Are Vascular Function and Oxygen Uptake Kinetics Related in People with Type 1 Diabetes?

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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Master of Science

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Abstract

Measures of aerobic fitness (VO$_2$ peak), vascular function, and oxygen uptake kinetics (VO$_2$ kinetics) were collected in people with type 1 diabetes (T1D) and controls to determine the relationship between calf blood flow and oxygen uptake kinetics. Outcomes were measured over two days, where calf blood flow was measured by strain gauge plethysmography and oxygen uptake kinetics was determined by 3 minute alternating square wave test at 80% of their predetermined ventilatory threshold. There were no significant differences between the two groups in measures of fitness, vascular function, or VO$_2$ kinetics (P > 0.05), and the relationship between VO$_2$ kinetics and maximal blood flow was not significant in this cohort (P > 0.05). With proper exercise and adequate glucose control, people with T1D may maintain vascular health and VO$_2$ kinetics similar to people without T1D.
Acknowledgments

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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>A₀, A₁</td>
<td>Amplitude values</td>
</tr>
<tr>
<td>AGEs</td>
<td>Advanced Glycation End Products</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic Nervous System</td>
</tr>
<tr>
<td>aPWV</td>
<td>Arterial Pulse Wave Velocity</td>
</tr>
<tr>
<td>AS</td>
<td>Arterial Stiffness</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Tri-Phosphate</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>BPM</td>
<td>Beats per Minute</td>
</tr>
<tr>
<td>CRF</td>
<td>Cardiorespiratory Fitness</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>ED</td>
<td>Endothelial Dysfunction</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>EPOC</td>
<td>Excess Post Exercise Oxygen Consumption</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow Mediated Dilation</td>
</tr>
<tr>
<td>HbA₁C</td>
<td>Glycated Hemoglobin</td>
</tr>
<tr>
<td>HF</td>
<td>High Frequency</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart Rate Variability</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High Sensitivity C-reactive Protein</td>
</tr>
<tr>
<td>LF</td>
<td>Low Frequency</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>MRT</td>
<td>Mean Response Time</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>RMSSD</td>
<td>Root Mean Squares of Successive Differences</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of Perceived Exertion</td>
</tr>
<tr>
<td>R-Squared</td>
<td>Coefficient of Determination</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>sc</td>
<td>Slow Component</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>τ</td>
<td>Tau; time constant; time to reach ~63% of final value</td>
</tr>
<tr>
<td>T1D</td>
<td>Type 1 Diabetes</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>TD</td>
<td>Time Delay</td>
</tr>
<tr>
<td>TP</td>
<td>Total Spectral Power</td>
</tr>
<tr>
<td>VLF</td>
<td>Very Low Frequency</td>
</tr>
<tr>
<td>VO₂</td>
<td>Volume of Oxygen</td>
</tr>
<tr>
<td>VT</td>
<td>Ventilatory Threshold</td>
</tr>
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Chapter 1: Introduction

Physical activity is highly recommended for people with type 1 and type 2 diabetes to improve quality of life and reduce risk of future all-cause mortality and cardiovascular disease. Reduced peak exercise capacity is often seen in people with diabetes compared with non-diabetic peers, which can be attributed to altered cardiovascular function during exercise, and could also contribute to a reduction in physical activity. However, many diabetes-related complications are implicated in reducing the total amount of physical activity in this population, including cardiovascular disease, retinopathy, and vascular dysfunction.

Not only do some people with diabetes exhibit reduced peak aerobic exercise capacity, but submaximal exercise responses also seem to be affected in many people with type 2 diabetes (T2D), as oxygen uptake and usage at the start of exercise (VO$_2$ kinetics) are abnormally slow in this population, possibly leading to premature muscle fatigue and reduced exercise tolerance. Investigating VO$_2$ kinetics could be important to describe exercise responses of people with type 1 diabetes (T1D). Humans frequently transition between different metabolic rates in everyday tasks such as climbing stairs, running to catch a bus, to more demanding tasks such as engaging in physical labor or sports. Fitness levels and degree of glucose control may vary greatly and VO$_2$ kinetics may also differ among people with T1D and affect their exercise tolerance. However, to date studies have not
compared VO₂ kinetics between people with T1D and control participants with similar fitness. The fitness of the participants may influence factors that determine VO₂ kinetics like vascular function, and muscle mass and type. Comparing VO₂ kinetics in participants with similar fitness may be a more appropriate measure of health than steady state exercise in the T1D population, as it gives insight into the effects that T1D has on muscle energetics and metabolic control.  

Current understanding suggests that slowed VO₂ kinetic responses are due to an imbalance of muscle oxygen delivery relative to oxygen uptake at the muscle, and that impaired vascular function might be one of the main mechanisms behind this imbalance. There is evidence of vascular dysfunction developing early on in T1D, and it has been linked to both macro and micro vascular complications, which are the leading causes of morbidity and mortality in this population. The cause of vascular dysfunction in T1D is not well understood, but hyperglycemia, oxidative stress, impaired vasodilation, low grade inflammation, and neuropathic abnormalities have been implicated in the development of vascular dysfunction.  

Despite these findings, the role of vascular dysfunction and its effects on exercise responses and tolerance through VO₂ kinetics has not been well investigated to date in the T1D population. This research will aim to quantify the relationship of vascular dysfunction to VO₂ kinetics through pulse wave velocity (PWV) to measure arterial stiffness, and vascular reactivity via strain gauge
plethysmography to measure both conduit and resistance vessel function. The health of the vasculature, specifically how it affects delivery of oxygen to the working muscles is a potential mechanism to slow VO$_2$ kinetics in T1D. We will compare vascular function and VO$_2$ kinetic values between people with T1D and healthy control participants, and also determine if VO$_2$ kinetics are correlated to vascular measures in these participants. This research will allow us to better understand the changes at different levels of the vasculature and their role in VO$_2$ kinetics in T1D.
Chapter 2: Review of the Literature

The following review of the literature will discuss the pathology of T1D, and specifically how it affects the vasculature. We will discuss the changes at different levels of the vasculature using a variety of methods and their role in exercise. Once we elucidate the impacts on the vasculature, we will transition into the possible impact of T1D on exercise performance and capacity. The focus will then shift to VO₂ kinetics, and the factors that slow kinetics due to impaired oxygen delivery or impaired use of oxygen at the muscle. Lastly, we will discuss possible means by which T1D specifically might change VO₂ kinetics, and the possible consequences of slowed kinetics such as reduced exercise capacity and exercise tolerance. Chapter 2 will conclude with a summary of the literature, project overview, and hypotheses.

2.1 Type 1 Diabetes

Diabetes mellitus is often accompanied by long term vascular, neurologic, and muscular complications. In T1D, these complications result from a loss of pancreatic β-cells due to autoimmune processes, leading to insulin deficiency that must be corrected with insulin injections to stabilize blood glucose levels, and if left untreated result in hypoinsulinemia and hyperglycemia 21. As T1D usually develops in adolescence and is not necessarily brought on by metabolic factors, the degree of glucose control and fitness in T1D can vary greatly.

Glucose control, or glycemic status, is quantified by measuring the amount of glycated hemoglobin (HbA₁c) in the blood, and gives an indication of plasma glucose
levels over the lifespan of red blood cells, which is approximately 3 months. Hemoglobin protein is glycated by exposure to glucose in the blood plasma, and longer or more severe hyperglycemia in the blood will result in a higher HbA1c value. Poor glucose management for extended periods of time can result in the development of disease related complications such as retinopathy, neuropathy, nephropathy, and cardiovascular disease. Long periods of hyperglycemia can affect cardiopulmonary and muscular responses to exercise in people of different fitness levels with T1D. Not only do glucose and insulin management impact people with T1D, but insulin resistance also affects this population regardless of glycemic control, and further contributes to muscular and vascular complications leading to reduced fitness.

Proper glucose management and regular physical activity are important factors to take into consideration in the T1D population, as research has shown that these two variables can improve diabetes related symptoms such as vascular function, and improve fitness. With good glucose control and regular physical activity, people with T1D may improve their health to a level comparable to their peers. However, there has not been any research to date looking at VO2 kinetics in a cohort of people with T1D that have comparable fitness to their peers as this may influence their vascular health and have an impact on kinetics.
2.2 Vascular Dysfunction

Vascular dysfunction develops early in T1D, affecting adolescents and young adults within a few years of diagnosis. Changes are seen at different levels of the vasculature, such as increased large vessel arterial stiffness, endothelial dysfunction, and reduced vascular conductance of resistance vessels. Many factors such as hyperglycemia, hypoinsulinemia, and low grade inflammation are implicated in contributing to vascular dysfunction, and are linked to micro and macrovascular complications, which are the leading source of morbidity and mortality in this population since vascular disease is widespread and involves a variety of factors in T1D, it can be difficult to identify a target and treat medicinally. However, evidence suggests that both good glycemic control and regular exercise improve blood glucose levels, ameliorate the effects of T1D on the vasculature, and improve fitness or VO$_2$ peak. Despite evidence of reduced physical fitness in people with T1D, studies have also shown that people with T1D whom have good glycemic control and exercise regularly are not only able to improve their fitness, but also achieve VO$_2$ peak values similar to healthy age matched controls. However, it is unknown how much of the increase in aerobic power is due to improvements at various levels of the vasculature, and how much gas exchange is limited by other factors. It is also unknown if changes in the vasculature are associated with changes in the VO$_2$ kinetics in this population. To measure vascular function, we will explore two measures that have shown changes due to T1D: arterial stiffness and vascular conductance.
2.21 Arterial Stiffness

Arterial stiffness (AS) is an early sign of arteriosclerosis and predicts cardiovascular events independently of other cardiovascular risk factors. Increases in arterial stiffness likely involve structural and functional changes in the arterial wall. Chronic low-grade inflammation and advanced glycation end products (AGEs) have both been independently associated with an increase in AS in Type 1 diabetes, and involved in the development of micro and macrovascular complications in T1D. AGEs are formed due to the glycation and oxidation of proteins, lipids, and nucleic acids as a result of hyperglycemia and oxidative stress. Low-grade inflammation is a metabolic state most often characterized by the presence of elevated levels of the downstream marker high sensitivity C-reactive protein (hsCRP).

Currently the gold standard, non-invasive measure of AS is arterial pulse wave velocity (aPWV), where a higher aPWV is reflective of higher AS. The pulse wave velocity is calculated by recording the ECG and pulse wave tracing at two sites, most commonly with a tonometry device. The transit time is recorded as the delay from the R wave from the ECG, representing early ventricular depolarization, to the foot of the pressure tracing measured by the tonometry device at either of the sites, indicating that blood flow has arrived. The distance between the two sites is measured, and the velocity is calculated by dividing the distance between the markers by the transit time. The consequences of increased arterial stiffness are increased pulsatile blood pressure (BP) caused by higher systolic BP (SBP) and...
lower diastolic BP (DBP), resulting in higher pulse pressure (PP), increasing left ventricular afterload and altered coronary perfusion \(^4^8\). Higher left ventricular afterload increases the workload of the left ventricle, and consequently increases the demand for blood supply, while altered coronary perfusion decreases blood supply to the heart, causing a possibly harmful combination.

Cardiorespiratory fitness (CRF) is independently correlated with AS in children and adults \(^4^9\)\(^,^5^0\), with lower fitness associated with higher AS. Exercise training lowers AS in those with increased baseline values \(^5^1\). Although evidence documenting how increased AS reduces aerobic exercise performance is sparse, it has been speculated that arterial stiffness could impair physiological responses to exercise in T2D by slowing VO\(_2\) kinetics \(^5^2\). Arterial stiffening provides evidence of vascular damage, helping to determine overall cardiovascular risk and can be used as a good characterization tool \(^4^6\).

2.22 Resistance Vessel Vascular Reactivity

Individuals with T1D often develop diabetes related complications such as peripheral vascular disease, retinopathy, and neuropathy, especially if they are in suboptimal glycemic control \(^1^8\). Vascular abnormalities have been identified in pediatric T1D cohort \(^3^4\), suggesting that vascular damage occurs early on in the disease and progresses further in adulthood \(^1^9\). Although the exact mechanism causing vascular damage is not known, improved glucose and insulin control are suspected to improve vascular function. However, research has shown that although
good glucose control may reduce the incidence of angiopathy, once structural changes occur in the vasculature they are irreversible and continue to progress. Studies have also shown that T1D in rats produce straighter, narrower capillaries, which impair capillary hemodynamics, reducing muscle O₂ delivery. Impaired blood flow at the level of the arterioles will limit oxygen delivery to the muscle, slowing VO₂ kinetics and increasing fatigue during exercise. In addition, another factor often overlooked in T1D is insulin resistance, which as mentioned previously, often accompanies T1D and plays a role in O₂ delivery to the muscle. With insulin resistance, insulin’s action to increase vascular perfusion and blood flow is decreased, further contributing to reductions in blood flow.

To measure the vascular reactivity and blood flow, strain gauge plethysmography device on the calf muscle can be used to measure the rate of blood inflow at rest and after ischemic exercise. The rate of inflow is an indicator of small or resistance vessel function. This technique has been shown to be ideal for obtaining accurate measurements of calf blood flow over multiple cardiac cycles. Peak blood flow after ischemic calf exercise is closely associated with peak whole body oxygen uptake across a range of fitness categories and ages. However, not much is known about the response of the resistance vessels in exercise in people with T1D. Previous research in T2D has suggested that slowed VO₂ kinetics in this population could be due to slowed blood flow to the exercising muscles. Although the mechanisms and manifestations of T2D and T1D are not identical, diminished
blood flow could reduce delivery of blood to the muscle, leading to slowed VO₂ kinetics in people with T1D.

2.4 Type 1 Diabetes and the Autonomic Nervous System

Another complication often seen in T1D is diabetic autonomic neuropathy, which has been shown to be closely related to vascular function. The autonomic nervous system (ANS) through the sympathetic and parasympathetic nervous systems innervate blood vessel walls and regulate wall tension. Both vascular and ANS dysfunction coexist in diabetes, and it is possible that there are interactions between vascular and ANS dysfunction in this population. ANS imbalance and enhanced sympathetic activity induces a sustained increase in blood pressure by causing peripheral vasoconstriction, reducing venous capacitance, and impairing vasodilation. Increases in muscle sympathetic nerve activity (MSNA) have also been associated with a decrease in endothelial function. A study on diabetic rats has shown that reduced blood flow to the nerves occurs very early after diabetic induction, suggesting that reduced nerve blood flow may be a factor influencing ANS dysfunction.

Not only does the ANS control blood pressure, but also another manifestation of ANS dysfunction can be seen in an impaired heart rate response. Specifically in people with diabetes, variation in heart rate at rest has been used to determine the presence of autonomic neuropathy. Previous research has also shown a reduced
heart rate response in people with T1D, which could contribute to detriments in fitness outcomes \textsuperscript{62,63} and possibly VO\textsubscript{2} kinetics. A simple way to measure ANS function is through heart rate variability (HRV) at rest, which is easy to administer and measures the differences in timing between peaks in the heartbeat waveforms (R to R interval)\textsuperscript{61}. Short-term recording of HRV include three main frequency domains: very low frequency (VLF), low frequency (LF), and high frequency (HF). Most researchers agree that vagal activity (and therefore the parasympathetic nervous system) is the main contributor to the HF component, and LF is a influenced largely, but not exclusively by sympathetic nervous system modulations\textsuperscript{61}. Previous studies have shown reductions of the absolute power of both LF and HF in diabetic neuropathy, suggesting that both the sympathetic and parasympathetic nervous systems may be involved \textsuperscript{63,64}. A reduction in HRV in people with diabetes seems to carry negative prognostic value and affect the vasculature \textsuperscript{65}. Therefore, proper function of both the ANS and vasculature can have a big impact on blood flow to the working muscles, and impact VO\textsubscript{2} kinetics in people with T1D.

\textbf{2.5 Oxygen Uptake Kinetics}

Due to the limited amount of non-oxidative energy stores in the muscle, there is a need for increased oxygen flux from the atmosphere to the mitochondria at the onset of exercise to provide for the energy needs of the working muscle. Delivery of oxygen to the muscles involves the inspiration and exchange of oxygen from the air into the bloodstream at the lungs and the distribution of the oxygenated blood from
the lungs to the working muscles by the cardiovascular system. Utilization of oxygen
to provide energy to the muscle through oxidative phosphorylation is a multi-
faceted process that is dependent on enzyme activity, substrate availability, and
oxygen delivery. At rest, the metabolic state is associated with high levels of ATP
and phosphocreatine (PCr), resulting in a high energetic state. At the onset of
exercise ATP and PCr are utilized, and the concentration of PCr decreases in a
similar, but inverse, exponential curve to that of VO$_2$ and has led some scientists to
believe that VO$_2$ kinetics are limited by PCr, or metabolic inertia at the working
muscle in healthy individuals$^{66}$.

Physical exercise requires transitions between different metabolic rates
depending on the task; placing demands on both the musculature and the O$_2$
transport pathway. The transitions between metabolic rates, and the speed at which
they are able to adapt between workloads are referred to as kinetics. Fast VO$_2$
kinetics elicit a smaller O$_2$ deficit, reduced glycolytic demand, and high exercise
tolerance, while conversely slow VO$_2$ kinetics result in a high O$_2$ deficit and poor
exercise tolerance$^{12}$. Despite many years of study, the mechanisms that control the
rate of increase of oxidative metabolism remain controversial. While some scientists
argue that the control of VO$_2$ kinetics is dependent solely on the exercising muscle
$^{12}$, others argue that the regulation of O$_2$ transport contributes to the control of
kinetics$^{66}$. The debate on the site of control for healthy individuals is still ongoing,
but research suggests that in people with T2D, VO$_2$ kinetics are slowed due to
decreased vascular blood flow$^{56}$. However, there has not been any research on VO$_2$
kinetics conducted in people with T1D to identify if oxygen delivery or oxygen utilization contributes to slowed kinetics.

2.51 Exercise Intensity Domains

The profile of the response to exercise, or the kinetic response, is dependent on the intensity of exercise performed. Specifically for exercise at moderate intensity, or below ventilatory threshold, a rise in pulmonary VO$_2$ at the onset of exercise begins within the first breath, continues for a few seconds (10 to 20 s) called Phase I, and then an exponential increase in VO$_2$ called Phase II, to end in steady state, Phase III. The quick phase I response is mostly attributed to the instantaneous cardiac output increase due to vagal withdrawal and increased venous return from the contracting muscles to the lungs, and does not reflect an increase in oxygen extraction in the active muscles. Phase I has frequently been assigned a fixed period of 15 s so that phase II would consistently start at the same time point for every participant, as a two component exponential equation for moderate intensity exercise is often used if Phase I is not the focus of the study. The exponentially increasing phase II response is the most variable component of VO$_2$ kinetics, but typically a steady state VO$_2$ is achieved within 2 to 3 minutes in healthy individuals. During heavy or severe exercise above the ventilatory threshold and below critical power, a slight increase of VO$_2$ is seen after 90-120 s called the slow component (sc) that is added on top of the phase II response. Both heavy and severe exercise intensities where the slow component is present will elicit fatigue relative quickly, and modeling the kinetics curve also becomes more complicated.
2.52 VO₂ Kinetic Modeling

Pulmonary VO₂ kinetics are most often modeled as a function of time and increases in an exponential manner. Pulmonary VO₂ kinetics will be measured 15s from the onset of exercise, to account for phase I as previously explained, until a steady state VO₂ response, or phase III, is reached. Restricting the stimulus to moderate intensity exercise will remove the need to include the slow component and also permit the participant to exercise for a longer duration. The formula that is widely utilized to determine the time constant (τ) of VO₂ kinetics is 68:

\[ \Delta VO_2(t) = A_0 + A_1 (1 - e^{-t/\tau}) \]

This model includes a baseline value for VO₂ \((A_0)\), \(A_1\) is the increase in amplitude of VO₂ for the primary component, \(t\) is the time elapsed from exercise onset, and \(\tau\) is the time constant to reach 63% of the required steady state VO₂. Calculating a reliable estimate of VO₂ kinetics requires repeated transitions from rest to exercise. A square wave protocol is used where the exercise intensity changes immediately from a low workload to the required intensity and cycles to obtain multiple transitions. The duration of each exercise cycle will be 3 minutes of moderate intensity exercise at 80% of the ventilatory threshold (80% VT), and 3 minutes of low load pedaling repeated a total of 6 times to ensure that there will be adequate time for the VO₂ response to reach steady state 68. Three minute exercise repeated 6 times, and the use of a monoexponential equation for moderate intensity exercise
modeled after the cardiodynamic phase has been previously shown to provide an adequate fit for VO₂ kinetics. 

### 2.53 Oxygen Debt/Deficit

During transitions from rest to exercise, energy use required to perform the task, and energy provided through oxidative means are not evenly matched. The measurement of VO₂ kinetics aims to determine how much time is required until the energy provided through oxidative means matches the energy needed for the task. The time to reach steady state ranges from 1 to 5 minutes, and can vary depending on age, fitness level, and disease state. While most healthy individuals can reach steady state within 1-2 min, people with T2D can take up to 3 min, and heart failure patients up to 5 min. The energy used not provided through oxidative means is known as the “signaling error”, which represents oxygen deficit. During oxygen deficit, the rest of the energy needed is provided through anaerobic systems, such as PCr and anaerobic glycolysis. The use of these unsustainable energy systems allow the individual to maintain the exercise intensity required, but accumulates oxygen debt throughout exercise that causes excess post-exercise oxygen consumption (EPOC). At the end of exercise, VO₂ does not immediately return to baseline values, but remains elevated and after moderate intensity exercise is symmetrical to the VO₂ on kinetics, and is termed VO₂ off kinetics. The factors contributing to VO₂ off kinetics reflect the return of many physiological processes to resting homeostasis, such muscle VO₂, partial refilling of O₂ stores in venous blood and muscle, and other energetic costs.
Increased aerobic glycolysis instead of anaerobic glycolysis improves exercise tolerance, as a study recently showed a correlation between the time constant (τ) of phase II kinetics and time to exhaustion at 85% of VO\textsubscript{2} peak\textsuperscript{70}. Decreased use of anaerobic glycolysis through faster VO\textsubscript{2} kinetics may also affect the perceived exertion and fatigue during exercise through a decrease in both inorganic phosphate and hydrogen accumulation, which are both associated with fatigue\textsuperscript{71}. Specifically in diabetes, a study showed that exercise effort at 35% VO\textsubscript{2peak} was greater in older women with T2D than in controls\textsuperscript{52}, and multiple studies have shown that people with T1D can have reduced fitness compared to controls\textsuperscript{37,38}.

2.54 Site of Control of VO\textsubscript{2} Kinetics

As mentioned briefly previously, research suggests that slowed vascular flow might contribute to slowed VO\textsubscript{2} kinetics in people with T2D\textsuperscript{72}. Although there are many similarities between T1D & T2D, there are a few differences that could cause the site of control to differ. One of these differences is the lack of insulin production in T1D that if not monitored well may lead to hypoinsulinemia. The reduced muscle mass associated with extended periods of hyperglycemia and hypoinsulinemia\textsuperscript{73} may slow VO\textsubscript{2} kinetics. Evidence of mitochondrial dysfunction in T1D has also been shown to be specifically correlated with the degree of insulin resistance, but it is unclear whether it is due to impaired blood flow to the mitochondria, or the function of the mitochondria itself\textsuperscript{25,74,75}. However, some scientists argue that even if muscle mass and mitochondrial capacity were decreased, skeletal muscle would still contain sufficient mitochondria to allow an approximately 150 fold increase in
oxygen uptake per kilogram of muscle in exercise conditions. Another study found that although mitochondrial capacity in young untrained women with T1D was affected by glycemic status, it was not impaired relative to healthy untrained young women. On the other hand research has shown vast vascular remodeling in T1D, leading to reduced blood flow and skeletal muscle O\textsubscript{2} delivery, which could viably be a source of slowed VO\textsubscript{2} kinetics in this population.

We will compare vascular, kinetics, and fitness outcomes between fit people with T1D and healthy control participants to observe if there are any differences between the two groups. We will also try to find a correlation between vascular measures and VO\textsubscript{2} kinetics to identify if reduced blood supply due to vascular dysfunction is related to slowing of VO\textsubscript{2} kinetics in this population.
**Objectives**

The primary objective of this research project is to determine if measurements of blood flow and VO₂ kinetics are correlated in the T1D population. The secondary objective is to examine if there are differences in vascular, fitness, and VO₂ kinetics outcomes between people with T1D and healthy control participants.

**Hypotheses**

The hypotheses for this study are as follows:

1. VO₂ kinetics and blood flow will be correlated, suggesting that inadequate blood flow is the limiting factor slowing VO₂ kinetics.

2. Measures of vascular function (reactive hyperemic blood flow) and oxygen uptake kinetics will not be significantly different in the T1D group compared with controls who have similar aerobic fitness (VO₂ peak).
Chapter 3: Design & Methods

3.1 Participants

A total of twenty-one participants were recruited to the study, 9 people with type 1 diabetes and 10 healthy control subjects in between the age of 20 to 40. To be included in this study, all participants were non-smokers, non-obese, normotensive (BP <140/100mmHg), with no previous history of cardiovascular or pulmonary disease. For this study, we recruited participants with T1D who were interested in fitness, and therefore will likely exercise often and be in good health. This will allow us to compare measures of vascular health and oxygen kinetics in the T1D participants to the control group with comparable fitness. The participants in the T1D group confirmed their diagnosis of type 1 diabetes, were free from any clinically diagnosed complications related to T1D as assessed by their own medical teams, and used either insulin pump therapy or daily injections, and received no sympatholytic and vasodilator drugs. Other exclusion criteria include conditions that impose safety concerns or could have impaired exercise performance such as past tobacco use, known hypothyroidism, or history of inflammatory disease. To characterize the control of glucose regulation amongst people with T1D and controls in this study, we collected HbA1C at the beginning of the test with A1Cnow assessment kits. We also collected blood glucose measurements for people with T1D at the start of both test days that were collected with their own devices. All participants in the group with diabetes had glucose levels between 5.6 and 13.9 mmol\textsuperscript{l\textsuperscript{-1}} with no signs of ketosis, according to published guidelines. If glucose levels were below 5.6 mmol\textsuperscript{l\textsuperscript{-1}} the participants ingested carbohydrates, and their
blood glucose was re-measured 15 minutes afterwards before proceeding to exercise testing. Glucose was also measured after the end of the VO₂ peak test to identify post-exercise hypoglycemia. Both men and women participated in the study, but in order to rule out the effects of female hormones on exercise and progesterone on ventilation, women were tested in the follicular phase of the menstrual cycle ⁷⁸. The participants with T1D were recruited from various clubs and events for people with T1D and word of mouth.

3.2 Experimental Design

All participants came to the Dr. Terry Kavanagh Heart Health Lab at the Goldring Center for Sport Performance for two visits. The first visit consisted of baseline assessments of anthropometric variables, arterial stiffness, vascular conductance, glucose measurements, and exercise capacity. During the second visit, participants underwent an autonomic nervous system function assessment, HbA1c measurement, a square wave exercise test to assess VO₂ kinetics, and a perceived exertion fitness test. Participants were asked to abstain from caffeine and alcohol consumption for 12 h and food consumption 3 h prior to assessments. Height (m) and weight (kg) were measured in exercise clothing with shoes removed and body mass index (BMI) was calculated.
3.3 Statistical Analysis

The goal of this study was to discover if there are differences between people with T1D and controls in their vascular measures and VO₂ kinetics, and observe which, if any vascular measures correlate with VO₂ kinetics. To analyze the differences between the two groups for vascular measures, ANS function, fitness, anthropometric measures, perceived exertion, and VO₂ kinetics we used the independent sample t test. A P-value of less than 0.05 was considered to be statistically significant. To measure if maximal blood flow was related to VO₂ kinetics we performed a Pearson's correlation analysis. A P-value of less than 0.05 was considered to be statistically significant.

3.4 Autonomic Nervous System Function

Heart rate variability (HRV) was measured as an index of autonomic nervous system control. All measurements were performed in accordance with the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology 61 in a quiet, temperature controlled room (22-26°C, 30-60% humidity) after 15 min of supine rest. Continuous 6 min supine and 5 min standing R-R intervals were recorded using heart rate monitoring (s810i model, Polar Electro Canada, Lachine, QC, Canada). Artifacts were corrected through the Polar Precision Performance Software Version 4.01.029 (Polar, Kempele, Finland) by applying a filter power set to a minimum beat protection of 6 beats per minute (bpm). Five minute sections of supine and standing data were selected and exported for analysis.
using HRV Analysis Software Version 1.1 (Biosignal Analysis and Medical Imaging Group, Kulplo, Finland), allowing for non-parametric and parametric spectral analyses. Frequency domain measures were determined by spectral analysis of the time course of R-R intervals using Fast Fourier transformation and included total spectral power (TP), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.50 Hz). Frequency domain measures were transformed using the natural logarithm and are expressed in ms². LF and HF were also expressed in normalized units (nu), calculated by dividing the LF and HF by TP – (total power). Sympathovagal balance was indicated by the ratio of the LF to the HF (LF/HF) with heart rate and the root mean square of the successive R-R interval differences (RMSSD), a measure of the short-term components of HRV, being the time domain measures reported.

3.5 Peripheral Arterial Stiffness

Triplicate measures of supine arterial blood pressure (BP) were obtained 1 min apart using the BpTRU automated sphygmomanometer BpTRU device (BpTRU Medical Devices, Coquitlam, BC, Canada) following 5 min of supine rest at the radial and carotid arteries. These sites were used to measure arterial stiffness in this study as we are also using other peripheral vasculature measures such as blood flow. Measures of arterial stiffness were assessed using a semi-automated device and software (SphygmoCor EM3, AtCOR Medical, Itasca, IL, USA). Briefly, arterial pressure waveforms were recorded by the application of a pencil-like probe (Millar
Instruments, Houston, TX, USA) to the surface of the arterial site. Arterial pressure pulse was captured by a high-fidelity transducer and recorded for subsequent analysis using both spatial and temporal landmarks. The arterial pressure waveform has two components: the incident wave generated by ventricular ejection that travels distally, and the reflected wave coming from peripheral points of resistance that travel centrally. The shape of the incident and reflected waves are determined by the structural and functional characteristics of the vasculature. Arterial stiffness was determined by analyzing the shape of the arterial waveform using specific spatial landmarks. Aortic augmentation index (AIx), a measure of systemic arterial stiffness was determined from central pressure waveforms collected from the carotid artery. AIx is calculated as the pressure wave about the systolic shoulder divided by pulse pressure. The shoulder is defined as the first concavity on the upstroke of the pressure wave, and separates the initial systolic pressure rise from the late systolic rise. Carotid AIx has been proposed to be indicator of the magnitude of wave reflections, which is closely related to arterial stiffness.

### 3.6 Blood Flow

Resting and maximal flow-mediated blood flows (ml 100ml⁻¹ min⁻¹) of the right calf was measured using venous occlusion strain-gauge plethysmography. Subjects rested in a supine position with their right foot elevated 20° and secured in a weighted pedal to ensure their knee was slightly bent and the lower limb was
parallel to the table. An indium-gallium strain gauge (Vasculab SPG16, Medasonics, Newark, CA, USA) was placed around the widest part of the belly of the calf muscle with approximately 10g of tension. Calf blood flow was isolated during testing by inflating an exclusion cuff proximal to the ankle, above systolic BP (180-200 mmHg). Venous occlusion was induced by rapid inflation of the occlusion cuff, located on the thigh proximal to the knee, to 60 mmHg for 7s followed by rapid (<1s) deflation for a period of 7s with four sequential measurements. Following resting measures, local calf ischemia was induced for 5 minutes by inflating the occlusion cuff to 180 mmHg, immediately followed by local calf plantar flexion against a 10kg mass using a pedal at a pace of approximately 60 per minute. At volitional fatigue (failure to maintain cadence and/or intolerable pain) the occlusion cuff was released and maximal blood flow was measured as described. Concurrent beat-to-beat BP was recorded from the third digit of the right hand using a photoplethysmographic device (Finometer, Finapres Medical Systems BV, Amsterdam, Netherlands). Both BP and blood flow data was analyzed using customized software (LabView 7.1, Austin, TX, USA). Resting and maximal blood flows were derived from the maximal slope form the series of time-calf volume curves with temporally aligned measures of systolic and diastolic BP used to determine mean arterial pressure (MAP) at each blood flow.

3.7 Exercise Test

A graded exercise test to exhaustion was performed on a cycle ergometer (Monark Ergomedic 828 E, Monark Exercise AB, Sweden). Following a brief warm
up and familiarization with the procedure, subjects cycled for 2 min at a resistance of 25W, with pedaling frequency self-selected between 60 and 80 rotations per min. After the first 2 min, workload was increased to 100 W, and then by 50W every 2 min until voluntary exhaustion. Expired gas was monitored and analyzed using an automated metabolic cart gas analyzer (VMAX 2900, CareFusion Corporation, CA, USA) and peak oxygen consumption (VO$_2$ peak) was determined from breath-by-breath samples averaged over 20s. Ventilatory threshold (VT) was determined by calculating the ventilatory equivalent ratio of volume of gas expired (V$_E$) and volume of O$_2$ expired (VO$_2$)\textsuperscript{83}. As VT is reached, the ratio of V$_E$ to VO$_2$ increases as V$_E$ increases at a faster rate with no rise in the ratio of V$_E$ to volume of carbon dioxide (VCO$_2$).

3.8 Square Wave Exercise Test

On the second day of testing participants performed one trial of square wave exercise, and were instructed on the procedure of the test. The exercise started with 3 minutes of unloaded pedaling at a pace of 80 revolutions per minute (rpm) followed by an immediate increase to constant workload at 80% of their ventilatory threshold (VT) still at a self-selected pace in between 60-80 rpm for the next 3 minutes. A time of 3 minutes per set intensity was predicted to provide enough time for the participants to transition to steady state (phase II) as shown in a previous study in people with T1D\textsuperscript{85}. As previously described, VO$_2$ kinetics will be measured in this study by tau, which is approximately 63\%, or $\frac{1}{4}$ of the required steady state...
This study found that the mean tau of the T1D group was 34.6s in sedentary youths, requiring approximately 140s to reach steady state (or 2 min and 20 seconds). We expected our cohort to be physically active and have similar or faster kinetics than the population in the aforementioned study, making three minutes enough time for the participants to reach steady state. This cycle repeated six times to obtain multiple transitions to obtain an estimate of VO$_2$ kinetics as described previously$^{68}$, and end with a cool-down of 2 minutes of unloaded pedaling. The constant load was a power output equivalent to 80% of their ventilation threshold (VT) as determined from their previous peak exercise test. Expired gas was analyzed using an automated gas analyzer (VMAX 2900, CareFusion Corporation, CA, USA) and oxygen consumption was determined from breath-by-breath samples.

### 3.9 Perceived Exertion Fitness Test

To compare the perceived exertion due to a functional task between individuals with T1D and controls, we conducted a portion of the FORCE test performed by the Canadian Armed Forces. The test began with two 10kg sand bags placed 2 metres away from each other and close to the wall and the participant standing over one of the bags. The participant was then asked to lift the sandbag to a height of 1 meter marked on the wall, place the bag back down, shuffle to the next bag, and repeat. The test lasted a total of 2 minutes, and the pace was set at one lift per 4 seconds. Controlling for the total time and pace allowed participants to have a similar absolute stimulus regardless of fitness. We maintained the weight of the
sandbags at 10kg regardless of participant weight and fitness, as the weight of both groups were similar, providing insight as to whether the T1D group finds the exercise more difficult than the control group. Once the test was completed, we measured rate of perceived exertion and dyspnea through the Borg RPE and dyspnea scales.

### 3.10 VO₂ Kinetics Data Analysis

Data manipulation for the square wave test was based on the methodology from Kolkhorst et al. VO₂ kinetic data was averaged from multiple trials (n=4-6) for each subject to reduce noise (see Appendix 3). Outlying data, defined as being outside three standard deviations (SD) from the average of the preceding and subsequent three data points, was eliminated. Kinetics was then modeled by overlaying the data from all trials using a mono-exponential, nonlinear regression model equation as described previously:

\[ \Delta VO_2(t) = A_0 + A_1 (1 - e^{-t/\tau}) \]

This model includes a baseline value for VO₂ \((A_0)\), \(A_1\) is the increase in amplitude of VO₂ for the primary component, \(\tau\) is the time constant for the primary component, and \(t\) is time. The time delay for the cardiodynamic phase will be set at an average of 15s as has been shown in a study with young adults. VO₂\(_{(t)}\) is the time-dependent variation of VO₂. According to previous studies, a mono-exponential model is ideal for modeling moderate intensity VO₂ kinetics. Curve fitting analysis was done using a nonlinear regression program (SigmaPlot 11.0, Systat Software Inc., IL, USA).
where the program calculated and minimized the residual sum of squares based on the initial predicted values. Initial values and constraints were based on visual inspection of the plotted data, and the program tried to reach convergence, or a curve that appropriately fitted the data, with as many iterations as needed.

### 3.11 Significance

This study allowed us to compare whether vascular function, ANS modulation, and VO₂ kinetics differ significantly between participants with T1D and control group participants who have similar aerobic fitness. We determined if vascular function is related to VO₂ kinetics in people with T1D, and if they perceive exercise to be more difficult than controls. With these findings we will gain a greater understanding of the ramifications of type 1 diabetes, and the role of vascular function and VO₂ kinetics in this population.
Chapter 4 - Results

4.1 Participant Recruitment

A total of 19 participants (9 T1D and 10 control) were recruited, consented (see Appendix A), and came to the Dr. Terry Kavanagh Heart Health Lab at the Goldring Center for Sport Performance. Participants were recruited either through poster advertisements (see Appendix B) or through recommendations from other participants. None of the participants recruited dropped out or were excluded, all of them completing the two sessions.

4.2 Participant Characteristics

The physical and demographic characteristics of the participants are presented in Table 1. There were no statistically significant differences between the two groups for any of the physical or demographic characteristics other than HbA1C, which confirmed the diagnosis of type 1 diabetes in the T1D group. Blood glucose was taken before and after exercise for both sessions for participants in the T1D group. Only 1 participant had glucose levels below 5.6 mmol/L by the end of exercise and took their own glucose tablet to normalize their blood glucose.

4.3 Exercise Capacity and VO₂ Kinetics

The average responses to exercise, perceived exercise exertion, and VO₂ kinetics are summarized in Table 2. VO₂ peak in the T1D and control groups were
not significantly different (p=0.884, p=0.974 for absolute and relative values respectively). The max heart rate (HR) achieved was 177 ± 8 and 183 ± 10 bpm for the control and T1D groups respectively and were not significantly different (p=0.25). The average ventilatory threshold (VT) was 2.0 ± 0.9 L/min for the control group and 1.9 ± 0.6 for the T1D group. The power output for the square wave test was set at 80% of the power output at the VT. The average square wave power output for the control group and T1D group were 118W and 116W respectively. Representative VO₂ kinetic responses for individuals from the T1D and control groups are illustrated in Figure 1. The average responses during the square wave test are summarized in Table 3. An independent samples t-test showed that there was no significant difference between the two groups for VO₂ kinetics at moderate intensity exercise (p=0.733).

Perceived exertion and dyspnea after the fitness test were RPE= 13 ± 2 and 13 ± 3, and RPD= 3 ± 1 and 3 ± 2 for the control and T1D group respectively. There were no significant differences between the two groups for both RPE and RPD (p = >0.05, p=0.317, 0.975). Contrary to expectation perceived exertion and dyspnea ratings were not correlated with the participants’ absolute fitness (r = -0.066, p = 0.795; r= -0.065, p= 0.799).

4.4 Vascular & Autonomic Nervous System Measures

The average responses of vascular & autonomic nervous system measures are summarized in Table 2. The average aortic index (AI₃) at 75bpm was -6.44 ±
10.5 % for the T1D group and 1.89 ± 10.3 % for the control group. There were no statistically significant differences between the two groups for pulse wave velocity and aortic index ($p=0.066$ AIx, $p=0.331$ PWV). Heart rate variability (HRV) was not significantly different for any measures (LF, HF, or the ratio LF/HF) between the T1D and control groups ($p > 0.05$). Resting and maximal blood flows were not different between the two groups ($p=0.551$, $p=0.206$ for resting and maximal values respectively).

4.5 Correlations

In addition to blood flow and VO$_2$ kinetics, correlations were analyzed between peak exercise capacity & VO$_2$ kinetics, and blood flow and glycosylated hemoglobin (HbA1C). A Pearson correlation revealed that there was a non-significant negative relationship between relative peak exercise capacity and VO$_2$ tau ($r=-0.378$, $p=0.149$). This result is in line with the findings that VO$_2$ kinetics are highly plastic and can be decreased with increased fitness with exercise training\textsuperscript{12}. Resting blood flow had no significant relationship with VO$_2$ kinetics. The relationship between tau and maximal blood flow, and relative peak exercise capacity and maximal blood flow are shown in Figures 2 and 3 respectively.

Maximal blood flow was not significantly correlated to VO$_2$ kinetics in both the whole group ($r= 0.10$, $p= 0.73$), and in the T1D group alone ($r = 0.08$, $p= 0.707$). Resting and maximal blood flows were not significantly related to A1C in the T1D group, ($r=-0.569$, $p= 0.183$ for resting, $r=0.177$, $p=0.704$ for maximal).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>T1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>4/6</td>
<td>3/6</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>26 ± 6</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.3 ± 9</td>
<td>170.1 ± 10.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.6 ± 18.2</td>
<td>74.2 ± 17.5</td>
</tr>
<tr>
<td>BMI</td>
<td>27.8 ± 4.6</td>
<td>25.4 ± 4.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>105 ± 10</td>
<td>114 ± 9</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72 ± 9</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>5.2 ± 0.4</td>
<td>7.4 ± 0.6 *</td>
</tr>
</tbody>
</table>

Values are means ± SD; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1C: amount of glycosylated hemoglobin.

*P<0.05 between Control & T1D group

---

Table 2. Peak Exercise Responses, Vascular and Autonomic Measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>T1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ peak (L/min)</td>
<td>2.81 ± 0.77</td>
<td>2.68 ± 0.89</td>
</tr>
<tr>
<td>VO₂ peak (mL<em>kg⁻¹</em>min⁻¹)</td>
<td>36.2 ± 9.4</td>
<td>35.8 ± 6.4</td>
</tr>
<tr>
<td>Resting BF (mL<em>100mL⁻¹</em>min⁻¹)</td>
<td>4.8 ± 2.3</td>
<td>4.2 ± 1.7</td>
</tr>
<tr>
<td>Maximal BF (mL<em>100mL⁻¹</em>min⁻¹)</td>
<td>50.9 ± 12.7</td>
<td>59.2 ± 12.5</td>
</tr>
<tr>
<td>AI₃ (%)</td>
<td>8.55 ± 8.86</td>
<td>0.94 ± 9.1</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>7.3 ± 1.1</td>
<td>6.8 ± 0.8</td>
</tr>
<tr>
<td>LF (nu)</td>
<td>71.4 ± 10.3</td>
<td>67.9 ± 9.0</td>
</tr>
<tr>
<td>HF (nu)</td>
<td>28.5 ± 10.3</td>
<td>27.5 ± 9.5</td>
</tr>
<tr>
<td>LF/HF</td>
<td>5.2 ± 2.7</td>
<td>4.1 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; BF: blood flow; PWA: pulse wave analysis; PWV: pulse wave velocity; LF, HF: low & high frequency respectively; LF/HF: ratio of low to high frequency; RPE, RPD: rating of perceived exertion & dyspnea respectively.

There were no statistically significant different between any of these measures
Table 3. Square Wave Test Measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>T1D</th>
</tr>
</thead>
<tbody>
<tr>
<td>τ (s)</td>
<td>29.6 ± 8.8</td>
<td>28.3 ± 4.3</td>
</tr>
<tr>
<td>VO₂</td>
<td>1.69 ± 0.69</td>
<td>1.53 ± 0.47</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>131 ± 10</td>
<td>140 ± 15 *</td>
</tr>
<tr>
<td>R-squared</td>
<td>0.54 ± 0.13</td>
<td>0.54 ± 0.10</td>
</tr>
</tbody>
</table>

Values are means ± SD; τ: time constant for oxygen uptake kinetics; VO₂: average VO₂ for the square wave test; HR: heart rate; R-squared; coefficient of determination
*P<0.05 between Control and T1D groups
Figure 1. Sample response to the onset of exercise to calculate tau for VO₂ uptake kinetics. The control (A) and T1D (B) participants had very similar VO₂ uptake kinetic response as the average of their respective groups.
Figure 2. Correlation of tau and maximal blood flow for control and T1D groups; control participants are the filled dots and T1D participants are the empty dots.

$r = 0.15, p=0.58$
Figure 3. Correlation of relative maximal VO$_2$ and maximal blood flow for control and T1D groups; control participants are the filled dots and T1D participants are the empty dots. 
$r = 0.38, p=0.12$
Chapter 5 – Discussion

In this study we found that when T1D and control groups had similar aerobic fitness (VO₂ peak) there were no significant differences for measures of vascular health and VO₂ kinetics. There was no significant correlation between VO₂ kinetics and maximal hyperemic blood flow at the calf.

5.1 Autonomic Nervous System Function

As hypothesized, there were no significant differences in the autonomic nervous system function of the participants between the T1D and control group. There was not a significant difference in average standing sympathovagal values (HF/LF) between the T1D and control groups. As previously described in Section 2, it is widely agreed that HF values are representative of the parasympathetic nervous system function, while LF values are largely representative of sympathetic nervous system influence on heart rate. The results in this study demonstrate that the T1D participants in this study have similar sympathovagal balance when compared to their peers. Although previous literature has shown that certain disease states such as T1D have resulted in a reduced sympathovagal balance, good glycemic control may reduce this complication as shown in this study.

However, we did find that the heart rate of the T1D group during the square wave test was significantly higher than the control group, which may suggest reduced parasympathetic and augmented sympathetic activity. Other factors such as
test anxiety, glucose control during the test, and fatigue may have contributed to higher HR. Overall, it is likely that the T1D group in this study have relatively good control over their glycemic levels, as shown by their A1C level of 7.4 ± 0.6, and this could contribute to regular sympathovagal balance. Similar values of sympathovagal balance were found in another study looking at the relationship between ANS and VO2 peak in young adults, where they found the HF/LF ratio of approximately 5.4 and 4.1 for control and T1D participants respectively, which are similar to this study. ANS function has also been shown to be linked with other aspects of vascular function and blood flow, therefore normal ANS function should contribute to normal vascular function.

5.2 Vascular Function

5.2.1 Arterial Stiffness

Arterial stiffness assessed by measuring pulse wave velocity and pulse wave analysis was not statistically different between the T1D and control groups. Although previous studies have shown that people with T1D, even without clinically diagnosed cardiovascular complications have increased arterial stiffness compared to control participants, this study showed no differences between the two groups. Aerobic exercise capacity (AEC) has been shown to be independently correlated with AS in adults, which was not measured in the previously mentioned study and exercise training decreases AS. These results show that although people with T1D on average may have higher AS values compared age matched controls, this may not
always be the case. Both the T1D and control groups had comparable cardiorespiratory fitness, so the relationship between CRF and AS may also be applicable for the T1D population, where increased fitness is associated with lower AS. This study cannot ascertain whether AEC and AS are independently correlated or if exercise training can decrease AS in people with T1D, but another study could look more in-depth at this relationship in the future.

Arterial stiffness may impact exercise and oxygen uptake kinetics in a T2D population and also has implications in the overall vascular health of an individual. Arterial stiffness is associated with blood pressure, where increased stiffness will result in increased pulsatile blood pressure due to an increase in systolic blood pressure and a decrease in diastolic blood pressure. If exercise training is able to decrease AS in people with T1D and have comparable values to age matched controls as shown in this study, this may help to reduce the incidence of cardiovascular complications for this population.

### 5.22 Resistance Vessel Vascular Reactivity

Resistance vessel vascular reactivity in this study was measured through assessing resting and maximal blood flow at the calf by strain gauge plethysmography. Both resting and maximal values were not significantly different between the T1D and control groups. Previous studies have shown that vascular damage in small blood vessels occurs early on, even in a pediatric cohort. Vascular damage then progresses through adulthood, possibly affecting blood flow by
reducing blood flow to the autonomic system nerves responsible for limb blood flow regulation. As vascular function worsens, it leads to well-known diabetes related complications such as retinopathy, nephropathy, and neuropathy. However, the T1D group in this study has shown comparable blood flow to controls, suggesting that their vascular function is currently normal and relatively unaffected by the disease.

In this study we cannot ascertain the degree of vascular damage, but the results suggest that the damage has not significantly affected blood flow and O2 delivery to the muscles, as blood flow values in the T1D group are similar to age matched controls. Other studies have suggested that decreased oxygen uptake kinetics may be related to vascular damage leading to decreased blood flow and O2 delivery in T2D, but in this study both blood flow and oxygen kinetics were comparable to age matched controls. These results indicate that it is possible to have similar blood flow and oxygen uptake kinetics to age matched control in people with T1D. Further investigation will need to be conducted to determine if fitness, glucose control, or any other factors are responsible for the improvement in blood flow and oxygen kinetics in this population. If these relationships are confirmed, it will help to consolidate the beneficial effect of exercise to improve fitness and prevent diabetes related complications in people with T1D.
5.3 Aerobic Exercise Capacity & Oxygen Uptake Kinetics

5.3.1 Aerobic Exercise Capacity

Aerobic exercise capacity (AEC) was not significantly different between the T1D and control groups. Aerobic exercise capacity was measured as the peak volume of oxygen consumed (VO₂ peak) with a graded exercise test on a cycle ergometer until exhaustion. The VO₂ values found in this study were also similar to other studies on people with T1D, such as a non-athlete T1D group with an average VO₂ of 34.8 ± 3.3 \(^{88,89}\). Increased AEC as a result of habitual physical activity has also been associated with lower risk of all-cause mortality and cardiovascular complications in the T1D population \(^{1,3}\). Similar VO₂ peak in both groups also simplifies the analysis of oxygen kinetics and blood flow, as differences in VO₂ do not have to be adjusted. Although the aim of this study was not determine if increases in AEC through training improve vascular measures in people with T1D, which has been shown previously \(^{88}\), it shows that they can have comparable values to age matched controls. Hence, if T1D participants are able to maintain AEC levels similar to their peers this might reduce the amount of diabetes related complications in the future.

In addition, the results also suggest that people with T1D can also have similar fitness levels in performance settings. Although glucose control might present an extra challenge when participating in high-level sports, it should not decrease participation due to being unable to attain comparable fitness to their peers. Future studies could aim to look at specific performance related measures in
a range of sports to determine performance related factors such as sprint speed, coordination, etc.

### 5.32 Oxygen Uptake Kinetics

\( \text{VO}_2 \) kinetics was not different between the T1D and control groups. \( \text{VO}_2 \) kinetics were measured with a square wave exercise test to determine the rate of increase of \( \text{VO}_2 \), with 3 minute intervals for rest and exercise at 80% of the ventilation threshold previously determined by the \( \text{VO}_2 \) peak test. All participants had reached steady state, or target \( \text{VO}_2 \) 30 seconds prior to the end of the 3 min exercise interval, showing that this model was appropriate to be used in this cohort. Although a previous study has shown slowed \( \text{VO}_2 \) kinetics in people with T2D (43.8 ± 9.6) 56, and in T1D (47 ± 6) 84, this study found that \( \text{VO}_2 \) kinetics were not different between the T1D and control group. Bauer and colleagues studied an older subject group (mean age 47 y) and less aerobically fit (T1D 24.6 ± 4.8; Controls 20.9 ± 5.1 ml · kg\(^{-1}\) · min\(^{-1}\)) with much slower \( \text{VO}_2 \) kinetics (\( \tau \text{\(\text{VO}_2\)} \) (s): T2D 43.8 ± 9.6; Controls 34.2 ± 8.2 s). They suggest that the slowed \( \text{VO}_2 \) kinetics in their T2D group could be contributed to impaired vasodilation due to vascular dysfunction on the basis of measures of muscle deoxygenation. The study by Kremser and colleagues studied a T1D group with similar age and fitness, but they used very low intensity exercise to calculate \( \text{VO}_2 \) kinetics (35-40% of \( \text{VO}_2 \) peak), which could have affected their results. However, this study found that there were no differences in blood flow and no impaired vasodilation in the T1D group compared to the control group, suggesting that reduced blood flow was not the limiting factor for the uptake of oxygen in this
T1D group. A recent study reported evidence suggesting that with tau values close to 20s, VO$_2$ kinetics is oxygen delivery independent, meaning that the delivery of oxygen to the muscles is no longer the limiting factor $^{13}$. At that point, the authors state that when there is adequate provision of oxygen to the muscle, the limiting factor becomes the ability for the muscles to uptake and utilize the oxygen provided.

Previous studies have found that there is a reduction in total contractile force in people with T1D, which is likely contributed to impaired growth or atrophy of glycolytic muscle fibers $^{73}$. Although the cause of this is unknown, there a variety of factors that could influence skeletal muscle in this population. Therefore it is unclear if the limiting factor for VO$_2$ kinetics in this population is oxygen utilization at the muscle, or delivery of oxygen to the muscles. Future studies could aim to recruit a large and varied sample of people with T1D of different fitness levels and glucose control. These studies could examine vascular and muscle function, and blood perfusion through NIRS to determine the limiting factor to VO$_2$ kinetics in this population. With a larger spectrum of participants, a relationship between VO$_2$ kinetics and blood flow may be seen as those with reduced blow flow may have slower kinetics.

### 5.33 Perceived Exercise Exertion

Perceived exercise exertion in this study was shown not to be different between the T1D and control group. Perceived exertion was measured with a fitness test derived from the FORCE test performed by the Canadian Armed Forces.
Although a previous study on women with type 2 diabetes showed that they perceive exercise to me more taxing than controls, the results from this study indicate that it might not be the same in people with T1D. This study theorized that several objective factors could influence the rating of perceived exertion (RPE) such as low aerobic fitness and increased tau. However, in this study the VO₂ peak and tau of the participants with T1D was similar to the controls, which may be a factor why the T1D participants would did not exercise more challenging. Although the results from this study cannot be generalized to the whole T1D population, it does suggest that with good glucose control and fitness comparable to age matched peers, perceived exercise effort should be similar in people with T1D than those without.

5.4 Conclusion

In conclusion, this study did not find a relationship between VO₂ kinetics and blood flow. This study also showed that when groups T1D and control groups with similar aerobic fitness (VO₂ peak) are compared no differences in VO₂ kinetics, and vascular function are observed. These results help to show that even with diabetes, people with T1D are able to have comparable cardiorespiratory fitness and vascular function to people without diabetes. Although this study cannot determine the causation, it can help encourage people with T1D to have good glucose control and exercise to maintain “regular” vascular function and be able to avoid future diabetes related complications.
Chapter 6 – Limitations and Future Research

6.1 Limitations

This study had a few limitations, mainly including the recruitment, sample size, and some measures. A main limitation to this study was recruitment, as a convenience sample was used for recruitment through putting up posters around the University of Toronto campus and gyms. This type of recruitment naturally includes people with T1D whom are interested in exercise and fitness, and are probably more fit, and would not include those that are not interested in exercise. The results showed that the T1D group had comparable cardiovascular fitness and vascular measures to the control group, but we cannot be certain that these results are representative or if they are biased due to the style of recruitment. Due to time constraints and difficulty in recruiting, a small group of 9 people with T1D was used for this study. A larger and more varied group of people would have been able to not only provide more statistical power and help with the correlations, but also give a better representation of the T1D population. Finally, there were some measures that might have had some limitations. Maximal blood flow measured by strain gauge plethysmography was determined in this study by maximal effort during ischemic calf exercise to increase metabolites in the calf and result in vasodilation when the pressure cuff was released. However, some participants may not have reached their true maximal effort in this test, possibly resulting in lower maximal blood flow values than their true maximum. However, the metabolites built up during the
period of ischemia prior to the exercise should produce a vasodilatory response close to their maximum.

6.2 Future Research

This study was able to provide some insight into oxygen uptake kinetics and vascular measures in the young T1D population. The relationship between oxygen uptake kinetics and blood flow was shown to be not significant in this study, perhaps due to the small sample size or homogeneity of this group in terms of glucose control. Future studies could further expand on some of these findings through longitudinal studies to examine the effect of exercise training on oxygen kinetics and vascular function, or through larger studies to look at the relationship between oxygen uptake kinetics and blood flow. As mentioned previously, another interesting topic could try to determine if oxygen utilization or delivery is the limiting factor of oxygen kinetics in this population by looking at muscle and vascular function. Studies could also use new technology to keep track of physical activity and determine if increased physical activity is related to improved vascular measures. As oxygen kinetics could be related to blood flow, vascular, muscle, and nervous system health in people with slow kinetics, it might be able to be used as a prognostic tool for diabetes related complications. Future studies could also aim to find what makes people with T1D motivated to exercise, how more people can maintain good glucose control and fitness.
References

(2004).


50. Gando, Y. *et al.* Cardiorespiratory Fitness Suppresses Age-Related Arterial Stiffening in Healthy Adults: A 2-Year Longitudinal Observational Study. *J.*


Appendix A: Participant Informed Consent

Consent Form to Participate in a Research Study

Study Title: Are Vascular Function and Oxygen Kinetics Related in People with Type 1 Diabetes?

Investigator: Daniel Merino (647) 609 3080

Background/Purpose:
People with diabetes differ widely in their ability to exercise and on difference is how quickly they adapt to a change in exercise intensity. One factor that might lead to slower adaptation to an increase in exercise intensity is how quickly use of oxygen and blood flow increase with exercise. Our purpose is to measure if blood vessel function is related to how quickly oxygen use rises with exercise. We will compare the function of blood vessels and the rise of oxygen use between people with T1D and healthy control participants.

Study Design:
You will come to the Goldring Centre for High Performance Sport at the University on two days for this study:

1) On the first day we will measure weight, height, and age. Measures of blood vessel function will require you to lie down, and in one of them you will need to do calf exercises while lying down. Blood samples will be taken from a finger prick that you will administer for the HbA1c and glucose measurements. The exercise capacity test will be carried out on a cycle ergometer where the resistance will be gradually increased until your max exercise capacity is reached. You will also have to wear a gas exchange mask to measure the amount of air taken in, and an electrocardiogram (ECG) device that will monitor your heart rate. These assessments will take approximately an hour and a half to complete.

2) On the second day you will complete a cycling test to assess how oxygen use rises, and a perceived exertion fitness test. You will lie down for fifteen minutes while wearing an ECG device. The cycling test will also be carried out on a cycle ergometer while equipped with an ECG device, and will consist of five repeated increases and decreases in exercise intensity. Finally, the perceived exertion fitness test will consist of lifting 10kg sand bags to a height of 1 meter on the wall, then shuffling over 2 meters and repeating the process. The test will last a total of 2 minutes, and a pace will be set to one lift every 10 seconds. These assessments will take approximately an hour and a half to complete.
**Risks:**
The risks that we know of include that you may find that some of the measurements are uncomfortable and tiresome. If you feel very uncomfortable about a particular measurement, you can let us know and skip it.

**Benefits:**
You will not receive any direct benefit from being in this study apart from reimbursement in the form of a $20 gift card. Information learned from this study may help influence interventions concerning type 1 diabetes and exercise in the future. You will receive a copy of the study results if you wish.

**Confidentiality:**
Protecting your privacy is an important part of this study. Every effort to protect your privacy will be made. If the results of this study are presented to the public, nobody will be able to tell that you were in the study. In rare cases the investigator may be required by law to allow access to research records. If you decide to participate in this study, the research team will ask you for information such as episodes of hypoglycaemia, and your most recent HbA1C scores.

Your name and contact information will be kept secure by the research team at the University of Toronto. It will not be shared with others without your permission. Your name will not appear in any report or article published as a result of this study. Information collected for this study will be kept as long as five years.

If you decide to withdraw from the study, the information collected up to that time will continue to be used by the research team and possibly be used in the results.

**Withdrawal from the Study:**
If you wish to withdraw your consent please inform the Investigator (Daniel Merino). All data collected up to the date you withdraw your consent will remain in the study records, to be included in study related analyses. If you do decide to quit the study, this will not affect you or your involvement in any other University of Toronto associated groups.

**Questions about the Study:**
If you have any questions, concerns or would like to speak to the study team for any reason, please call: **Daniel Merino, study investigator at 647 609 3080, or Dr. Scott Thomas, Principal Investigator at 416 978 6957.**

If you would like to contact the research ethics board (REB), you can contact Daniel Gyewu at 416 946 5606
**Consent**
This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to the use of my information as described in this form. I agree to take part in this study.

__________________________  ______________________  ____________
Print Study Participant’s Name  Signature  Date

My signature means that I have explained the study to the participant named above. I have answered all questions.

__________________________  ______________________  ____________
Print Name of Person Obtaining Consent  Signature  Date
Appendix B: Recruitment Poster

Do you have Type 1 Diabetes?

- We are recruiting people with T1D for a two visit study at the Goldring Centre.

OUR GOAL IS TO SEE IF VASCULAR HEALTH CAN IMPACT EXERCISE.

If interested email: Dan Merino
dan.merino@mail.utoronto.ca
Appendix C: Oxygen Kinetics Processing

To process the oxygen uptake kinetics data, a few steps were taken:

- Performed square wave exercise test, aiming for 6 repetitions, and recorded the time started for each cycle
- Obtained the VO₂ data after the square wave exercise test and imported the time and VO₂ data into excel
- Removed the first 15s of all of the exercise steps to account for the time delay
- Visually inspected and removed any data points that were 3 standard deviations away from the surrounding 3 data points
- Imported data into SigmaPlot to process the data through the aforementioned equation, and fit the data to an average kinetics curve for each participant
- The program processed the data until convergence was reached

Sample square wave test response