vascular smooth muscle responsiveness (i.e. endothelium-independent dilation) are often done in conjunction with these assessments (89). In such tests of endothelial function, healthy arteries generally dilate ~5-15%, and brachial artery blood flow increases in the range of 5- to 6-fold (176), while markedly reduced responses have been observed in persons with T2DM (125).

For instance, Regensteiner et al. (2003) demonstrated that women with T2DM had blunted FMD and forearm blood flow responses following 5 minutes of blood pressure cuff-induced ischemia, compared to healthy controls (125). In a similar protocol, Enderle et al. (1998) demonstrated reduced post-ischemic forearm blood flow and impaired FMD in persons with T2DM, with no differences between groups in response to sublingual glyceroltrinitrate (GTN), an exogenous NO donor (39). Likewise, Kingwell et al. (2003) demonstrated reduced leg blood flow responses to endothelium-dependent (ACh) but not endothelium-independent (sodium nitroprusside; SNP, an exogenous NO donor) vasodilators in persons with T2DM versus healthy controls (76). These studies indicate that the dysfunction is specific to the endothelium and its ability to produce NO, and that the vascular smooth muscle responsiveness is preserved in persons with T2DM.

In contrast however, Williams et al. (1996) found reduced forearm blood flow responses to both an endothelium-dependent (methacholine chloride) and endothelium-
independent (SNP) vasodilator, but no impairment in response to a mechanistically different endothelium-independent vasodilator (verapamil) (179). Since vasodilation was impaired in response to both endogenous and exogenous NO donors, but not to verapamil (a calcium channel blocker that acts directly on the vascular smooth muscle), it could be concluded that the endothelial dysfunction in T2DM may be due to both a reduced production and an increased degradation of NO.

That there is an impaired vascular responsiveness to exogenous NO donors in some studies (179) but not others (39; 76) may be related to the degree of metabolic control. It has been shown that there is a progressive reduction in endothelial function from the early stages of insulin resistance to overt T2DM in the coronary circulation, whereas endothelium-independent dysfunction (reflective of structural changes) was significantly reduced only in those with overt T2DM (121). It has been suggested that this demonstrates a progressive dysfunction with worsening metabolic control (86). Consistent with this concept, vascular reactivity to exogenous NO donors was preserved in studies of persons with T2DM having adequate metabolic control (39; 76), but was impaired in the presence of lesser metabolic control (178) with HbA1c values of ~6-9% and 11% respectively (see “Leftward Shift of the Hb-O₂ Dissociation Curve” for identification of how HbA1c is indicative of the degree of metabolic control).

Interestingly, an attenuated response to methacholine chloride, with no difference in response to verapamil, is also observed in healthy individuals in a state of acute hyperglycemia (180), suggesting that the elevated blood glucose in persons with T2DM may contribute to their endothelial dysfunction. Although the precise etiology of endothelial dysfunction in T2DM is uncertain, three possible mechanisms have been
proposed: 1) an increased production of ROS which would contribute to increased NO degradation, 2) an increase in advanced glycosylation end products, which also serve to degrade NO, and 3) activation of protein kinase C, which could result in increased generation of vasoconstrictor prostanoids and/or phosphorylation of endothelial cell muscarinic receptors (stimulation of these results in NO production) (180) (Figure 5). Thus endothelial dysfunction in T2DM is probably a function of both a reduced NO production and an increased NO degradation.

Again, a dysfunctional endothelium would be expected to impair exercising MBF, and the finding of a significant correlation ($r = 0.54$) between the percentage increase MBF in response to leg exercise and ACh in persons with T2DM is consistent with this notion (76).

**Enhanced Sympathetic Nervous System-Mediated Vasoconstriction**

A second possible peripheral contributor to the reduced exercising MBF in persons with T2DM is an increase in sympathetic nervous system (SNS)-mediated vasoconstriction. SNS activity both contributes to basal vascular tone and causes constriction in the arterial resistance vessels via the action of norepinephrine (NE) on $\alpha$-receptors (108). Over-activity of the SNS has not yet been directly investigated as a mechanism of reduced exercising MBF in persons with T2DM, however there are 2 lines of evidence to suggest that this may be the case.

First, there is evidence for heightened SNS-mediated vasoconstriction at rest in persons with T2DM (67). Intraarterial infusion of phentolamine, an $\alpha$-antagonist, elicited a greater degree of vasodilation in persons with T2DM versus controls, indicating a
higher basal vascular tone (67). Additionally, the T2DM participants had a greater percent decrease in forearm blood flow in response to intrabrachial artery infusion of NE, in the presence of similar SNS activation (estimated from $^3$H-NE kinetics). This demonstrates a greater responsiveness of the vasculature to a given amount of NE at rest. If this increased SNS-mediated vasoconstriction is present during exercise, then it may represent a mechanism of reduced exercising MBF.

Second, SNS-mediated vasoconstriction is necessary during exercise, even in healthy individuals, for the maintenance of MAP (134). In healthy humans, local factors in the muscle such as NO blunt this vasoconstrictor effect, such that the restraint of blood flow to active muscle is minimized; this phenomenon is termed “functional sympatholysis” (169). If NO availability is reduced in persons with T2DM, either via reduced production or enhanced degradation, as suggested by studies of endothelial function (76), then this blunting effect may also be reduced such that the SNS constrains MBF to a greater degree than in healthy individuals.

Thus there is evidence to suggest that heightened SNS-mediated vasoconstriction may contribute to the reduced exercising MBF in persons with T2DM; this mechanism remains to be studied further.

*RBC-O$_2$ Sensor Dysfunction*

Finally, and most relevant to the current investigation, evidence suggests that the impairment in MBF during exercise in T2DM may be due at least in part to a defect in the RBC-O$_2$ Sensor mechanism of blood flow regulation. This concept will be described more fully in Section 3 of this review.
Lastly, altered hemorheological properties may contribute to the impaired MBF in persons with T2DM. Hemorheology is the study of the flow and deformation behaviour of blood and its individual components (e.g. RBCs, plasma) (8). It is concerned primarily with blood viscosity and the mechanical properties of RBCs (including deformability and aggregation behaviours), both of which can influence proper tissue perfusion (8). Altered flow behaviour has been observed in persons with T2DM, which may contribute to the reduced bulk MBF in this population (86; 105).

Simplistically, viscosity can be thought of as the “thickness” of a fluid, where a thicker fluid has a greater resistance to flow (or a reduced conductance for flow; recall, $VK = \frac{\pi r^4}{8L\eta}$, where $\eta$ is viscosity) (148). More precisely, viscosity is defined as the ratio between shear stress (i.e. the force per unit area acting parallel to a surface) and shear rate (i.e. the movement of material – or deformation – resulting from shear stress) (8; 150). In other words, the lower the flow resulting from a given shear stress (or force), the greater the viscosity; the thicker the fluid, the less flow for a given force. Blood is a “non-Newtonian” fluid, meaning that this ratio (shear stress / shear rate) is not constant; blood responds differently to varying amounts of applied force (105). Specifically, blood exhibits “shear-thinning” behaviour, whereby the viscosity decreases as shear rate is increased (8) (Figure 6). Thus it is important to measure blood viscosity across the range of shear rates found in the vascular system (150). Blood viscosity has been found to be greater in persons with T2DM than in healthy controls across the physiological range of shear rates (92; 126; 150); on average, blood viscosity is ~20% higher in persons with
Figure 6. Shear-rate and viscosity relationship for blood in healthy individuals (solid line) and persons with T2DM (dashed line). Blood is a non-Newtonian fluid with shear thinning behaviour, meaning that its viscosity increases exponentially as shear rates approach zero, and its viscosity approaches zero at high shear rates. Blood viscosity is ~20% higher in individuals with T2DM across the physiological range of shear-rates (150).

T2DM versus controls (150). This means that all else being equal (i.e. for a given driving pressure for flow (MAP) and a given degree of blood vessel dilation), MBF will be less in persons with T2DM due to an increase in blood viscosity.

Blood viscosity is dependent on the composition of the blood (i.e. hematocrit and protein content), as well as the behaviour of RBCs within the blood (8). RBC behaviour refers to both their degree of deformability (i.e. changes in shape in response to applied forces, important for remaining streamlined during flow through large vessels and for passing through the narrow vessels of the microcirculation) and aggregation (i.e.
grouping of RBCs, causing disruptions in flow streamlines) (8). In persons with T2DM, increased glycosylation of RBC membrane proteins (i.e. nonenzymatic linkage of glucose to proteins (152)) reduces their deformability, thereby increasing the apparent viscosity of the blood (118; 144). In addition, increased RBC aggregation (144) and the presence of abnormal levels of plasma proteins, namely a decline in albumin and elevation of several globulins (105; 106), contribute to the increase in viscosity in T2DM (144). These changes in T2DM appear to be relatively independent of the onset and duration of diabetes, but depend on the existing degree of metabolic control (105; 144).

While the existence of altered hemorheological properties in T2DM is certain, their relative importance with respect to MBF in vivo is unclear. For instance, although whole blood viscosity was shown to be higher in T2DM than control subjects, there was no correlation between viscosity and exercise performance in a graded treadmill exercise protocol (126), indicating that the increased viscosity did not cause a functional limitation. In general, the relative importance of hemorheological properties in pathological conditions is still uncertain (8), however it is important to acknowledge that these characteristics exist and therefore have the potential to contribute to the overall dysfunction.

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Therefore in persons with T2DM it is probable that there are several contributing factors to the impairment in exercising MBF. These factors may include a cardiac contribution (i.e. reduced CO), vascular contribution (impaired endothelial function, enhanced SNS-mediated vasoconstriction, and/or RBC-O₂ Sensor dysfunction), and
altered hemorheological properties (reduced deformability and increased aggregation of RBCs, and increased blood viscosity).

O$_2$ Diffusive Capacity

As indicated in Section 1, convective O$_2$ delivery does not determine PcellO$_2$ alone, but rather interacts with the diffusive capacity of the muscle for O$_2$. Recall that diffusive flux occurs according to Fick’s Law (Figure 2), whereby the diffusive flux of O$_2$ into the muscle is a function of both the pressure gradient for O$_2$ from the skeletal muscle capillaries to the inside of the myocytes, and the diffusive conductance of the muscle. The high part of the pressure gradient for diffusive flux is determined by the bulk delivery of O$_2$ to the muscle (i.e. convective O$_2$ delivery), as well as factors affecting the affinity of hemoglobin (Hb) for O$_2$ at a given bulk O$_2$ delivery. Diffusive conductance is determined by the surface area available for diffusion, as established by the ratio of the capillary surface area to the muscle fibre surface area (100), and the thickness of the barrier for diffusion or the distance across which the O$_2$ must diffuse (175). Importantly, for a given convective O$_2$ delivery, and a given rate of O$_2$ consumption, PcellO$_2$ will be higher if diffusive conductance is higher and vice versa. In persons with T2DM, the diffusive flux of O$_2$ may be hindered by: 1) a leftward-shift in the Hb-O$_2$ dissociation curve (affecting the pressure gradient for diffusion), and 2) a reduced diffusive conductance brought about by capillary rarefaction. Both of these factors would be expected to interact with the established impairment in convective O$_2$ delivery to result in a lower PcellO$_2$ and reduced exercise tolerance.
**Leftward Shift of the Hb-O₂ Dissociation Curve**

“Glycosylated Hb”, or HbA1c, is a variant of Hb that is formed via the non-enzymatic binding of glucose to the Hb protein (152). The rate of formation of HbA1c is directly proportional to the ambient glucose concentration (48), and thus the proportion of Hb that is glycated is indicative of the average blood glucose levels over the past ~2-3 months, the average RBC lifespan (48). As a metabolic disorder involving poor blood glucose regulation, it is expected for HbA1c levels to be higher in persons with T2DM. An HbA1c of <9% is considered adequate glycemic control in the context of diabetes, whereas ~5% is considered normal for healthy individuals (73). Importantly, the glycation of Hb increases its affinity for O₂, effectively causing a leftward shift of the Hb-O₂ dissociation curve (35).

To elaborate, 2,3-diphosphoglycerate (2,3-DPG) is present in human RBCs, and it controls the ease with which Hb releases O₂, such that an increase in the concentration of 2,3-DPG reduces the O₂ affinity of Hb and vice versa (91). The HbA1c variant of Hb, however, has an impaired binding of 2,3-DPG due to its change in conformation (35). As a result, the offloading of O₂ from HbA1c is hindered and the HbA1c variant of Hb has an increased affinity for O₂ (35). Thus the poorer the metabolic control, the greater the proportion of Hb that is glycated, and the greater the change in PcapO₂ that is required for O₂ to offload from the RBCs. In other words, the “high” part of the pressure gradient for the flux of O₂ from the blood to the myocyte is reduced in T2DM due to an increased proportion of HbA1c and its greater affinity for O₂.
Capillary Rarefaction

Histological analysis of skeletal muscle in T2DM reveals a reduction in capillary density (95). Termed “structural capillary rarefaction”, this reduced number or combined length of capillaries effectively reduces the surface area available for diffusion (86). Even in the absence of structural capillary rarefaction, there is evidence of “functional capillary rarefaction” in persons with T2DM, whereby the anatomical capillary density itself is not changed, but the number of perfused capillaries is reduced. For instance, Padilla and colleagues (2006) used intravital microscopy to examine the capillaries in the spinotrapezius muscle of Goto-Kakizaki (GK) Type II diabetic rats, a rat model considered to be highly representative of the Type II Diabetic condition in humans (118). They found a ~29% reduction in the number of perfused capillaries in the T2DM versus control rats (i.e. a reduced functional capillary density), even in the absence of any visible structural changes. Additionally, there were no visible differences (e.g. luminal diameter) in the perfused versus non-perfused capillaries in the T2DM rats. This provides evidence for a functional capillary rarefaction in T2DM, and it has been demonstrated that prolonged vessel nonperfusion can progress to its structural loss (120). A reduction in capillary density, either functionally or structurally, is significant because it not only effectively reduces the surface area for diffusion, but increases the distance that O₂ must diffuse in order to reach the muscle fibres that are no longer supported by immediately adjacent capillaries.

In terms of cause and effect, it is difficult to determine what causes the microvascular abnormalities in T2DM muscle. Capillary density has been shown to be inversely related to fasting plasma glucose and fasting insulin, and positively related to
insulin sensitivity in persons without T2DM (87), suggesting that the metabolic environment of persons with T2DM contributes to the generation of microvascular changes. On the other hand, a reduction in capillary density contributes to the pathogenesis of insulin resistance, as insulin-mediated capillary recruitment is important for the uptake of glucose from the blood; a reduction in capillary density or impaired recruitment could contribute to insulin resistance by reducing glucose uptake (174). It has been suggested that these processes are not mutually exclusive in T2DM, but rather act cyclically, such that a reduction in insulin sensitivity serves to cause a decrease in capillary recruitment and/or structural capillary rarefaction, which serves to worsen insulin resistance, which causes further damage to the microvasculature, and so on (86).

Regardless of the cause, both structural and functional abnormalities at the microvascular level serve to reduce the diffusive conductance for the flow of O₂ into the cells, and therefore may contribute to the exercise intolerance in persons with T2DM.

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Therefore the diffusive capacity of the muscle for O₂ is reduced in persons with T2DM via an increased Hb-O₂ affinity (affecting the pressure gradient for diffusion) and capillary rarefaction (affecting the surface area for diffusion and distance across which O₂ must travel to supply some muscle fibres). This means that for a given convective O₂ delivery, in order to attain the same O₂ flux into the myocyte (i.e. to match the same rate of “outflow” – VO₂) PcellO₂ must be lower. Given that the convective O₂ delivery is in fact lower at a given VO₂ in persons with T2DM, a reduced diffusive conductance would necessitate an even greater reduction in PcellO₂ in order for the pressure gradient to
compensate to maintain diffusive flux. Thus concomitant impairments in convective O\textsubscript{2} delivery and diffusive O\textsubscript{2} capacity may interact to impose a reduced PcellO\textsubscript{2} and to accelerate fatigue processes in this population.

**Metabolic O\textsubscript{2} Consumption**

A discussion of the O\textsubscript{2}-related limitations to exercise tolerance would be remiss without mention of the actual consumption of O\textsubscript{2} within the cell. Thus in brief, the rate of O\textsubscript{2} consumption in mitochondrial respiration is determined by the combined drive from the phosphate energy state and redox state of the cell, and PcellO\textsubscript{2}, as mentioned earlier (the “Net Drive” concept). In addition, the mitochondrial respiration rate is affected by the number of mitochondria and the availability of the relevant enzymes (165). According to the law of mass action, the same flux can be achieved for a lower [substrate]/[product] ratio if the concentration of enzymes is increased. Thus increasing the mitochondrial content of the muscle (as occurs with aerobic training) (164), or increasing the activity of the relevant enzymes for a given mitochondrial content, would allow the same amount of ATP to be produced aerobically for lower concentrations of ADP and Pi.

Conversely, in T2DM, there is evidence for a reduced mitochondrial volume (75; 131), a functional impairment in the available mitochondria such that the decrement in activity of the electron transport chain is greater than that in mitochondrial content (131), and an imbalance between glycolytic and oxidative enzyme capacities (75; 149). Each of these factors could serve to limit mitochondrial O\textsubscript{2} consumption (e.g. slowed VO\textsubscript{2}
kinetics), or to cause a greater perturbation of the intracellular environment in response to a given workload (i.e. increase the phosphate energy state) and thereby contribute to the development of fatigue.

Many of the studies of mitochondrial oxidative function in T2DM are limited, however, by failing to account for physical activity level (75), and muscle fibre type composition (149). Aerobic exercise has been shown to stimulate increases in mitochondrial size and content (164) as well as increases in activity of oxidative enzymes (146); thus the decrements in these factors observed in T2DM could be due to a sedentary lifestyle. Similarly, there is generally a trend towards a reduced type I and increased type II fibre content in T2DM (44; 95); since glycolytic and oxidative capacities vary by fibre type, the imbalance between glycolytic and oxidative capacities observed in T2DM could be due to their differences in muscle morphology and not be reflective of a functional impairment per se (122). In a comprehensive review, Rabol and colleagues (2006) concluded the following:

“Several studies show reduced activity of oxidative enzymes in skeletal muscle of type 2 diabetics. The reductions are independent of muscle fibre type, and are accompanied by visual evidence of damaged mitochondria. In most studies, the reduced oxidative enzyme activity is explained by decreases in mitochondrial content; thus, evidence of a functional impairment in mitochondria in type 2 diabetes is not convincing. These impairments in oxidative function and mitochondrial morphology could reflect the sedentary lifestyle of the diabetic subjects.” (122)

Accordingly, it is important to acknowledge that alterations in the mitochondrial oxidative capacity in skeletal muscle of persons with T2DM could contribute to the slowed VO$_2$ kinetics (127) and altered skeletal muscle energy metabolism (143) observed in persons with T2DM.
As has been demonstrated, the exercise intolerance experienced by persons with T2DM is extremely complex and could involve factors related to the convective delivery of O₂ to skeletal muscle, the diffusive capacity of the muscle for the movement of O₂ into the myocytes, and the availability and functioning of metabolic machinery to consume that O₂ inside the myocytes. It is important to recognize however that although these conditions of impairment exist, no study has selectively reversed them and measured the effect on exercise tolerance. Table 1 contains a summary of the potential contributions to exercise intolerance in T2DM discussed herein.

**Table 1. Summary of Potential Contributions to Exercise Intolerance in T2DM**

<table>
<thead>
<tr>
<th>Potential Contributions to Exercise Intolerance in T2DM</th>
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<tbody>
<tr>
<td><strong>Convective O₂ Delivery</strong></td>
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<tr>
<td>• Slowed microvascular blood flow kinetics (9)</td>
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<tr>
<td>• Lower resting PmvO₂, and faster PmvO₂ kinetics at the onset of exercise (11; 117)</td>
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<tr>
<td>• Reduced steady state MBF (76; 81)</td>
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<tr>
<td>• Central Contributors:</td>
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<tr>
<td>o Slowed HR kinetics (127)</td>
</tr>
<tr>
<td>o Reduced CO during submaximal exercise (136)</td>
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<tr>
<td>o Reduced steady state HR (7)</td>
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<tr>
<td>• Peripheral Contributors:</td>
</tr>
<tr>
<td>o Endothelial dysfunction (30)</td>
</tr>
<tr>
<td>o Heightened SNS-mediated vasoconstriction (67)</td>
</tr>
<tr>
<td>o RBC-O₂ Sensor dysfunction (157)</td>
</tr>
<tr>
<td>• Hemorheological Contributors:</td>
</tr>
<tr>
<td>o Increased blood viscosity (150)</td>
</tr>
<tr>
<td>o Reduced RBC deformability and increased aggregation (118)</td>
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<tr>
<td><strong>Diffusive O₂ Capacity</strong></td>
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<tr>
<td>• Increase in HbA1c, causing an increased affinity of Hb for O₂ (i.e. a leftward shift of the Hb-O₂ dissociation curve) (35)</td>
</tr>
<tr>
<td>• Functional and/or structural capillary rarefaction (i.e. ↓ capillary-to-muscle-fibre surface area ratio) (118)</td>
</tr>
<tr>
<td><strong>Metabolic O₂ Consumption</strong></td>
</tr>
<tr>
<td>• Reduced mitochondrial size / volume (75)</td>
</tr>
<tr>
<td>• Functional impairment in mitochondria (131)</td>
</tr>
<tr>
<td>• Imbalance between glycolytic and oxidative capacities (149)</td>
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</tbody>
</table>
We will now turn our attention to the RBC-O₂ Sensor mechanism of MBF regulation and its particular role as a contributor to the reduced steady state MBF and as a target for intervention in persons with T2DM.

**The RBC-O₂ Sensor – Function and Impairment in T2DM**

The RBC-O₂ Sensor - Function

The matching of O₂ supply to demand requires that MBF be tightly regulated (167). In 1995, Ellsworth and colleagues proposed a mechanism of blood flow regulation in which the RBC functions as a roving O₂ sensor that evokes ATP-mediated vasodilation as it moves through areas of high O₂ demand – the RBC-O₂ Sensor mechanism of MBF regulation (37). Since its initial suggestion, this mechanism has gained much empirical support as a means of matching O₂ delivery to metabolic need in skeletal muscle (for a comprehensive review see Ellsworth and colleagues, 2009 (36)). Figure 7 illustrates the sequence of events by which the RBC-O₂ Sensor is purported to function in terms of matching O₂ supply to O₂ demand in active skeletal muscle. (Note: Figure 7 includes parts A through D and spans the following three pages).
**Figure 7.** Illustration of the RBC-O₂ Sensor Mechanism.

(A) **Resting Muscle: \( O_2 \) supply = \( O_2 \) demand.**
Schematic representation of a blood vessel supplying muscle tissue (oval). Red squares in blood vessel represent arbitrary “units” of blood; purple dashes adjacent to Hb represent attached \( O_2 \) molecules. For illustrative purposes, this diagram shows 3 \( O_2 \) molecules being consumed by the muscle at rest, so one \( O_2 \) molecule must come off of each of the 3 available Hb molecules. At rest, there is an equilibrium between \( O_2 \) consumption and delivery, such that blood leaves the muscle with a given fraction of its \( O_2 \) content left.

(B) **Onset of Exercise: \( O_2 \) supply < \( O_2 \) demand.**
At the onset of exercise, the \( O_2 \) consumption in the muscle increases, so the \( P_{cell}O_2 \) decreases. This increases the pressure gradient for the movement of \( O_2 \) from the capillary to the cell (\( \Delta P \uparrow \)), and so the rate of diffusion from the blood into the muscle increases. Since more \( O_2 \) is now going out of the blood and into the muscle, this means that the amount of \( O_2 \) left in a given unit of blood that has passed through the muscle will decrease at a given blood flow. Thus in the figure, 2 \( O_2 \) molecules have come off of each Hb, and the blood leaves the muscle with a lower Hb-\( O_2 \) saturation.
Figure 7 (C) RBC-O₂ Sensor Function: ATP-Mediated Vasodilation.
When Hb loses O₂, it undergoes a conformational change that causes the RBCs to produce and release ATP (72); the amount of ATP released is in proportion to the degree of saturation (157) (i). This occurs via a pathway (ii) involving G₁ protein activation of adenylyl cyclase (AC), which catalyzes the conversion of ATP to cAMP, and is followed downstream by increased ATP release (36) (Adapted from Sprague et al., 2007 (157)). Note that this pathway is also activated by mechanical deformation of RBCs (155), as occurs with increases in blood flow velocity and/or reductions in vessel diameter (e.g. when RBCs traverse the microcirculation) (8). Importantly, ATP is an indirect vasodilator (iii); it diffuses into the plasma where it can bind to receptors (P₂Y purinergic receptors) on the vascular endothelium, thereby stimulating production and release of nitric oxide (NO) and other vasodilators (e.g. prostacyclin, endothelium derived hyperpolarizing factor) and evoking a local and conducted vasodilation and increase in blood flow (19; 102). Vertical double-headed arrow represents vasodilation; graded block arrow signifies conduction of this vasodilation. ? = unidentified mechanism; + = stimulation.
Figure 7 (D) Steady State Exercise: $O_2$ supply = $O_2$ demand.

At steady state, the vasodilation has caused an increase in blood flow relative to at rest, such that more “units” of blood are moving through the muscle in the same amount of time. Therefore less $O_2$ must be taken from each unit of blood, reducing the stimulus for ATP release, and again there is equilibrium between $O_2$ consumption and $O_2$ delivery such that the blood leaves the muscle with a given fraction of its $O_2$ content left.
There are several lines of evidence in support of the RBC-O₂ Sensor mechanism; for brevity, they will be number-listed here:

I. *ATP increases in venous effluent of active muscle (42; 51), and the [ATP] is proportional to the exercise intensity (51).* This demonstrates that the purported vasodilatory stimulus of the RBC-O₂ Sensor mechanism (i.e. ATP) is present during exercise. Additionally, this illustrates that the magnitude of the stimulus for vasodilation (ATP) correlates with the magnitude of the metabolic demand (exercise intensity), and thereby provides support for the role of ATP in matching blood flow to metabolic needs in active skeletal muscle.

II. *Intraluminal application of ATP causes a dose-dependent increase in vessel diameter that is conducted upstream of the site of application (26; 37; 51; 102; 133); this increase in diameter is accompanied by an increase in RBC flux (26; 37).* A local and conducted ATP-mediated vasodilation has been demonstrated in hamster arterioles (37; 102), venules (26) and capillaries (37), and in the human leg and forearm via femoral (51) and brachial (133) artery ATP infusion respectively. In other words, in various vessel types, local application of ATP has been shown to cause both local and conducted vasodilation as evidenced by measurement of blood vessel diameter upstream of the site of ATP application. From these studies, a few important points are evident. First, the amount of ATP infused (~10⁻⁶ M) (26; 102), and capable of evoking the conducted vasodilation, was of the same order of magnitude as would be expected to be released from RBCs in response to a low O₂ environment (37). Second, the infusion of ATP into the femoral artery at rest in humans produced similar
increases in thigh blood flow as those observed during dynamic exercise (51). Third, that the ATP-mediated dilation was conducted upstream is critical as it has been shown that only when vasodilation extends beyond the local site of initiation does a substantial increase in blood flow result (80). Together, these observations provide support for the role of ATP in vasoregulation.

III. The vasodilatory response to intraluminal ATP infusion occurs within milliseconds; this is a physiologically relevant time course (14; 157). This supports the potential physiological relevance of this mechanism.

IV. The increase in vessel diameter caused by intraluminal ATP application is blocked by systemic pretreatment with a NO-synthase inhibitor in some (26; 102), but not all (133) studies. This provides support for the proposed role of NO in this mechanism of blood flow regulation, while also indicating that NO is not the exclusive mediator of the response.

V. Only in the presence of RBCs does a low O\textsubscript{2} environment result in both vessel dilation and an increase in effluent ATP (34; 61). Dietrich and colleagues (2000) exposed rat cerebral arterioles to a low O\textsubscript{2} environment in the presence of RBCs or a dextran solution of identical viscosity (34). They demonstrated that only in the presence of RBCs did the vessel diameter and ATP effluent increase, suggesting that the ATP was from the RBCs and that they are necessary for the dilation to occur. Similarly, Hanson and colleagues (2008) exposed isolated rabbit striated muscle arterioles to a reduced
PO\textsubscript{2} in the presence or absence of RBCs (61). It was demonstrated that vessel diameter increased in response to a reduced PO\textsubscript{2} in the presence of RBCs, but actually decreased when the arterioles were perfused with the buffer solution. These studies provide evidence for the RBC as the source of dilation-inducing ATP, which is important as other potential sources of ATP are also present; for example, the endothelium has been shown to release ATP in response to shear stress (16). Since an increase in effluent ATP and vessel diameter in response to a reduced PO\textsubscript{2} only occurs in the presence of RBCs, this provides support for an essential role of the RBC in the ATP-mediated vasodilation.

VI. 

\textit{RBCs release ATP in response to conditions that are associated with reduced O\textsubscript{2} supply relative to demand: a reduced PO\textsubscript{2} and a decreased pH} (37). RBCs that are exposed to low PO\textsubscript{2} and low pH solutions (both of which reduce blood O\textsubscript{2} content) exhibit greater ATP efflux than those exposed to normoxic, normal pH solutions (37). In addition, it has been shown that RBCs release ATP in response to mechanical deformation (155), which occurs with an increase in blood flow velocity and/or reduction in vessel calibre such as in the microcirculation (8). This demonstrates that the conditions inside active skeletal muscle are conducive to the stimulation of ATP release from RBCs.

VII. 

\textit{ATP efflux and MBF are tightly coupled to the oxygenation state of Hb} (50-52; 72). Gonzalez-Alonso and colleagues (2001) demonstrated that exercising leg blood flow (LBF) responds to changes in Hb saturation but not to PO\textsubscript{2}, by measuring LBF during
one-legged knee extensor exercise (~40% peak power output) under conditions of normoxia, hypoxia, and carbon monoxide (CO) breathing in combination with either normoxia or hyperoxia (52). Hypoxia causes a decrease in O₂ content by reducing the O₂ saturation of Hb, while CO breathing reduces O₂ content by binding with Hb at an affinity of ~200x that of O₂ and maintaining Hb saturation (52). Thus these experimental manipulations afforded comparison of LBF under conditions of similar Hb saturation, but vastly different PO₂. It was found that the exercising LBF was similar under conditions of hypoxia, CO + normoxia, and CO + hyperoxia, despite large differences in PO₂ (~47-538 mmHg), and that LBF was closely associated with reductions in arterial oxyhemoglobin. In a similar study, Gonzalez-Alonso and colleagues (2002) again used hypoxia and CO to manipulate arterial O₂ content with and without affecting the saturation of Hb during incremental one-legged knee-extensor exercise (51). They determined that arterial O₂ content and LBF were similar during exercise in the CO + normoxia and hypoxia conditions, although the concentration of ATP in the venous effluent was significantly reduced in the CO + normoxia condition, indicating that ATP release is related to Hb saturation. In addition, it was shown that plasma ATP concentration was inversely related to venous O₂ content (51). In an even more recent study, the same research group used similar manipulations to demonstrate a strong inverse relationship between arterial O₂ content and leg blood flow (r² = 0.86) during knee extensor exercise at ~50% peak power (50). These studies agree with data from an isolated cell study, in which it was found that ATP efflux correlated more strongly with Hb saturation (r² = 0.88) than with PO₂ (r² = 0.54) (72). Together, these studies demonstrate that a reduction of PO₂ in the
absence of a conformational change in Hb is insufficient to stimulate ATP release, and that the oxygenation state of the RBC (i.e. Hb O2 saturation) plays a pivotal role in ATP-mediated skeletal muscle vasoregulation.

VIII. Direct activation of G\textsubscript{i} proteins (116) or adenylyl cyclase (155) results in increases in intracellular cAMP and ATP release from RBCs, and incubation of RBCs with pertussis toxin, a G\textsubscript{i} protein inhibitor prevents ATP release (116). This provides support for the proposed pathway for regulated ATP release from RBCs (157), and in particular demonstrates that increases in intracellular cAMP are required for this ATP release.

Thus the RBC-O\textsubscript{2} Sensor is a feedback mechanism of blood flow regulation that is capable of initiating an increase in blood flow and O\textsubscript{2} delivery to areas of increased metabolic demand (157). Accordingly, a defect in this mechanism could result in inadequate O\textsubscript{2} delivery to meet muscle metabolic needs and consequently a decrement in exercise tolerance.

The RBC-O\textsubscript{2} Sensor – Impairment in T2DM

There is evidence for impairment in the RBC-O\textsubscript{2} Sensor mechanism in persons with T2DM, and this may contribute to observations of reduced MBF and exercise intolerance (76; 81) in this population.

First, it has been demonstrated that RBCs of persons with T2DM are less deformable than those of healthy individuals due to increased glycosylation (118; 144).
Since mechanical deformation of RBCs is one of the stimuli for the release of ATP (155), this reduced deformability would be expected to reduce ATP efflux from RBCs under given blood flow velocity conditions.

Second, and perhaps more relevant to the matching of O₂ delivery to metabolic demand, RBCs of persons with T2DM have also been shown to have a diminished ATP release in response to decreased O₂ tension, and this impairment has been linked to a reduced expression of the membrane-bound Gi protein (61; 154; 155), an identified component of the signal-transduction pathway for ATP release from RBCs (157). For instance, Gi expression has been shown to be reduced in both animal models of T2DM (45; 101), and in the RBCs of humans with this disease (154; 156).

In a seminal study by Sprague and colleagues (2006), it was found that the expression of Gi was selectively reduced in RBCs of humans with T2DM, and that while incubation of RBCs from healthy humans with mastoparan 7 (MAS7), an activator of Gi, caused a ~7-fold increase in ATP release, this ATP release was significantly attenuated from RBCs of persons with T2DM (156). Moreover, incubation of RBCs with MAS7 resulted in a significant increase in cAMP only in RBCs of healthy humans. This provides support for the proposed mechanism of low O₂-content-induced ATP release, and for its impairment in persons with T2DM.

Furthermore, maximum ATP release was delayed from the RBCs of persons with T2DM versus healthy humans (~12 vs 8 mins respectively). Unfortunately only the time to maximum ATP release was reported and the overall time course was not shown; the time to maximum ATP release being on the order of minutes is far removed from the in
*vivo* time course of blood flow adjustment (141), and so the relevance of these findings is somewhat ambiguous. As a feedback mechanism of MBF regulation however, a slowed ATP release from RBCs may explain the sluggish microvascular blood flow response that is observed at the onset of exercise in persons with T2DM (9).

Importantly, the degree of impairment of MAS7-induced ATP release from RBCs was inversely related to the degree of glycemic control as determined by the concentration of HbA1c \( (r^2 = 0.72) \) (156). It has previously been demonstrated that exercise intolerance (assessed by exercise capacity on a treadmill test) is also inversely related to the concentration of HbA1c in persons with T2DM \( (r^2 = 0.54) \) (32). This indicates that the worse the glycemic control, the greater the impairment in ATP release from RBCs and the greater the exercise intolerance in persons with T2DM. Together, these observations suggest that the metabolic environment characteristic of T2DM plays a role both in the impaired ATP release from RBCs and in the exercise intolerance; it remains to be directly investigated whether the impairment in ATP release is causative of the reduced exercise tolerance.

Thus it is evident that RBCs of persons with T2DM have an impaired release of ATP in response to both mechanical deformation and exposure to reduced oxygen tension. This impairment is related to a reduced expression of G\(_i\) in RBC membranes and consequent reduction in intraerythrocyte cAMP, and is associated with the degree of glycemic control. This defect in the RBC-O\(_2\) Sensor mechanism may contribute to observations of impaired MBF in persons with T2DM.
Rationale for Caffeine as an Intervention

It has been established that there is impairment in the RBC-O$_2$ Sensor mechanism of MBF regulation in persons with T2DM, and that this may contribute to observations of reduced MBF and exercise intolerance in this population. As such, this mechanism represents a target for therapeutic intervention.

As discussed, the impairment in RBC-O$_2$ Sensor functioning in persons with T2DM is related to a reduced expression of $G_i$, a protein involved in the signal transduction pathway for the release of ATP from RBCs (61; 155). Mature RBCs, however, lack the capacity to synthesize new proteins and thus it is not possible to stimulate additional $G_i$ synthesis or expression as a mode of therapy (157). Consequently, a pharmacologic intervention would need to be targeted downstream of the $G_i$ component of the signal transduction pathway, namely to stimulate AC or otherwise increase cAMP within the RBC. Caffeine, a methylxanthine, is a drug that has the potential to meet these criteria.

First, caffeine is a phosphodiesterase (PDE) inhibitor that inhibits some forms of cyclic nucleotide phosphodiesterases (CNPs) (38). CNPs are enzymes that hydrolyze cAMP; thus inhibiting CNPs may result in increased cAMP and a corresponding increased ATP efflux from the RBC. This hypothesis was tested in an in vitro study by Ellsworth and colleagues (2007), in which rabbit RBCs were exposed to 70 μM caffeine, a concentration that would be expected in an individual who had consumed ~1-2 cups of coffee (38). They found that this amount of caffeine resulted in a ~70% increase in ATP release from the RBCs, and that this quantity of ATP was similar to that released in
response to exposure to a reduced PO$_2$ environment. Additionally, the increased ATP release was accompanied by a significant (22%) increase in intraerythrocyte cAMP, supporting the proposed mechanism of action of caffeine. In another study, incubation of chicken RBCs with caffeine (only 5 μM) resulted in a slower rate of cAMP degradation and an $\sim$30% increase in half-life (53). This also supports the relevance of caffeine as a CNP inhibitor that could augment ATP efflux from RBCs in vivo.

These findings are actually somewhat surprising, since CNP inhibition has been cited as occurring only at concentrations higher than those encountered in vivo (43). It has been shown however that only partial inhibition of PDE activity is necessary to raise the level of intracellular cAMP (10), and furthermore that because of the cascade nature of cAMP function only small changes in cAMP levels may be required to produce substantially larger effects in the cell (119). Additionally, it has been suggested that a metabolite of caffeine could also be a PDE inhibitor (119), and thus some of the increase in intraerythrocyte cAMP observed after caffeine administration may be attributable to its metabolites. For instance, therapeutic levels of theophylline (50 or 100 μmol/L), a drug of the same class as caffeine and also a product of caffeine metabolism, were shown to significantly inhibit cAMP hydrolysis (by 10-12.5% or 14.3-20.4% respectively) in human lung tissue (119). These findings may help to explain why caffeine does in fact appear to inhibit CNPs and increase intraerythrocyte levels of cAMP despite the contention that CNP inhibition cannot occur at physiological levels of caffeine (43).

A second mechanism by which caffeine may exert effects on the level of intraerythrocyte cAMP is indirect via the increased release of epinephrine (54; 55; 70). Importantly, epinephrine activates adenylyl cyclase, the component of the signal
transduction pathway for ATP release that increases intracellular levels of cAMP (155). Thus an increase in intracellular cAMP, and resultant ATP efflux, in the presence of caffeine in vivo may be brought about secondary to epinephrine release. Figure 1B is an illustration of these mechanisms by which caffeine is proposed to augment the RBC’s ability to function as an O₂ sensor.

In support of the assertion that caffeine may increase MBF, Marsh and colleagues (1993) showed that administration of theophylline (mean plasma concentration ~70 μmol/L) slowed the breakdown of PCr during progressive forearm exercise compared with a control condition (96). This altered metabolism was accompanied by a significant increase in the endurance of the forearm muscle (~19% improvement in maximum power attained), and is consistent with an improvement in oxidative metabolism (96), which could be attained via enhanced O₂ delivery (i.e. MBF) (165).

Thus caffeine has the potential to enhance RBC release of ATP both by inhibiting the breakdown of intracellular cAMP and by stimulating its production. Given the nature of the RBC-O₂ Sensor impairment, and the theoretical capacity of caffeine to circumvent this impairment, this provides a rationale for testing the efficacy of caffeine as a RBC-O₂ Sensor therapy in persons with T2DM. This represents only one potential function for caffeine however, and the actions of caffeine are widespread throughout the body (43; 93); thus it is important to have an understanding of its movement within the body (i.e. pharmacokinetics) and its other known cardiovascular-specific effects.
(5) Caffeine – Pharmacokinetics, Effects on the Cardiovascular System, and Logistics as an Intervention

Pharmacokinetics of Caffeine

Upon ingestion, caffeine is absorbed directly from the stomach into the bloodstream, and the rate of absorption appears to be independent of the amount for doses up to 10 mg/kg (i.e. approximate maximal dose consumed by humans) (93). It reaches maximal plasma concentrations in ~1 hour, and has a half-life of 4-6 hours; relatively high plasma concentrations are maintained for ~3-4 hours post-ingestion (54). The peak plasma caffeine concentration reached is ~5-10 μM for each 1 mg/kg oral dose (93). Caffeine is both water and lipid soluble (54), and as such it is readily distributed throughout all tissues of the body (93). In fact, by ~30-60 minutes post-ingestion, intracellular and plasma levels of caffeine are not significantly different (93).

Caffeine is metabolized in the liver by cytochrome P450 subtypes 1A2 and 2E1 (i.e. CYP1A2, CYP2E1), and its primary metabolites are dimethylxanthines that result from demethylation reactions: paraxanthine, theobromine and theophylline (93). The cytochrome P450 system saturates at caffeine doses of ~5mg/kg; thus any greater dose may result in disproportionate increases in plasma caffeine concentration (54). Some of the effects of caffeine may actually be attributable to its metabolites, although this is probably irrelevant for practical purposes (54). Some caffeine is excreted in the urine, although this amount represents a very small proportion of the administered dose (<5%) (93).
Effects of Caffeine on the Cardiovascular System

Caffeine has been recognized as an ergogenic aid – a substance that enhances physical or mental performance – for several decades (54). Its utility as an ergogenic aid has been demonstrated in a wide range of activities, varying in intensity, mode and duration (for a systematic review of the effect of caffeine on physical performance, please see (54)). Despite the recognition of caffeine’s beneficial effect on exercise performance however, the mechanisms by which it exerts these effects remain largely uncharacterized. Knowledge of the cellular and molecular mechanisms of caffeine’s action stems principally from in vitro studies, and the relevance of these findings in vivo is unclear, largely because the caffeine concentrations used in these studies are often much higher than those measured in humans following normal caffeine consumption (54; 93). Furthermore, the majority of in vivo studies of caffeine’s effect are largely observational with respect to performance outcome measures, without specific exploration of the mechanisms behind the improvement (54).

Nonetheless, three primary mechanisms of action for caffeine have been described: mobilization of intracellular calcium from the sarcoplasmic reticulum, inhibition of phosphodiesterases leading to increases in cAMP in tissues such as muscle, and antagonism of adenosine receptors (110). The general consensus is that the most physiologically relevant mechanism of caffeine is its action as a competitive inhibitor of adenosine receptors (43; 54; 93); the binding of caffeine to these receptors does not generate any kind of response, but rather simply occupies the binding site for adenosine and thereby prevents it from exerting its actions (93). Adenosine receptors are pervasive throughout the body (88), and thus stimulation or inhibition of these receptors can cause...
widespread effects. Yet in this regard, few studies have examined the effects of caffeine on the cardiovascular system specifically (29), and what observations are available suggest that caffeine may in fact cause vasoconstriction (29; 55; 124), which could impair MBF during exercise. With respect to the cardiovascular system, some key roles for stimulation of adenosine receptors have been described, and as an adenosine-antagonist caffeine would be expected to counteract these effects. Specifically, it appears that caffeine may exert vasoconstrictor effects via its actions on various adenosine receptors on the vascular smooth muscle (124; 151), sympathetic nerve endings (55), and the kidney (29; 56; 57).

First, it has been demonstrated that adenosine may be responsible for ~20-40% of the maintenance of steady state exercise hyperemia via its actions on the smooth muscle of the vasculature (for review, see (97)). During exercise, adenosine is formed primarily from AMP that is released from exercising muscle fibres when there is insufficient O2 to regenerate ATP, and it causes dilation mainly via its action on the A2A subtype of adenosine receptors that are present on the extraluminal surface of the arterial smooth muscle (97). Thus as an adenosine-antagonist, caffeine would be expected to attenuate the increase in MBF during exercise. In agreement with this assertion, Radegran and Calbet (2001) found that intravenous infusion of theophylline resulted in ~20% and 17% reductions in femoral artery blood flow and vascular conductance respectively, during one-legged exercise at ~48% of peak power output (124). Similarly, Smits and colleagues (1990) demonstrated that caffeine infusion attenuated the increase in forearm blood flow elicited by concomitant adenosine infusion (151). Thus caffeine may attenuate the adenosine-mediated vasodilatory response during exercise.
Second, in addition to its effect on A2A receptors, adenosine contributes to an increased vascular conductance via reducing sympathetic nerve activity to muscle (85; 145). Adenosine attenuates sympathetic nerve activity by preventing the release of norepinephrine (NE) (i.e. pre-synaptic inhibition) via stimulation of P1 purinergic receptors on the adrenergic nerve endings (145). Again, as a non-selective adenosine receptor antagonist, caffeine would be anticipated to counteract these effects, thereby leading to an increased sympathetic nerve activity and corresponding reduced vascular conductance.

As expected, Graham and colleagues (2000) found that there was indeed an increase in sympathetic nerve activity to active muscle with caffeine administration (6 mg/kg) during 1hr of cycling exercise at 70% VO2max, as evidenced by an increase in leg NE spillover (55). The impact of this increased activity was unclear however, since it was only present during the last 30 minutes of exercise, but leg vascular conductance was reduced compared with the placebo condition to the same extent throughout the entire trial; the increase in sympathetic nerve activity did not alter the vascular conductance in the exercising muscle. In addition, despite the reduction in leg vascular conductance, leg blood flow was not different between the caffeine and placebo trials; MAP was elevated in the caffeine condition. The lack of a difference in leg blood flow, even with a reduced vascular conductance, suggests that this enhanced sympathetic nervous activity did not result in a functional impairment. Nonetheless, this provides evidence for an augmented vasoconstrictor stimulus due to caffeine during exercise, which could negatively impact muscle perfusion in some situations. It should be noted however that not all studies show
enhanced sympathetic nerve activity to muscle with caffeine administration (56), and the cause of these discrepancies is not clear.

Third, adenosine plays a role in modulating MAP via its influence on renin activity; adenosine reduces renin activity via its stimulation of A₁ receptors (6), which leads to a reduction in the production of angiotensin II (ANG II) (132). The renin-angiotensin system is one of the most complicated endocrine pathways in the body (148). Hence simplistically, it can be understood that ANG II has important short- and longer-term effects on MAP via increasing sympathetic nervous system activity to the heart and blood vessels (i.e. increasing CO and reducing total vascular conductance) and increasing blood volume (due to an increased sodium reabsorption at the kidney) (13). Thus by indirectly reducing ANG II, stimulation of adenosine receptors could serve to reduce MAP, and conversely by antagonizing adenosine receptors caffeine could result in an ANG II-mediated increased MAP (i.e. via an increased CO and reduced systemic vascular conductance). Consistent with this intricate series of events, Daniels and colleagues (1998) demonstrated that caffeine accentuated increases in ANG II during exercise (55 min of cycling at 65% VO₂max), and that this increase in ANG II was accompanied by both an increase in MAP and relative reduction in forearm vascular conductance in comparison to the placebo condition (29). Unfortunately, blood flow to the exercising legs was not measured in this study, and thus it is not clear what, if any, effect a caffeine-induced increase in ANG II may have on exercising muscle perfusion. Even so, the effects on caffeine on the renin-angiotensin system may contribute to the hemodynamic response to exercise by increasing CO and reducing total vascular conductance.
A fourth means by which adenosine affects the cardiovascular system is via attenuation of the release of epinephrine from the adrenal medulla (171); thus caffeine would be expected to increase epinephrine release, and this is commonly observed (55-57; 70; 171). Epinephrine exerts both vasoconstrictor and vasodilatory effects, depending on the dose as it targets different receptors (i.e. α- and β2-receptors mediate constriction and dilation respectively); small doses increase total vascular conductance, while large doses reduce it (65). Thus an increase in circulating epinephrine with caffeine administration may result in reduced systemic vascular conductance.

In addition to epinephrine’s effect on the vasculature, it has positive chronotropic and inotropic actions on the heart (predominantly via β1-receptors) (65). Therefore caffeine may cause an increase in HR and contractility of the heart, secondary to augmented epinephrine release; this could augment CO and therefore contribute to an increase in MAP. An increase in HR however is seen in only some (96), but not other (29; 55) studies with caffeine administration. This discrepancy may be due to baroreflex-mediated sympathetic withdrawal due to the increase in MAP brought about by a reduction in total vascular conductance (130). This is an illustration of the complexity of caffeine’s effects due to the pervasiveness of adenosine receptors in the body, and the importance of studying its function in an integrated fashion.

Therefore it is clear that in addition to the potential vasodilatory function of caffeine described earlier (i.e. enhancement of the RBC-O2 Sensor mechanism), there is evidence that caffeine may cause vasoconstriction via inhibition of various adenosine receptors on vascular smooth muscle, adrenergic nerve endings, and the kidney. Thus the actions of caffeine in vivo with respect to the cardiovascular system are quite complex.
While there is a strong rationale for testing caffeine’s efficacy as a means of improving MBF and exercise tolerance in persons with T2DM, it is important to be cognizant of the many alternate actions this drug may be simultaneously exerting.

**Caffeine as an Intervention – Logistics**

From a purely logistical standpoint, caffeine is appealing as a drug intervention. It is already arguably the most widely consumed drug worldwide (54), with the average per capita daily intake estimated to be ~200 mg/day (43) (for perspective, the average cup of coffee generally contains about 100 mg of caffeine (43)). It is found in many commonly consumed products, including coffee, tea, chocolate, energy drinks, and over-the-counter drugs (54), and is thus readily available, affordable, and with social acceptance (93). Additionally, even high doses are well tolerated by most individuals, and caffeine has little or no acute adverse health effects (93).

Thus if caffeine does improve exercise tolerance in persons with T2DM, it will be a practical intervention to enable individuals to more-comfortably engage in physical activity.

*(6) Studying T2DM – Issue of Co-Morbidities and Medications*

One of the difficulties in undertaking studies of T2DM is that many individuals with this disease have co-morbidities and/or are on medication for their diabetes or for these co-morbidities. Therefore to ascribe observed responses to T2DM *per se*, it would
be important to exclude persons with co-morbidities or who are on medications that could influence outcomes of interest. In contrast, it could be argued that those persons with T2DM who have co-morbidities are actually more representative of persons with T2DM as a whole. For example, up to 80% of persons with T2DM also have hypertension and are commonly on medication to control their blood pressure (142). Similarly with respect to medications, it could be argued that it would be important to test participants who are taking common medications, since the reality is that individuals’ physiology is influenced by those medications in everyday life. In fact, the majority of T2DM patients are unable to manage their blood sugar without oral antidiabetic agents (79). Thus including these individuals in a study would perhaps make it more clinically relevant.

Both approaches are taken in research, with some researchers testing only unmedicated persons with T2DM (75), some testing people with T2DM after abstaining from taking their medications for a standardized period of time (67; 76; 180), and others testing people with T2DM while on their medications and simply disclosing what these medications are (7; 127; 143). The latter approach was taken in the present study, with participants tested while on their medications. As such, it is important to have an understanding of the mechanisms of action of medications commonly prescribed to persons with T2DM, and whether these medications can influence the cardiovascular response to exercise. Table 2 summarizes some of the medications that are commonly taken by persons with T2DM, and a brief description of their modes of action.
### Table 2. Common Medications Taken by Persons with T2DM

<table>
<thead>
<tr>
<th>Category</th>
<th>Example Trade Name(s)</th>
<th>Primary Mode of Action</th>
<th>Other Information</th>
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</thead>
<tbody>
<tr>
<td><strong>Antidiabetic Drugs</strong></td>
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<tr>
<td>Insulin secretagogues</td>
<td>Tolbutamide</td>
<td>Stimulate insulin secretion from the pancreas (act directly on the insulin-producing islet β-cells)</td>
<td>May reduce peripheral vascular function (15)</td>
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<tr>
<td>(Sulphonylureas, rapid-acting secretagogues)</td>
<td>Tolazamide</td>
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<td></td>
<td>Glyburide</td>
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<td>Glipizide</td>
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<td></td>
<td>Glimepiride</td>
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<tr>
<td>α-glucosidase inhibitors</td>
<td>Acarbose</td>
<td>Delay digestion and absorption of carbohydrate from intestine</td>
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<td></td>
<td>Miglitol</td>
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<td></td>
<td>Voglibose</td>
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<tr>
<td>Thiazolidinediones</td>
<td>Rosiglitazone</td>
<td>Improve insulin action (via multiple actions on gene regulation)</td>
<td>Can cause fluid retention with increased plasma volume, a reduced hematocrit, and a decrease in Hb concentration</td>
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<td></td>
<td>Pioglitazone</td>
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<tr>
<td>Insulin</td>
<td>Lispro (Humalog)</td>
<td>Regular actions of endogenous insulin: reduce hepatic glucose output and increase peripheral glucose uptake</td>
<td>Indicated for initial stabilization of patients with severe hyperglycemia or for advanced T2DM where oral hypoglycemic therapy has failed (28)</td>
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<tr>
<td><strong>Antihypertensive Drugs</strong></td>
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<tr>
<td>ACE Inhibitors</td>
<td>Ramipril</td>
<td>Block conversion of Angiotensin I to Angiotensin II (ANG II) in the plasma; results in reduced SNS stimulation of heart and blood vessels (i.e. ↓ CO, ↑ vascular conductance) and a reduced blood volume (due to a ↓ Na⁺ reabsorption at the kidney); improves endothelial function</td>
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<td></td>
<td>Enalapril</td>
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<tr>
<td>Angiotensin receptor-blockers</td>
<td>Losartan</td>
<td>Same effects as ACE Inhibitors, but by acting directly on ANG II receptors</td>
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<td></td>
<td>Valsartan</td>
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<td></td>
<td>Candesartan</td>
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<tr>
<td>Calcium Antagonists</td>
<td>Verapamil</td>
<td>Reduce smooth muscle contractile properties (i.e. increase vascular conductance)</td>
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<td></td>
<td>Diltiazem</td>
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<tr>
<td>Diuretics</td>
<td>Esidrix, Zaroxylyn</td>
<td>Reduce blood volume and CO</td>
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<tr>
<td>β-blockers</td>
<td>Atenolol</td>
<td>Reduce CO (i.e. inflow to arteries) Block hormone-stimulated AC activity, without affecting basal enzyme activity (53) (may have relevance to RBC-O₂ sensor function)</td>
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<td></td>
<td>Propranolol</td>
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<td>Metoprolol</td>
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<tr>
<td>α₁-blockers</td>
<td>Phentolamine</td>
<td>Block action of NE on α-receptors (i.e. block SNS-mediated vasoconstriction; increase vascular conductance)</td>
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<td></td>
<td>Prazosin</td>
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</tr>
<tr>
<td><strong>Lipid-Lowering Drugs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>Lipitor</td>
<td>↓ cholesterol production via inhibition of key enzyme (HMG-CoA reductase); also ↓ blood triglycerides and contributes to small ↑ in HDL-cholesterol</td>
<td>Reduce oxidative stress</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelet treatment</td>
<td>Low-dose Aspirin</td>
<td>Inhibits thromboxane prostaglandins (which bind platelets together)</td>
<td>Reduces blood viscosity</td>
</tr>
</tbody>
</table>

Sources: (13; 94; 112; 161).
(7) Summary and Conclusions

In summary, T2DM is a common disease with considerable consequences to general health and well-being. Physical activity is extremely important as a treatment modality in this population, however people with T2DM often experience fatigue and a reduced exercise capacity which negatively influences their ability or willingness to maintain an active lifestyle. A number of probable contributors to this reduction in exercise tolerance have been identified, and it is likely that the cardiovascular system is partly responsible.

Limitations to exercise tolerance that are due to the cardiovascular system are due to the muscle oxygenation environment that is established by the convective delivery of O\textsubscript{2} from the lungs to the muscle capillaries, and the diffusive capacity for the movement of O\textsubscript{2} from the capillaries to the inside of the cells; emerging evidence suggests that both are impaired in persons with T2DM. The reduction in the convective delivery of O\textsubscript{2} to muscle in T2DM is likely due to a combination of central factors (i.e. reduced CO), peripheral elements (e.g. endothelial dysfunction, enhanced SNS to active muscle, and impaired RBC-O\textsubscript{2} Sensor function), and altered hemorheological properties (i.e. reduced RBC deformability, increased RBC aggregation and increased blood viscosity). The diffusive capacity of the muscle for O\textsubscript{2} is also reduced in persons with T2DM due to an increased affinity of the glycated variant of Hb for O\textsubscript{2}, and to both functional and structural capillary rarefaction. Furthermore, defects in the intracellular machinery for the consumption of O\textsubscript{2} may contribute to the O\textsubscript{2}-related limitations to exercise tolerance (i.e. reduced mitochondrial size / volume, functional mitochondrial impairment, and imbalance between glycolytic and oxidative capacities).
Of particular importance to the present study, the RBC-O₂ Sensor, a mechanism of MBF-regulation in which the RBC acts as a roving O₂ sensor and elicits ATP-mediated vasodilation in areas of high metabolic demand, has been shown to be dysfunctional in persons with T2DM. Preliminary *in vitro* work suggests that caffeine may be a viable intervention to correct this dysfunction, however caffeine exerts a multitude of other, and perhaps conflicting, effects *in vivo*. The net effect of caffeine’s vasodilatory and vasoconstrictor influences remains to be elucidated in persons with T2DM.

Lastly, in studying persons with T2DM, various approaches are taken with respect to co-morbidities and medications. Since co-morbidities and medication use are so predominant in this population, it could be argued that testing persons with co-morbidities and while on their medications may be the most clinically relevant approach.

The cardiovascular system’s contribution to exercise intolerance in persons with T2DM is quite complex. Much future research is needed in this area, both to characterize basic responses to exercise in persons with T2DM and to gain an understanding of their underlying physiology, and to guide the development of appropriate interventions.
Chapter 3: METHODS

Subjects

Twelve men volunteered for this study; of these men, four with Type II Diabetes and four healthy controls were determined to be suitable for participation during the screening process. These eight subjects participated in the study after having received full written and verbal explanations of the experimental protocol and any potential risks, and giving written consent on forms approved by the Office of Human Research of the University (Appendix A). This study was approved by the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

Subject characteristics are listed in Table 3. With respect to medications, of the T2DM participants all 4 were on statins, 2 were on antihypertensive medications, 2 were on Metformin, and 2 were on low-dose ASA (see Appendix B for a complete list of medications). No control participants were on any medications. T2DM subjects were tested while continuing their regular medication regimens, as precedent in other studies (7; 127; 143). Subject recruitment initiatives are listed in Appendix C. A sample information brochure and flyer used for recruitment purposes are also provided in Appendix C.

Screening

Preliminary Screening Process

In order to ensure that subjects were suitable for study inclusion, and that their health status was such that participation would not pose any greater risk than that
inherently involved in exercise of the required intensity, subjects were extensively screened prior to participation. At the outset, when participants expressed interest in the study, they were asked a series of questions over the phone (Appendix D). Also at this time, with verbal consent, the first portion of a Medical Information Questionnaire (Appendix E) was filled out. This questionnaire, containing relevant medical history, was then forwarded to the prospective participant’s family physician for completion, and then to Dr. Robert Hudson (the physician in charge of patient care for this study) for final assessment about the suitability of subject participation. A comprehensive list of inclusion / exclusion criteria is provided in Appendix F.

### Table 3. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>T2DM Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>43.8 ± 2.6</td>
<td>50.5 ± 3.0</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>183.8 ± 2.2</td>
<td>174.9 ± 3.9</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>91.1 ± 4.7</td>
<td>106.9 ± 3.3*</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>27.1 ± 1.8</td>
<td>35.1 ± 1.6*</td>
</tr>
<tr>
<td><strong>WC, cm</strong></td>
<td>98.3 ± 3.4</td>
<td>115.4 ± 4.0*</td>
</tr>
<tr>
<td><strong>Fasting Blood Glucose, mmol/L</strong></td>
<td>5.4 ± 0.2</td>
<td>8.1 ± 1.2</td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td>5.2 ± 0.1</td>
<td>7.6 ± 0.9*</td>
</tr>
<tr>
<td><strong>PAR Score, MET hr/wk</strong></td>
<td>260.4 ± 14.4</td>
<td>248.4 ± 10.1</td>
</tr>
<tr>
<td><strong>Avg. Caffeine Consumption, mg/wk</strong></td>
<td>2090 ± 971</td>
<td>3927 ± 815</td>
</tr>
<tr>
<td><strong>Duration of T2DM, years since diagnosis (range)</strong></td>
<td>--</td>
<td>1-12</td>
</tr>
<tr>
<td><strong>MVC, kg</strong></td>
<td>57.6 ± 3.6</td>
<td>47.6 ± 3.6</td>
</tr>
<tr>
<td><strong>Relative Intensity, %MVC</strong></td>
<td>30.7 ± 1.7</td>
<td>37.5 ± 2.9</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. BMI = Body Mass Index; PAR = Physical Activity Recall; WC = Waist Circumference; MVC = Maximum Voluntary Contraction. *P<0.05 compared with Control subjects.
In-Laboratory Screening Process and Familiarization

Following the preliminary screening, subjects visited the lab for an in-lab screening session. During this visit, subjects received a full explanation of the experimental procedure including a description of all the measurements to be performed, and then read and signed Consent Forms. Subjects arrived at the lab for this visit in an overnight-fasted state so that fasting blood glucose measurements could be made. The screening visit included the following procedures:

**Anthropometric Measurements**

Measures of the subject’s height, body weight, and waist circumference were taken for purposes of subject characterization and for determination of the appropriate dose for the caffeine trial (explanation in section titled “Caffeine / Placebo Administration”).

Waist circumference was measured according to the measurement protocol of the Canadian Society for Exercise Physiology (CSEP) (104). Briefly, the upper border of the iliac crest of each hip bone was palpated and marked with an indelible marker. A tape measure was then positioned around the abdomen with the bottom of the tape at the top of the marker landmarks, and the measurement was taken to the nearest 0.5cm at the end of a normal expiration.
**Fasting Blood Glucose and Maximum Voluntary Contraction**

Fasting blood glucose and maximum voluntary contraction were determined as described in the “Measurements” section.

**Seven-Day Physical Activity Recall**

The Seven-Day Physical Activity Recall (PAR) was administered as a measure of subjects’ physical activity levels. The Seven-Day PAR is an interviewer-administered assessment of both leisure and occupational physical activity that has been validated in numerous studies (71; 162). The scoring of the interview results in an estimate of total energy expenditure across the 7 days, expressed in Metabolic Equivalents (METs). A MET value is defined as the ratio of the rate of energy expended during an activity to the rate of energy expenditure at rest; 1 MET is the rate of energy expenditure at rest (3). By convention, 1 MET is equivalent to 3.5 ml O$_2$·kg$^{-1}$·min$^{-1}$ or 1 kcal·kg$^{-1}$·hr$^{-1}$ (20), and MET values are considered a practical way comparing energy expenditure across persons of different body weight. Appendix G contains a sample script for the Seven-Day PAR.

**Caffeine Consumption Questionnaire**

The Caffeine Consumption Questionnaire (Appendix H) was used to determine subjects’ typical caffeine consumption for the purpose of subject characterization. This questionnaire was modified with permission from Landrum, 1992 (82).
Doppler and Echo Ultrasound Screening

Subjects were positioned supine in the posture assumed during the actual experimental trials (“Experimental Setup”), and it was determined whether an adequate image of the brachial artery and an adequate blood velocity signal could be obtained, using Echo and Doppler ultrasound respectively. One potential subject was excluded since his brachial artery was found to bifurcate high up in the upper arm, as this precluded measurement of total forearm muscle blood flow using the techniques in this study. Additionally, two potential subjects were excluded as clear brachial artery blood velocity signals could not be obtained (i.e. without interference from adjacent vein blood flow).

Familiarization Trial

Lastly, participants underwent one exercise trial, as described below (“Experimental Protocol”) for purposes of familiarization. One potential subject was excluded because he could not maintain the required work rate for 1 minute.

Experimental Setup

Subjects were positioned supine with both arms extended at heart level for the duration of the experiment. Subjects were further instrumented with the measurement apparatus as outlined below, and were instructed to lie quietly to ensure that blood flow remained at baseline levels. The experiment was performed in a cool environment (~18-
22°C) to minimize skin blood flow and thereby isolate muscle blood flow responses (166). The experimental setup is shown in Figure 8.

**Figure 8.** Experimental setup.
Measurements

Forearm Blood Flow (FBF) - Brachial Artery Diameter and Mean Blood Velocity

An image of the brachial artery was obtained using a 10-MHz linear Echo ultrasound probe, operating in two-dimensional B-mode (GE Vingmed System 5, GE Medical Systems), and recorded in Digital Imaging and Communications in Medicine (DICOM) format. Images of the brachial artery were obtained during 60 seconds of resting baseline and during the last 15 seconds of each minute of exercise.

Brachial artery Mean Blood Velocity (MBV) was measured with a 4-MHz Doppler ultrasound probe, which was fixed to the skin proximal to the elbow joint (Model 500V TCD, Multigon Industries, Mt. Vernon, NY). The angle of the ultrasound transducer crystal relative to the skin was 57 degrees. MBV measures were performed continuously and recorded on a personal computer data acquisition system.

The Echo ultrasound probe was also used to image the brachial artery at the exact spot where the Doppler ultrasound probe was to be positioned in order to measure the angle of the artery relative to the skin and therefore relative to the probe. This was because the blood velocity estimated by Doppler ultrasound is dependent on the angle at which the ultrasound intersects the blood vessel (i.e. the angle of insonation) (47), and therefore knowledge of this angle is critical in determining absolute values of MBV (168). Following data collection, the appropriate two-point units conversion (volts to cm/sec) for the specific angle of insonation was entered into the software so that absolute values of MBV could be determined. See Appendix I for a more detailed explanation.

All measurements were performed by the same operator.
Forearm Deep-Venous Blood Sampling

Upon arrival to the laboratory, participants had a 20 gauge catheter inserted in retrograde fashion into a forearm vein in the exercising arm at the anticubital fossa. Prior to catheterization, the forearm vein was imaged using Echo ultrasound to confirm that the vein drained the forearm muscle (i.e. was not superficial). Catheterization was performed by a researcher trained in venous access. Baseline blood samples were taken from this catheter for measurements of:

1. Baseline plasma / serum caffeine concentration – This measurement was to confirm that subjects did indeed abstain from caffeine consumption in the 48 hours prior to the study as instructed (“Experimental Protocol”). For the plasma caffeine concentration, samples were taken in green-topped vacutainer tubes (with sodium heparin) and were put on ice until the conclusion of the experimental trial, after which they were spun (refrigerated centrifuge, 10 minutes) and the plasma separated and frozen at -80°C. Logistical issues prevented analysis of these samples, so partway through the study additional samples for the determination of serum caffeine concentration were taken. These samples were taken in red-topped vacutainer tubes (silicone coated) and were stored at room temperature until analysis.

2. HbA1c – This measurement was for the purpose of subject characterization. Samples were taken in lavender-topped vacutainer tubes (spray-coated K$_2$EDTA; plastic).
3. Baseline Venous Oxygen Content ($C_{O_2}$), Hemoglobin (Hb) Concentration, and Lactate ($La^-$) Concentration – The measurements of $C_{O_2}$, Hb concentration, and baseline $La^-$ concentration were used to calculate forearm oxygen consumption and lactate efflux (see “Data Analysis”). All of these measures were obtained from a single blood sample, taken in a heparinized syringe (sodium heparin). These measures were repeated throughout the exercise trial, and each time an initial 1 ml sample was discarded and followed by a 1 ml sample for analysis.

The intravenous catheter was kept patent by flushing with a sterile 0.9% saline solution, and was used for further blood draws throughout the duration of the study for additional measurements of plasma / serum caffeine concentration (1 hr after the subject consumed the caffeine or placebo capsule), $C_{O_2}$ (for the calculation of VO$_2$) and lactate concentration (for the calculation of lactate efflux). The total blood volume taken over the course of the entire experiment did not exceed 110 mL, including all discards and samples, which represents about a third of the amount of blood taken when one donates blood.

Measurements of venous oxygen content and lactate concentration were prevented in the placebo trial of one T2DM subject and in both trials of another T2DM subject due to difficulties with catheterization. In these instances, blood samples were taken using butterfly needles (BD Vacutainer Safety-Lok Blood Collection Set) from the back of the hand for the measurement of caffeine concentration and HbA1c.

Instrumentation for venous blood measurements is shown in Figure 9.
Central Hemodynamic Monitoring

Heart rate (HR) was measured beat-by-beat with an electrocardiogram 3-lead placement. Mean Arterial Pressure (MAP), Cardiac Output (CO) and Stroke Volume (SV) were measured beat-by-beat with finger photoplethysmography on the resting arm (Finometer Midi, Finapres Medical Systems). Arterial Oxygen Saturation ($S_aO_2$) was also measured continuously on the resting arm using a pulse oximeter (Nellcor N-395). Problems with the finger cuff, or possible clinical differences reducing the accuracy of the Finometer, prevented accurate measurement of CO and SV in at least 3 of the 4
subjects in the T2DM group; therefore these data are of minor value to this study and will be shown only in Appendix J. Data were recorded on a personal computer data acquisition system.

**Fasting Blood Glucose**

Fasting blood glucose was measured using a blood glucose monitoring system (Accu-Chek Compact Plus). Briefly, after cleaning the area with an alcohol swab, a sterile single-use lancet was used to prick the subject’s finger. A drop of blood was transferred to a test strip in the Accu-Chek Compact Plus, which displayed the blood glucose concentration, in mmol/L, shortly thereafter. The accuracy of the blood glucose monitoring system was confirmed by performing performance checks with standardized control solutions.

**Maximum Voluntary Contraction**

Subjects were instructed to squeeze a calibrated handgrip dynamometer as hard as they could, and to hold the contraction for one second, for the determination of their Maximum Voluntary Contraction (MVC), measured in kilograms. Subjects were given three attempts, and the largest value was taken to represent their true MVC. The MVC was used as a means of quantifying what the absolute work rate of the exercise trial represented in relative terms compared to subjects’ maximum ability.

**Time to Task Failure**

As described below (“Experimental Protocol”), the exercise trial continued until “task failure” (defined as an inability to maintain the appropriate cadence or contraction
intensity for three consecutive contractions, as determined by oscilloscope output). The amount of time taken to reach task failure was used to indicate “exercise tolerance”, such that a longer time to task failure at the given work rate represented a higher exercise tolerance. If task failure was not reached by 20 minutes, the exercise trial was terminated (i.e. time to task failure was not quantified).

Experimental Protocol

Subjects participated in the study after a minimum 3-hr fast, and having been instructed to abstain from exercising or consuming alcohol for 12 hours before the study start time, and from consuming caffeine within 48 hours of the study. Participants were provided with an appointment card to remind them of these stipulations at the end of their in-lab screening session. All subjects participated in the study on two separate occasions, once with each treatment condition (i.e. Caffeine and Placebo).

Caffeine/Placebo Administration

Caffeine (USP grade; American Chemicals Limited) and placebo (lactose) capsules were administered in a double-blind fashion, such that both the subject and researcher were unaware of whether the caffeine or placebo capsule was administered in any given trial, and the treatment order was randomized. This was facilitated by Mr. Chris Gray, the Kingston General Hospital Pharmacy’s Investigation Drug Service Technician. All capsules were prepared at Kingston General Hospital (KGH) Pharmacy and were taken with bottled water in the laboratory. See Appendix K for a sample capsule order form.
Caffeine was administered indexed for body weight since this has been shown to result in consistent plasma concentrations (54). The dose of caffeine was 5mg/kg body weight since the hepatic P450 system, the system responsible for metabolizing caffeine, saturates at a caffeine dose of about 5 mg/kg, and therefore doses greater than this could result in disproportionate plasma levels (54). Furthermore, this dose of caffeine would be expected to result in plasma concentrations sufficient to cause some phosphodiesterase inhibition (119) and an increase in circulating epinephrine (171), which are the proposed mechanisms of action for caffeine in this study. Importantly, this amount of caffeine is similar to that in 2-4 cups of coffee (depending on the individual’s body weight) (43), and therefore would be a reasonable dose for persons to consume outside of a laboratory situation, making any findings clinically relevant.

Capsules were given ~1 hour prior to the onset of exercise since plasma caffeine concentrations reach a maximum level in approximately 1 hour (54). It is worth noting that the half-life of caffeine is approximately 4-6 hours (54), and thus the circulating caffeine concentration would remain elevated and relatively constant for the exercise duration of 20 minutes or less. Subjects’ plasma/serum caffeine concentrations were measured at baseline before consuming the caffeine (or placebo) capsule, and one hour later at the onset of the exercise trial.

Ultimately, 3 of the 4 Control subjects and 2 out of 4 T2DM subjects had the treatment order Caffeine/Placebo, with the remaining 3 subjects having the treatment order Placebo/Caffeine.
Specific Protocol

Upon arrival at the laboratory, subjects were positioned supine, catheterized, and baseline blood samples were obtained as described above, after which they consumed either the caffeine or placebo capsule. A timer was started immediately thereafter (and clock time noted) so that the exercise trial could be initiated ~1 hour after capsule consumption. At the start of the one-hour time period, subjects practiced a few contractions as a reminder of how the exercise was to be done and then rested quietly while being instrumented with the rest of the measurement apparatus as described above.

About 1 hour after capsule consumption, one minute of resting baseline measures were taken, after which the subjects began exercise, consisting of squeezing the handgrip dynamometer in time with a beeper (2 seconds of contraction to 2 seconds of relaxation) at an intensity equivalent to 17.5 kg (the handgripper was calibrated prior to every trial). Subjects were able to view oscilloscope output (i.e. live computer feedback displaying force output) to guide their contractions. A designated researcher, elected the “coach”, monitored the subjects’ contractions to ensure that they were of the appropriate strength and duration, and provided verbal feedback and encouragement. The subjects continued to undergo this handgripping exercise until “task failure” was reached, defined as being unable to maintain the appropriate cadence or contraction intensity for three consecutive contractions as determined by oscilloscope output, or until 20 minutes of exercise was completed. If task failure was reached in <20 minutes, subjects were enthusiastically encouraged to continue for a few additional contractions so that there could be no ambiguity that true task failure was achieved (i.e. force output would drop off rapidly), and the exact point of task failure was identified after the completion of the exercise trial.
During the exercise, measurements of brachial artery blood velocity and diameter, MAP, HR, CO, SV and S\textsubscript{o}O\textsubscript{2} were made. Venous blood was sampled from the intravenous catheter at the end of every minute of exercise for the quantification of VO\textsubscript{2} and lactate efflux.

In addition, the coach recorded the subjects’ ratings of perceived exertion (17) at the end of each minute of exercise, where “1” was defined as “resting intensity” and “10” was defined as “maximal effort of which you can probably only maintain for a few more contractions”. The RPE was used to communicate to the researchers approximately how much longer the subject would be able to continue the exercise.

Throughout the exercise trial, subjects were not given any time feedback; they were informed that blood samples would be taken at “various times” throughout the trial. Additionally, subjects were not provided any feedback regarding their performance (i.e. time to task failure) before the conclusion of the study (i.e. completion of both exercise trials).

At the conclusion of the trial, subjects were asked to fill out a brief questionnaire (Appendix L) asking whether they believed they could identify which experimental condition they experienced (Caffeine or Placebo), and identifying their beliefs as to how this would impact their performance.

The timeline of the experimental protocol is shown in Figure 10.

Subjects were compensated $75 plus an additional $10/hr for each hour exceeding 6-hrs in the laboratory, and parking expenses were reimbursed.
Figure 10. Timeline of experimental protocol.
Data Analysis

Forearm Blood Flow

Brachial artery diameter was quantified offline from the DICOM files using custom edge-detection software (183). A minimum of two values for brachial artery diameter were taken from the baseline measurements and were averaged together to give one representative value for brachial artery diameter. This averaging process was repeated for each minute of exercise (from the last 15 seconds of each minute) such that one value for brachial artery diameter was acquired for every minute of exercise.

For brachial artery MBV, after the correction for the angle of insonation was applied as described in the “Measurements” section, the baseline value was averaged over a 60 second period. During exercise, averages were taken across 5 duty cycles (~20 sec) for every minute of exercise. Therefore one value for MBV was obtained for every minute of exercise.

These values of brachial artery blood velocity were combined with the values of brachial artery diameter to calculate FBF according to the following equation:

\[ FBF = MBV \times 60 \text{ s/min} \times \pi \times \frac{\text{diameter}}{2}^2 \]

where FBF is calculated as ml/min, and diameter is brachial artery diameter in cm (141). Thus one value for FBF was obtained for baseline and for each minute of exercise.
Forearm Vascular Conductance

Forearm vascular conductance (FVK) was calculated as:

\[ FVK = \left( \frac{FBF}{MAP} \right) \times 100 \]

where FBF is in ml/min, MAP is in mmHg, and FVK is in mL·min\(^{-1}\)·100mmHg\(^{-1}\) such that the values for FVK are quantitatively similar to those for FBF. The value for MAP used in this calculation was an average across the same 5 duty cycles as used for the MBV measurement.

Heart Rate, Mean Arterial Pressure, Cardiac Output, Stroke Volume

Data for HR, MAP, CO and SV were averaged into 5-second time bins.

Venous Blood Constituents

Serum Caffeine Concentration

Vacutainer tubes for the determination of serum caffeine concentration were transported to the Core Laboratory at KGH at the conclusion of each experimental trial. The samples were then packaged and transported to the Hospital for Sick Children in Toronto, Ontario, where the serum caffeine concentration was determined using High Performance Liquid Chromatography (HPLC). Note: The plasma samples are still being stored at -80°C for analysis at a later time.
**HbA1c**

Vacutainer tubes for the determination of HbA1c were transported to the Core Laboratory at KGH at the conclusion of each experimental trial. These samples were analyzed at the Core Laboratory using a liquid chromatography method.

**CvO₂, Hb, La⁻**

Samples for the determination of CvO₂, Hb, and La⁻ were analyzed in the lab using StatProfile M Blood Gas Analyzer (Nova Biomedical, Mississauga, Canada). Although analysis was initiated immediately when blood samples were taken, it sometimes took several hours for all of the samples to be run through the machine.

**Forearm Oxygen Consumption (VO₂)**

Forearm oxygen consumption (VO₂) was calculated using the Fick equation:

\[ VO₂ = FBF \times \frac{(CₐO₂ - CᵥO₂)}{100} \]

where VO₂ is calculated as ml of O₂/min, CᵥO₂ is in ml O₂/dl blood, 100 is a conversion factor to convert ml O₂/dl blood to ml O₂/ml blood, FBF is in ml of blood/min, and CₐO₂ is in ml O₂/dl blood and was calculated as:

\[ CₐO₂ = (1.34 \times Hb) \times \left( \frac{SₐO₂}{100} \right) + 3 \]

where 1.34 is the oxygen binding capacity constant for Hb and is measured in ml O₂/g of Hb, Hb is the concentration of Hb in grams/dl blood, SₐO₂ is measured in percent, and 3 is the ml of dissolved O₂ in 1 dl of blood at an assumed PO₂ of 100 mmHg. The baseline value for Hb concentration was used for all calculations of CₐO₂. For SₐO₂, the baseline
value was averaged over a 60 second period, and averages were taken across 5 duty cycles (~20 sec) for every minute of exercise (i.e. the same time periods as for measurement of MBV).

**Lactate Efflux**

Lactate efflux was calculated according to the equation:

\[
\text{La}^-\text{Efflux} = \frac{[(\text{exercising La})/1000 \times \text{FBF}] - [(\text{baseline La})/1000 \times (\text{baseline FBF})]}{}
\]

where La\(^-\) Efflux is in mmol/min, exercising and baseline La\(^-\) are in mmol/L of blood, 1000 is a factor to convert mmol/L into mmol/ml, and FBF is in ml of blood/min.

**Time to Task Failure**

If task failure was reached in <20 minutes of exercise, time to task failure was determined by identifying the first three consecutive contractions that were of insufficient duration or contraction intensity.

**Across-Subject Averaging**

Values for FBF, FVK, VO\(_2\), C\(_a\)O\(_2\), C\(_v\)O\(_2\), Lactate efflux and concentration, MAP, HR, CO, and SV were averaged across subjects within groups (i.e. T2DM and control) and treatment conditions (i.e. caffeine and placebo) into time bins (baseline, 0-5, 5-10, 10-15, 15-20 minutes) represented by specific time points (baseline, 2.5, 7.5, 12.5, 17.5 minutes). These time points were used to visualize group trends in relation to individual responses.
For each subject, values for FBF, FVK, VO$_2$, (a-v)O$_2$ difference, Lactate efflux and concentration, MAP, HR, CO, and SV were averaged across one minute of resting baseline, the first minute of exercise, and the final minute of exercise achieved, for each treatment condition (i.e. caffeine and placebo). These values were then averaged across subjects within groups (i.e. T2DM and control) and treatment conditions (i.e. caffeine and placebo). The values at the final minute of exercise achieved were taken to represent “steady state”. These time points (baseline, first minute of exercise, and steady state) were used for statistical analysis.

All data are expressed as mean values ± standard error (SE).

Statistical Analysis

Group characteristics were compared using unpaired $t$ tests.

A three-factor mixed ANOVA was used to compare the effects of group (T2DM, control), treatment condition (caffeine, placebo), and time (baseline, first minute of exercise, steady state) on FBF, FVK, VO$_2$, Lactate Efflux and Concentration, MAP, (a-v)O$_2$ difference, HR, CO and SV. Repeated contrasts were used for detecting specific differences.

A two-way ANOVA was used to compare the effects of group (T2DM, control) and treatment condition (caffeine, placebo) on Time to Task Failure (TTF). An unpaired $t$ test was used to compare the percent change in TTF with caffeine versus placebo between groups (i.e. T2DM versus control).
Linear regression analysis was performed for average exercising FBF (from the first minute of exercise until task failure) versus TTF, for those subjects who reached task failure in <20 minutes. Similarly, linear regression analysis was performed for average exercising FVK and VO_2 versus TTF.

Statistical significance for all tests was set at P < 0.05. All statistical tests were conducted using PASW Statistics 17 (SPSS), except for the unpaired *t* tests and linear regression analyses, which were conducted using SigmaStat 3.0.
Chapter 4: RESULTS

Subject characteristics are included in the “Methods” section (Table 3). On average, the T2DM and control groups did not differ with respect to age, height, fasting blood glucose levels, physical activity levels (as determined by the seven-day physical activity recall score), average weekly caffeine consumption, or maximum voluntary contraction (P>0.05). On average however, the T2DM group had a greater body weight, BMI, and waist circumference than the control participants (P<0.05). As expected, on average the T2DM group also had a greater HbA1c percent than the control group (P<0.05).

For the subjects in which serum caffeine concentrations were measured, the values fell within the expected range (43) for a caffeine dose of 5mg/kg (Table 4).

Table 4. Serum Caffeine Concentration

<table>
<thead>
<tr>
<th>Time Point - Treatment Condition</th>
<th>Control Subjects</th>
<th>T2DM Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-capsule, μmol/L (n = 6)</td>
<td>Samples not obtained</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Start of exercise trial - Placebo condition, μmol/L (n = 3)</td>
<td>Samples not obtained</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Start of exercise trial - Caffeine condition, μmol/L (n = 3)</td>
<td>Samples not obtained</td>
<td>46 ± 7.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of subjects

Figures 11 to 18 show the individual subject and group mean FBF, FVK, VO$_2$, O$_2$ content, Lactate Efflux, Lactate Concentration, MAP and HR responses over time, under the caffeine and placebo treatment conditions. These figures serve to show the group trends in relation to individual subject responses.
Figures 19 to 26 show the mean FBF, FVK, VO\textsubscript{2}, (a-v)O\textsubscript{2} difference, Lactate Efflux, Lactate Concentration, MAP and HR responses by group (T2DM, control) and treatment condition (caffeine, placebo), at baseline, the first minute of exercise, and at steady state. As expected, each of these variables increased from baseline during exercise (P<0.05), however there were no differences between T2DM and control subjects (P>0.05) or between the caffeine and placebo treatment conditions (P>0.05) for any of the variables measured at any of the time points.

Four subjects reached task failure in <20 minutes (two T2DM and two control subjects); the other four subjects were instructed to stop exercising after 20 minutes of exercise were completed. For those subjects who reached task failure, the mean Time to Task Failure (TTF) was not different between T2DM and control subjects (8.74 ± 2.67 versus 9.54 ± 0.77 for T2DM versus control respectively; P=0.873). Similarly, the mean TTF was not different between the caffeine and placebo treatment conditions (P=0.295), although there was a trend towards a longer TTF in the caffeine condition (10.00 ± 2.02 versus 8.24 ± 1.79 minutes for caffeine and placebo respectively). The average percent improvement in time to task failure was ~18% with caffeine versus placebo (not significantly different between T2DM and control subjects; P=0.492; data not shown). Figure 27 illustrates the TTF by treatment condition for those four subjects who reached task failure in <20 minutes.

There was a strong positive relationship between average exercising FBF and TTF ($r^2 = 0.763; \ P=0.005$; Figure 28). Similarly, there was a strong positive relationship between average exercising FVK and TTF ($r^2 = 0.779; \ P=0.004$; Figure 29). In addition,
there was a moderate positive correlation between average exercising VO\textsubscript{2} and TTF, although this relationship was not significant ($r^2 = 0.278; P=0.282; \text{Figure 30}$).

The results of the Post-Trial Questionnaire are shown in Table 5. Overall, 50% of the time participants were able to correctly identify the caffeine trial, with only one participant incorrectly guessing that the placebo trial was the caffeine trial. In general, ~44% of the time participants were unsure as to which treatment they had received. Most participants claimed that the treatment received had no effect on how they performed the exercise test, although two participants gave the explanation “I exercised longer” as their reason for believing they had consumed the caffeine capsule. Therefore, there is no reason to suspect that subjects’ expectations about the treatment they received had any effect on the outcomes of interest in this study.

SV and CO data are shown in Appendix J.

<table>
<thead>
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<th>Table 5. Post-Trial Questionnaire Results</th>
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<td>Group</td>
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Caff = Caffeine, Pl = Placebo; ID = Identification of Treatment Condition
Figure 11. Individual subject and group mean FBF responses at rest (Time 0) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 12. Individual subject and group mean FVK responses at rest (Time 0) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 13. Individual subject and group mean VO₂ responses at rest (Time 0) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 14. Individual subject and group mean O$_2$ Content responses at rest (Time 0) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 15. Individual subject and group mean Lactate Efflux responses during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 16. Individual subject and group mean Lactate Concentration responses at rest (Time 0) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 17. Individual subject and group mean MAP responses at rest (-60 to 0 sec) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 18. Individual subject and group mean HR responses at rest (-60 to 0 sec) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 19. Mean FBF at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls \( n = 4 \); T2DM \( n = 4 \). *\( P < 0.05 \) compared to baseline values; †\( P < 0.05 \) compared to the first minute of exercise.
Figure 20. Mean FVK at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls $n = 4$; T2DM $n = 4$. *$P<0.05$ compared to baseline values; †$P<0.05$ compared to the first minute of exercise.
Figure 21. Mean VO$_2$ at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls $n = 4$; T2DM Caffeine $n = 3$; T2DM Placebo $n = 2$. *P<0.05 compared to baseline values; †P<0.05 compared to the first minute of exercise.
Figure 22. Mean (a-v) O$_2$ difference at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls $n = 4$; T2DM Caffeine $n = 3$; T2DM Placebo $n = 2$. *$P<0.05$ compared to baseline values.
Figure 23. Mean Lactate Efflux at the first minute of exercise and steady state. Data are means ± SE. Controls $n = 4$; T2DM Caffeine $n = 3$; T2DM Placebo $n = 2$. †$P < 0.05$ compared to the first minute of exercise.
**Figure 24.** Mean Lactate Concentration at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls $n = 4$; T2DM Caffeine $n = 3$; T2DM Placebo $n = 2$. *P<0.05 compared to baseline values; †P<0.05 compared to the first minute of exercise.
Figure 25. Mean MAP at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls n = 4; T2DM n = 4. *P<0.05 compared to baseline values; †P<0.05 compared to the first minute of exercise.
Figure 26. Mean HR at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls \( n = 4 \); T2DM \( n = 4 \). *P<0.05 compared to baseline values; †P<0.05 compared to the first minute of exercise.
Figure 27. Time to Task Failure by Treatment Condition for individual subjects (small circles) and group means (large circles). Time to Task Failure did not differ between the T2DM and Control subjects (P=0.873) or between the Caffeine and Placebo treatment conditions (P=0.295). n = 4. Data are means ± SE.
Figure 28. Relationship between average exercising FBF and Time to Task Failure. Square symbols with horizontal standard error bars represent average exercising FBF for those subjects ($n = 4$) who did not reach task failure by 20 minutes of exercise.
Figure 29. Relationship between average exercising FVK and Time to Task Failure. Square symbols with horizontal standard error bars represent average exercising FVK for those subjects ($n = 4$) who did not reach task failure by 20 minutes of exercise.
Figure 30. Relationship between average exercising VO\textsubscript{2} and Time to Task Failure. Square symbols with horizontal standard error bars represent average exercising VO\textsubscript{2} for those subjects (Control \( n = 2 \); T2DM Caffeine \( n = 2 \); T2DM Placebo \( n = 1 \)) who did not reach task failure by 20 minutes of exercise.
Chapter 5: DISCUSSION

This study investigated the cardiovascular response to moderate-intensity forearm handgrip exercise, in persons with T2DM and healthy controls, following consumption of either a caffeine or placebo capsule. It was hypothesized that persons with T2DM would have reduced MBF, VO$_2$, and exercise tolerance responses to exercise compared to control subjects, and that caffeine would act to attenuate these impairments. Interestingly, the major findings were in disagreement with these hypotheses.

The major findings of this study are: 1) there were no differences in steady state exercising FBF and VO$_2$ between persons with T2DM and healthy controls in the exercise model utilized, 2) there was no difference in exercise tolerance between the T2DM and control groups, as determined by measuring time to task failure at the same absolute work rate, 3) caffeine had no effect on FBF or VO$_2$ in either group, nor did caffeine affect time to task failure in either group, although there was a trend towards an improvement with caffeine versus placebo, and 4) there was a strong linear relationship between exercising FBF and exercise tolerance (i.e. as determined by time to task failure), suggesting that differences in FBF may be the underlying mechanism regarding differences in exercise tolerance. These results suggest that, contrary to findings in leg exercise, there is no impairment in the cardiovascular response to exercise in persons with T2DM, and further that caffeine consumption does not provide any benefit.
Steady State Exercising FBF

**FBF - T2DM versus Control Participants**

FBF did not differ between persons with T2DM and healthy controls in the present study, and the similar FBF levels were achieved by similar FVK and MAP (Figures 19, 20 and 25). That there was no difference in exercising FBF between the groups, either during the first minute of exercise or at steady state, is somewhat surprising given the recent findings of slowed microvascular blood flow kinetics (9) and reduced steady state muscle blood flow (MBF) in persons with T2DM (76; 81) during leg exercise.

For instance, Kingwell and colleagues (2003) found that steady state leg blood flow was significantly lower in T2DM participants versus controls (matched for age, VO$_2$peak, and body weight) during moderate-intensity exercise (25-min supine cycling at 60% VO$_2$peak) (76). Similarly, Lalande and colleagues (2008) demonstrated that steady state femoral blood flow, indexed to lean thigh mass, was significantly lower in T2DM participants versus healthy controls (matched for a variety of characteristics including age, BMI, and fat-free mass) during low-intensity leg extensor exercise (contralateral leg extensions with 1.5-kg ankle weights at a rate of 60±5rpm for ~15min) (81). Given these findings, it was anticipated that an impairment in exercising FBF would be observed, and the actual similarity in FBF responses across groups was unexpected.

There are three possible explanations for this apparent discrepancy: 1) that an impairment in exercising MBF in persons with T2DM exists in the leg but not in the arm, 2) that there is in fact no impairment in exercising MBF in persons with T2DM, or 3) that
an impairment in forearm MBF truly does exist in this population but we were unable to detect it in the present study.

1) An impairment in MBF may exist in the leg but not the arm in persons with T2DM.

It is possible that there is an impairment in exercising MBF in the leg but not in the arm in persons with T2DM, as limb differences are present in MBF regulatory mechanisms (129).

First, since the forearm is of small muscle mass, central hemodynamic responses play virtually no role in the MBF response to exercise (141), and thus the forearm exercise model was used in this study to better isolate local peripheral contributions to the expected dysfunction. That an impairment in exercising MBF has been observed in the leg (76; 81), but not the forearm, suggests that the impairment in MBF is centrally mediated and is therefore only apparent during large muscle mass exercise. Consistent with this notion, central impairments in the form of slowed HR kinetics (127), and reduced HR at submaximal exercise intensities (7) have been observed in persons with T2DM during lower body exercise. Importantly however, in the aforementioned study by Lalande and colleagues (2008), the reduced steady state leg blood flow in the T2DM group was observed in the presence of CO similar to that of the control group (81), indicating that central factors are not the only contributors to impaired MBF in this population. Thus while the lack of a relevant central contribution to the FBF response may partly explain the absence of impairment in the present study, it is likely that there are other reasons for which FBF did not differ between groups.

A second difference in MBF regulatory mechanisms between the limbs concerns the intrinsic reactivity of the blood vessels. It has been shown that arms exhibit greater
vascular reactivity than the legs, in that arms exhibit greater relative increases in blood flow in response to both pharmacological and physiological stimuli (111). For instance, the leg exhibits smaller relative increases in blood flow than the forearm in response to endothelium-dependent (acetylcholine and substance P) and –independent (sodium nitroprusside) vasodilators, and in response to a 10-minute period of ischemia (111). If the intrinsic vasoreactivity is blunted in the legs compared to the arms, it is perhaps unsurprising that they should be more susceptible to dysfunction. In other words, if the vasoreactivity in the arms is such that the capacity for responsiveness is greater than that employed under normal conditions, then a reduction in this capacity may occur without any impact on function. In contrast, where there is lower inherent vasoreactivity in the leg, any decrement in vascular function may immediately be manifest in impaired MBF regulation.

If in fact dysfunctional MBF regulation is only present in the leg, but not in the forearm in persons with T2DM, then the implication would be that the forearm may not be an appropriate model to use in this population, despite the fact that it provides easier access to measures that are critical in understanding the nature of the dysfunction (e.g. quantification of VO$_2$ and lactate efflux).

2) **There may not truly be an impairment in MBF in persons with T2DM.**

Second, it is possible that there is no impairment in exercising MBF in persons with T2DM. Documentation of such impairment has only occurred in two rigorous studies thus far (76; 81), and these findings need to be replicated as it is possible that, despite well-executed studies, some unidentified confounding variable influenced the
results. We must be careful not to interpret findings from two studies, in a total of 37 individuals (including T2DM and control participants), as dogma.

3) *An impairment in exercising FBF may actually be present in persons with T2DM, but we were unable to detect it in this study.*

Lastly, it is possible that an impairment in exercising FBF truly does exist in this population, but that we were unable to detect it in the present study due to a small sample size. Please see the “Limitations” section for a more detailed explanation.

From the currently available data, it is not clear which, if any, of these postulates is correct; future research in more subjects is necessary.

**FBF – Effect of Caffeine**

This was the first study, to our knowledge, to directly assess the effect of caffeine on MBF during exercise in humans, although other studies have measured MBF following caffeine consumption as a secondary outcome variable (55), or have quantified hemodynamic responses to caffeine at rest (22; 84). Caffeine is known to have vasoconstrictor influences (i.e. via inhibition of adenosine receptors on vascular smooth muscle (151), and enhancement of sympathetic nerve activity to skeletal muscle (145)), and is postulated to have contradictory vasodilator influences (i.e. via enhancement of ATP release from RBCs (38); see “Introduction” section for more detail). It was hypothesized that the vasodilatory influence of caffeine would dominate in the T2DM individuals in the present study, due to the basal impairment in the ability of RBCs to produce and release ATP in this population (61; 154; 155); however caffeine was found to have no effect on FBF or FVK in either persons with T2DM or healthy controls.
(Figures 19 and 20). In fact, if anything, it appears that caffeine may have suppressed FBF somewhat rather than augmented it as predicted (Figure 19, and mean FBF for subjects who did not reach task failure by 20 minutes of exercise in Figure 28). In general, it appears that with respect to MBF the effect of caffeine is dependent on the conditions studied; caffeine has been shown to cause vasodilation, vasoconstriction, or no effect on MBF under different circumstances.

For instance, caffeine was shown to cause vasodilation during a hyperinsulinemic-euglycemic glucose clamp in a study concerning the effect of caffeine on insulin sensitivity (74). While it is normal for blood flow to increase during a hyperinsulinemic-euglycemic clamp due to the vasodilatory actions of insulin (158), the increase in blood flow was greater under conditions of caffeine than placebo, indicating that caffeine itself had an effect on vascular tone. Similarly, caffeine has been shown to magnify vasodilator responses induced by mental stress (83). For instance, Lane and Williams (1987) measured FBF responses during psychological stress (mental arithmetic task under conditions of time pressure and competition) in healthy volunteers following consumption of caffeine (250 mg) or placebo (84). They found that caffeine magnified the FBF response to mental stress, and the authors concluded that caffeine and stress may interact in everyday life to potentiate cardiovascular reactivity. If caffeine exerts similar effects in response to physical stress as it does to psychological stress, then it may also increase MBF during exercise, a physical stressor.

In contrast, caffeine has been shown to cause vasoconstriction under other conditions. For example, consumption of 200 mg of caffeine was found to reduce FBF and FVK from 30 minutes to two hours post-consumption at rest (22). Similarly, Daniels
and colleagues (1998) demonstrated that caffeine consumption attenuated the increase in FBF and FVK during leg exercise (55 min of cycling at 65% VO$_2$max) (29). Unfortunately, blood flow to the exercising legs was not measured in this study, and thus it is not clear what effect, if any, caffeine had on the perfusion of the exercising muscles.

Furthermore, in some instances caffeine has been shown to have no effect on MBF. For instance, Graham and colleagues (2000) measured leg MBF with or without caffeine administration as a corollary to their primary metabolic outcomes of interest during 1 hour of exercise (cycling at 70% VO$_2$max) (55). Interestingly, they found that leg blood flow was not different between the caffeine and placebo conditions, despite an increase in sympathetic nerve activity in the caffeine trial (as evidenced by increased leg norepinephrine spillover). This suggests that in order to counteract the vasoconstrictor effects of caffeine, its vasodilatory properties may also have been active.

Therefore it is evident that the effect of caffeine on MBF is varied and may be highly dependent on the particular situation and/or on the characteristics of the individuals consuming it. Thus while no benefit to caffeine consumption with respect to FBF was found in the present study, the rationale for its use as an intervention to improve MBF and exercise tolerance is still valid and this should be tested in other exercise conditions.

**Steady State Exercising VO$_2$**

**VO$_2$ – T2DM versus Control Participants**

Due to prior observations of reduced steady state exercising MBF in persons with T2DM (76; 81), and due to the fact that VO$_2$ is partly dependent on oxygen delivery as
determined by MBF (165), it was hypothesized that steady state exercising VO\textsubscript{2} would be lower in the T2DM participant group versus the healthy controls. Just as there were no differences in steady state exercising FBF between the T2DM and control participants however, so too were there no differences observed in VO\textsubscript{2} between the groups (Figure 21).

While it has long been recognized that VO\textsubscript{2max} is reduced in persons with T2DM versus healthy controls (9), rarely has the VO\textsubscript{2} response to submaximal exercise been quantified. In 1995, Regensteiner and colleagues observed reduced VO\textsubscript{2} at submaximal work loads in persons with T2DM, compared to healthy age- and activity-matched controls, during a graded treadmill protocol (126). Similarly, Baldi and colleagues (2002) observed reduced VO\textsubscript{2} at some submaximal work loads during a graded bicycle ergometer test in persons with T2DM versus controls matched for age and body composition (7). In these studies however, the work loads were increased every one or two minutes respectively; given that there are consistent observations of slowed VO\textsubscript{2} kinetics in persons with T2DM (9; 18; 127), it is likely that the VO\textsubscript{2} had not yet adjusted to steady state levels at the time of measurements. Consistent with this notion, in another study it was demonstrated that although VO\textsubscript{2} kinetics were slowed in response to constant-load bicycle exercise, the same steady state VO\textsubscript{2} was ultimately reached in persons with T2DM and healthy controls (127).

Therefore the finding of similar steady state VO\textsubscript{2} between T2DM participants and controls in this study is consistent with the literature, and is expected given the similar FBF responses between the groups and the established linear relationship between work rate and VO\textsubscript{2} (60).
**VO$_2$ – Effect of Caffeine**

Contrary to the original hypothesis, caffeine had no effect on VO$_2$ in either the T2DM or control participants (Figure 21).

As discussed, it was initially suspected that VO$_2$ would be reduced in the T2DM group in the placebo condition, due to an impairment in FBF. This was hypothesized because VO$_2$ can be limited by inadequate O$_2$ delivery (165); an impairment in exercising FBF could be anticipated to impair VO$_2$ and to mandate an increased reliance on anaerobic means of energy generation. Furthermore, it was predicted that caffeine would attenuate, or fully correct, the expected impairment in FBF, and therefore it would also be expected to increase the VO$_2$. Given that there was in fact no basal impairment in either FBF or VO$_2$ in the T2DM versus the control group in the placebo condition, the finding that caffeine had no effect on VO$_2$ is understandable.

**Other Relevant Hemodynamic Variables**

*Lactate Efflux*

Lactate efflux did not differ between the T2DM and control groups in the present study (Figure 23), which suggests that the oxygenation state of the exercising forearm muscle was uniform across the groups.

To elaborate, lactate efflux can be used to provide insight into the oxygenation state of the muscle since lactate production is determined by the magnitude of the difference between the rate of glycolysis and the rate at which pyruvate (the end product of glycolysis) is utilized in the mitochondria via oxidative phosphorylation. In other words, the greater the rate of glycolysis for a given VO$_2$ (i.e. a given amount of oxidative
phosphorylation), the greater the production of lactate. The oxygenation state of the cell (PcellO$_2$) affects the rate of glycolysis, such that a lower PcellO$_2$ results in greater levels of Pi and ADP, both of which stimulate glycolysis (167). Thus at a given VO$_2$, and therefore a given utilization of pyruvate in the mitochondria, more lactate will be produced if the PcellO$_2$ is lower. If more lactate is produced in the muscle, then according to the principles of diffusion more lactate will exit the muscle into the blood where it can be measured. Therefore a greater lactate efflux at a given VO$_2$ would indicate a reduced oxygenation of the muscle. Since lactate efflux and VO$_2$ were similar between groups in the present study, this suggests that the oxygenation state of the muscle was also similar.

Additionally, lactate efflux did not differ between the caffeine and placebo treatment conditions (Figure 23). This was somewhat unexpected since blood lactate levels are often shown to increase with caffeine consumption (24; 55-57). The increase in blood lactate with caffeine administration has commonly been interpreted as resulting from a reduced flux of pyruvate through oxidative phosphorylation due to increased fat oxidation (and therefore greater flux of pyruvate to lactate), however there is little evidence that caffeine does in fact spare carbohydrate in favour of oxidizing a greater proportion of fat (54). In a review concerning the effects of caffeine on metabolism, exercise, and performance, Graham (2001) suggests that these increases in circulating lactate are due to an inhibition of its clearance rather than an increase in its production (54). Therefore in the present study, it is possible that the magnitude of lactate production by such a small exercising muscle mass was simply not great enough to result in measurable differences in the presence of reduced clearance.
**HR and MAP**

As expected, HR and MAP were found to increase moderately during the handgrip exercise (average increase of ~19 beats/min and ~20 mmHg), and there were no differences across groups (T2DM, control) or treatment conditions (caffeine, placebo) (Figures 25 and 26). The magnitude of change in these variables was similar to that seen in previous studies of forearm handgrip exercise of comparable intensity (163; 173).

**Exercise Tolerance**

**Time to Task Failure**

There were no differences in exercise tolerance between the T2DM and control groups, as determined by time to task failure, showing that the T2DM participants were not limited in the specific exercise task utilized in this study. In addition, there was a trend towards an increase in time to task failure with caffeine treatment (~10 versus 8 min for caffeine versus placebo), however this trend did not reach statistical significance (Figure 27).

For several decades, caffeine has been recognized as an ergogenic aid (i.e. a substance that enhances physical or mental performance) (54), although the mechanisms underlying its ergogenicity remain largely elusive. The most commonly cited mechanism of caffeine’s ergogenic effect is that it enables glycogen conservation via increased epinephrine-mediated mobilization of free fatty acids and a “Randle” effect (i.e. increasing fat oxidation and sparing glycogen) (54). However, it has been noted that “there is a serious lack of support [for this theory] and, more recently, there are studies that clearly illustrate that it is not correct in many circumstances” (54). For example, this
mechanism does not explain the beneficial effects of caffeine in short-term exercise bouts where glycogen is unlikely to be limited (e.g. (70)). Further, as an adenosine receptor antagonist, phosphodiesterase inhibitor, and a substance that mobilizes intracellular calcium from the sarcoplasmic reticulum (110), the effects of caffeine are likely to be widespread throughout the body and its overall effects may be attributable to several concurrent actions. Thus while caffeine has frequently been shown to have a beneficial effect on exercise performance, the reason for this effect often remains uncertain.

In the present study, it was hypothesized that caffeine would have a beneficial effect on exercise tolerance and that this would be related to its effect on exercising FBF. The findings of a trend toward an improvement in exercise tolerance with caffeine consumption, as well as a strong positive relationship between FBF and exercise tolerance, appear to support this hypothesis (see “Relationship between FBF and Exercise Tolerance”). It is important to remember however that many other effects of caffeine are also operating, and therefore we cannot attribute the effect of caffeine in this study solely to its potential influence on FBF.

Importantly, caffeine has been shown to improve time to task failure in other exercise modalities and subject populations. For instance, Denadai and Denadai (1998) showed that ingestion of caffeine in the same dosage as in the present study (5 mg/kg) resulted in a greater time to exhaustion during bicycle ergometer exercise at an intensity of approximately 10% below the anaerobic threshold in young untrained male subjects (33). Interestingly, in the same study there was no difference in time to exhaustion between the caffeine and placebo conditions at an intensity of about 10% above the anaerobic threshold. This illustrates the complexity of the effects of caffeine, and
indicates that its effects may differ depending on the mode or intensity of exercise. In other words, the lack of a statistically significant effect on exercise tolerance with caffeine administration in the present study does not negate its utility as a potential treatment intervention.

In a clinical population study, Notarius and colleagues (2006) found that intravenous infusion of caffeine significantly increased exercise time during graded cycling exercise in subjects with heart failure, but not in healthy controls (113). This supports the notion that the percent improvement may be greater if there is a basal impairment, as was hypothesized in the present study.

In yet another study, Marsh and colleagues (1993) showed that consumption of theophylline, a drug of the same class as caffeine, improved forearm muscle endurance in healthy men (~19% improvement in maximum power attained in a progressive forearm exercise test) (96). While MBF was not measured in this study, findings of a slowed breakdown of PCr compared to the placebo condition are consistent with an improvement in oxidative metabolism (96), which could be attained via enhanced O₂ delivery (i.e. MBF) (165). This suggests that the beneficial effect of methylxanthines such as caffeine may be related to enhanced responsiveness of the cardiovascular system.

These are only some of the many studies to show an effect of caffeine on exercise tolerance (for an excellent review, see (54)).

**Relationship between FBF and Exercise Tolerance**

In the present study, a strong positive relationship was observed between FBF and exercise tolerance, as quantified by time to task failure (Figure 28). This relationship
suggests that differences in FBF may be an underlying mechanism explaining the observed differences in exercise tolerance. Support for this notion comes from studies of sex differences in muscle fatigability, and the elimination of such differences through muscle occlusion.

In brief, when a muscle contracts, the pressure inside the muscle increases, which causes partial occlusion of the blood vessels that are supplying it (140). The greater the tension developed with the contraction, the greater the intramuscular pressure, and the greater the decrement in MBF. As a result, since men generally have greater muscle mass than women, a muscle contraction at the same relative intensity would impede MBF to a greater extent (163). If MBF is related to fatigability (or exercise tolerance), then men would be expected to reach task failure sooner than women when exercising at the same relative intensity, and this sex difference would be expected to be eliminated if MBF were externally occluded (i.e. since both sexes would then have the same occlusion of blood vessels). Clark and colleagues (2005) tested this hypothesis in a study of human quadriceps muscles (sustained knee extension contraction at 25% of maximal force) (25). They showed that indeed women took longer to reach task failure than men under conditions of normal muscle blood flow, but that this difference was eliminated under conditions of ischemia (external pressure cuff on proximal thigh). Russ and Kent-Braun (2003) showed similar findings in a study of human dorsiflexor muscles (137). These studies provide evidence for the role of MBF in exercise tolerance.

In contrast, Gonzales and colleagues (2007) found sex differences in exercise tolerance despite similar blood flow between the groups (time to task failure in dynamic handgrip exercise at the same absolute work rate and increased by 0.25 W/min) (49).
This demonstrates that exercising MBF is not the only factor contributing to exercise tolerance.

Nonetheless, the relationship in the present study is striking and shows a clear association between exercising FBF and exercise tolerance. A similar relationship was observed between FVK and time to task failure (Figure 29), indicating that the different flows were the result of differences in vascular conductance.

Limitations

Small Sample Size

The primary limitation of the present study was the small sample size. Based on leg blood flow data from a previous study (76), a priori power calculations determined that a sample size of 9 subjects per group would give a power of 99% for detecting a difference in steady state exercising blood flow between persons with T2DM and control subjects (GPower 3.0.10 Software). Furthermore, with 80% power and P=0.05, it was estimated that 9 participants per group would be required to show an effect size of 1.2. An effect size of this magnitude would represent a within-group difference in FBF of 70ml/min with caffeine treatment, if the variability in exercising FBF was similar to that in a previous study (69).

In the present study, we were able to collect data from 4 participants per group, and a retrograde power calculation revealed that the achieved power for detecting a difference between steady state exercising FBF in control versus T2DM individuals was only ~0.065, which is well below the desired power of 0.800. Furthermore, with respect to some of the calculated variables, such as VO₂ and Lactate Efflux, we were only able to
obtain data for two subjects in the T2DM group; such a small sample size cannot realistically be expected to represent the T2DM population as a whole and to be capable of identifying differences between groups if they exist. Thus it is possible that the non-significant differences between groups and treatment conditions may be due in part to a Type 2 error (i.e. accepting the null hypothesis – no effect – when the null is false).

Careful examination of the available data however does not suggest that a Type 2 error has been made; data from both groups and treatment conditions are virtually superimposed (Figures 11 through 26). In other words, for the most part there do not appear to be trends towards differences that are unable to be detected statistically because of low power, but rather it does appear that in fact there are no differences between the groups and treatment conditions in the given exercise condition studied herein. Nonetheless, given previous findings of reduced steady state exercising MBF in persons with T2DM (76; 81), and the strong rationale for the administration of caffeine as an intervention (see “Introduction” section), it would be prudent to follow-up on this study by collecting more data in additional subjects.

Subjects not Matched on Some Characteristics

A similar limitation of the present study was that subjects in the T2DM and control groups were not matched on characteristics such as age, weight, BMI, waist circumference, and MVC. On average, the T2DM group was older, heavier, with a larger waist circumference, and smaller MVC (even though the groups did not differ statistically on all of these characteristics; see Table 3). If anything, however, these characteristics would be expected to be detrimental to the T2DM group, since age (77), and obesity (64)
in particular have been shown to reduce exercising MBF in humans, and no such impairment was observed in the T2DM participants. Therefore it would appear that the lack of matching on these characteristics was of little consequence to the present study.

**Work Rate**

A third limitation to the present study was that the absolute work rate chosen (17.5 kg) was not appropriate for testing exercise tolerance in some participants. Only half of the participants (in both groups) reached task failure by twenty minutes of exercise, thereby making it difficult to evaluate the performance implications of caffeine as an intervention to make exercise easier.

While constant work rate exercise testing is recognized as a way of measuring exercise tolerance that can be used to assess the effectiveness of an intervention (21), it has been suggested that the work rate chosen should result in task failure in 4-7 minutes (21). If the work rate is too low, participants may be able to exercise indefinitely and therefore prevent measurement of the effectiveness of the intervention, as occurred with some participants in this study. Conversely, if the work rate is too high, the exercise will be tolerated for only a short period of time and any improvements with the intervention will result in only small improvements in exercise time (21). This is important as it has been suggested that the minimal clinically important difference for a constant work rate test is ~1.75 minutes (21). In the present study, only one participant reached task failure within the suggested 4-7 minute range; the work rate may have been too low to effectively assess exercise tolerance in the majority of the participants.
To this end, an alteration in the experimental design may be suggested. Specifically, it would be beneficial to have separate exercise tests to investigate the hypotheses concerning exercise tolerance and other steady state outcomes of interest (e.g. FBF, VO$_2$). In the present study, it was necessary to have participants work at an identical absolute work rate so that direct comparisons of these steady state variables could be made across the groups. As has been shown however, this was problematic as it was not possible to select a work rate that caused most individuals to reach task failure within a small range of time. In the future, it would therefore be prudent to have subjects exercise at a lower work rate, which all subjects could maintain for a period of about 5 minutes, in order to make steady-state comparisons of the relevant hemodynamic variables. A separate exercise test, such as a ramp test to task failure or a constant work rate test with the work rate being chosen on an individual subject basis, could then be used as a measure of exercise performance under conditions of caffeine or placebo. In this way, it would be possible to measure the outcomes of interest without compromising them by the study design.

Future Directions

The majority of research concerning exercising MBF and O$_2$ consumption in persons with T2DM is quite recent, occurring within about the last ten years. As a field that is still in its infancy, there are many questions that remain to be answered.

First, there is a need to develop a comprehensive understanding of the basic cardiovascular responses to exercise in persons with T2DM. Current knowledge concerning exercising MBF and exercise tolerance in persons with T2DM comes from
only a small number of studies from a few research groups, and it will be important to replicate and expand upon these findings. Definitively, is MBF impaired in this population during exercise, and if so do central factors, peripheral factors, or both contribute? Is the impairment specific to certain vascular beds, or is it present systemically? Where in the time course of the response does the impairment occur; is it only at the onset of exercise, or does it remain at steady state? What is the magnitude of the impairment, and is it dependent on the intensity of the exercise? What are the functional implications of the impairment?

Second, it will be important to identify the pathogenesis of dysfunctional cardiovascular responses to exercise, and to develop treatment interventions to attenuate or reverse these impairments. Is the impairment a function of the duration of disease, or does the acute metabolic environment affect the cardiovascular responses to exercise? Does correction of blood glucose regulation repair the dysfunction, and does reversal of the dysfunction improve exercise tolerance? Further investigation of the effect of caffeine on exercising MBF and exercise tolerance also has merit, beginning with the basic question: does caffeine increase ATP efflux from RBCs in humans in vivo during exercise? If caffeine is shown to have a beneficial effect in laboratory situations, can its effects be extrapolated to commonly consumed products containing caffeine, or to the chronic use of caffeine?

Given the widespread prevalence of T2DM (161), and the importance of regular physical activity as a treatment modality (4; 147; 160), this is an area of research that holds much intrigue.
Chapter 6: SUMMARY AND CONCLUSIONS

Exercise is a critical component of the medical management of T2DM (4; 147; 160), however persons with this disease commonly experience chronic fatigue, weakness, and a reduced exercise tolerance (32), which affects their ability or willingness to participate in regular physical activity (9). Recent findings suggest that this difficulty exercising may be related to an impaired adjustment of MBF to meet the metabolic demands of exercising muscle (76; 81), and further *in vitro* evidence suggests that caffeine may act to reverse this impairment (38). With this as a foundation, the present study investigated the MBF, VO$_2$, and exercise tolerance responses to forearm handgrip exercise in persons with T2DM and healthy controls, following consumption of caffeine or a placebo.

It was hypothesized that persons with T2DM would have reduced MBF, VO$_2$, and exercise tolerance responses to exercise compared to control subjects, and that caffeine would attenuate these impairments. In contrast to this hypothesis, no differences were seen in any of the measured variables between groups (T2DM, control) or treatment conditions (caffeine, placebo). It is possible that the discrepancy between the findings of this and previous studies is due to the exercise model utilized (forearm handgrip), some extraneous variable influencing the results in this or previous studies, or an inadequate sample size resulting in Type II error. Interestingly however, a strong positive relationship was found between forearm MBF and time to task failure in the exercise task, suggesting that differences in exercising MBF may be an underlying mechanism regarding differences in exercise tolerance. The major findings of the present study
suggest that, in the exercise model utilized, persons with T2DM do not have impaired cardiovascular responsiveness, and further that caffeine has no benefit.

Given the emerging evidence for impairment in persons with T2DM in the literature however, and the enormous relevance of such evidence, as well as the limitations of the present study, it is recommended that future work be undertaken to gain a comprehensive understanding of the basic cardiovascular responses to exercise in persons with T2DM, and if necessary to guide the development of appropriate interventions.
REFERENCES


APPENDIX A

CONSENT FORM
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.

____________________  ________________________
Signature Principal Investigator    Date (day/month/year)
APPENDIX B
SUBJECT MEDICATIONS
<table>
<thead>
<tr>
<th>Subject</th>
<th>Medications</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>D01</td>
<td>Low-dose ASA</td>
<td>Antiplatelet treatment</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin (Lipitor)</td>
<td>Statin</td>
</tr>
<tr>
<td>D02</td>
<td>Ramipril</td>
<td>ACE Inhibitor</td>
</tr>
<tr>
<td></td>
<td>Hydrochlorothiazide</td>
<td>Thiazide Diuretic</td>
</tr>
<tr>
<td></td>
<td>Lipitor</td>
<td>Statin</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>Biguanide</td>
</tr>
<tr>
<td>D03</td>
<td>Rosuvastatin (Crestor)</td>
<td>Statin</td>
</tr>
<tr>
<td>D04</td>
<td>Lipitor</td>
<td>Statin</td>
</tr>
<tr>
<td></td>
<td>Low-dose ASA</td>
<td>Antiplatelet treatment</td>
</tr>
<tr>
<td></td>
<td>Cilazapril</td>
<td>ACE Inhibitor</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>Biguanide</td>
</tr>
<tr>
<td></td>
<td>B12</td>
<td>Vitamin</td>
</tr>
</tbody>
</table>
APPENDIX C

SUBJECT RECRUITMENT

- List of Recruitment Initiatives
- Recruitment Materials (Information Brochure & Flyer)
Table 7. *Subject Recruitment Initiatives*

<table>
<thead>
<tr>
<th>Subject Recruitment Initiatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Distribution of ~150 poster advertisements at various locations in Kingston, Ontario, including:</td>
</tr>
<tr>
<td>o Doctors’ offices, dentists, physiotherapists, nutritionists</td>
</tr>
<tr>
<td>o Kingston General Hospital (KGH)</td>
</tr>
<tr>
<td>o Diabetes Education Centre at Hotel Dieu Hospital</td>
</tr>
<tr>
<td>o Queen’s University campus</td>
</tr>
<tr>
<td>o Staff rooms of schools in the Limestone City School Board, and Algonquin and Lakeshore Catholic District School Board</td>
</tr>
<tr>
<td>• Distribution of &gt;600 information brochures at the same locations</td>
</tr>
<tr>
<td>• Advertisement posted on the Limestone City School Board website</td>
</tr>
<tr>
<td>• Advertisement in the online version of the Kingston Whig Standard (local newspaper)</td>
</tr>
<tr>
<td>• Print advertisement in the Queen’s Gazette (newspaper of Queen’s University, circulated on campus and to Queen’s alumni)</td>
</tr>
<tr>
<td>• Print advertisement in “Spectrum”, the newsletter publication of KGH (also available online)</td>
</tr>
<tr>
<td>• E-mail advertisement to all members of the School of Kinesiology and Health Studies at Queen’s University, including graduate students and faculty</td>
</tr>
<tr>
<td>• E-mail advertisement through undergrad network at Queen’s (to all departments / service areas that work with undergrads), with request to please post in their areas</td>
</tr>
<tr>
<td>• Information / exhibitor booth at Diabetes Education Symposium, hosted by the Canadian Diabetes Association</td>
</tr>
<tr>
<td>• Manual search through patient files of Dr. Robert Hudson, the physician in charge of patient care for the study. When potential participants were identified, Dr. Hudson’s secretary contacted them to inquire as to whether they would consent to having a graduate student contact them with more information about the study.</td>
</tr>
<tr>
<td>• Dr. Hudson contacted other family physicians in the area to ask for assistance in recruiting some participants. Outcome: was sent a list of 12 eligible patients who were then contacted by phone.</td>
</tr>
<tr>
<td>• Word of mouth</td>
</tr>
</tbody>
</table>
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-inflammatory 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.

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.

Human Vascular Control Laboratory

STUDY PARTICIPANTS WANTED!

Human Vascular Control Laboratory
Queen's University
PhysEd Centre, Room 303A
69 Union Street
Kingston, ON, K7L 3N6
Phone: (613) 533-6000 x76425
vascular.lab@queensu.ca
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 who are not taking any medications.

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.

**LEG STUDY**

You will do:
- Increasing intensities of leg kicking exercise (bending and straightening your legs) 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?

**ARM STUDY**

The study requires three 1.5-2 hour visits to the lab over a 2-3 week period (including a screening visit).

**LEG STUDY**

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.
Research Opportunity for MEN:

Who?
30-65 year old non-smoking males with or without Type 2 Diabetes

What/When/Where?
• Three 1.5-2 hour visits over a 2-3 week period
• Forearm exercise: rhythmically squeezing a hand-gripping device until you are too tired to continue (~15 minutes), after consuming either a caffeine or sugar pill
• 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 II Diabetes have a hard time performing physical activity, and to develop interventions that will help make exercise easier.

Want more information?
Please contact Veronica at 533-6000 ext. 78425 or vascular.lab@queensu.ca.

Please Help Spread the Word!
Please share this announcement with friends and coworkers. We need 15 additional men before the end of May, and would greatly appreciate your help!
APPENDIX D

PRELIMINARY SCREENING QUESTIONS
Pre-Screening Questions and Information

General Information

1. How old are you? (*30-65 years*)
2. Do you have Type II diabetes?
3. Are you currently taking any medications? List.
4. Do you perform any regular physical activity? Describe.
5. Are you comfortable with giving blood samples?
6. Are you a smoker?
7. Do you currently experience back pain, or have you experienced back pain in the past?
8. 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?

*PAR (Physical Activity Readiness) Questions were used to determine whether it would be advisable to undertake the screening session (i.e. including familiarization with the experimental protocol) prior to receiving the full medical history from the prospective participant's family doctor.
APPENDIX E

MEDICAL INFORMATION QUESTIONNAIRE
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

A. Has your doctor ever said you have heart trouble?  ____  ____

B. Do you get pains, pressure or tightness in your chest?  ____  ____

C. Do you often feel faint or experience dizziness?  ____  ____

D. Has your doctor ever told you that you have high blood pressure?  ____  ____

E. (If has diabetes) Do you know if you have any complications from your diabetes?  ____  ____

F. Is there a good reason, not mentioned above, why you should avoid exercise?  ____  ____

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

   i. Heart or blood vessels  ____  ____
   ii. Nerves or brain  ____  ____
   iii. Breathing or lungs  ____  ____
   iv. Hormones, thyroid, or diabetes  ____  ____
   v. Muscles, joints, or bones  ____  ____
   vi. Other (please list) _________________________________________________
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 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 or 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:

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?

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.
References for Study Details:


APPENDIX F

INCLUSION / EXCLUSION CRITERIA FOR SUBJECT PARTICIPATION
Type II Diabetes Studies – Inclusion / Exclusion Checklist

Both Groups:

☐ Male
☐ 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 to exclude:
  o Insulin
  o Insulin secretagogues
  o Verapamil
  o Diltiazem
  o Sulphonylureas
☐ Drugs that are ok to include:
  o Metformin
  o ACE inhibitors
  o Diuretics
  o Ca\(^{2+}\) channel blockers (except for Verapamil and Diltiazem)
  o ARBs
  o α-blockers
  o Lipid-lowering drugs
  o β-blockers (arm study only)
  o Rosiglitazone, pioglitazone

Participants with Type II Diabetes:

☐ Ages 30-65
☐ Any levels of physical activity

Control Participants:

☐ Ages 35-65
☐ As physically inactive as possible (unless directly matched with an “active” participant with diabetes)
Seven-Day PAR Instructions / Script

The following is a sample script for the of the seven-day PAR Interview, as administered in this study.

- Now we are going to do a Physical Activity (PA) questionnaire, where I ask you about your PA over the last 7 days. This is simply a recall of actual activities for the past week, and isn’t a history of what you “usually” do. It’s not a test, and it will not affect the exercise that you do as part of this study, we’re just interested in physical activity levels so that we can match our participants based on PA.
- I’m going to start off by asking you some questions about the past week.
- Questions on page 1 of Seven-Day PAR.

Over the course of this interview, I'll be asking questions about yesterday, and then working backwards through the previous 7 days.

- So first, let’s talk about the time you spent sleeping in the past week.
  - By “sleeping”, I mean the time you went to bed one night and the time that you got out of bed the next morning. You may not necessarily have been asleep the entire time you were in bed. You may have been reading, watching TV, or doing paperwork. Time spent in sexual activity is not counted as “sleep”.
  - Today is (i.e. Monday), so yesterday was (i.e. Sunday). What time did you go to bed (Sunday) night and get up (Monday) morning. Record to the nearest ¼ hour. Do this for each of the 7-d recall. Calculate total time spent sleeping after completing the interview. Did you have any naps on (Sunday)? Did you have any disruptions to your sleep – any times when you got out of bed for 15 minutes or more?
    - Repeat for all other days

- Now I’m going to ask you about physical activities done in the past 7 days. In talking about PA, we will classify activities into 3 categories:
  - The “moderate” category is similar to how you feel when you’re walking at a normal pace, walking as if you were going somewhere
  - The “very hard” category is similar to how you feel when you are running
  - The “hard” category just falls in between in other words, if the activity seems harder than walking but not as strenuous as running, it should go in the hard category
- These cards give examples of some activities that fall into each of these categories (sample activities were shown).
- I’m going to ask you about the PAs you engaged in during three segments of the day, which includes morning, afternoon, and evening.
  - “Morning” is considered from the time you get up in the morning to the time you have lunch
  - “Afternoon” is from lunch to dinner
  - And “evening” is from dinner until the time you go to bed
  - NOTE: If a meal is skipped, “morning” is from the time a person wakes up to 12:00 pm, afternoon from 12:00-6:00pm, and evening from 6pm to bed.
• For this interview we are not considering light activities such as desk work, standing, light housework, strolling, and stop-and-go walking such as grocery shopping or window shopping.
• We are interested in occupational, household, and sports activities that make you feel similar to how you feel when you are walking at a normal pace.
• Remember that this is a recall of activities for the past week, not a history of what you usually do.
  o We’ll start with yesterday. Today is (i.e. Monday), so yesterday was (i.e. Sunday). Think about what you did in general yesterday morning. Did you do any PA (Sunday morning)? How long did you do that activity? How much of that time was spent standing still or taking breaks? Did that activity feel similar to how you feel when you are walking or running or is it somewhere in between? Did you do any PA (Sunday afternoon)? (Duration, intensity). Did you do any PA (Sunday evening)? (Duration, intensity).
  o If people are giving too much information, it is appropriate to ask “how much time in general?” – i.e. remind them they do not need to account for every minute of the day. For an activity to be counted, it must add up to at least 10 min in one intensity category for one segment of the day (round to 15 min).

1. **At the end of each day:** Are there any PAs you might have forgotten? Did you do any PA at work? Any other recreational or sport activities? Housework or gardening? Were there any other walks that you might have taken?
2. **On the last day of recall:** Take a moment to think back over the course of the week and think of any activities you may have forgotten.
3. **Last question:** The last question I’m going to ask you is, “Compared to your PA over the past 3 mo, was last week’s PA more, less, or about the same?”
4. Thank you.

Prompting questions (examples):

- What were you doing [day] morning?
- You said that you got up at 6am. Did you go anywhere after that?
- Did you watch any particular TV show?
- What did you make for dinner?
- What did you do that evening?
- Did you take any walks that you may have overlooked?
- Did you do any vigorous home repair or gardening?
- Are there any activities that you are unsure about?

**Scoring**

<table>
<thead>
<tr>
<th>Time Range</th>
<th>Round To</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min to 22 min</td>
<td>15 min</td>
<td>0.25</td>
</tr>
<tr>
<td>23 min to 37 min</td>
<td>30 min</td>
<td>0.5</td>
</tr>
<tr>
<td>38 min to 52 min</td>
<td>45 min</td>
<td>0.75</td>
</tr>
<tr>
<td>53 min to 67 min</td>
<td>60 min</td>
<td>1.0</td>
</tr>
<tr>
<td>68 min to 1 hr 22 min</td>
<td>1 hr 15 min</td>
<td>1.25</td>
</tr>
</tbody>
</table>
Scoring Continued

Table 8. Scoring of 7-Day PAR

<table>
<thead>
<tr>
<th>Category</th>
<th>MET range</th>
<th>Hours</th>
<th>MET Value</th>
<th>Total MET hr/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep</td>
<td>1.0</td>
<td>?</td>
<td>× 1.0</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>3.0-4.9</td>
<td>?</td>
<td>× 4.0</td>
<td></td>
</tr>
<tr>
<td>Hard</td>
<td>5.0-6.9</td>
<td>?</td>
<td>× 6.0</td>
<td></td>
</tr>
<tr>
<td>Very Hard</td>
<td>&gt;7.0</td>
<td>?</td>
<td>× 10.0</td>
<td></td>
</tr>
<tr>
<td>Remaining Hours (Light)</td>
<td>1.1-2.9</td>
<td>?</td>
<td>× 1.5</td>
<td></td>
</tr>
</tbody>
</table>

TOTAL MET hr/wk:  

Note: This script was adapted from reference (2).
Physical Activity Recall

1. Were you employed in the last seven days (paid or volunteer)?

   □ YES    □ NO  → Go to question 4

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

   Subject ID: __________    Interviewer Initials: __________
<table>
<thead>
<tr>
<th>Sleep</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
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<tr>
<td>Hard</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Hard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Moderate</td>
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<td></td>
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<tr>
<td>Hard</td>
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<td></td>
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<td>Very Hard</td>
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<tr>
<td>Moderate</td>
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<td>Hard</td>
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<td></td>
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<tr>
<td>Very Hard</td>
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<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 mins = 1.25 hrs

Subject ID: __________   Interviewer Initials: ___________
7-Day PAR: Interview Evaluation Form

Were there any problems with the 7-Day PAR interview? (circle one)

1. Yes  2. No

Explain:

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

Do you think this was a valid 7-Day PAR interview?

1. Yes  2. Maybe  3. No

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

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________

Other comments/concerns:

_________________________________________________________________________________________________________
_________________________________________________________________________________________________________
APPENDIX H

CAFFEINE CONSUMPTION QUESTIONNAIRE
**Caffeine Consumption Questionnaire**

Please fill in the table below by indicating your best estimate of the number of times per week on average that you consume a particular substance. Only report items that you consume at least once per week.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Average # of Times per Week</th>
<th>X Caffeine Amount (mg)</th>
<th>=</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee (5 oz. Servings)†</td>
<td></td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Regular, percolated</td>
<td></td>
<td>110</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Regular, drip-brewed</td>
<td></td>
<td>150</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Regular, instant</td>
<td></td>
<td>66</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Decaffeinated, brewed</td>
<td></td>
<td>4.5</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Decaffeinated, instant</td>
<td></td>
<td>2</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Tea (5 oz. Servings)</td>
<td></td>
<td>45</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Cocoa (5 oz. Servings)</td>
<td></td>
<td>13</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Chocolate (1.5 oz. Servings; ~ 1 bar)</td>
<td></td>
<td>30</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Soft Drinks (1 can; 12 oz.)</td>
<td></td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Coke or Cherry Coke</td>
<td></td>
<td>46.5</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Diet Coke, Diet Cherry Coke</td>
<td></td>
<td>46.5</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Minute Maid Drinks</td>
<td></td>
<td>46.5</td>
<td>=</td>
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</tr>
<tr>
<td>Mello Yello, Diet Mello Yello</td>
<td></td>
<td>52.5</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Dr. Pepper</td>
<td></td>
<td>61</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Pepsi</td>
<td></td>
<td>37.2</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Diet Pepsi</td>
<td></td>
<td>35.2</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Mountain Dew, Diet Mountain Dew</td>
<td></td>
<td>54</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>RC Cola (or other brand)</td>
<td></td>
<td>36</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Jolt</td>
<td></td>
<td>71.2</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Energy Drinks (e.g., Red Bull); 250 mL, 8.8 oz can</td>
<td></td>
<td>80</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Over-the-Counter Drugs (tablets/wk)</td>
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<td>=</td>
<td></td>
</tr>
<tr>
<td>Vivarin (alertness aid)</td>
<td></td>
<td>200</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>NoDoz (alertness aid)</td>
<td></td>
<td>100</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Caffedrin (alertness aid)</td>
<td></td>
<td>200</td>
<td>=</td>
<td></td>
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<tr>
<td>Excedrin (analgesic)</td>
<td></td>
<td>65</td>
<td>=</td>
<td></td>
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<tr>
<td>Vanquish (analgesic)</td>
<td></td>
<td>33</td>
<td>=</td>
<td></td>
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<tr>
<td>Darvon compound-65 (analgesic)</td>
<td></td>
<td>32</td>
<td>=</td>
<td></td>
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<tr>
<td>Anacin (analgesic)</td>
<td></td>
<td>32</td>
<td>=</td>
<td></td>
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<tr>
<td>Dristan (cold/flu)</td>
<td></td>
<td>16.2</td>
<td>=</td>
<td></td>
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<tr>
<td>Dextrim (appetite suppression)</td>
<td></td>
<td>200</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
<td></td>
<td>=</td>
<td></td>
</tr>
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<td>=</td>
<td></td>
</tr>
<tr>
<td>Other:</td>
<td></td>
<td></td>
<td>=</td>
<td></td>
</tr>
<tr>
<td><strong>GRAND TOTAL:</strong></td>
<td></td>
<td></td>
<td>=</td>
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</tr>
</tbody>
</table>

†Note: For size reference, 5 oz = “small”; 2 x 5 oz = “medium”; 3 x 5 oz = “large”; and 4 x 5 oz = “extra large” at Tim Hortons.

APPENDIX I

DOPPLER ULTRASOUND

QUANTIFYING THE ANGLE OF INSONATION
“Ultrasound” refers to high frequency waves (>20 kHz) that are of the same physical nature as sound, but are above the range of human hearing (78). Doppler ultrasound functions by directing ultrasound energy of a known frequency to diagonally intersect the blood vessel of interest (168); in our case, this ultrasound energy is 4-MHz (i.e. 4 million cycles per second). Stationary objects, such as the vessel wall, reflect the ultrasound back at the same frequency, whereas moving objects, such as RBCs, scatter the ultrasound so that the reflected frequency is shifted (168). The reflected sound waves are in the audio range (168), and are transduced into electrical energy (in Volts) that can be recorded in a computer data acquisition system for analysis and interpretation (78). The frequency shift in the reflected energy (and thus the recorded Voltage) is proportional to the RBC velocity, and is represented by the equation:

\[ \Delta f = \frac{2(V \cos \theta) f_t}{c} \]

where \( \Delta f \) is the Doppler frequency shift, \( V \) is the magnitude of the RBC’s velocity (i.e. the scatterer of the ultrasound), \( \theta \) is the angle between the ultrasound and the direction of motion (i.e. the angle of insonation of the ultrasound beam), \( f_t \) is the frequency of the transmitted ultrasound, and \( c \) is the speed of propagation of ultrasound in blood (47).

Rearranging this equation to calculate blood velocity gives (168):

\[ V = \frac{\Delta f \cdot c}{2f_t \cdot \cos \theta} \]

From this it is evident that measurement of the Doppler shift (\( \Delta f \)) allows calculation of RBC velocity (\( V \)), given that the frequency of the transmitted ultrasound (\( f_t \)) and the angle of insonation (\( \theta \)) are known. In practice, a two-point calibration (i.e. \( \Delta f \rightarrow \text{Volts} \) to
cm/sec) is entered into the computer recording software to compute the MBV for a given angle of insonation and a given transmitted ultrasound frequency.

The ultrasound probe used in this study has an inherent 57° angle of insonation (Figure 31A), and a two-point calibration allows for calculation of the actual MBV for any given frequency shift observed (the two points of reference being $\Delta f$ of 0 Hz = 0.5 Volts = 0 cm/s, and $\Delta f$ of 8,841.5 Hz = 2.036 Volts = 312.5 cm/s). Importantly, any change in the angle of the blood vessel relative to the skin, and thus relative to the probe, alters this angle such that the angle of insonation is actually less than or greater than 57° (Figure 31 B & C). An angle of less than 57° would result in a larger frequency shift for the same blood velocity, and would therefore overestimate the true blood velocity, while conversely an angle of greater than 57° would underestimate it.

Therefore in this study the angle of the blood vessel relative to the probe was determined by imaging the blood vessel with Echo ultrasound, and an adjusted units conversion (i.e. volts to cm/sec) was entered into the software program prior to data analysis.
Figure 31. Doppler Ultrasound Probe angle of insonation. (A) When the blood vessel is parallel to the skin, the angle of insonation of the transmitted ultrasound is 57° for the probe used in this study. (B) If the true angle of insonation is less than 57°, MBV will be overestimated based on the 57° assumed angle of insonation. (C) Conversely, if the true angle of insonation is greater than 57°, MBV will be underestimated. Therefore the angle of the blood vessel relative to the skin was measured to quantify the actual angle of insonation and an adjusted units conversion (i.e. volts to cm/sec) was applied to the software program prior to data analysis. $f_t =$ frequency of the transmitted ultrasound; MBV = mean blood velocity; RBC = red blood cell
APPENDIX J

STROKE VOLUME and CARDIAC OUTPUT DATA
Figure 32. Individual subject and group mean SV responses at rest (-60 to 0 sec) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 33. Individual subject and group mean CO responses at rest (-60 to 0 sec) and during forearm handgrip exercise (17.5 kg, 2 sec contraction to 2 sec relaxation). Inset numbers represent number of subjects included in mean points (i.e. identifying subject drop-out as exercise continued); Gray = T2DM, Black = Control. Data are means ± SE.
Figure 34. Mean SV at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls $n = 4$; T2DM $n = 4$. 
Figure 35. Mean CO at baseline, the first minute of exercise, and steady state. Data are means ± SE. Controls $n = 4$; T2DM $n = 4$. 
APPENDIX K

SAMPLE CAPSULE ORDER FORM
Caffeine Trial

Treatment Group: □ C □ D

Subject Code: __ __ __
Subject Code-Treatment: __ __ __ - __ , __
Subject Initials: __ __ __
Date of Dose Request: ___ / ____/ _____
Date of Data Collection Session: ___ / ____/ _____

Data Collection Session #: □ 1 □ 2 □ Both

► Subject Weight at screening: ___ ___ ___ kg (rounded up or down)

Dose Calculation
► ___ (kg) X 5 (mg/kg) = ___ mg
X ________________________ Dr. R. Hudson

Dose confirmed by IDS: _____________ Date: ___/___/____

Caffeine 5 mg/kg OR Placebo capsule (1)
Take ONE capsule as directed

Please FAX to the Kingston General Hospital
Investigational Drug Service at (613) 548-1386 at least 1 hour prior to the projected dosing time. Call KGH 613-549-6666 ext#3654 or pager #512 to notify the IDS technician of the subject’s enrollment.
APPENDIX L

POST-TRIAL QUESTIONNAIRE
Post-Trial Questionnaire

Trial #: ______

In this trial, do you believe that you consumed:

☐ Caffeine
☐ Placebo
☐ Not Sure

Reason for answer:
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

How do you think this affected your performance?

☐ It allowed me to exercise longer.
☐ It made me stop exercising sooner.
☐ It had no effect on how I would otherwise have been able to perform.