METABOREFLEX-INDUCED FLOW IMPROVEMENT IS ABSENT IN OLDER MALES WITH TYPE II DIABETES

by

Michael Bravo

A thesis submitted to the School of Kinesiology and Health Studies

In conformity with the requirements for

the degree of Master of Science

Queen’s University

Kingston, Ontario, Canada

January, 2012

Copyright ©Michael Bravo, 2012
Abstract

Background: Exercise is widely recognized as the cornerstone of management of type II diabetes (T2D). However, it is also known that people with T2D have poor adherence to exercise regimens, which is largely thought to be because of poor exercise tolerance. Recent studies have suggested that this exercise intolerance may be caused by a reduction in exercising muscle blood flow. One physiological mechanism which could potentially contribute is the muscle metaboreflex (MMR). This mechanism is thought to be a pressure-based flow-improving mechanism, but as a result of reduced efficacy of vasodilators and sympatholytic agents, might in fact be restraining the flow-improvement in persons with T2D.

Hypothesis: Persons with T2D would not improve exercising muscle blood flow upon MMR activation. This absence of flow-improvement will be due to an augmented vasoconstriction in the exercising muscle.

Methods: T2D (n=7) and CTL (n=6) participants performed rhythmic forearm handgrip exercise at an intensity equivalent to 20% MVC for 9 minutes with and without the application of ischemic plantar flexion (IPF). Forearm blood flow (FBF), mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), total peripheral resistance (TPR) and forearm vascular conductance (FVK) were quantified for the last thirty seconds of each of four time points during the protocol. Plasma norepinephrine was measured via deep venous and arterialized venous blood sampling.

Results: Steady state exercising FBF was increased in CTL but not in T2D during MMR activation (mean ± SE mL/min: CTLControl 161.16 ± 5.95, CTLMMR 212.72 ± 9.49, T2DControl 156.71 ± 13.08, T2DMMR 144.22 ± 10.55). This occurred despite similar increases in MAP, CO, HR, and TPR (across groups and treatment conditions, NS). FVK increased in CTL
during the MMR protocol compared to the Control protocol, but decreased in the T2D group using the same comparison (mean ± SE mL/min/100 mm Hg: CTLControl 144.74 ± 5.63, CTLMMR 176.76 ± 11.99, T2DControl 143.29 ± 13.44, T2DMMR 103.53 ± 8.44).

Conclusions: In the exercise model utilized, persons with T2D do not demonstrate the MMR-induced flow improvement seen in CTL. This impaired muscle blood flow in T2D is the result of MMR induced exercising limb vasoconstriction.
Acknowledgements

First and foremost, I would like to thank and acknowledge my supervisor, Michael Tschakovsky, for his guidance, counseling and mentorship over the last two and a half years. You have taught me how to think, how to question and how to perceive science as a frontier of exciting discovery rather than a collection of data. Your faith and support will undoubtedly guide my future development as a scientist.

I would like to thank my lab mates and friends of the HVCL, for your unwavering support and dedication even in the face of weekend data collection sessions and Monster-fuelled all-nighters. I would especially like to acknowledge the superb work of the gentlemen of BWO: Mikhail Kellawan, Robert Bentley and Jeremy Walsh. I cannot thank you enough for all the sacrifice and hard work you put in to my thesis and for that I am eternally grateful. I would also like to thank Veronica Poitras for being the pioneer/guinea pig in the world of HVCL T2D research and for her guidance, consultation and friendship. You have no idea the help and assurance you gave me over these last 22 months.

A huge thank you to my favourite Winnepegger, Valerie Carson, for her assistance, guidance and friendship. You did much to assuage my fear of all things statistics!

Another thank you to the bro-est of the bros, David Lysecki, without whom I would have gone crazy during my time in Kingston. We had an epic run over the last two years and I hope to bro it with you as often as possible. What bros we are!

Finally, I would like to thank my parents, Frank and Dorothy, for your unwavering support, your love and your affection. Without it, I would be lost beyond belief. Few people are as blessed as I am to have you as parents (just three others, technically), and I appreciate all you have done for me during this long journey. It will never be forgotten, even when the thoughts of Shady Pines cross my mind.
# Table of Contents

Abstract ................................................................................................................................. ii
Acknowledgements ................................................................................................................ iv
Chapter 1 - Introduction ......................................................................................................... 1
Chapter 2 - Literature Review ............................................................................................... 6
  2.1 Introduction to Type II Diabetes .................................................................................... 6
  2.2 How does exercise help in the management of T2D? ................................................... 6
  2.3 What are the problems with exercise as a means of managing T2D? ......................... 7
  2.4 What could be causing this exercise intolerance in T2D? ........................................... 8
    2.4.1 What are the impairments during the transition to exercise? ................................. 9
    2.4.2 What are the impairments to steady-state exercise blood flow in T2D? ............... 10
  2.5 What happens to the vasodilatory response in T2D? ................................................. 12
  2.6 How does sympathetic restraint affect blood flow? ................................................... 14
  2.7 What is the muscle metaboreflex? ............................................................................... 15
  2.8 How is the MMR activated? ....................................................................................... 16
  2.9 How does the MMR maintain or increase flow to exercising muscle? ....................... 16
  2.10 What could exaggerate the effect of the MMR in T2D? ............................................. 17
    2.10.1 What are the differences in the diabetic muscle? ............................................... 18
    2.10.2 How does the altered reliance on anaerobic means affect the onset of the MMR? ... 18
  2.11 Study Rationale ......................................................................................................... 21
Chapter 3 - Methods ............................................................................................................ 22
  3.1 Subjects ....................................................................................................................... 22
  3.2 Seven-day Physical Activity Recall ............................................................................ 24
  3.3 Fasting Blood Glucose ............................................................................................... 25
  3.4 Maximum Voluntary Contraction .............................................................................. 25
  3.5 Forearm Handgrip Exercise ....................................................................................... 26
  3.6 Ischemic Calf Exercise ............................................................................................... 26
  3.7 Arterialized Blood Sampling ....................................................................................... 27
  3.8 Forearm Deep Vein Blood Sampling ........................................................................... 28
  3.9 Central Hemodynamic Monitoring ............................................................................ 29
  3.10 Brachial Artery Mean Blood Velocity and Diameter ................................................ 29
  3.11 Subject Screening and Familiarization .................................................................... 30
    3.11.1 Anthropometric Measurements ........................................................................ 30
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.12 Subject Instrumentation</td>
<td>31</td>
</tr>
<tr>
<td>3.13 Experimental Protocol</td>
<td>32</td>
</tr>
<tr>
<td>3.14 Experimental Protocol</td>
<td>34</td>
</tr>
<tr>
<td>3.14.1 Control Protocol</td>
<td>34</td>
</tr>
<tr>
<td>3.14.2 MMR Protocol</td>
<td>34</td>
</tr>
<tr>
<td>3.15 Data Acquisition</td>
<td>35</td>
</tr>
<tr>
<td>3.16 Data Analysis</td>
<td>35</td>
</tr>
<tr>
<td>3.16.1 General Data Analysis Approach</td>
<td>35</td>
</tr>
<tr>
<td>3.16.2 Forearm Blood Flow</td>
<td>35</td>
</tr>
<tr>
<td>3.16.3 Heart Rate, Mean Arterial Pressure, Cardiac Output</td>
<td>36</td>
</tr>
<tr>
<td>3.16.4 Forearm Vascular Conductance</td>
<td>36</td>
</tr>
<tr>
<td>3.16.5 Total Peripheral Resistance</td>
<td>37</td>
</tr>
<tr>
<td>3.16.6 Venous Blood Analysis</td>
<td>37</td>
</tr>
<tr>
<td>3.16.7 Group Analysis</td>
<td>37</td>
</tr>
<tr>
<td>3.17 Statistical Analysis</td>
<td>38</td>
</tr>
<tr>
<td>Chapter 4 - Methods</td>
<td>39</td>
</tr>
<tr>
<td>Chapter 5 - Discussion</td>
<td>51</td>
</tr>
<tr>
<td>5.1 Subject Characteristics</td>
<td>52</td>
</tr>
<tr>
<td>5.2 Muscle Metaboreflex Analysis</td>
<td>53</td>
</tr>
<tr>
<td>5.2.1 The absence of MMR-induced blood flow improvement in persons with T2D</td>
<td>53</td>
</tr>
<tr>
<td>5.2.2 Why is there heterogeneous flow response in the T2D group?</td>
<td>55</td>
</tr>
<tr>
<td>5.2.3 How does MAP change during the MMR and is this change similar between groups?</td>
<td>56</td>
</tr>
<tr>
<td>5.2.4 How is MAP increased?</td>
<td>56</td>
</tr>
<tr>
<td>5.2.5 How does vascular conductance change during the MMR and is this change similar between groups?</td>
<td>57</td>
</tr>
<tr>
<td>5.3 Steady State Exercising FBF</td>
<td>59</td>
</tr>
<tr>
<td>5.3.1 Baseline and exercise time points</td>
<td>59</td>
</tr>
<tr>
<td>5.3.2 Medication Wash-out Period</td>
<td>60</td>
</tr>
<tr>
<td>5.3.3 Exercising Muscle Mass</td>
<td>61</td>
</tr>
<tr>
<td>5.3.4 Does the amount of muscle mass matter?</td>
<td>62</td>
</tr>
<tr>
<td>5.3.5 Is there a difference in limb vasodilatory response?</td>
<td>63</td>
</tr>
<tr>
<td>5.4 Limitations</td>
<td>64</td>
</tr>
<tr>
<td>5.4.1 Sample Size</td>
<td>64</td>
</tr>
<tr>
<td>5.4.2 Medical Comorbidities</td>
<td>64</td>
</tr>
</tbody>
</table>
5.5 Future Directions ........................................................................................................ 65
Chapter 6 - Summary and Conclusion ........................................................................ 67
References ..................................................................................................................... 69
Appendix A - Consent and Participant Data Form ...................................................... 77
Appendix B - Subject Medications .............................................................................. 92
Appendix C - Inclusion/Exclusion Criteria ................................................................ 94
Appendix D - Subject Recruitment Initiatives ............................................................ 96
Appendix E - Seven-Day Physical Activity Recall .................................................... 98
Appendix F - ACSM Contraindications to Exercise Testing ....................................... 103
Appendix G - Individual Subject Data ........................................................................ 105
Appendix H - Repeatability Data ................................................................................ 136
List of Figures

Figure 1. Schematic of the muscle metaboreflex ................................................................. 2
Figure 2. Schematic of vasodilator and vasoconstrictor influence ........................................... 4
Figure 3. Potential contributors to decreased exercise tolerance ............................................ 9
Figure 4. Net drive hypothesis .............................................................................................. 20
Figure 5. Subject Instrumentation Set-up ............................................................................... 32
Figure 6. Protocol schematic ................................................................................................. 33
Figure 7. Absolute Forearm Blood Flow ............................................................................... 41
Figure 8. Absolute Mean Arterial Pressure .......................................................................... 42
Figure 9. Absolute Cardiac Output ....................................................................................... 43
Figure 10. Absolute Heart Rate ......................................................................................... 44
Figure 11. Absolute Total Peripheral Resistance ................................................................... 45
Figure 12. Absolute Forearm Vascular Conductance ............................................................ 46
Figure 13. Relationship between cardiac output and heart rate for CTL group ...................... 47
Figure 14. Relationship between cardiac output and heart rate for T2D group ..................... 48
Figure 15. Relationship between cardiac output and mean arterial pressure during the MMR protocol .................................................................................................................................. 49
Figure 16. Relationship between change in flow from baseline to exercise and actual workload performed .................................................................................................................................. 50
Figure 17. Relationship between change in cardiac output and change in stroke volume ...... 51
Figure 18. Change in flow from exercise in the CTL group .................................................. 106
Figure 19. Change in flow from exercise in the T2D group ................................................... 107
Figure 20. Change in flow from exercise in CTL group – grouped by individual ................... 108
Figure 21. Change in flow from exercise in T2D group – grouped by individual .................... 109
Figure 22. Flow boxplot demonstrating median change in flow with 25th and 75th percentiles... 110
Figure 23. Change in mean arterial pressure from exercise in the CTL group ....................... 111
Figure 24. Change in mean arterial pressure from exercise in the T2D group ....................... 112
Figure 25. Change in mean arterial pressure from exercise in CTL group – grouped by individual. .................................................................................................................................. 113
Figure 26. Change in mean arterial pressure from exercise in T2D group – grouped by individual. .................................................................................................................................. 114
Figure 27. Mean arterial pressure boxplot demonstrating median change in MAP with 25th and 75th percentiles ....................................................................................................... 115
Figure 28. Change in cardiac output from exercise in the CTL group. .................................................. 116
Figure 29. Change in cardiac output from exercise in the T2D group. .................................................. 117
Figure 30. Change in cardiac output from exercise in CTL group – grouped by individual. ..... 118
Figure 31. Change in cardiac output from exercise in T2D group – grouped by individual. ........ 119
Figure 32. Cardiac output boxplot demonstrating median change in MAP with 25th and 75th
percentiles. ................................................................................................................................. 120
Figure 33. Change in heart rate from exercise in the CTL group. .................................................... 121
Figure 34. Change in heart rate from exercise in the T2D group. .................................................... 122
Figure 35. Change in heart rate from exercise in CTL group – grouped by individual. ............... 123
Figure 36. Change in heart rate from exercise in CTL group – grouped by individual. ............. 124
Figure 37. Heart rate boxplot demonstrating median change in MAP with 25th and 75th
percentiles. ................................................................................................................................. 125
Figure 38. Change in mean arterial pressure from exercise in the CTL group. ......................... 126
Figure 39. Change in total peripheral resistance from exercise in the T2D group. ..................... 127
Figure 40. Change in total peripheral resistance from exercise in CTL group – grouped by
individual. ................................................................................................................................. 128
Figure 41. Change in total peripheral resistance from exercise in CTL group – grouped by
individual. ................................................................................................................................. 129
Figure 42. Total peripheral resistance boxplot demonstrating median change in MAP with 25th
and 75th percentiles. ................................................................................................................ 130
Figure 43. Change in forearm vascular conductance from exercise in the CTL group. ............. 131
Figure 44. Change in forearm vascular conductance from exercise in the T2D group. ............. 132
Figure 45. Change in forearm vascular conductance from exercise in CTL group – grouped by
individual. ................................................................................................................................. 133
Figure 46. Change in forearm vascular conductance from exercise in CTL group – grouped by
individual. ................................................................................................................................. 134
Figure 47. Forearm vascular conductance boxplot demonstrating median change in DVK with
25th and 75th percentiles. ........................................................................................................ 135
Figure 48. Repeatability data for flow at baseline. ....................................................................... 137
Figure 49. Repeatability data for mean arterial pressure at baseline. ....................................... 138
Figure 50. Repeatability data for cardiac output at baseline. ..................................................... 139
Figure 51. Repeatability data for flow at heart rate. ..................................................................... 140
Figure 52. Repeatability data for total peripheral resistance at baseline. .................................. 141
Figure 53. Repeatability data for forearm vascular conductance at baseline. ......................... 142
Figure 54. Repeatability data for flow during exercise. .......................................................... 143
Figure 55. Repeatability data for mean arterial pressure during exercise. .......................... 144
Figure 56. Repeatability data for cardiac output during exercise. ........................................ 145
Figure 57. Repeatability data for heart rate during exercise. ................................................ 146
Figure 58. Repeatability data for total peripheral resistance during exercise. ...................... 147
Figure 59. Repeatability data for forearm vascular conductance during exercise. ............... 148
List of Tables

Table 1. Subject characteristics. ..........................................................................................................................23

Table 2. Subject medications. ............................................................................................................................93
Chapter 1

Introduction

Type II diabetes mellitus (T2D) is one of the fastest developing worldwide epidemics. There are currently nearly 300 million people suffering from this disease and at present, 10% of the Canadian population suffers from T2D (42). It has long been known that physical activity is one of the cornerstones for the management of T2D (2). However, it is also recognized that persons with T2D have low adherence to exercise programs, even when prescribed by doctors (58). This has been attributed to poor exercise tolerance by T2D (68, 69). It has been suggested that a number of physiological mechanisms could underlie this disproportionately poor exercise intolerance in persons with T2D, such as an impaired cardiac response to exercise, a deficit in the skeletal muscle diffusion or impairments to oxygen delivery (69). Of those that have been investigated, the mechanism which has been most researched is impaired oxygen delivery.

At the onset of exercise, increases in aerobic ATP production requires an increase in oxygen delivery to the exercising muscle. This increase in oxygen delivery can be accomplished via an increase in two factors: the vascular conductance to the exercising muscle and the mean arterial pressure. An increase in either or both of these factors, will result in an increase in the flow of oxygen to the exercising muscle.
Figure 1. Schematic of the muscle metaboreflex.

1: Metabolites accumulate in the exercising muscle and increase the firing of muscle afferents to the NTS. 2: The NTS receives muscle afferent input and responds by increasing sympathetic output. 3: Sympathetic output causes an increase in cardiac output and muscle sympathetic nervous activity.

In moderate- to high-intensity exercise there can be an activation of the muscle metaboreflex (MMR), a nervous system pressor response (44). The MMR-induced pressure increase is evoked in response to the accumulation in the interstitial space of metabolites such as H+ and inorganic phosphate (Pi) (44). These metabolites stimulate muscle afferent nerves which project to the nucleus tractus solitarii in the brain which then processes the incoming signals and responds with an increase in efferent sympathetic nerve activity (72).
There are two main effects of this nervous activity: one evokes peripheral vasoconstriction, specifically via an increase in muscle sympathetic nervous activity (MSNA) (44); the other is an increase in cardiac output (Lee et al. 2000, Saltin et al. 1985). The constriction of the vasculature would seem to be counter-productive to maintaining muscle blood flow to exercising muscles – why would a physiological process potentially hamper blood flow by reducing conductance? However, the action of local vasodilators (11) as well as sympatholytic agents (which blunt the response to MSNA at the level of the exercising muscle) are believed to allow the maintenance of vascular conductance at the exercising muscle sufficient to benefit from the increase in arterial pressure. As a result, there is an increase in blood flow to the exercising muscle (64).

Although cardiac output does play a role in blood flow regulation during exercise, it is important to recognize that there must be sufficient vascular conductance to the exercising muscle to benefit from this increase in CO and decrease in conductance elsewhere in the body. Not only are vasodilators (Figure 2, Item 1) at work but there is also the significant contribution of sympatholytic factors at play, i.e., agents which blunt the MSNA by inhibiting its target receptors (71) (Figure 2, Item 5). These agents tend to have a vasodilatory influence, which ultimately reduces the overall MSNA-induced vasoconstriction. However, it has been demonstrated that certain vasodilators and sympatholytic agents, such as ATP, have a reduced response in T2D subjects (13, 18). As a result, there may in fact be a reduction in vasodilatory influence by both vasodilators and
sympatholytic agents, resulting in a reduction in the exercising muscle vascular conductance in T2D patients as a result of MMR activation.

**Figure 2. Schematic of vasodilator and vasoconstrictor influence.**

1 - Vasodilator influence from various dilator substances in the blood. 2 + 3 - Vasoconstrictor influence - MMR induced increases in MSNA results in a vasoconstrictor influence at the level of the muscle vasculature. 4 - Vasoconstrictor influence – the baroreflex exerts a vasoconstrictor influence by attempting constricting vasculature to return MAP to homeostatic levels. 5 – Vasodilator influence – sympatholytic agents blunt the effects of the MSNA by preventing the activation of alpha-adrenergic receptors.

Furthermore, T2D subjects also tend to be obese (20) and obesity has been linked to elevated levels of MSNA (25). These T2D subjects might not only be experiencing these vasodilatory complications, but may also be subject to higher levels of MSNA, which when
coupled to the MMR-induced elevation in MSNA, may result in excessive vasoconstrictor influence at the level of the exercising muscle. Therefore, we propose that activation of the muscle metaboreflex may actually lead to reduction in exercising muscle blood flow in T2D patients.

Thus, the objective of this thesis is to determine whether evoking the muscle metaboreflex (MMR) in subjects with type II diabetes allows for the same pressure-induced flow increase as seen in healthy controls, or whether there may in fact be a flow impairment.
Chapter 2

Literature Review

2.1 Introduction to Type II Diabetes

Type II diabetes is diagnosed if a person exhibits at least one of three conditions: a fasting plasma glucose level of greater than 7.0 mmol/L; or casual plasma glucose ≥ 11.1 mmol/L; or 2 hour plasma glucose in a 75 g oral glucose tolerance test of ≥ 11.1 mmol/L (8). The elevated levels of plasma glucose point to the major complication of T2D – a reduction in insulin action (i.e. insulin resistance) and secretion (i.e. insulin deficiency) (76). Insulin resistance is most often described as a reduction in the efficacy of insulin to lower plasma glucose levels by storing glucose as glycogen in the liver and muscle(8). Inadequate insulin secretion is considered to be an absence of the first phase of insulin release and can result in hyperglycemia, also known as glucose toxicity (76). The long-term effects of insulin resistance and secretion deficiencies can lead to retinopathy, hypertension, atherosclerotic cardiovascular disease, peripheral vascular disease, and other pathologies (2).

2.2 How does exercise help in the management of T2D?

While there are a number of pharmacological interventions that can be prescribed for T2D patients, it is recognized that one of the ideal treatment methods of T2D is physical activity (Albright et al. 2000, Stewart 2002, Sigal et al. 2003). Physical activity (PA) has been shown to target many of the major symptoms of T2D. For example, Hubinger et al.
(1987) (30) demonstrated that after mild-to-moderate exercise, blood glucose levels decreased in T2D patients. Furthermore, Hubinger et al. (30) also demonstrated that these lowered blood glucose levels extended into the post-exercise period, up to 7 hours after the bout of exercise (30). Exercise has also been shown to elevate insulin-independent muscle glucose uptake (9). Physical activity has also been shown to mitigate the insulin resistance that exists in T2D patients. It has been demonstrated that T2D patients performing mild-to-moderate bouts of exercise demonstrated elevated insulin sensitivity for up to 24 hours post-exercise (10). Apart from these pathology-related improvements caused by exercise, it has been demonstrated by a number of research groups that T2D patients who exercise also demonstrate lower resting and submaximal exercise heart rates, increased stroke volume and cardiac output, enhanced oxygen extraction and lower resting and exercise blood pressure (2, 5, 69). (2, 15, 69)

2.3 What are the problems with exercise as a means of managing T2D?

While it is generally recognized that physical activity is highly effective for the management of T2D, many patients tend not to adhere to the exercise prescription given to them by their doctors. In one study by Thomas et al., only 34% of T2D patients performed any kind of exercise and of those, only 9% performed sufficient exercise to cause a "large" change in heart rate or breathing (82). The authors found that the most significant barrier to performing physical activity was a lack of confidence in being able to perform the exercise and feelings of "tiredness" (82). Another study demonstrated slowed VO₂ kinetics during the transition to moderate exercise which, according to the authors, suggests that
there may be an accumulated oxygen deficit during the initiation of exercise that could affect the willingness of individuals to perform exercise (6). Another study also demonstrated that higher levels of glycosylated hemoglobin (the major marker of diabetes) correlated with a lower exercise capacity in diabetics (16). Considering that the T2D patients admit to a decreased compliance to prescribed exercise which is attributed to tiredness, and that this tiredness may have an underlying physiological cause, it is clear that an investigation into the possible causes of this tiredness is vital to ameliorating the exercise tolerance issues of type II diabetics.

2.4 What could be causing this exercise intolerance in T2D?

There are a number of identified potential contributors to exercise intolerance. Some researchers have identified a reduced ability for oxygen to diffuse into the myocyte as a result of decreased capillary density (46). These results have also been found in animal models, confirming a potential diffusive obstruction (62, 63). Others have focused on the reduction of the capacity for metabolic oxygen consumption by analyzing mitochondrial enzyme content (33, 70). However, a number of studies have focused on the potential impairment to convective oxygen delivery in T2D patients. Of these studies, there are two main categories of investigation: those addressing muscle blood flow during the transition to exercise, and those addressing steady-state exercising muscle blood flow.
Figure 3. Potential contributors to decreased exercise tolerance.
1 - A reduced diffusive conductance; 2 – A reduction in metabolic oxygen consumption; 3 – A reduction in convective oxygen delivery

2.4.1 What are the impairments during the transition to exercise?

Firstly, Regensteiner and colleagues (1998) (68) evaluated the response to the onset of constant-load exercise at three different intensities in premenopausal women with uncomplicated type II diabetes. They found that subjects with T2D had slower VO₂ and heart rate kinetics when compared to both lean and overweight control subjects. This confirms the previous findings from the same research group which found similar lower VO₂ responses (i.e. a 20% decrease) during submaximal graded exercise tests in T2D patients when compared with controls. Clearly, these studies demonstrate a decreased ability to adjust to a given oxygen demand and thus indicate a great oxygen deficit that would exist within the myocyte at the onset of exercise.

Further confirming this was a study in T2D patients by Bauer et al. (2007) (6) which not only measured VO₂ kinetics, but also used near-infrared spectroscopy to quantify the amount of deoxygenated hemoglobin/myoglobin [HHb] at the level of the myocyte to give an indication as
to the microvascular blood flow response during the transition to moderate exercise. They found that there was an “overshoot” in the initial response to [HHb] in those with diabetes when compared to healthy normal subjects, indicating a clear impediment to the initial muscle blood flow response and thus a greater reliance on oxygen extraction in order to attempt to match oxygen delivery to demand (6). The authors suggest that it is this abnormal blood flow response which may contribute to the impaired exercise tolerance seen in T2D patients.

Although there are few studies looking into the muscle blood flow responses at the onset of exercise in T2D patients, there conclusions are at least clear: there is a reduced kinetic VO$_2$ response in T2D patients during their transition to exercise. This would suggest a greater reliance on anaerobic means to maintain force production and a greater reliance on oxygen extraction to compensate for this decreased response. However, it is not clear from these studies whether or not these reduced responses have a long-term effect during steady-state exercise.

### 2.4.2 What are the impairments to steady-state exercise blood flow in T2D?

While Regensteiner and colleagues (1998) (68) have identified that T2D subjects will eventually reach the same steady-state exercising VO$_2$ when compared to healthy controls, this does not necessarily mean that muscle blood flow also reaches the same levels. In fact, there have been a number of studies which have demonstrated a reduction in the skeletal muscle blood flow response during steady-state exercise.

Early investigation into this potential complication was undertaken by Young and colleagues who had people with diabetes perform cycle ergometer exercise at 75 W in different positions (90). They found that muscle blood flow (determined with the 133Xe clearance technique) in the vastus lateralis and tibialis anterior was significantly reduced in the last 15
seconds of exercise (90). Using the same technique, Menon and colleagues demonstrated that although resting muscle blood flow was similar in both healthy normals and those with T2D, the subjects without diabetes had a significantly increased postexercise muscle blood flow. However, the main problem with these studies is the fact that in both cases, subjects with type 1 and 2 diabetes were included and there was no exclusion for certain factors, such as smoking (54, 90).

However, there have been some better-controlled and more significant studies which have clearly demonstrated reduced steady-state muscle blood flow in T2D subjects. Of particular note is a study by Kingwell and colleagues (2003) (35) which demonstrated an impairment in leg blood flow response to cycling exercise in subjects with type II diabetes when compared to age- and weight-matched controls. This was due to increased leg vascular resistance and in spite of raised blood pressure in the T2D subjects compared to the controls in response to 25 minutes of leg exercise at 60% VO$_2$max (34).

A further study by Lalande et al. (2008) (39), demonstrated an impairment to leg blood flow independent of cardiac output, suggesting that the major complication in T2D subjects was related to a reduction in vascular function. Specifically, Lalande and colleagues used MRI during subject leg exercise and found a 25% reduction in femoral blood flow indexed to lean thigh mass (39).

Taken together, these two studies point to a potential limitation in the peripheral vasculature of T2D patients to dilate in response to exercise – i.e. a reduction in the capacity to increase vascular conductance in response to increased metabolic demand. The reduced dilatory response points to two potential explanations: either the vasodilation is impaired;
there is a greater effect of vasoconstriction from the sympathetic nervous system; or there is some combination of both of these factors at play.

2.5 What happens to the vasodilatory response in T2D?

Of these two explanations, there has been quite a lot of research into the idea of a potential vasodilatory impairment. Specifically, there have been a number of studies which have focused on the function of endothelial-specific vasodilatory capabilities of T2D patients. Most of these studies focus on either a reduced endothelial-independent vasodilation (31), or a reduced endothelial-dependent vasodilation (19). In fact, a review of the literature addressing a potential vasodilatory impairment in T2D patients reveals dozens, if not hundreds of studies addressing this issue.

For example, Sprague and colleagues (2006) (79) have demonstrated a reduced ability to release ATP from erythrocytes and cause endothelium-dependent ATP-mediated vasodilation (79). They identified a reduction in the ability to release ATP from the red blood cell, which binds to purinergic receptors on the luminal surface of the endothelium which catalyze the production of nitric oxide, a major upstream-vasodilator (49). Another study focused on both endothelium-dependent and independent vasodilation in T2D patients and found that both of these mechanisms were impaired in the diabetic group (53). Researchers infused both acetylcholine and glyceryl trinitrate in separate trials and found a significantly reduced blood flow when compared to healthy controls (53). Finally, Williams et al. (1996) (88) demonstrated a clear reduction in the ability of diabetic individuals to vasodilate upon administration of an NO-agonist during a reactive hyperemia protocol.
Although this is a sample of just three or four studies, there are hundreds of similar experiments that have been performed. However, there are a number of problems with many of them when it comes to their direct application to the investigation of the vasodilatory response in diabetics during exercise. Firstly, many of these studies have not focused on the period during exercise, but instead on other methods of causing vasodilation, such as flow-mediated dilation (31) during reactive hyperemia or with alpha-adrenergic agonist infusion at rest, or both (19). Secondly, an overwhelming majority of these studies (if not all of them) have been performed specifically at rest in order to come to their conclusions. As a result, there is little literature which is specific to the nature of the vasodilatory response and potential impairment during exercise. Furthermore, these investigations do not shed light on impaired exercise hyperemia because they focus on isolated function of individual vasodilatory mechanisms. It has been well-documented that there are a number of redundant vasodilatory mechanisms involved in exercise vasodilation (11). As a result, the demonstrated impairment in particular pathways governing the vasodilatory mechanisms does not describe the impaired exercise hyperemia seen in subjects with T2D. Thus, it is of importance to consider the possibility of the early onset of “sympathetic restraint” in the skeletal muscle vascular bed of subjects with T2D, thus causing the vasoconstriction and the increase in vascular resistance which is mediating the observed reduction of blood flow.
2.6 How does sympathetic restraint affect blood flow?

In normal muscle, there are two determinants governing oxygen delivery to a particular muscle – the arterial pressure and the vascular resistance of the vessel supplying the muscle. At the onset of exercise, there is an increase in demand for oxygen at the level of the exercising muscle, which necessitates an increase in blood flow. In order to facilitate this increase in blood flow, there would either have to be an increase in arterial pressure, a decrease in vascular resistance or a combination of the two in order to increase blood flow. Generally, there tends to be an immediate increase in local vasodilatory factors which would decrease vascular resistance and increase the overall blood flow to the isolated muscle (85). However, there is also a central contribution to muscle blood flow when the vascular conductance has increased to its peak. Once $F VK_{peak}$ has been reached, there is evidence demonstrating a continued increase in forearm blood flow, which indicates that there must be a central component contributing to the overall increase in blood flow (75). Furthermore, when the exercising muscle mass is large enough, this increase in blood flow is such that it exceeds the capacity of the heart to pump blood and maintain arterial blood pressure, which the arterial baroreflex detects and thus induces both a systemic vasoconstrictor response and central hemodynamic response (67). The central response is an increase in heart rate and subsequently cardiac output, while the vasoconstrictor response is one of systemic vasoconstriction (43). The net effect is a restrained ability to increase conductance combined with an increase in cardiac output, resulting in a net increase in arterial pressure. Thus, we see that both with small-mass and large-mass
exercise, there is a tight interplay between vascular conductance and cardiac output in order to maintain arterial pressure. While these responses may be enough to maintain systemic arterial pressure at its original level, this blood pressure may not be sufficient to provide enough blood flow to the exercising muscle nor to maintain perfusion of that exercising muscle. As a result, there is a secondary response termed the muscle metaboreflex (or ergoreflex or chemoreflex), which acts to increase perfusion pressure and blood flow to the exercising muscle.

2.7 What is the muscle metaboreflex?

The muscle metaboreflex was first observed in the 1930s by Alam and Smirk (1). The underlying physiology was more fully described by Mark et al. 1985 in an experiment in which subjects performed 30% MVC static handgrip exercise. They noticed that there was a gradual increase in muscle sympathetic nervous activity (MSNA) which was different than the almost immediate onset of heart rate and arterial pressure. They also found that with post-handgrip circulatory arrest, in which they occluded the arteries with a pneumatic arm cuff, they were able to maintain chemical stimulation of muscle afferents in the arm and the sympathoexcitatory response in the leg was sustained even though muscle relaxation should have eliminated central command and mechanical stimulation of the muscle (29). As a result, they described a chemically-stimulated feedback pathway which increased vasoconstriction and elevated mean arterial pressure during exercise. However, an increase in sympathetic vasoconstriction may at first seem counter-intuitive as it would vasoconstrict in the very muscle that required increased blood flow.
2.8 How is the MMR activated?

The contracting muscle itself produces a number of metabolic by-products that begin to accumulate in the blood, such as lactate, inorganic phosphate (Pi) and potassium (37). Some of metabolites, such as H⁺, are known to activate the free-endings of group IV muscle afferents which have central nervous projections (55). The activation of these group IV muscle afferents results in an increase in their firing rate. This information is processed at the level of the nucleus tractus solitarii, the site of the cardiovascular control center, in the medulla, which responds with an increase in sympathetic efferent activity in the form of systemic MSNA (65, 72). This leads to an increase in the signal for vasoconstriction in all major tissues, including the exercising muscle. By reducing the overall conductance to tissues in the body, there is a subsequent increase in mean arterial pressure (65, 72).

2.9 How does the MMR maintain or increase flow to exercising muscle?

Although there is an increase in systemic vasoconstriction, there are two pathways which reduce the effect of this vasoconstriction at the level of the exercising muscle: vasodilation and sympatholysis, the exercise-induced blunting of the MSNA. As previously discussed, at higher intensities of exercise, the release of vasodilators represents a vasodilatory influence counteracting the vasoconstrictor influence of elevated MSNA from the MMR. However, there is a secondary action by some of these vasodilator substances – the blunting of the effects of the MSNA at the level of the free-nerve ending. For example, ATP has been shown not only to be a vasodilator but also a sympatholytic agent which counteracts the effects of MSNA by activating vascular smooth muscle cell potassium
channels that modulate the activity of the alpha-adrenergic vasoconstriction (71). As a result, ATP not only causes vasodilation but also blunts constriction. These two effects would lead to a protection of vascular conductance to the exercising muscle at a level sufficient to benefit from the increase in mean arterial pressure caused by the systemic vasoconstriction and elevated cardiac output (13). Thus, the maintenance of dilation to the exercising muscle coupled with the metaboreflex-induced increase in arterial pressure leads to an increase in flow to the exercising muscle and thus an increase in oxygen delivery. As beneficial as this mechanism can be for maintaining blood flow in a healthy individual, the muscle metaboreflex may produce significant problems for type II diabetics by potentially reducing muscle blood flow and subsequently leading to exercise tolerance if sympatholytic and vasodilatory mechanisms do not effectively counteract muscle sympathetic vasoconstriction. In order to elucidate the potential mechanisms that may cause a negative effect, it is important to understand the characteristics of the diabetic muscle.

2.10 What could exaggerate the effect of the MMR in T2D?

It is important to understand that any change in the composition of the diabetic muscle or how it responds to an increased oxygen demand, such as during exercise, will ultimately dictate the extent to which other physiological mechanisms may come into play. As such, it is important to understand how the diabetic muscle is different.
2.10.1 *What are the differences in the diabetic muscle?*

There are significant differences between normal, healthy muscle and the diabetic muscle. First, clear reductions in mitochondrial volume and function have been demonstrated in T2D patients. Kelley et al. 2002 (33) demonstrated a 35% reduction in the size of the mitochondria in T2D subjects and Ritov et al. 2005 (70) demonstrated a reduction in the electron transport chain activity of the mitochondria in T2D subjects compared to normal and obese individuals. Taken together, these data confirm a reduction in the Krebs-dependent pathway and oxidative phosphorylation pathway in the production of ATP that was postulated by Simoneau et al. (78). This also means these individuals rely more heavily on glycolytic and aerobic enzyme pathways, which has a significant effect when it comes to discussing the effect of the MMR.

2.10.2 *How does the altered reliance on anaerobic means affect the onset of the MMR?*

Since the onset of the muscle metaboreflex is governed by the production of metabolites such as $H^+$, it is important to identify the methods by which these substrates are produced. To that effect, a brief review of the “net drive hypothesis” is necessary.

Oxygen diffusion into the cells is governed by the same flow equation which governs many other physiological processes:

\[
\text{Flow} = \text{Gradient} \times \text{Conductance}
\]

In the case of oxygen diffusion from the artery to the myocyte, the partial pressures of the oxygen in the artery and myocyte ($P_{\text{cell}O_2}$) act as the two factor determining the gradient which determines oxygen flow into the muscle cell. Since the partial pressure of oxygen and
the conductance (the diffusive conductance of the muscle) are generally constant, it is mainly the \( P_{\text{cell}O_2} \) which governs the flow of oxygen into the myocyte.

The “net drive hypothesis” is a concept which describes the ability of the muscle to produce ATP through the collective contributions of oxygen \( (P_{\text{cell}O_2}) \), the phosphate energy state of the cell \( ([ADP][Pi]/[ATP]) \) and the redox energy state \( ([NADH]/[NAD^+]) \) (84). Any change in any of these contributors will cause the others to compensate by increasing their relative contribution in an effort to maintain ATP production sufficient to meet the ATP demand of the muscle (84). If we consider that there is an oxygen deficit at the onset of exercise in T2D, as discussed previously discussed, then there must be a subsequent increase in both redox state and phosphate energy state in the myocyte. As a result, there could potentially be an increase in \( H^+ \), and thus the onset of the muscle metaboreflex at lower exercise intensities in a diabetic individual compared to a healthy normal individual. This would cause an increase in vasoconstriction in these subjects and could result in the impairment to blood flow which has been contributing to the exercise tolerance that has been demonstrated in T2D patients.
Figure 4. Net drive hypothesis.

The net drive hypothesis describes the attempt to maintain ATP production for the use in force production. The oxygen content of the cell, the phosphate potential, and the redox potential all contribute to the production of ATP and any decrease in one of these factors necessitates an increase in the others to maintain force production.

Of further concern is the fact that T2D subjects tend also to be obese (20) and obesity has been linked to elevated levels of MSNA (23-25, 76). As a result, these T2D subjects might not only be experiencing these vasodilatory complications, but may also be subject to higher levels of MSNA, which when coupled to the MMR-induced elevation MSNA, may result in even greater vasoconstriction at the level of the exercising muscle. As a result, the muscle metaboreflex, although it generally acts as a flow-restorative mechanism in healthy individuals, might in fact be self-defeating in type II diabetics and lead to the exercise intolerance which makes management of this disease so difficult.
2.11 Study Rationale

The determination of the effect of the muscle metaboreflex on exercising muscle blood flow in type II diabetics will elucidate any potential negative impact this nervous response might have in a hyperadrenergic population. It will also provide insight into how this reflex might contribute to impaired exercise tolerance of T2D patients and will allow a more detailed model of exercise and muscle metabolism in this clinical population to be developed. To our knowledge no studies have formally attempted to explore this idea, with only one published abstract in the current literature, but no follow-up study (21). While there has been a lot of research into the endothelial response during exercise in T2D patients as well as the effect of the muscle metaboreflex in obese non-diabetics (19, 31, 91), there does not seem to be any research into the specific effects of the MMR in T2D subjects. As such, this will be one of the first experiments to explore this concept.

Objective:

To determine whether evoking the muscle metaboreflex (MMR) impairs exercising muscle blood flow in T2D patients compared to controls.

Hypotheses:

1. Exercising muscle blood flow in T2D patients will not improve and may be impaired upon activation of the MMR.
2. The absence of flow improvement will be due to an augmented vasoconstriction in the exercising muscle.
Chapter 3

Methods

3.1 Subjects

Twenty two men volunteered for this study (10 controls, 12 persons with T2D); of these men 13 were included in the experiment. Of the 13 men, seven were men with type 2 diabetes and six were control subjects. Those individuals not included in the study were either: unable to complete the exercise during the screening; on medications included in the exclusion list (Appendix C); or had some pre-existing condition which prevented them from participating in the exercise (Appendix F). These thirteen men participated in the study after receiving full written and verbal explanations of the experimental protocol and any potential risks, as well as filling out a medical screening questionnaire. Each subject gave written consent on forms approved by the Office of Research Services at Queen’s University (Appendix A). Subject characteristics are described in table (Table 1).

All subjects provided a list of their medications with dosages. The medications for each subject are listed in (Appendix B). Subjects with medications that would affect the cardiac response to exercise were excluded. A list of the medications used for inclusion and exclusion is included and is based on previous research in our lab (Appendix C). Subjects were asked to continue their medication regimens. Subjects were recruited primarily through the Canadian Diabetes Association, advertisements and word of mouth through the community. Subject recruitment initiatives are listed in (Appendix D).
Table 1. Subject characteristics.
Asterisk indicates significantly different from CTL group (*, P<0.05).

<table>
<thead>
<tr>
<th></th>
<th>CTL Group</th>
<th>T2D Group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.33 ± 7.87</td>
<td>57.14 ± 9.69</td>
<td>0.970</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>97.83 ± 26.50</td>
<td>98.14 ± 18.05</td>
<td>0.981</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>184.17 ± 6.31</td>
<td>178.29 ± 5.74</td>
<td>0.106</td>
</tr>
<tr>
<td>Maximum Voluntary Contraction (kg)</td>
<td>55.03 ± 8.72</td>
<td>40.91 ± 9.13*</td>
<td>0.016</td>
</tr>
<tr>
<td>Actual Workload (kg)</td>
<td>12.46 ± 2.08</td>
<td>10.13 ± 2.60</td>
<td>0.107</td>
</tr>
<tr>
<td>Forearm Volume (mL)</td>
<td>1342.67 ± 397.73</td>
<td>1185.57 ± 187.06</td>
<td>0.369</td>
</tr>
<tr>
<td>Forearm Circumference (cm)</td>
<td>29.17 ± 3.16</td>
<td>28.17 ± 1.40</td>
<td>0.494</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>103.00 ± 18.19</td>
<td>106.50 ± 11.70</td>
<td>0.700</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mmol/L)</td>
<td>4.78 ± 0.88</td>
<td>8.18 ± 2.30*</td>
<td>0.040</td>
</tr>
<tr>
<td>Physical Activity (MET hr/wk)</td>
<td>281.07 ± 34.20</td>
<td>273.82 ± 36.96</td>
<td>0.722</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.54 ± 5.49</td>
<td>30.75 ± 4.65</td>
<td>0.448</td>
</tr>
</tbody>
</table>
3.2 Seven-day Physical Activity Recall

The seven-day physical activity recall (PAR) was used to quantify the amount physical activity performed by each subject over a week-long period. This physical activity questionnaire has been shown to be reliable and valid for assessing physical activity by previous studies and has been used previously in experiments with persons with type 2 diabetes as the participants (32, 80).

The PAR itself is an interview-based questionnaire, in which the interviewer asks a series of directed questions that are meant to ascertain the amount of time spent by each subject sleeping, sitting, and performing physical activity at various levels (moderate, hard and very hard). The interview itself lasts about 30-45 minutes and the interviewer follows a precise script in order to maintain consistency across all subjects as well as retrieve specific information needed to perform the activity level calculations. An example of the different levels of physical activity and their description is presented in Appendix E.

In order to determine the amount of physical activity performed by each individual, the subject is familiarized with the physical activity categories through explanation by the interviewer. The amount of time spent during each activity in the day is assigned into a category of physical activity intensity. Each category of physical activity, as well as sleeping, is assigned a MET value (1 MET = 1 kcal/kg/hr). A measure of physical activity per day is determined by multiplying the MET value by the subject’s body weight to give a final value in units of kcal/day or kcal/week. To facilitate this, the data from the PAR was inputted into a custom-made Microsoft Excel-based PAR calculator (Appendix E) which sums the METs for each subject and produces a final numerical figure describing the subject’s physical activity for the day and for the week.
Following the PAR, the Medical Screening Form was then completed by the participant (Appendix A). The Medical Screening Form was meant to further inform the investigators as to the medications each participant was currently prescribed, as well as any cardiovascular disease or other co-morbidities which might preclude their inclusion in the experiment, in accordance with the listed ACSM contraindications to exercise testing (Appendix F). Subjects who indicated on the medical screening form that they were current smokers were not included in the study.

### 3.3 Fasting Blood Glucose

Subjects were asked to come to the lab following an overnight fast in order to take an accurate overnight fasting blood glucose measurement. A small blood droplet was obtained using a contact-activated lancet (1.5 mm x 30G, BD Microtainer, Franklin Lakes, NJ, USA). The blood glucose was measured using a blood glucose monitoring system (Accu-Chek Compact Plus, Roche Diagnostics, Mannheim, Germany).

### 3.4 Maximum Voluntary Contraction

Participants were then asked to perform three maximum voluntary contractions (MVC) using forearm muscles with the same handgrip dynamometer to be used during the experiment. This was done in order to determine the 20% MVC contraction intensity to be used during the experimental protocol. Specifically, the subject was asked to grip the dynamometer in a comfortable position and after a cue, squeeze the dynamometer as hard as possible using their forearm muscles only. After a minute of complete rest, the subject was asked to repeat the procedure. This was performed three times and the peak value of the three was determined and used as the maximum value for the experiment.
3.5 Forearm Handgrip Exercise

Participants were then asked to perform a shortened version of both experimental trials. First, subjects were familiarized with the metronome, which provides both auditory and visual feedback to guide their rhythmic forearm contractions. The exercise consisted of 1s contraction 2s relaxation duty cycle where contraction intensity was 20% MVC. Once subjects had successfully performed consistent handgrip contractions of the right duration and magnitude, they were then asked to perform the alternating handgrip-plantar flexion protocol in time with the metronome. Once the subject had practiced this protocol, the automated blood pressure cuff was applied to their calf and inflated to give the participant an understanding of the intensity of pressure they would feel as they performed the exercise. A tourniquet was also applied to the right forearm and bicep to determine the suitability for venous blood sampling.

Force output was determined with the handgrip dynamometer which has a strain gauge which senses distortion of a metal bar by the force exerted on it which provides information on the relative force of each contraction. This information was used to target specific exercise intensity (20% MVC). Subjects had constant visual feedback during the exercise protocol in order that they could reproduce the target exercise intensity with each contraction.

3.6 Ischemic Calf Exercise

Calf exercise was performed using a custom-built pedal system which allowed for 2.0 W of exercise to be performed. Ischemia was induced by surrounding the calf with a blood pressure cuff and using an auto-inflator to rapidly apply suprasystolic (~250 mm Hg) pressures at the appropriate times.
3.7 Arterialized Blood Sampling

Upon entry into the lab on arterialized sample experimental days, a 20 gauge catheter (BD Instyle Autoguard, Becton Dickinson Canada Inc., Oakville, Ontario) was inserted in a retrograde fashion into the radial vein (near the wrist) of the right arm. Catheterization was performed by a researcher with phlebotomy training. The catheter was placed at the level of the wrist and the hand was heated with a heating pad in order to recruit venous anastomoses so that arterial blood bypassed the capillary exchange sites (52). Blood samples were only taken once $S_aO_2 \geq 90\%$, based on analysis performed by a StatProfile M Blood Gas Analyzer (Nova Biomedical, Mississauga, Canada). The blood constituents in the sampled blood using this approach represents arterial levels of the same constituents because hand-heating maintains high flow through arterio-venous anastomoses and prevents blood constituent exchange with tissue (1). Epinephrine and norepinephrine have been shown to correlate highly with arterial values using this method (52). Blood samples were taken in the last 1-1.5 minutes of each time point (BSL, EX, IPF9, IPF12) in order to get an estimate of the circulating NE and E concentrations.

As for procedure, two 6 mL pink-topped blood collection tubes containing spray-coated K2 EDTA (BD, Becton Dickson Canada Inc., Oakville, Ontario) were filled with blood samples during baseline period, during the handgrip exercise period and approximately 1 – 1.5 minutes prior to the end of each time period. A total of 72 mL of blood was drawn for analysis with an additional 18 mL drawn during the discard phase for a total of 90 mL drawn during one day of the experiment. Two further blood samples, taken before the experimental protocols began, were used for determination of HbA1c and [Hb] levels. A total of 212 mL of blood was drawn over the
two experimental days. HbA1c% was used for subject characterization. These samples will be analyzed at the CORE Laboratory at Kingston General Hospital.

Blood samples will be sent for the determination of [NE] and [E] concentrations to the Muscle Biochemistry Lab in the School of Kinesiology and Health Studies using the BA E-6500 Fast Track ELISA kit (Labor Diagnostika Nord, Nordhorn, Germany).

Both the HbA1c% and the [NE] and [E] are not currently reported as these tests are currently underway.

3.8 Forearm Deep Vein Blood Sampling

Upon entry into the lab on venous sample experimental days, a 20 gauge catheter (BD Instyle Autoguard, Becton Dickinson Canada Inc., Oakville, Ontario) was inserted in a retrograde fashion into the median vein at the level of the antecubital fossa (usually beginning just proximal to the junction of the median cephalic vein and the median basilica vein) of the right arm (contralateral to the exercising arm). Blood drawn at the antecubital fossa was used to determine the local forearm norepinephrine concentration after muscle-metaboreflex activation. Measuring changes in norepinephrine in this way allows a characterization of the muscle metaboreflex response because increased MSNA alters circulating norepinephrine levels which reflect the changes in the balance of uptake and release of NE in the forearm. These measures provide an relevant index of the increased sympathetic vasoconstriction evoked by the MMR and are used to confirm its effect. Prior to catheterization at the level of the antecubital fossa, the vein was imaged used Echo ultrasound to ensure the vein drained the forearm muscle. The two [NE] values
obtained were later combined to determine the overall amount of norepinephrine released form the nerve terminal of the forearm.

3.9 Central Hemodynamic Monitoring

Heart rate, blood pressure, cardiac output, blood flow velocity, brachial artery diameter and force output were recorded continuously with a data acquisition and analysis program. Heart rate was measured using two 3 lead ECGs positioned on the chest and abdomen. Blood pressure and cardiac output were monitored using a Finometer device (Finometer MIDI, Finapres Medical Systems, Amsterdam). This device uses a pneumatic cuff which is placed around the middle finger on the right hand and uses photoplethysmography to determine aortic flow for each beat (i.e. stroke volume). It does this based on the Modelflow method in which arterial pressure measured at the finger is used to compute an aortic flow waveform which is then integrated to provide an estimate of left ventricular stroke volume (Bogert et al. 2007).

3.10 Brachial Artery Mean Blood Velocity and Diameter

Blood flow velocity was determined using Doppler ultrasound operating at 4 MHz using a Multigon Industries device (Model 500V TCD, Multigon Industries, Mt. Vernon, NY). Blood flow velocity was continuously recorded and displayed on both the recording computer screen and the ultrasound computer screen. The Doppler probe was positioned and fixed to the skin over the brachial artery once all traces of venous blood flow were removed and the position was marked with permanent marker to ensure the same probe position for subsequent laboratory visits.
Brachial artery diameter was obtained using Echo ultrasound imaging technology operating at 10 MHz (Echo Ultrasound, GE Vigmed System V). This image data was recorded on a separate computer in DICOM format for later offline analysis using custom edge-detection software. The Echo probe was positioned and fixed to the skin over the brachial artery once all traces of venous blood have been removed and the position was marked with permanent marker to ensure the same probe position for subsequent laboratory visits. The Echo ultrasound probe was placed immediately proximal to the Doppler ultrasound probe. The software finds and tracks the artery walls within an investigator defined region of interest (Woodman et al. 2001). This allows continuous measurements of artery diameter from baseline through the 16 minute protocol.

3.11 Subject Screening and Familiarization

Once a volunteer expressed interest in the study, they visited the lab for their familiarization visit. During this visit, subjects received a full explanation of the experimental protocol including all of the measures to be taken, special instructions for the experiment, and then read and signed the consent form. The seven-day physical activity recall (PAR) questionnaire was administered and the Medical Screening Form was completed (Appendix E).

3.11.1 Anthropometric Measurements

Anthropometric measurements were taken in the lab and recorded on a Participant Data Form (see Appendix G). The subject’s date of birth, weight (kg), height (cm), waist circumference (cm), forearm volume (mL) and forearm circumference (cm) were recorded on the Participant Data Form. Waist circumference was measured in accordance with the NIH protocol, as recommended by (51). Specifically, subjects were asked to raise their shirts and the tape
measure was placed at the superior borders of the iliac crest. Subjects were then asked to take a deep breath in and then exhale. The measurement was recorded once the subject had fully exhaled.

Finally, Doppler and echo ultrasound measurements were performed on the subject’s left brachial artery in order to determine whether an acceptable quality of measurement could be achieved for the determination of blood flow. Acceptable echo ultrasound measurements were images that had clear endothelial borders which could be detected by the custom-designed edge-detection software. Acceptable Doppler ultrasound measurements were those in which a 20% MVC did not detect venous blood flow during rhythmic contractions at 20% MVC.

3.12 Subject Instrumentation

Subjects entered the lab and lay supine on the experimental bed with their arms out to the side at heart level. The experimental protocols were performed in a cool environment (~18.5°C) to minimize skin blood flow. Subjects were asked to lie quietly to maintain blood flow at baseline levels. There was a minimum of 15 minutes between protocols in order to allow for hemodynamic variables to return to baseline levels and to allow for hormone washout.
Instruments used during data collection are represented by coloured boxes and their general placement noted.

Once the subject was comfortable, the catheter was first placed in the appropriate vein, depending on the experimental day (arterialized vs. deep venous). Once completed, instrumentation and set-up began (Figure 5). The right arm was used for measurement of mean arterial pressure using a Finometer device and the right forearm and antecubital fossa were used as venous blood sampling sites. The Finometer device was placed on the middle finger between the first and second knuckle and secured with Velcro. The tip of the right index finger was used for pulse oximetry determination. The left arm was used for blood flow determination using echo and Doppler ultrasound. Three ECG leads were placed on the chest for heart rate determination and the subject was provided a computer screen for visual feedback of both force and metronome timing. Diagrams of the experimental set-up are presented in figure (Figure 5).

### 3.13 Experimental Protocol

Exercise testing was performed over two separate visits. Each day of exercise testing involved two main protocols: a control protocol and the ischemia + plantar flexion (or MMR) protocol. The order of the protocols were counter-balanced and assigned randomly to each subject.
so that all subjects performed both the control and MMR protocol, but in different orders on each of the experimental visits in order to eliminate an order effect. There was also a minimum of 15 minutes rest between each protocol on each day to allow sufficient time to return all cardiovascular variables to resting levels. Subjects were instructed to avoid alcohol, caffeine or strenuous physical activity for at least 12 hours prior to the experiment and were asked to fast for 4 hours prior to the experiment.

The control protocol used solely forearm isometric handgrip exercise, while the MMR protocol added ischemic plantar flexion during the handgrip exercise (Figure 6). This allowed the stimulation of the MMR using the IPF exercise, while the effect of the MMR was observed in the forearm, as seen in previous experiments (83).

Figure 6. Protocol schematic.
Each protocol lasted seventeen minutes. The Control protocol involved solely forearm handgrip exercise from the third minute lasting for 9 minutes. The MMR protocol also used handgrip exercise but introduced ischemic plantar flexion (IPF) 3 minutes into exercise (6 minutes into the duration of the protocol).
3.14 Experimental Protocol

3.14.1 Control Protocol

The control protocol consisted of handgrip exercise only in order to provide a measure to compare to the MMR treatment in order to determine the effect of the MMR by itself (Figure 6). The control protocol involved two minutes of rest, followed by nine minutes of hand-grip exercise performed at an intensity of 20% of the subject’s MVC at a duty cycle of 1s contraction: 2s relaxation. The duty cycle was maintained by a metronome which had auditory and visual feedback for the subject. The subject was also provided visual feedback of their handgrip intensity and a target for them to reach for each squeeze of the hand grip dynamometer. Either the experimenter who was taking blood samples or the experimenter running the computer was responsible for providing feedback and motivation to the participant to keep them on timing and at the right intensity level of exercise. After the nine minutes of exercise, the subject stopped exercising and 5 minutes of recovery data was recorded.

3.14.2 MMR Protocol

The MMR protocol consisted of handgrip exercise with ischemic plantar flexion in order to elicit the muscle metaboreflex (Figure 6). This protocol involved two minutes of rest, followed by 3 minutes of handgrip exercise alone. After three minutes of handgrip exercise, the subject was then asked to add the plantar flexion while a blood pressure cuff was inflated to occlude the leg performing exercise. This combination of handgrip exercise and ischemia was performed for a period of six minutes for a total of nine minutes of exercise, similar to the control protocol. The handgrip exercise was performed at the same duty cycle, but during the 2s of relaxation, the
subject would perform the plantar flexion. Plantar flexion was performed at a work rate of 2.0 W. At the end of the nine minutes, the subject stopped exercising and the blood pressure cuff was released and 5 minutes of recovery data was recorded.

### 3.15 Data Acquisition

Central hemodynamic variables recorded included heart rate (HR), cardiac output (CO), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP). Data were collected by Beatscope Easy software (Finapres Medical Systems, Amsterdam, The Netherlands). Mean blood velocity (MBV), MAP, hand-grip dynamometer force data, and pulse oximetry data were collected on a beat-by-beat basis using Chart 4.0 software (ADInstruments, Sydney, Australia). Brachial artery diameter data was collected on a separate recording computer using DICOM medical imaging software.

### 3.16 Data Analysis

#### 3.16.1 General Data Analysis Approach

A 30 s average was obtained and analyzed at 4 time points: 1.5-2 minutes into baseline, 2.5-3 minutes into exercise (steady state hand-grip exercise), 5.5-6 minutes into exercise (3 minutes after ischemia + plantar flexion) and 8.5-9 minutes into exercise (the end of ischemia + plantar flexion). These values were averaged between the two experimental protocol days. These final values were used for data analysis.

#### 3.16.2 Forearm Blood Flow

Forearm blood flow was calculated according to the following calculation:
FBF = brachial artery blood velocity (cm/s) x 60 s x \pi x radius^2

The mean blood velocity (MBV) was determined using Doppler ultrasound. MBV was averaged over a thirty second period after the angle of insonation had been corrected. This was accomplished by using an image of the brachial artery (obtained using Echo ultrasound) at the spot of the Doppler ultrasound probe in order to determine the angle of the artery relative to the probe. This angle was determined and used to adjust the two-point units conversion in the software so that absolute MBV values could be determined. The radius was determined from offline analysis of the Echo ultrasound recording. Images were obtained every five seconds over a thirty second period and averaged to determine the diameter. The diameter was halved and used as the radius for specific time points in the protocol (Figure 6). Once value of FBF was determined for each of these time points.

### 3.16.3 Heart Rate, Mean Arterial Pressure, Cardiac Output

Data for heart rate, mean arterial pressure and cardiac output were recorded on a beat-by-beat basis and then averaged over a thirty second period.

### 3.16.4 Forearm Vascular Conductance

Forearm vascular conductance was calculated as

\[ FVK = \frac{FBF}{MAP} \times 100 \]

Where FBF is in mL/min, MAP is in mmHg and FVK is in mL/min/100 mmHg such that the values for FVK are similar to those for FBF. The values for FVK used in this calculation were averages of thirty second periods at the aforementioned time points in the experiment.
3.16.5 Total Peripheral Resistance

Total peripheral resistance was calculated as:

\[ TPR = \frac{MAP}{CO} \]

Where MAP is in mm Hg, CO is in L/min and TPR is in mm Hg/L/min. The values for TPR used in this calculation were averages of thirty-second periods at the aforementioned time points in the experiment.

3.16.6 Venous Blood Analysis

Venous blood was centrifuged at 4°C at a rate of 2500 rpm for 10 minutes. The blood serum from each vial was then separated into three separate microcentrifuge tubes and placed into a -80°C freezer for analysis at a later time. HbA1c and [Hb] samples were stored as whole blood samples upright in a -80°C freezer and sent in a batch to the CORE Laboratory at KGH at the conclusion of the study and analyzed simultaneously. The samples were analyzed using a liquid chromatography method. Data will be provided by the CORE Laboratory upon completion of the analysis.

3.16.7 Group Analysis

Values for SBP, DBP, MAP, CO, HR, Flow, TPR, and FVK were averaged across subjects and within groups (i.e. CTL and T2D) and treatment conditions (i.e. CTL and MMR). The time points were intended to characterize the response of each group over the duration of each experimental protocol. These time points \{BSL, EX (3min), IPF (9) and IPF(12)\} were used for statistical analysis.

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

Group characteristics were compared using independent t tests for each category.

A three-factor mixed-model ANOVA was used to compare the effects of group (CTL, T2D), treatment (Control, MMR), and time (BSL, EX, IPF9, IPF12) on Flow, MAP, CO, HR, TPR, and FVK. Repeated contrasts were used for detecting specific contrasts. Testing whether there is a difference in how the metaboreflex affects blood flow in a specific group can be examined by a within group comparison of control vs metaboreflex condition. Thus, the within group results were used for the determination of such a difference. Furthermore, a comparison of whether exercising blood flow was different in T2D vs CTL was determined using between group comparisons.

Linear regression analysis was performed for heart rate and cardiac output for all subjects within groups and protocols.

Statistical significance for all tests was set at P<0.05 with a power of 0.8. All statistical tests were conducted using IBM SPSS Statistics 20.
Chapter 4

Results

Subject characteristics are included in the “Methods” section (Table 1). On average, the CTL and T2D groups did not differ with respect to age, weight, height, BMI, waist circumference, forearm volume, forearm circumference, or physical activity levels (as determined by the 7-day PAR score). On average, the T2D group had a significantly lower maximum voluntary contraction, higher fasting blood glucose and higher HbA1c.

Figures 7-12 show the group mean absolute data for FBF, MAP, CO, HR, TPR and FVK for each protocol. Data used encompasses the last thirty seconds of baseline, after 3 minutes of exercise (EX), at 3 minutes of ischemic plantar flexion (IPF9) and at 6 minutes of ischemic plantar flexion (IPF12). FBF and FVK was only significantly higher at IPF12 in the MMR protocol in the CTL and not in the T2D (P<0.05). For the T2D group, FVK was significantly lower in the MMR protocol compared to the Control protocol at IPF12 (P<0.05).

Figures 13, 14, 17, 18, 22, 23, 27, 28, 32, 33, 37, 38, 42 and 43 (Appendix G) show the individual data for the change from exercise in both protocol and both groups. These graphs are meant to characterize the group response to the activation of the muscle metaboreflex at each time point. The CTL group had significantly higher flow at the IPF12 point in the MMR protocol (P<0.05). Similar responses in MAP, CO and HR were found in both groups (P<0.05). Figures 15, 16, 19, 20, 24, 25, 29, 30, 39, 40, 44, and 45 (Appendix G) present the same data but in terms
of each individual’s responses to each time point, to characterize how the muscle metaboreflex affected each individual.

Figures 13 and 14 demonstrate a strong, positive relationship between average CO and HR for each group during both protocols (CTL: $r^2 = 0.994$, $P=0.03$, T2D: $r^2 = 0.096$, $P=0.03$) as expected. Figure 15 demonstrates a positive relationship between CO and MAP for each group during the MMR protocol ($r^2 = 0.57$, $P=0.03$). Figure 43 demonstrates similar relationships between the change in flow per kilogram of work performed between both groups at the EX time point.
Figure 7. Absolute Forearm Blood Flow.

Group mean FBF responses at baseline (BSL) and during forearm handgrip exercise both with ischemic plantar flexion (MMR – open symbols) and without ischemic plantar flexion (Control – closed symbols). Asterisks represent significantly different from BSL (*, P<0.05); Crosses represent significantly different from Control protocol (+, P<0.05); Hash marks represent significantly different from T2D group within the same protocol (#, P<0.05).
Figure 8. Absolute Mean Arterial Pressure.

Group mean MAP responses at baseline (BSL) and during forearm handgrip exercise both with ischemic plantar flexion (MMR – open symbols) and without ischemic plantar flexion (Control – closed symbols). Asterisks represent significantly different from BSL (*, P<0.05); Crosses represent significantly different from Control protocol (+, P<0.05); Hash marks represent significantly different from T2D group within the same protocol (#, P<0.05).
Figure 9. Absolute Cardiac Output.

Group mean CO responses at baseline (BSL) and during forearm handgrip exercise both with ischemic plantar flexion (MMR – open symbols) and without ischemic plantar flexion (Control – closed symbols). Asterisks represent significantly different from BSL (*, P<0.05); Crosses represent significantly different from Control protocol (+, P<0.05); Hash marks represent significantly different from T2D group within the same protocol (#, P<0.05).
Figure 10. Absolute Heart Rate.

Group mean HR responses at baseline (BSL) and during forearm handgrip exercise both with ischemic plantar flexion (MMR – open symbols) and without ischemic plantar flexion (Control – closed symbols). Asterisks represent significantly different from BSL (*, $P<0.05$); Crosses represent significantly different from Control protocol (+, $P<0.05$); Hash marks represent significantly different from T2D group within the same protocol (#, $P<0.05$).
Figure 11. Absolute Total Peripheral Resistance.

Group mean TPR responses at baseline (BSL) and during forearm handgrip exercise both with ischemic plantar flexion (MMR – open symbols) and without ischemic plantar flexion (Control – closed symbols). Asterisks represent significantly different from BSL (*, $P<0.05$); Crosses represent significantly different from Control protocol (+, $P<0.05$); Hash marks represent significantly different from T2D group (#, $P<0.05$).
Figure 12. Absolute Forearm Vascular Conductance.

Group mean FVK responses at baseline (BSL) and during forearm handgrip exercise both with ischemic plantar flexion (MMR – open symbols) and without ischemic plantar flexion (Control – closed symbols). Asterisks represent significantly different from BSL (*, P<0.05); Crosses represent significantly different from Control protocol (+, P<0.05); Hash marks represent significantly different from T2D group within the same protocol (#, P<0.05).
Figure 13. Relationship between cardiac output and heart rate for CTL group.

Closed circles are data points from the Control protocol while open circles are from the MMR protocol. $r^2 = 0.459$, $P=0.001$. 
Figure 14. Relationship between cardiac output and heart rate for T2D group.

Closed circles are data points from the Control protocol while open circles are from the MMR protocol. $r^2 = 0.496$, P<0.001
Figure 15. Relationship between cardiac output and mean arterial pressure during the MMR protocol.
Closed circles are data points from the CTL group while open circles are from the T2D group. $r^2 = 0.57$, $P=0.03$. 
Figure 16. Relationship between change in flow from baseline to exercise and actual workload performed.

Circles represent the T2D group and squares represent the CTL group. Closed symbols are data points from the MMR protocol and open symbols are data points from the Control protocol.
Figure 17. Relationship between change in cardiac output and change in stroke volume.
Closed circles are data points from the MMR protocol while open circles are from the Control protocol. $r^2 = 0.04$, $P=0.355$.

Chapter 5

Discussion
This study investigated the cardiovascular response to moderate-intensity forearm handgrip exercise in persons with T2D and healthy controls during the application of the muscle metaboreflex (MMR). The model used allowed the evaluation of the effectiveness of the MMR in improving exercising muscle blood flow (MBF) in persons with T2D. It was hypothesized that a) the MMR would increase MBF in the CTL group but not in the T2D group and b) that persons with T2D would have reduced FBF at the end of the MMR compared to the pre-MMR exercise levels and that the controls (CTL) would not experience this reduction in FBF.

The major findings of this study are: 1) the MMR protocol caused a significant, 18 mm Hg rise in MAP in both subject groups; 2) this resulted in a pressure-induced rise in MBF in the CTL group but not in the T2D group; 3) the pressure-induced increase was limited to the CTL group despite similar increases in CO in both groups. These results suggest that there may be a FBF impairment during metaboreflex-inducing exercise in persons with T2D due to forearm vascular constriction resulting in an inability to take advantage of the MAP increase that is characteristic of the MMR. Thus, while the MMR is a flow increasing mechanism in healthy controls, it is not in persons with T2D.

5.1 Subject Characteristics

In comparing subject characteristics, there were no significant differences between groups in all but three of the descriptors, suggesting that the subjects were well matched. The only characteristics that were significantly different between groups were those of the maximum voluntary contraction, the fasting blood glucose and the glycosylated hemoglobin measures. The
fasting blood glucose values are expectedly and significantly higher in the T2D group and are characteristic of the disease and its expression.

However, the significant difference in the MVC has implications for the comparison of hemodynamic variables between the two groups. Since this protocol was based on a relative measure of handgrip intensity, this significantly higher value in the CTL group would mean many of the variables would not be immediately comparable between groups, as it is well established that exercising muscle blood flow is tightly matched to the metabolic demand of the exercise being performed (3, 11, 38). In previous studies including persons with T2D which used relative exercise intensities, this has not been an issue because the absolute maximum has not been significantly different between groups (35, 39, 89). However, a review of the time-tension integral reveals that the T2D group worked at a slightly higher intensity than intended, resulting in no significant difference between groups in the absolute workload performed (Table 1).

Finally, the lack of differences in any other of the subject characteristics indicates that any difference in the effect of the protocol can be wholly attributed to the disease and not any other factor. This is especially important in the case of age, which is known to cause a reduction in exercising MBF in humans (17, 36).

5.2 Muscle Metaboreflex Analysis

5.2.1 The absence of MMR-induced blood flow improvement in persons with T2D
This was the first study, to our knowledge, to directly assess the effect of the muscle metaboreflex on exercising muscle blood flow in persons with T2D, although one previous conference presentation investigated the relationship between the MMR and glycemic control in persons with T2D (21). The muscle metaboreflex is commonly thought to be a flow-improving reflex, with evidence from both animal (4, 56, 61) and human models (64, 83) to this effect. As there is a build-up of metabolic byproducts, which stimulates afferent muscle fibers, resulting in an increase in MSNA (65,72). This stimulates vasoconstriction in all tissues, including the exercising muscle. However, because of the contribution of sympatholytic agents, which act to blunt sympathetic activity at the level of exercising muscle, as well as vasodilators, there tends to be a net systemic vasoconstriction except in the exercising muscle (13). This raises blood pressure but maintains conductance to the exercising muscle, resulting in a flow improvement during exercise (13). However, previous investigations have revealed both endothelial-dependent and –independent vasodilation in persons with T2D (19, 53). This would then suggest that the expected MMR-induced flow improvement might be compromised by the inability of the vasculature of persons with T2D to maintain high conductance. Thus, persons with T2D would not be able to take advantage of this increased pressure, resulting in no improvement (and potentially impairment) in exercising muscle blood flow (MBF).

To that end, it is clear that in the control group in this experiment, the application of the muscle metaboreflex causes a significant increase in muscle blood flow (MBF) in the exercising arm (Figure 7). Conversely, in the T2D group there was no increase in flow during MMR activation, with flows being maintained at the same levels seen in the control protocol (Figure 1). The CTL group flow increase is achieved by the IPF9 time point and further improved by the
IPF12 time point (Figure 7). A closer look at individual subject data (Figure 7) also reveals that there is a significant increase in exercising muscle blood flow caused by the MMR at both the IPF9 and IPF12 time points in all CTL group subjects. These data are supported by previous studies (64, 83) which observed similar increases in blood flow upon activation of the MMR.

The individual data for the T2D group seems to indicate a heterogeneous response, with two of the diabetics showing an increase in flow of more than 25 mL/min, 4 showing no difference, and one subject demonstrating a decrease in blood flow of more than 25 mL/min (Figure 19).

5.2.2 Why is there heterogeneous flow response in the T2D group?

This heterogeneity of response within the T2D group is not surprising. Firstly, it has been suggested that metaboreflex responsiveness is exaggerated with impairments in glycemic control (21). In a population of persons with T2D with a wide range of glycemic control, as is the case in our experiment, this would have an effect on the amount of blood flow restraint caused by the MMR. Secondly, it has been demonstrated that there may be a subgroup of the general population which does not respond with an increase in blood flow to MMR activation (64). These researchers identified approximately half of their subjects as non-responders to MMR activation, with non-responders being identified as individuals who exhibited similar increases in blood pressure as the responders, but did not elevate blood flow (64). In the light of this data, there are two potential interpretations of our results: 1) either there was a mix of responders and non-responders in the T2D but not the CTL group or 2) T2D has the effect of causing responders to act more like non-responders because of disease-related effects on the muscle vasculature. The
latter suggestion seems to be more plausible, especially considering the criteria describing non-responders have been known to also characterize persons with T2D, including altered expression of β-receptors (50, 66, 86) and increased sensitivity to norepinephrine (27, 28). In summary, it is possible that the disease affects of T2D might account for the variability in flow response seen in the persons with diabetes and the absence of T2D further explains why there is a homogenous response in the CTL group.

5.2.3 How does MAP change during the MMR and is this change similar between groups?

Despite the persons with T2D matching the muscle blood flow necessary to maintain arm exercise, there is evident a clear and identifiable lack of metaboreflex-induced flow elevation (Figure 7). This lack of flow increase occurs despite similar increases in MAP between groups of approximately 18 mm Hg (Figure 8). There was a consistent increase in mean arterial pressure across all subjects in both groups (Figure 23, Figure 24). Only the T2D group had achieved a significantly higher MAP by the IPF9 time point, indicating that the MMR was activated within at least 3 minutes of the initiation of the protocol, while it was not significantly higher until the IPF12 time point in the CTL group (Figure 8). Furthermore, this increase of 18 mm Hg is characteristic of the muscle metaboreflex, which is directly related to the exercise intensity performed in the ischemic exercising muscle (13).

5.2.4 How is MAP increased?
Each group achieved this increase in MAP via an increase in CO, as demonstrated by the correlation between these variables in both the T2D and CTL group (Figure 15). This achievement of an increase in MAP via CO is typical of submaximal exercise, when there is sufficient cardiac reserve to accommodate for an increase in pressure without net systemic vasoconstriction (15, 64, 83). This would also explain the lack of a significant change in TPR in either group during the MMR protocol (Figure 11), because there is no necessity to redirect blood flow from other regions.

It should also be noted that the increase in CO was primarily achieved through an increase in HR (Figure 9, Figure 11). This is consistent with recent literature which suggested that when the MMR is activated by exercise muscle ischemia – similar to that used in our experiment – there is a HR increase leading to cardiac output increases (14). This is most likely because the increase in norepinephrine and epinephrine release caused by MMR activation stimulates beta receptors in the cardiac muscle (40, 41, 43, 75). The similarity in response between groups also suggests that there is no impairment in cardiac output in T2D during the muscle metaboreflex in this type of small muscle mass exercise (Figure 9).

5.2.5 How does vascular conductance change during the MMR and is this change similar between groups?

However, this similarity in MAP increase implies that even with no change in vascular conductance, there should at least be a passive increase in exercising muscle blood flow during MMR activation. However, since this increase was not observed, it implies that in the persons with T2D there must be a vasoconstriction occurring during muscle metaboreflex activation. This vasoconstrictor response is noted in the T2D group only at the IPF12 point between protocols
(Figure 12). This data should be interpreted cautiously – while the differences are different between protocols at the same time point, when compared to the value at exercise within a given protocol (i.e. EX and IPF12 during MMR, or EX and IPF12 during Control), there is no significant difference (Figure 12). This might be indicative of a lack of statistical power with this small sample size. This would also explain why although we see a clear rise in FVK in the CTL group during the MMR protocol, there is no significant difference compared to the Control protocol in this group. Furthermore, there is no difference between the MMR IPF12 conductance values of the T2D and CTL groups, which would suggest large variances, despite a clear directional change (Figure 12). A closer look at individual data reveals either a decrease in FVK or a reduction in the increase of FVK in the T2D group, while there is a consistent increase in FVK in the CTL group (Figure 12). These data suggest that there is a stronger vasoconstrictor influence in the T2D group during the MMR protocol, while the CTL group are experiencing purely vasodilation.

A vasoconstrictor influence would agree with the second hypothesis that there would be augmented vasoconstriction in persons with T2D which would contribute to a reduced or absent blood flow increase during the MMR protocol. There are a number of possibilities to explain this observation. First, it may be that there is a greater activation of sympathetic vasoconstriction (22,26). This can be explained by the observations that at similar VO$_2$ there is an increase in blood lactate levels (35) and that blood pH stimulates the purinergic (P2X) receptors that are responsible for stimulation of type IV muscle afferents (22, 26, 47, 48). These muscle afferents are the primary nerves which cause MMR-induced systemic vasoconstriction and increased cardiac output primarily through increased norepinephrine release at nerve terminals (41, 43, 44,
73). Thus, the MMR-induced vasoconstriction and CO increase would be activated much earlier and to greater extents in persons with T2D compared to healthy individuals. This possibility will be addressed once the analysis of the NE samples have been completed. Further analysis of blood catecholamine samples taken during the experiment will reveal differences in epinephrine and norepinephrine levels between groups and protocols and shed more light on the magnitude of the vasoconstrictor influence caused by MMR activation.

Secondly, a reduction in the efficacy of vasodilators in persons with T2D has been demonstrated. It is well-established that there is both endothelial-dependent and endothelial-independent vasodilatory impairment due to reduced efficacy of vasodilators and sympatholytic agents in persons with T2D, such as NO, ATP, and others (19, 31, 49, 53, 79, 88). Furthermore, some of these vasodilators also act as sympatholytic agents, acting to reduce the magnitude of sympathetic responses. Their reduced action in persons with T2D would act not only to prevent vasodilation but also would fail to blunt vasoconstriction. Thus, the decreased sympatholysis and increased vasoconstrictor influence as observed in our experiment is supported by the literature.

5.3 Steady State Exercising FBF

5.3.1 Baseline and exercise time points

FBF was not significantly different either at baseline or exercise values between groups, nor was there any difference in HR, MAP, FVK, TPR or CO (Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12). Additionally, when individual flows were indexed to the amount of exercise performed, there was no difference in the relative flow per kilogram of work performed between subjects (Figure 16). These results are in agreement with a previous experiment by
Womack et al. (89), which demonstrated similar flows between lean controls and persons with uncomplicated diabetes. There was no difference in absolute handgrip force or blood flow velocities at baseline or either of the two exercise intensities (89). A rat study by Copp and colleagues (2010) also demonstrated no difference in the exercising muscle blood flow between rats with and without type 2 diabetes (12).

Conversely, these results seem to conflict with the findings of two of the most cited studies investigating exercise responses in persons with T2D in the leg. Lalande and colleagues (2008) observed a significant decrease in exercising leg blood flow indexed to lean muscle mass in persons with T2D during low-intensity exercise (1.5 kg leg extensions at 60 rpm) for 15 minutes. This occurred despite similar levels of cardiac output when compared to the healthy controls, indicating a peripheral vascular impairment (39). Similarly, Kingwell and colleagues (2003)(35) demonstrated significantly decreased leg blood flow in persons with T2D during exercise at 60% VO$_{2}$max. These investigations have formed the foundation for much of the subsequent research in this field. The major differences between this study and those previously published are numerous and provide some explanation as to why these discrepancies might be observed in the current investigation.

5.3.2 Medication Wash-out Period

Firstly, the Lalande, Kingwell and Womack studies had participants discontinue use of their diabetes-related medication at least 24h prior to the onset of the study, while the current study had subjects continue their medication regimen. Since a number of the subjects were being treated by drugs like metformin, which has been shown to improve endothelial function (45), it is
plausible that the lack of a difference in exercising muscle blood flow was a result of a medication effect. Metformin has also been shown to reduce the blood levels of endothelin-1 in rats, a potent vasoconstrictor (7, 74). Another study contradicting the findings of the Lalande (74) and Kingwell studies was performed by Mohler and colleagues (2006)(57). In this study, subjects did not withdraw their medication regimen and demonstrated no difference in the change in blood volume (an indirect index of flow) between healthy controls and persons with T2D performing both plantar-flexion and treadmill-walking exercise (57).

Many of the major studies which have found impairments in vascular function or skeletal muscle have had subjects withdraw from their medication regimen, sometimes as much as four weeks in advance of the study (33-35, 39, 89). However, it is important to consider that since most individuals with T2D will perform exercise while under a medication regimen, it is important to perform investigations in the same context. In addition, in order to fully appreciate the contribution of the MMR to exercise intolerance in a medicated population such as this, withdrawal of medication would be counterproductive to identifying contributors to exercise intolerance.

5.3.3 Exercising Muscle Mass

Another potentially contributing factor to the differences between this study and previously published literature such as Lalande et al. (2008)(39) and Kingwell et al. (2003) (35) is that these experiments investigated the effect of exercise on leg muscle while the current study was performed in an arm-exercise model. There are two main factors to take into consideration:
1) the amount of muscle mass being recruited by the exercise and 2) the type of muscle mass being recruited.

5.3.4 Does the amount of muscle mass matter?

One key issue relating to an impairment is the magnitude of the change in hemodynamic responses to different quantities of muscle mass. It has been well-documented in this lab and others that small muscle mass exercise does not present such a challenge as to necessitate a response in central hemodynamic factors (75, 77, 85). However, large muscle mass recruitment (e.g. leg exercise) has been shown to rely on central contributors to MBF and that these central contributors are demonstrably impaired in people with T2D (5, 6, 68, 69). For example, Baldi and colleagues (2003) demonstrated a reduction in VO$_2$max as well as heart rate at various relative workloads in persons with T2D, indicating an overall impairment in central hemodynamic response to exercise.

However, many studies, especially Lalande et al. (2008) (39), seem to suggest a peripheral contributor to decreased blood flow because the decrease in muscle blood flow in people with T2D demonstrated occurred despite similar cardiac output responses between groups. Furthermore, in a study of older men with T2D compared to healthy, age-matched controls, Wilkerson et al. (2011)(87) saw a reduction in VO$_2$max, but not a reduction in VO$_2$ kinetics during ramp and step increases performed on a cycle ergometer. These results directly contradicts the findings of Baldi and colleagues (2003) (5), who demonstrated a reduction in arterio-venous extraction in people with T2D during a similar cycle ergometer test.
At least at this time, with such contradictory results being presented, the evidence to suggest a central hemodynamic response impairment is inconclusive at best and should be read cautiously. While the reduced blood flow demonstrated in leg exercise in subjects with T2D may have some central contribution which contradicts the results of the current data, it can also be said that the observation of no difference in MBF during exercise between groups in the current study fits somewhat with the evidence presented by a number of authors. Thus, it seems that no conclusion can be made about a muscle mass effect contributing to a reduction in muscle blood flow in T2D.

5.3.5 Is there a difference in limb vasodilatory response?

A third factor potentially explaining study differences is that there is evidence of a difference in vasodilatory reactivity between the arm and the leg. Specifically, recent studies have demonstrated differences in limb vascular responsiveness, or reactivity, as indicated by both infusion-models (59, 60) and flow-mediated dilation (FMD) models (81). For example, there is some suggestion of an age-related change in vascular reactivity in the arm compared to the leg, with it being more reactive at a young age (59). A study using FMD demonstrated no relationship between the FMD responses in the brachial artery and the superficial femoral artery, indicating that conclusions made about the forearm could not be readily applied to the leg and vice-versa (81). This would seem to suggest that the difference in vascular reactivity observed in this study compared to the leg-model studies is not surprising and in fact expected. No investigation has addressed a potential difference in the vascular reactivity of the leg and arm when including persons with type 2 diabetes as subjects. Thus, there is the possibility that the
results from forearm model studies can only be applied to forearm exercise and are thus not able to be used to characterize the effects of type II diabetes on a systemic level. This would not invalidate the methodology but may just restrict the comparisons of results to other leg-model based studies.

5.4 Limitations

5.4.1 Sample Size

One of the major limitations of the present study is the small sample size of subjects in each group (n=7 for T2D and n=6 for CTL). This is mainly attributed to difficulties in subject recruitment complicated by strict inclusion/exclusion criteria as well as difficulties in subjects dropping out of the study. Although all of the significant relationships demonstrated had a power of at least 0.8, it is clear that an increase in sample size to the a priori determination of 10 per group might detect potential differences and give higher resolution to the data analysis. This would be especially important for the FVK data analysis, which might reveal a more significant relationship, given the trends evident in the individual subject data. It would be prudent to follow-up this study by collecting more data in additional subjects.

5.4.2 Medical Comorbidities

The absence of a requirement for a medical examination prior to participation in the study may have led to recruitment of subjects with subclinical comorbidities. Inclusion and exclusion criteria were based on previous studies from this lab as well as individual assessment of any additional medications that might confound data analysis. However, the inclusion of these
subjects with comorbidities may provide additional relevance of this study to the general population of persons with T2D.

5.5 Future Directions

Firstly, the study of the pathophysiology of T2D and its affect on exercise capacity is still in its infancy, with a majority of studies having been performed within the last decade. It is important to consider that few, if any, conclusive determinations can be made with so little data to draw from.

This is especially important in the realm of blood flow during exercise in persons with T2D. There are multiple conflicting studies, with some demonstrating a flow impairment during exercise while others, including this study, observing no flow impairment. Another possibility is that the muscle blood flow impairment demonstrated in the Lalande (2008) and Kingwell (2003) studies is not necessarily a reflection of a true impairment in exercising muscle blood flow. No other studies have investigated potential impairments in exercising muscle blood flow in persons with T2D nor have either of these two studies been corroborated or reproduced by any lab. Furthermore, making conclusions based on two studies in which only 17 persons with T2D have participated, compared to the Womack investigation in which 22 persons with T2D were included, seems contentious at best. With the paucity of experiments performed and the lack of agreement between these experiments, it is imprudent to make definite conclusions about T2D and its effect on exercise and it is clear that further investigation is necessary to more fully characterize this disease.
Most important is the fact that the conflicting studies are separated by the specific limb used for the exercise. Thus, it would be prudent to analyze the vascular reactivity in the different limbs of persons with T2D to determine whether the observed differences in flow impairment can be attributed to the ability of a specific limb to adapt to exercise. Furthermore, an investigation using this experiment’s methodology could be combined with a vasodilator blockade to quantify the contribution of vasodilation and NE during MMR activation and would confirm that there is a vasodilatory impairment, which is preventing the MMR-induced flow improvement seen in matched controls.

As for this experiment specifically, an analysis of blood catecholamine levels during the MMR will reveal the magnitude of effect of the muscle metaboreflex in terms of vasoconstrictor influence. This will allow a direct comparison between groups and provide a standard for future studies in the investigation of sympathetic vasoconstriction in T2D. Also, a more in-depth analysis of the current data with higher resolution (e.g. every 5 seconds instead of 4 distinct time points) would provide a more detailed characterization of the exercise and MMR responses in persons with T2D. This might provide additional insight into the mechanism underlying the flow restraint observed in this study and reveal additional avenues of research.
Chapter 6

Summary and Conclusion

Although exercise is recognized as the ideal prescription for the treatment and management of type 2 diabetes, it is also known that people with T2D have poor exercise tolerance (68, 69). Previously demonstrated exercising muscle blood flow deficits (35,39) might potentially be explained by the impact of the muscle metaboreflex, a pressor-response meant to improve exercising muscle blood flow. This mechanism might act to vasoconstrict the arteries of the exercising muscle in persons with T2D because of reduced efficacy of vasodilators and sympatholytic agents. This study sought to determine whether this was in fact occurring.

The major findings of this study are 1) the MMR protocol caused a significant, 18 mm Hg rise in MAP in both subject groups; 2) this resulted in a pressure-induced rise in MBF in the CTL group but not in the T2D group; 3) the pressure-induced increase was limited to the CTL group despite similar increases in CO in both groups. It was also noted that there was a significant decrease in forearm vascular conductance during the MMR protocol compared to the Control protocol in the T2D group. These results suggest that there may be a FBF impairment during metaboreflex-inducing exercise in persons with T2D due to forearm vascular constriction resulting in an inability to take advantage of the MAP increase that is characteristic of the MMR.

With the emerging evidence in this field of research and its relevance for a population which is growing in number each year, further research must be performed to not only confirm these results, but also to propose treatments for these individuals. Further work which elucidates the underlying physiological principles that govern blood flow in this population, as well as
research focused on potential interventions to overcome these impairments and allow for exercise as the main treatment for management of this disease are necessary.
Chapter 7

References


43. MacPhee S, Shoemaker J. Kinetics of O2 uptake, leg blood flow, and muscle deoxygenation are slowed in the upper compared with lower region of the moderate-intensity exercise domain. Journal of Applied ....


72. Rowell LB, O'Leary DS. Reflex control of the circulation during exercise: chemoreflexes


Appendix A

Consent and Participant Data Forms
CONSENT FORM
FOR RESEARCH PROJECTS ENTITLED:
Investigation into Peripheral Vascular Control in Humans

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Purpose of the Study:

The purpose of this study is to improve our understanding of how the flow of blood through your arms and/or legs is controlled.

Benefits For You:

There are no direct benefits to you by participating in this study.

Subject Initials _____ Witness Initials_____ DD/MM/YY ____/____
Version 3.3, 19/11/2010
Description of Experiment and Risks:
What will happen? During this study, you will take part in some of the specific experimental procedures outlined below. These procedures have been checked. Depending on the specific experimental protocol, the combination of these procedures will be different. The investigator will explain to you in detail how each of these procedures will be combined in the particular experiment involving your participation. Please initial by each box that is marked.

- **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 entirely safe. 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:**

  1. A cuff that can be inflated with air is wrapped around your upper arm, just as would occur if you had your blood pressure measured at the doctor’s office. This cuff is inflated to a pressure higher than your systolic blood pressure (the pressure in your blood vessels when the heart beats), and gradually deflated over a number of seconds to measure systolic blood pressure and diastolic (the pressure in your blood vessels when the heart is relaxed) blood pressure. Meanwhile, your wrist is secured in a wrist brace and a small pressure sensor is placed over your radial artery at the wrist. This pressure sensor is able to detect the increases and decreases in size of your radial artery that occur with each heart beat, and what the pressure sensor measures is compared to the pressure that the upper arm cuff measures (this calibrates the sensor). From then on, the pressure sensor at the wrist measures blood pressure continuously, while the upper arm cuff may be inflated intermittently. OR

  2. 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:** These techniques are non-invasive and pose no risk.

Subject Initials _____ Witness Initials______ DD/MM/YY __/__/__

Version 3.3, 19/11/2010
**LIMB BLOOD FLOW AND BLOOD VESSEL DIAMETER MEASUREMENTS:** The blood flowing through your brachial (above the elbow), radial (above the wrist), 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.

**ELECTROMYOGRAPHY (EMG):** This measures the electrical activity of your muscles. Electrodes will be placed on muscles of interest for a given study. **RISKS:** This procedure is entirely safe. 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.

**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 risks.

**VENOUS BLOOD SAMPLING:** Blood samples from veins are used to measure one or a number of the following substances in your blood: oxygen content, oxygen and carbon dioxide partial pressure, potassium, sodium, hemoglobin, hematocrit, pH, lactate, glucose, insulin, insulin-like growth factor 1 (IGF-1), brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), glycosylated hemoglobin (HbA1c), low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol, norepinephrine, epinephrine, nitrite, nitrate. 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 ml into a syringe. We also need to take multiple 1 ml samples of blood from a vein at the elbow. 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.

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___

Version 3.3 19/11/2010
from the vein. We will take a volume of blood that is in total no more than ~120 ml. This represents approximately 1/3 of the volume of blood taken when you donate blood (370-400 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. It is also possible that this pain may fere down the arm (a “shooting” pain sensation), if there has been nerve irritation in the catheterization process. 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.

- **FOREARM AND LEG VOLUME MEASUREMENTS:** The volume of your forearm or calf can be measured by a thin, stretchable rubber band placed around your respective limb that is filled with mercury. A very small electrical current runs through this gauge and changes in the length of this mercury-filled rubber band are detected by changes in this current that occur in proportion to changes in the length of the rubber band. **RISKS:** This technique is non-invasive and poses no risk.

- **BLOOD OXYGEN CONTENT:** A plastic clip is placed over your left 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 poses no risks.

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___

Version 3.3 19/11/2010
- **MUSCLE MASS:** Circumference and length measurements of segments of your arm or leg will be taken via manual placement of a tape measure on your limbs by the investigator.

OR

At Kingston General Hospital, you will lay on a table and a scan of your body will be performed using a technique called “dual-energy x-ray absorptiometry” (DXA). This technique uses a small amount of x-ray energy to scan a “picture” of your body and identify how much muscle there is on your arms and legs.

**RISKS:** Radiation levels with DXA are considered trivial by radiation regulatory agencies. The technique uses less radiation than a dental X-ray, roughly equivalent to the background amount a person would be exposed to when flying from Cincinnati to the West Coast. This is a mere fraction of the radiation dose we are all exposed to every week, from just being alive.

- **FOREARM OR LEG OCCLUSION:** In order to completely block the blood flow through your forearm or leg, a pressure cuff will be inflated around your arm or around your upper or lower leg for 1-10 min or inflated and deflated rhythmically depending on the protocol. You may feel a strong pressure and some mild tingling with cuff inflation but it should not be uncomfortable. If there is pain, immediately notify the investigator and the cuff will be deflated and repositioned. Upon cuff release there will be a large rush of blood into your forearm or leg. This may feel warm and you may experience mild tingling but no discomfort. **RISKS:** This technique is non-invasive and poses no risk.

- **FOREARM COMPRESSION:** A stylus will be positioned over your artery pulse to control the amount of flow through the artery. The arterial compression provided by the stylus will be varied to create different blood flow profiles. Increases in stylus downward pressure with result in decreases in blood flow, while controlled release of stylus downward pressure will result in increases in blood flow. The blood flow to your limb will never be completely occluded by the arterial compression. In some cases, manual finger pressure will be used instead of the stylus.

OR

A cuff will be positioned around your forearm or leg, and can be inflated and deflated at will to increase and decrease blood flow to your limb. **RISKS:** The brachial artery and nerve run close together, thus the compression of this particular artery may result in a tingling sensation and some temporary numbness in the forearm. The compression of the artery can also become somewhat

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___

Version 3.3 19/11/2010
uncomfortable over time. These symptoms will subside within 5 minutes of compression release. There are no risks to your forearm from temporarily stopping blood flow to the forearm.

**FOREARM OR HAND HEATING:** In order to increase the blood flow through your brachial artery and/or radial artery, your forearm or hand will be enclosed in a water bath that is circulated with warm water. The warm water will result in the dilation of your skin blood vessels. The water bath consists of a cylinder that is circulated with heated water. Your arm will rest inside the tube enclosed in a plastic glove that prevents your skin from being in direct contact with the water. A temperature sensor will be fixed to your skin and your skin temperature will be maintained between 41 and 42°C. The water for the bath is heated remotely to a temperature not exceeding 45°C and is circulated into the bath via a water pump. The water in the bath will feel quite warm, but not too hot. If at any time you feel discomfort the warm water inflow will be stopped and replaced with cooler water to allow the bath temperature to drop to a more comfortable level. Your forearm may be heated for a total of one to two hours. **RISKS:** When the skin blood vessels fill with blood for an extended period while undergoing arterial compression it causes a temporary swelling as some fluid escapes from the blood vessels into the surrounding tissue. This minor swelling should resolve itself within 24 hours. Elevation of the arm will help to speed up the process. Your skin may appear red after removal from the bath. This is due to the increased skin circulation. The redness should resolve within 24-36 hours.

**CONGESTION OF YOUR FOREARM OR LEG VEINS:** One inflatable cuff will be placed around your upper arm or above the knee and another may be placed around your wrist or ankle. The wrist cuff will be inflated to a pressure that prevents blood flow to your hand for a period of 10-15 minutes at a time. This should not be uncomfortable. If it is, notify the investigator and the position of the cuff will be adjusted until inflation without discomfort is achieved. These cuffs will be inflated to pressures that feel like a mild to moderate squeeze. This will prevent blood from flowing out of your limb back to the heart, but allow blood to flow in to your arm. Your limb will fill with blood and if the cuff inflation is maintained for a number of minutes, you may feel a sensation of swelling. This is because some of the plasma (water portion of your blood) will leak out of the small blood vessels and into the space between other cells in your limb. This is similar to when you stand up in the morning and stay upright during the day. In that case, gravity makes it difficult
for blood to flow back to the heart from the legs, and they slowly swell over the course of the day as plasma leaves the blood vessels. When the cuff is released, the limb will slowly return to normal as the plasma moves back into the blood vessels. **RISKS:** The movement of fluid out of the blood vessels into your limb may in extreme cases cause discomfort. This discomfort should resolve itself within minutes of deflating the cuff, and the swelling should subside within 24 hrs. Elevating the arm above the heart for 15 minutes should speed this process.

**INTERMITTENT COMPRESSION OF THE FOREARM OR LEG:** You will have an inflatable cuff placed around your forearm or leg. We can rapidly inflate and deflate this cuff to different pressures that are able to squeeze the blood out of the veins in your limb. Inflation is maintained for only a brief period of time (a few seconds). The sensation of limb compression will feel like a strong grip, but should not be painful. If it is uncomfortable, notify the investigator and the position of the cuff can be adjusted. **RISKS:** There are no risks associated with this procedure.

**ALTERNATING FOREARM SUCTION AND COMPRESSION:** Your forearm will be enclosed in a plexiglass box and sealed with a neoprene sleeve around the upper arm. Suction or compression of your forearm can be created by rapidly adding or removing air in the box via a connected automated air compressor. The sensation of suction and compression should not be painful. Notify the investigator if there are any feelings of discomfort. **RISKS:** There are no risks associated with this procedure.

**EXERCISE MANEUVERS THAT ALTER BLOOD PRESSURE:** You may be asked to perform one of the following MANEUVERS to temporarily increase your blood pressure: 1) squeezing a handgripper with your forearm for a few minutes with or without blood flow to your forearm being prevented 2) contracting your leg muscles with or without blood flow to your leg being prevented. **RISKS:** When muscle contractions are performed while the blood flow to the limb is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no risk to your muscles in performing this exercise.

**STROOP TEST:** In order to create a mental stress a “STROOP” test will be performed. A series of words for colours will be displayed such as “RED”. However, the word will be displayed in a different colour, perhaps the colour green. You must read out the colour in which the word is written in, not the

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___
Version 3.3 19/11/2010
word itself. Therefore, upon seeing “RED” written in green text you will respond by saying “Green”. You will be asked to perform the task as fast as you can. Part of the study evaluates the score you achieve on the test and it is very important that your score achieves the normal range for persons of your age and education. Your performance will be measured by how much of the list you read through in two minutes time, as well as how many mistakes you make. **RISKS:** **There are no risks posed by this procedure.**

- **ANGER TEST:** In order to create emotional stress an anger test will be performed. Prior to the testing day, you will have been asked to fill out an anger questionnaire in order to recall a past event that made you very angry. We will use the questionnaire to elicit momentary anger. You will be asked to describe the event while re-experiencing the event in your imagination, as well as report on thoughts, feelings, and physical aspirations about the situation. The test will last two minutes. **RISKS:** You will feel momentary anger that will subside following the interview. It is possible that this anger interview might contribute to renewing problems between yourself and this individual. If you believe that this might in any way be problematic, you are encouraged to withdraw from participation in this study.

- **CONTROL TEST:** A control test will be performed in order to understand if verbalization is contributing to the blood vessel response. You will simply count from ‘one’ in Mississippi’s. Your verbalization will start as “one Mississippi, two Mississippi, three Mississippi...” and will continue for two minutes. **RISKS:** **There are no risks posed by this procedure.**

- **LOWER BODY NEGATIVE PRESSURE:** You will lay on your back and your lower body will be enclosed in an air-tight box. Various levels of suction will then be applied to the box to simulate how the blood normally shifts in the body during activities like standing up. This will cause your heart rate to increase and your blood vessels to constrict to maintain blood pressure. This is a normal response that you experience every morning when you get up out of bed. **RISKS:** **There is a small chance that you may begin to faint with this procedure.** We will be monitoring your blood pressure continuously. If you experience any of the following symptoms, notify the investigator immediately: nausea, narrowing field of vision, sweating. Changes in your blood pressure that we detect will most likely indicate that fainting is imminent well before you experience any of these symptoms. By shutting off the suction, blood will rapidly return.

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___

Version 3.3 19/11/2010

85
to your heart and symptoms of fainting will be reversed. You may feel nauseous for a few hours after this procedure if you came close to fainting. This should resolve itself without any complications.

- **COLD PRESSOR TEST:** In this test, you will place your hand or foot in an ice water bath for a few (1-3) minutes. This will cause your heart rate to increase and your blood vessels to constrict as the cold will activate your sympathetic nervous system (the part of your nervous system involved in the “fight or flight” response). **RISKS:** There are no risks posed by this procedure. However, it can be quite painful. You have the right at any time to withdraw your hand or foot from the ice water bath if you feel unable to continue.

- **HANDGRIP EXERCISE:** You will be asked to perform handgrip squeezing exercise. The duration of this exercise can vary from a few seconds to 10-20 minutes, and at an intensity that can vary from very mild to maximal contraction force. Exercise may take place in combination with any of the above-mentioned techniques which can control the blood flow to your limbs, congest the limbs, and which can alter your blood pressure. **RISKS:** When forearm muscle contractions are performed while the blood flow to the forearm is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no 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 seconds to 10-20 minutes, and at an intensity that can range from very mild to maximal contraction force. Exercise may take place in combination with any of the above-mentioned techniques which can control the blood flow to your limbs, congest the limbs, and which can alter your blood pressure. **RISKS:** When leg muscle contractions are performed while the blood flow to the forearm is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no damage or risk to your leg from this. 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.
How long will it take?

On an initial visit we will use ultrasound to get an image of the blood vessels in your limbs in order to determine whether you are eligible to participate in the main study.

For the main study: preparing all of the techniques for measuring your response and creating the correct experiment conditions usually takes ~45 minutes. The actual experiment will take ~1-3 hours.

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 immediately and we can temporarily break from data collection. However, if everything is comfortable, please maintain a very quiet posture. Even very slight movements interfere with our experiments.

Special Instructions:

Participants are asked to not drink alcohol or caffeine during the 12 hours prior to the study. Also, we ask that you do not consume any food during the 4 hours preceding the experiments. 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 short time to allow you to readjust to the upright posture. These precautions should be enough to prevent any sensations of dizziness. Please be aware that sensations of dizziness are not normal and you should let us know if you experience any discomfort before you leave the laboratory.

Attached Medical Screening Form:

This questionnaire asks some simple questions about your health. This information is used to guide us with your entry into the study. Current health problems indicated on this form which are related to cardiovascular diseases (including high blood pressure) and liver or kidney problems will exclude you from the study only if the particular experiment in question requires healthy subjects.

Subject Initials: ____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___
Version 3.3 19/11/2010
Safety precautions for the study will include the following:
Subjects who enter the study will be identified as either healthy men and women, insulin resistant, or type II diabetic.

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 seated upright. These precautions allow us to quickly identify if you are becoming faint and simply stopping the experimental manipulation will allow you to quickly recover.

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. Your data will be kept in locked files 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 Physical and Health Education at Queen's University.

Study Compensation
You will receive $11 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.

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: ___/___/___
Version 3.3 19/11/2010
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)
KHS 306, Kinesiology and Health Studies Building
Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-6000, ext, 74697

Jean Cote, Ph.D.
Director, School of Kinesiology and Health Studies
KHS 206, Kinesiology and Health Studies Building
Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-3054

Dr. Albert F. Clarke, Chair
Office of Research Services
Fleming Hall, Jemmett Wing 301
Queen’s University, Kingston, ON, K7L 3N6
Tel: 533-6081

By signing this consent form, I am indicating that I agree to participate in this study.

____________________  ______________________
Subject Signature          Signature of Witness

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

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

Subject Initials: _____ Witness Initials: _____ Date: DD/MM/YY: __/__/___
Version 3.3 19/11/2011
Medical Screening Form

Name: ________________________________

Regular Physician (Name & Address): ________________________________

______________________________

SELF REPORT CHECK-LIST

Past Health Problems:

Rheumatic Fever ■ □ Bleeding disorders ■ □
Heart Murmur ■ □ Blood clots (pulmonary embolism) ■ □
High Blood Pressure ■ □ Varicose Veins ■ □
High Cholesterol ■ □ Disease of Arteries ■ □
Congenital Heart Disease ■ □ Emphysema, Pneumonia, ■ □
Heart Attack ■ □ Asthma, Bronchitis ■ □
Heart Operation ■ □ Kidney and liver disease ■ □
Any other Heart Problem ■ □ Back Injuries ■ □
Diabetes (diet or insulin) ■ □ Heartburn ■ □
Low blood sugar (hypoglycemia) ■ □ Epilepsy ■ □
Ulcers ■ □ Nervous System Disorders (Neuropathy) ■ □
Bleeding from Intestinal Tract ■ □ Stroke ■ □
Enteritis/colicis/diverticulitis ■ □ Other (describe on back of page) ■ □

Present Health:

List current problems: 1. List any medications taken now or in the last 3 months: 1.
2. 2.
3. 3.

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

List Symptoms:

Irregular Heart Beat ■ □ Cough up blood ■ □
Chest Pain ■ □ Back Pain / Injury ■ □
Short of Breath ■ □ Leg Pain / Injury ■ □
Persistent Cough ■ □ Dizziness, light-headedness ■ □
Wheezing (asthma) ■ □ Fainting or “blacking out” ■ □
Fatigue ■ □

Current Exercise Training Status:
I consider my exercise training status to be: High □, Average □, Low □.
List the types of activities that you do on a regular basis: ______________________________________________________

Habits:
Smoking: Never □, Ex-smoker □, Regular □ Average # cigarettes/day: ___
Alcohol: Do you drink alcohol regularly? ____________________________
Drugs: Do you use any other drugs? ____________________________

Signature of Subject: ____________________________ Witness: ____________________________
Participant Data Form

Name:

D.O.B.:

Age:

Forearm Volume:

Forearm Circumference:

Weight:

Height:

Maximum Voluntary Contraction: 1. 2. 3.

Waist Circumference:

Date of Onset of Diabetes:

☐ Run through of experiment
☐ Explanation of day of study requirements:

- No exercise 12 hours before experiment
- No caffeine 12 hours before experiment
- No food 4 hours before experiment
- Take elevator to the lab – not stairs
- Bring t-shirt, short
Appendix B

Subject Medications
Table 2. Subject medications.

<table>
<thead>
<tr>
<th>Subject Identifier</th>
<th>CTL Group</th>
<th>Subject Identifier</th>
<th>T2D Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>None</td>
<td>A</td>
<td>None</td>
</tr>
<tr>
<td>J</td>
<td>None</td>
<td>B</td>
<td>Metformin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tevaramopril – ACE Inhibitor</td>
</tr>
<tr>
<td>K</td>
<td>None</td>
<td>C</td>
<td>Crestor – Statin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ezetrol – Cholesterol Lowering</td>
</tr>
<tr>
<td>M</td>
<td>None</td>
<td>E</td>
<td>Ramipril – ACE inhibitor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metformin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Indocin – NSAID (only for back spasm)</td>
</tr>
<tr>
<td>N</td>
<td>None</td>
<td>F</td>
<td>Ventolin – B receptor agonist</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Symbicont – anti-asthma (only during prolonged heavy exercise)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metformin</td>
</tr>
<tr>
<td>P</td>
<td>None</td>
<td>G</td>
<td>Diavan – ARB</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crestor – Statin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metformin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pantaprazole - PPI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>Potassium Chloride – Dietary</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cyanocobalamin – Vit B12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium Carbonate – Antacid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lipitor – Statin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ergocalciferl – Vit D2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pantoprazaole – PPI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Linsinopril – ACE inhibitor</td>
</tr>
</tbody>
</table>
Appendix C

Inclusion/Exclusion Criteria
Subject Inclusion and Exclusion List

Both Groups:

- Male
- Age 30-70 (so long as matched)
- Any level of physical activity (so long as matched)
- Non-smokers or Former Smokers (>5 years)
- 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:
  - Insulin
  - Insulin secretagogues
  - Verapamil
  - Diltiazem
  - Sulphonylureas
- Drugs that are ok to include:
  - Metformin
  - ACE inhibitors
  - Diuretics
  - Ca$^{2+}$ blockers (except for Verapamil and Diltiazem)
  - ARBs
  - α-blockers
  - β-blockers
  - Lipid-lowering drugs
  - Rosiglitazone, pioglitazone
Appendix D

Subject Recruitment Initiatives
School of Kinesiology and Health Sciences

PARTICIPANTS NEEDED FOR
RESEARCH IN EXERCISING MUSCLE

We are looking for volunteers to take part in a study of non-smoking men, aged 40 or older.

As a participant in this study, you would be asked to perform rhythmic hand grip exercise using forearm muscles while also performing leg exercise (pushing a pedal). The exercise will last for 9 minutes.

Your participation would involve 3 sessions, each of which is approximately 1 – 2 hours.

In appreciation for your time, you will receive a $60 honorarium.

Contact Info

For more information about this study, or to volunteer for this study, please contact:

Michael Bravo
at
613-533-6000 Ext. 78425 or
Email: michael.bravo@queensu.ca

This study has been reviewed by, and received ethics clearance through the Office of Research Services, Queen’s University.
Appendix E

Seven-Day Physical Activity Recall
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 1/4 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 à 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 o 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.
5. Compared to your physical activity over the past three months, was last week's physical activity more, less or about the same?

At the end of the interview:

Explanation: Moderate, Hard and Very Hard Intensity Levels

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

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

2. How many days in the last seven did you work?

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

Go to question 4

Date:

Day of the week form completed: Sunday

Month / day / year

Human volunteer;

Control Laboratory
<table>
<thead>
<tr>
<th>Subject ID</th>
<th>10-22 mins</th>
<th>23-77 mins</th>
<th>78+ mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>One week ago</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
</table>

Yesterday
Naps [±] 30 min
Please list below any activities reported by the participant that you do not know how to classify:

1. Yes
2. Maybe
3. No

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

Explain:

1. Yes
2. No

Were there any problems with the 7-Day PAR Interview (circle one):

7-Day PAR: Interview Evaluation Form
Appendix F

ACSM Contraindications to Exercise Testing
American College of Sports Medicine Contraindications to Exercise Testing

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

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

Individual Subject Data
Figure 18. Change in flow from exercise in the CTL group.

Individual changes in flow from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 19. Change in flow from exercise in the T2D group.

Individual changes in flow from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 20. Change in flow from exercise in CTL group – grouped by individual.

Individual changes in flow from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 21. Change in flow from exercise in T2D group – grouped by individual.

Individual changes in flow from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars represent a different subjects responses at four time points.
Figure 22. Flow boxplot demonstrating median change in flow with 25th and 75th percentiles.

A) Change in flow from baseline to exercise time points for both groups. B) Change in flow from exercise to IPF9 and exercise to IPF12 time points for both groups. Light grey boxes represent Control protocol while dark represent MMR protocol. Crosses represent different from Control protocol (+, P<0.05).
Figure 23. Change in mean arterial pressure from exercise in the CTL group.

Individual changes in mean arterial pressure from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 24. Change in mean arterial pressure from exercise in the T2D group.

Individual changes in mean arterial pressure from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 25. Change in mean arterial pressure from exercise in CTL group – grouped by individual.

Individual changes in mean arterial pressure from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 26. Change in mean arterial pressure from exercise in T2D group – grouped by individual.

Individual changes in mean arterial pressure from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 27. Mean arterial pressure boxplot demonstrating median change in MAP with 25th and 75th percentiles.

A) Change in mean arterial pressure from baseline to exercise time points for both groups. B) Change in mean arterial pressure from exercise to IPF9 and exercise to IPF12 time points for both groups. Light grey boxes represent Control protocol while dark represent MMR protocol. Crosses represent different from Control protocol (+, P<0.05).
Figure 28. Change in cardiac output from exercise in the CTL group.

Individual changes in cardiac output from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 29. Change in cardiac output from exercise in the T2D group.

Individual changes in forearm vascular conductance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 30. Change in cardiac output from exercise in CTL group – grouped by individual.

Individual changes in cardiac output from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 31. Change in cardiac output from exercise in T2D group – grouped by individual.

Individual changes in cardiac output from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 32. Cardiac output boxplot demonstrating median change in MAP with 25th and 75th percentiles.

Change in cardiac output from baseline to exercise time points for both groups. B) Change in cardiac output from exercise to IPF9 and exercise to IPF12 time points for both groups. Light grey boxes represent Control protocol while dark represent MMR protocol. Crosses represent different from Control protocol (+, P<0.05).
Figure 33. Change in heart rate from exercise in the CTL group.

Individual changes in heart rate from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 34. Change in heart rate from exercise in the T2D group.

Individual changes in heart rate from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 35. Change in heart rate from exercise in CTL group – grouped by individual.

Individual changes in heart rate from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 36. Change in heart rate from exercise in CTL group – grouped by individual.

Individual changes in heart rate from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 37. Heart rate boxplot demonstrating median change in MAP with 25th and 75th percentiles.

A) Change in heart rate from baseline to exercise time points for both groups. B) Change in heart rate from exercise to IPF9 and exercise to IPF12 time points for both groups. Light grey boxes represent Control protocol while dark represent MMR protocol. Crosses represent different from Control protocol (+, P<0.05).
Figure 38. Change in mean arterial pressure from exercise in the CTL group.

Individual changes in mean arterial pressure from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 39. Change in total peripheral resistance from exercise in the T2D group.

Individual changes in total peripheral resistance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 40. Change in total peripheral resistance from exercise in CTL group – grouped by individual.

Individual changes in total peripheral resistance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Individual changes in total peripheral resistance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.

Figure 41. Change in total peripheral resistance from exercise in CTL group – grouped by individual.
Figure 42. Total peripheral resistance boxplot demonstrating median change in MAP with 25th and 75th percentiles.

A) Change in total peripheral resistance from baseline to exercise time points for both groups. B) Change in total peripheral resistance from exercise to IPF9 and exercise to IPF12 time points for both groups. Light grey boxes represent Control protocol while dark represent MMR protocol. Crosses represent different from Control protocol (+, P<0.05).
Figure 43. Change in forearm vascular conductance from exercise in the CTL group.

Individual changes in forearm vascular conductance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 44. Change in forearm vascular conductance from exercise in the T2D group.

Individual changes in forearm vascular conductance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each vertical bar represents a different subject. Crosses represent significant group differences compared to Control protocol (+, P<0.05).
Figure 45. Change in forearm vascular conductance from exercise in CTL group – grouped by individual.

Individual changes in forearm vascular conductance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 46. Change in forearm vascular conductance from exercise in CTL group – grouped by individual.

Individual changes in forearm vascular conductance from exercise to IPF9 and IPF12 time points in both the Control and MMR protocols. Each grouping of four bars bar represents a different subjects responses at four time points.
Figure 47. Forearm vascular conductance boxplot demonstrating median change in DVK with 25th and 75th percentiles.

A) Change in forearm vascular conductance from baseline to exercise time points for both groups. B) Change in forearm vascular conductance from exercise to IPF9 and exercise to IPF12 time points for both groups. Light grey boxes represent Control protocol while dark represent MMR protocol. Crosses represent different from Control protocol (+, P<0.05).
Appendix H

Repeatability Data
Figure 48. Repeatability data for flow at baseline.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of baseline. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 49. Repeatability data for mean arterial pressure at baseline.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of baseline. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 50. Repeatability data for cardiac output at baseline.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of baseline. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 51. Repeatability data for flow at heart rate.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of baseline. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 52. Repeatability data for total peripheral resistance at baseline.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of baseline. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 53. Repeatability data for forearm vascular conductance at baseline.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of baseline. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 54. Repeatability data for flow during exercise.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of exercise. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 55. Repeatability data for mean arterial pressure during exercise.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of exercise. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 56. Repeatability data for cardiac output during exercise.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of exercise. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 57. Repeatability data for heart rate during exercise.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of exercise. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 58. Repeatability data for total peripheral resistance during exercise.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of exercise. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.
Figure 59. Repeatability data for forearm vascular conductance during exercise.

Each of the first four vertical bars represents the average response for all subjects during the last thirty seconds of exercise. The final bar represents the overall average response for all trials. The coefficient of variation is presented in the top right corner as mean ± SD.