MECHANISMS OF FATIGABILITY IN INDIVIDUALS WITH PREDIABETES AND THE EFFECT OF DIETARY NITRATE SUPPLEMENTATION

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MECHANISMS OF FATIGABILITY IN INDIVIDUALS WITH PREDIABETES AND THE EFFECT OF DIETARY NITRATE SUPPLEMENTATION

by

Blaine Arney, B.S, M.S.

A Dissertation submitted to the Faculty of the Graduate School,
Marquette University,
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

Milwaukee, Wisconsin

August 2024
ABSTRACT
MECHANISMS OF FATIGABILITY IN INDIVIDUALS WITH PREDIABETES AND THE EFFECT OF DIETARY NITRATE SUPPLEMENTATION

Blaine Arney, B.S., M.S.
Marquette University, 2024

Prediabetes is characterized as elevated blood glucose levels below the clinical threshold for type 2 diabetes. Previous research identified an increased fatigability (exercise-induced reduction in limb force or power) of the lower limb muscles in individuals with prediabetes which can impair physical function and lead to reduced physical activity. The mechanisms of fatigability in people with prediabetes are not known but are likely related to suggested vascular dysfunction in prediabetes. An important healthcare goal is to determine effective treatments to offset the increased fatigability in people with prediabetes to improve daily function. The purpose of this dissertation was to determine (1) vascular contributions to the increased fatigability in people with prediabetes and (2) whether dietary nitrate supplementation is an effective treatment to improve lower limb fatigability.

Study 1 investigated resting vascular function and exercise-induced blood flow and muscle oxygenation responses during dynamic, fatiguing knee-extension exercise in people with prediabetes compared with age-, body mass index-, and physical activity-matched controls. Males and females with prediabetes had an attenuated exercise-induced blood flow compared with controls; however, fatigability was not different between groups. Resting vascular function and muscle oxygenation responses during exercise did not differ between people with prediabetes and controls. Fatigability (reduction in power) was associated with reductions in electrically evoked muscle contractile properties, exercise-induced blood flow, and deoxygenated myo/hemoglobin responses during exercise, but not reductions in voluntary activation. Thus, the mechanisms of fatigability in both groups were muscular in origin with contributions from muscle oxygen delivery and extraction.

Study 2 determined the effect of dietary nitrate supplementation (via beetroot juice) on fatigability in males and females with prediabetes. Nitrate supplementation had no effect on fatigability and measures of resting vascular function, exercise-induced blood flow, and muscle oxygenation. These results suggest dietary nitrate supplementation is not an effective strategy to improve fatigability in people with prediabetes. Collectively, lower limb fatigability in people with prediabetes and controls was related to contractile mechanisms involving lower exercise-induced blood flow and oxygen extraction. Dietary nitrate supplementation was not effective in improving fatigability indicating other interventions targeting blood flow and oxygen extraction may be more effective.
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Blaine Arney. B.S., M.S.

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CHAPTER 1: INTRODUCTION AND REVIEW OF THE LITERATURE

Prediabetes is characterized as hyperglycemia below the clinical cut off for Type 2 Diabetes (T2D) (ElSayed et al., 2023a). It is estimated that over 95 million (1 in 3) American adults and 720 million individuals worldwide have prediabetes (Centers for Disease Control and Prevention, 2022; Sun et al., 2022). Prediabetes is indicated as a primary risk factor for developing T2D (Brannick & Dagogo-Jack, 2018; Echouffo-Tcheugui et al., 2023; ElSayed et al., 2023a). Indeed, if not treated, it is estimated that ~30% of individuals with prediabetes will progress to T2D in three years (Knowler et al., 2002). Additionally, a more recent Cochrane systematic review estimated 12-year incidence rates of T2D in individuals with prediabetes to be 31-70% depending on the presence of 1 or more clinical criteria for prediabetes (Richter et al., 2018). Prediabetes furthermore independently increases a person’s risk for cardiovascular events, coronary heart disease, stroke, and all-cause mortality (Cai et al., 2020; Echouffo-Tcheugui et al., 2023; Huang et al., 2016). Because T2D is among the top 10 causes of mortality in the U.S. with prevalence rates rising (Cho et al., 2018), and cardiovascular disease (CVD) is the leading cause of mortality in the U.S. (Ahmad & Anderson, 2021), it is imperative to target people with prediabetes with early intervention to restore normal glucose regulation and prevent further disease progression.

Lifestyle intervention, particularly increased physical activity, is the frontline treatment for prediabetes due to the acute and chronic effects of physical activity on glucose regulation (Echouffo-Tcheugui et al., 2023; ElSayed et al., 2023b; LaMonte et al., 2005). However, recent data suggests individuals with prediabetes have an increased performance fatigability of limb muscles (i.e., acute reduction in muscle power and
strength in response to exercise) (Senefeld, Harmer, et al., 2020). Fatigability of limb muscles can impair physical function (Shang et al., 2021) and decrease exercise tolerance, leading to reduced physical activity (Taylor et al., 2010; Xu et al., 2018; Yen & Li, 2021), decreased ability to perform activities of daily living (Tapp et al., 2006), and overall decreased quality of life in people with prediabetes (Tapp et al., 2006; Taylor et al., 2010; Xu et al., 2018). Despite the significant implications of an increased performance fatigability, the mechanisms of fatigability in people with prediabetes are still not known. It is important to determine the mechanisms leading to the demonstrated increased performance fatigability in people with prediabetes to aid in identifying appropriate treatment strategies and interventions for the prevention of T2D and CVD. To fill this knowledge gap, a major goal of this dissertation is to investigate performance fatigability and its mechanisms in the lower limb muscles in people with prediabetes, targeting the vascular contributions and oxygen delivery during dynamic fatiguing exercise.

A promising, cost-effective intervention for the treatment of increased performance fatigability in people with prediabetes is dietary nitrate (NO\textsuperscript{3−}) supplementation. Indeed, increasing nitric oxide (NO) bioavailability, a critical signaling molecule for numerous physiological functions (e.g., vasodilation), through NO\textsuperscript{3−} supplementation has led to increased exercise performance in both healthy and diseased populations (Jones et al., 2021; Woessner et al., 2018). For this reason, an additional goal of this dissertation is to explore the potential beneficial effects of dietary nitrate supplementation on performance fatigability in people with prediabetes. The following chapter provides an in-depth review of the etiology and disease progression of
prediabetes, treatment of prediabetes, performance fatigability in people with prediabetes and the potential mechanisms for the greater fatigability of limb muscles compared to healthy controls. Last, the review explores the potential beneficial effects of dietary NO3− supplementation on performance fatigability in people with prediabetes. A table of acronyms used in this dissertation is provided here for reference (Table 1.1).
<table>
<thead>
<tr>
<th>Terms</th>
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<td>Adenosine Triphosphate</td>
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<td>Homeostatic Model of Insulin Resistance</td>
<td>HOMA-IR</td>
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<td>Hydrogen</td>
<td>H^{+}</td>
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<tr>
<td>Impaired Glucose Tolerance</td>
<td>IGT</td>
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<tr>
<td>Inorganic Phosphate</td>
<td>P_{i}</td>
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<td>Maximal Oxygen Consumption</td>
<td>VO_{2}\text{max}</td>
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<td>Maximal Voluntary Concentric Contraction</td>
<td>MVCC</td>
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<td>Maximal Voluntary Isometric Contraction</td>
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<td>Near-Infrared Spectroscopy</td>
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<td>Nitrate</td>
<td>NO_{\text{3}}</td>
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<td>Nitrite</td>
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<td>Peak Post-Occlusive Reactive Hyperemia</td>
<td>RH_{\text{peak}}</td>
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<td>Phosphorus-31 Magnetic Resonance Spectroscopy</td>
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<td>Potentiated Twitch Torque Amplitude</td>
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<td>Tissue Oxygen Saturation</td>
<td>StO_{2}</td>
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<td>Total[heme]</td>
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<tr>
<td>Type 2 Diabetes</td>
<td>T2D</td>
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Table 1.1. List of acronyms used in this dissertation.
1.1 Etiology and Disease Progression of Prediabetes

People with prediabetes have elevated blood glucose levels, although below that of people with T2D. Clinical ranges for prediabetes were established by the American Diabetes Association (ElSayed et al., 2023a) and include one or more of the following criteria: fasting plasma glucose (FPG) of 100 mg/dL to 125 mg/dL, also termed impaired fasting glucose (IFG); 2-hour plasma glucose value from 75 g oral glucose tolerance test of 140 mg/dL to 199 mg/dL, also termed impaired glucose tolerance (IGT); glycated hemoglobin (HbA1c) of 5.7% to 6.4%. However, it should be noted that there is not consensus on the clinical ranges for prediabetes with the World Health Organization criteria for FPG being 110 mg/dL to 125 mg/dL and International Expert Committee criteria for HbA1c being 6.0-6.4% (Echouffo-Tcheugui et al., 2023; International Expert Committee, 2009). Each criteria is thought to reflect varying sites of glucose regulation within the body. For example, IFG is understood to represent impaired hepatic glucose regulation, mainly as a result of hepatic insulin resistance and increased endogenous glucose production, whereas IGT is thought to represent skeletal muscle insulin resistance resulting in impaired glucose uptake (Echouffo-Tcheugui et al., 2023). Glycated hemoglobin represents a 2–3-month average of blood glucose levels and is thought to reflect a combination of hepatic and skeletal muscle insulin resistance.

The development of prediabetes and T2D is secondary to the onset and progression of insulin resistance (i.e., decrease cellular sensitivity to insulin) and beta cell dysfunction (i.e., insulin production) and loss (Echouffo-Tcheugui et al., 2023; Petersen & Shulman, 2018; Tabak et al., 2012). In healthy adults, the endocrine hormone insulin is
released by beta cells in the pancreas in states of increased glucose availability. Insulin then binds to insulin receptors on the plasma membranes of various target cells (e.g., myocytes, adipocytes, and hepatocytes) in order to maintain glucose homeostasis through various cell specific mechanisms (Petersen & Shulman, 2018). For example, the binding of insulin to receptors on myocytes leads to the indirect cellular uptake of glucose through translocation of the glucose transporter GLUT4 to the plasma membrane. Glucose is then taken into the myocyte where it is either converted and stored as glycogen or utilized for intracellular metabolism. Insulin resistance is thought to develop as a result of both genetic and environmental (i.e., poor dietary habits and physical inactivity) factors, with increased adiposity and lipotoxicity being a key contributor (Petersen & Shulman, 2018; Roden & Shulman, 2019). The development of insulin resistance results from decreased cellular membrane content of insulin receptors in various cell types such as skeletal muscle and/or cellular insulin signaling defects (Petersen & Shulman, 2018). Without proper intervention (e.g., lifestyle modification) following the onset of insulin resistance, insulin resistance can worsen, and glucose levels can rise to hyperglycemic levels (Tabak et al., 2012).

A multistage model of T2D development was proposed and describes the development of T2D in five stages (Tabak et al., 2012; Weir & Bonner-Weir, 2004). In brief, stage 1 (Compensation) is characterized by insulin resistance leading to compensatory increases in insulin secretion and maintained normoglycemia. Stage 2 (Stable Adaptation) occurs when beta cell function, and consequently insulin secretion, is no longer able to fully match the insulin resistance and plasma glucose levels gradually begin to rise. This stage can last for several years with an individual beginning stage 2 in
a normoglycemic condition, but eventually progressing to a prediabetic state before the conclusion of the stage. In stage 3 (Unstable Early Decompensation), beta cells are unable to compensate for insulin resistance, a result of further beta cell dysfunction and loss of beta cell volume, leading to a rapid rise in plasma glucose and the onset of T2D. This rapid rise in glucose will eventually plateau leading to stage 4 (Stable Decompensation) where beta cells are able to secrete enough insulin to maintain stable hyperglycemic values, but beta cell volume can continue to decrease until insulin production is no longer maintained and supplemental insulin is required in the 5th stage (Severe Decompensation) (Tabak et al., 2012; Weir & Bonner-Weir, 2004).

Early intervention for individuals with prediabetes is crucial in order to prevent marked beta cell dysfunction and loss that is hallmark in the development of T2D. Remission of prediabetes (return to normoglycemia without reliance of medication) is attainable and commonly reported with lifestyle intervention (Galaviz et al., 2022; Knowler et al., 2002). However, returning to normoglycemia in people with T2D is much more difficult with complete remission resulting from intensive lifestyle intervention only occurring in ~1% of individuals (Gregg et al., 2012).

1.2 Treatment of Prediabetes

Frontline treatment for prediabetes, with the goal of lowering plasma glucose and preventing T2D, is lifestyle modification in the form of increased physical activity and dietary intervention (Echouffo-Tcheugui et al., 2023). Physical activity is thought to improve glucose management through both acute responses and chronic adaptions (ElSayed et al., 2023b; Kanaley et al., 2022; LaMonte et al., 2005). Acute responses
include insulin-independent skeletal muscle glucose uptake during exercise through exercise-induced GLUT4 translocation (Flores-Opazo et al., 2020) and enhanced post-exercise insulin sensitivity (Bajpeyi et al., 2009; Larsen et al., 2020; Voldstedlund et al., 2024). Chronic adaptations of aerobic exercise training include improved beta cell function and mass (Curran et al., 2020; Malin et al., 2018; Malin et al., 2013), increased GLUT4 content (Flores-Opazo et al., 2020), improved vascular function and capillarization (LaMonte et al., 2005; Magalhaes et al., 2019; Naylor et al., 2016), and improved mitochondrial function (Meex et al., 2010). With the acute responses being transient in nature (lasting from 2 to 72 hours) and chronic adaptations elicited through regular physical activity, the current physical activity recommendations include 3 or more days of moderate to vigorous aerobic activity, with no more than 2 consecutive days between bouts and a goal of at least 150 minutes per week of moderate intensity activity (Kanaley et al., 2022). The addition of 2 to 3 days of resistance training is also advised due to the glucose lowering effects of resistance training alone (Kobayashi et al., 2023; Liu et al., 2019) and the additive effect of adaptations to both aerobic and resistance training (Church et al., 2010; Kanaley et al., 2022; Sigal et al., 2007).

Numerous trials have documented the efficacy of lifestyle modification of increased physical activity alone, as well as the combination of physical activity and improved diet (Jastreboff et al., 2022; Knowler et al., 2002; Kosaka et al., 2005; Pan et al., 1997; Ramachandran et al., 2006; Tuomilehto et al., 2001). To highlight the efficacy of combined treatments, the U.S. Diabetes Prevention Program (Knowler et al., 2002) included 3234 individuals with prediabetes that were randomized to one of three interventional groups, placebo, metformin, or intensive lifestyle. Metformin is a
commonly prescribed medication for glucose management and is thought to reduce glucose levels by lowering hepatic glucose production and reducing intestinal glucose absorption (Rena et al., 2017). The intensive lifestyle group participated in a 16-session course designed to educate participants on diet, exercise, and behavior modification. The goal of this instruction was to achieve weight loss of at least 7% of body weight by improving diet and performing 150 minutes per week of moderate intensity physical activity. At the conclusion of the follow-up period, an average of 2.8 years, the relative reduction in incidence rates for developing T2D compared to the placebo group was 58% in the lifestyle group (i.e., exercise and diet) and 31% in the metformin group (Knowler et al., 2002). Furthermore, remission of prediabetes occurred in 40% of the lifestyle group and 20% in the metformin group (Knowler et al., 2002). A similar study had previously identified that physical activity alone resulted in a relative risk reduction for T2D of 46% (Pan et al., 1997). These results highlight the importance of lifestyle modification in the treatment of individuals with prediabetes.

Although lifestyle modification is the frontline treatment for prediabetes, pharmaceutical therapy is advised in conjunction with lifestyle intervention in individuals with an increased number of T2D risk factors and poorer glucose management (Echouffo-Tcheugui et al., 2023; ElSayed et al., 2023b). Figure 1.1 provides current recommendations for the treatment of prediabetes (Echouffo-Tcheugui et al., 2023). Of the available medications for glucose management, metformin is most commonly prescribed (Echouffo-Tcheugui et al., 2023). Clinical trials have identified lower incidence rates of T2D in individuals with prediabetes when treated with metformin alone compared to a placebo (Knowler et al., 2002; Ramachandran et al., 2006). Although
effective, the overall incidence reduction of T2D and long-term beneficial effects of metformin seem to be suboptimal compared to lifestyle modification, hence the current recommendations of combined lifestyle and pharmaceutical therapy if warranted. However, it is worth noting that previous studies suggest metformin therapy in conjunction with exercise may attenuate the cardiorespiratory and insulin sensitizing benefits of exercise (Boule et al., 2011; Konopka et al., 2019; Malin et al., 2012). These data further support the independent role of exercise therapy for improvements in glucose management and overall health.

**Figure 1.1. Current recommendations for the treatment of prediabetes.** The figure was reprinted from (Echouffo-Tcheugui et al., 2023).

1.3 Performance Fatigability, Neuromuscular Function, and Vascular Function in Individuals with Prediabetes

*Performance Fatigability and Neuromuscular Function in Individuals with Prediabetes*

Increased physical activity is an essential treatment for those with prediabetes; however, it appears that people with prediabetes can have neuromuscular impairments
leading to decrements in physical function (Lee et al., 2013; Shang et al., 2021) and increased performance fatigability (Senefeld, Harmer, et al., 2020). Such decrements could result in decreased physical activity levels and poor exercise adherence in people with prediabetes (Taylor et al., 2010; Yen & Li, 2021). In a longitudinal study by Shang et al. (2021), physical function (i.e., Five Time Sit-to-Stand Test and walking speed) was assessed in 2,013 older individuals with normoglycemia, prediabetes, and T2D at baseline and every 3 to 6 years for 12 years. Prediabetes was associated with reduced physical function at baseline and that the rate of decline in physical function was greater in people with prediabetes compared to normoglycemic adults (Shang et al., 2021). However, it is important to note that not all studies confirm a reduced physical function in people with prediabetes (Godino et al., 2017) suggesting that physical function can be variable between people with prediabetes.

Additionally, individuals with prediabetes have been shown to have an increased performance fatigability (Senefeld, Harmer, et al., 2020) which could lead to increased exercise intolerance and impair physical function in people with prediabetes. In this context performance fatigability was quantified as an exercise-induced reduction in power of the lower limb muscles. However, the concept and taxonomy of fatigue is multifaceted and fatigue is thought to exist in two distinct domains, perceived fatigue and performance fatigability (Kluger et al., 2013).

Perceived fatigue is defined as “subjective sensations of weariness, increasing sense of effort, mismatch between effort expended and actual performance, or exhaustion” (Kluger et al., 2013). Perceived fatigue is a common symptom in multiple neurological conditions such as Parkinson’s disease, multiple sclerosis, and stroke.
(Kluger et al., 2013), but also can present as a symptom for other diseases such as T2D (Kalra & Sahay, 2018) and multiple forms of cancer (Ma et al., 2020). Perceived fatigue is debilitating for these populations and its mechanisms and treatments have received considerable attention. However, recent studies investigating the relationship between perceived fatigue and performance fatigability in T2D, and prediabetes have suggested no relationship between the two domains for these populations (Senefeld, Harmer, et al., 2020). For this reason and with the mechanisms and treatments of performance fatigability in people with prediabetes being a central aim of this dissertation, the remainder of this review will focus on performance fatigability.

Performance fatigability, also known as fatigability or muscle fatigue, is broadly defined as “the decline in an objective measure of performance over a discrete period” (Enoka & Duchateau, 2016). The measure of performance could include, but is not limited to, speed during walking, power output during cycling, or force production during isolated, single limb exercise. A reduction in any of these measures of performance would be an indication of performance fatigability. Single limb exercise is often used in lab-based research to study the mechanisms of performance fatigability due to the ability to investigate physiological responses to exercise in task specific, isolated muscles. When considering these types of fatiguing tasks, performance fatigability can be more narrowly defined as an acute, exercise-induced reduction in muscle force or power (Gandevia, 2001; Hunter, 2018).

The mechanisms of performance fatigability are multifactorial and there are numerous sites within the neuromuscular system in which fatigue of the muscle may originate. These mechanisms can be divided into neural mechanisms (processes proximal
to the neuromuscular junction that lead to suboptimal activation of the muscle) and muscular mechanisms (processes at or distal to the neuromuscular junction that attenuate contractile function of the muscle) (Figure 1.2) (Enoka & Duchateau, 2016; Gandevia, 2001; Hunter, 2018). Both neural and muscular mechanisms ultimately lead to a reduced motor performance at the muscle, the site at which force output occurs.

**Figure 1.2. Potential sites within the neuromuscular system in which performance fatigability can originate.** The figure was reprinted from Hunter (2018). Volitional contraction of skeletal muscle is foremost dictated by the degree of activation from the motor cortex. Activation from the motor cortex leads to descending drive through the corticospinal tract to the ventral horn of the spinal cord where motoneurons are activated which innervate skeletal muscle. Any decrements in activation along this neural pathway during fatiguing contractions (e.g., decreased cortical excitability) will manifest in performance fatigability and are termed neural mechanisms. Action potentials propagate
from the motoneuron to the muscle across the neuromuscular junction depolarizing t-tubules and leading to the release of calcium (Ca\(^{2+}\)) and resultant muscle contraction. Decrements as a result of fatiguing contractions at any sites contributing to these processes (e.g., neuromuscular junction instability, attenuated blood flow, altered muscular metabolism) will result in performance fatigability and are termed muscular mechanism. Lastly, complex interactions between neural and muscular mechanisms exist through inhibitory feedback originating from group III (mechanosensitive) and IV (metabosensitive) afferents which can modulate motoneuron activation.

Performance fatigability and its mechanisms are largely dictated by the demands of the fatiguing task (e.g., contraction type and duty cycle), the exercising muscle group, and the population conducting the fatiguing task (e.g., older adults and clinical populations) (Hunter, 2018). The interplay of these factors is demonstrated when considering age-related changes in fatigability. When conducting high-velocity, dynamic contractions, studies have shown that older adults are more fatigable than young adults (Dalton et al., 2010; McNeil & Rice, 2007; Paris et al., 2022; Sundberg, Kuplic, et al., 2018), although this age-related fatigability is less pronounced in the upper limb (Senefeld et al., 2017). In contrast, when conducting isometric or slow-velocity, dynamic contractions, older adults are more fatigue resistant compared with young adults (Callahan et al., 2009; Christie et al., 2011; Kent-Braun, 2009; Yoon et al., 2013). These variations in age-related fatigability are important when considering population specific decrements in motor performance and effective, individualized rehabilitation programs.

Very few studies have been conducted to understand the magnitude and mechanisms of fatigability in individuals with prediabetes (Senefeld, Harmer, et al., 2020; Senefeld, Singh-Peters, et al., 2020): of these studies, only one has compared people with prediabetes to healthy controls (Senefeld, Harmer, et al., 2020). Senefeld et al. (2020) conducted a study in which healthy controls, individuals with prediabetes, and
individuals with T2D performed a 6-minute (1 contraction every 3 seconds), high-velocity, dynamic fatiguing task of the knee extensor muscles lifting a load equivalent to 20% of maximal voluntary contraction (MVC) torque. Importantly, participants were matched for age, sex, body mass index (BMI), and physical activity levels to control for these potentially confounding factors. Fatigability (quantified as the percent reduction in limb power) was greater in people with prediabetes than healthy controls (31.8% vs. 22.1% power reduction) and people with T2D had greater fatigability than both people with prediabetes and healthy controls (44.8% vs. 31.8% vs. 22.1% power reductions). Furthermore, using electrical stimulation of the quadriceps muscles, this study investigated the potential neuromuscular mechanisms of the greater fatigability in people with prediabetes. The reduction in the ability to voluntarily activate the quadriceps muscles following the fatiguing task did not differ between groups. However, individuals with prediabetes had a greater reduction in the electrically evoked potentiated twitch torque amplitude of the quadriceps muscles compared with the healthy controls (32.5% vs. 21.3% reduction). This reduction in twitch torque was associated with the magnitude of fatigability suggesting muscular mechanisms were the primary factor contributing to the greater fatigability identified in people with prediabetes.

One of the potential leading factors contributing to the greater fatigability and attenuated muscle contractile function following fatiguing exercise in people with prediabetes is the greater accumulation of the fatigue-inducing metabolites hydrogen (H⁺) and inorganic phosphate (Pi). Studies have identified strong relationships between H⁺ and Pi accumulation during exercise, as measured by phosphorus-31 magnetic resonance spectroscopy (31P-MRS), and fatigability and impaired contractile function in healthy and
aged people (Broxterman et al., 2017; Hureau et al., 2022; Lanza et al., 2007; Sundberg et al., 2019; Wilson et al., 1988). Furthermore, studies investigating contractile properties of isolated, human single muscle fibers have shown similar results with impaired force and power production being induced by exposure to elevated levels of H\(^+\) and P\(_i\) (similar to in-vivo H\(^+\) and P\(_i\) levels during fatiguing exercise) (Sundberg & Fitts, 2019; Sundberg, Hunter, et al., 2018). Briefly, both elevated H\(^+\) and P\(_i\) are thought to decrease myofilament Ca\(^{2+}\) sensitivity (Debold et al., 2016; Sundberg & Fitts, 2019). Elevated H\(^+\) is thought to reduce the force per cross-bridge of myosin and actin through an impaired low- to high-force transition during the cross-bridge cycle and increasing myosin and actin binding times in which slows cross-bridge shorten velocity (Debold et al., 2016; Sundberg & Fitts, 2019). Lastly, P\(_i\) is postulated to decrease Ca\(^{2+}\) release from the sarcoplasmic reticulum and cause an early dissociation of myosin from actin during the cross-bridge cycle, both leading to reduced force production (Debold et al., 2016; Sundberg & Fitts, 2019).

With a greater accumulation of H\(^+\) and P\(_i\) likely contributing to the greater magnitude fatigability in people with prediabetes, the mechanisms leading to the greater metabolite accumulation are of interest. Possible mechanisms include vascular dysfunction leading to an attenuated exercise limb blood flow and muscle perfusion (Bock et al., 2020; Groen et al., 2019; Kingwell et al., 2003; Novielli & Jackson, 2014; Senefeld et al., 2019; Wasserman et al., 2018), reduced mitochondrial function (Meex et al., 2010; Mogensen et al., 2007; Petersen et al., 2004; Schrauwen-Hinderling et al., 2007; Turner et al., 2022) and/or reduced skeletal muscle contractile efficiency (ATP utilized for a given amount of power) (Lewis et al., 2019), with evidence for each
mechanism primarily derived from studies in individuals with T2D. Of these mechanisms, a leading candidate is an attenuated exercise-induced limb blood flow and muscle perfusion due to the well-documented macro- and microvascular dysfunction in prediabetes (Caballero et al., 1999; Sorensen et al., 2016; Su et al., 2008; Vehkavaara et al., 1999; Wasserman et al., 2018). The ability of the cardiovascular system to provide adequate blood flow and oxygen supply to the working muscle during exercise is a key determinant of fatigability (Amann, Eldridge, et al., 2006; Amann, Romer, et al., 2006; Broxterman et al., 2015; Hammer et al., 2020; Millet et al., 2009; Poitras et al., 2018). A mismatch between oxygen supply and the metabolic demand of the working muscle induces a greater reliance on anaerobic energy production during exercise and the accumulation of the fatigue-inducing metabolites H+ and Pi and thus a greater fatigability (Hepple, 2002; Hogan et al., 1999; Sundberg & Fitts, 2019). Thus, it is possible that inadequate blood supply or muscle perfusion is a key mechanism for the greater fatigability of limb muscles in people with prediabetes.

**Vascular Function in Prediabetes**

Blunted vascular responses to various vasodilatory stimuli (i.e., vascular dysfunction) along the vascular tree have been reported in individuals with prediabetes. Using intra-arterial infusions of endothelium-dependent (acetylcholine [ACh]) and - independent (sodium nitroprusside [SNP]) vasodilators, Vehkavaara et al. (1999) identified an attenuated forearm blood flow response to ACh in individuals with IFG compared to healthy controls, but not SNP suggesting impaired microvascular endothelial function in people with prediabetes. With ACh-induced vasodilation being an endothelial, NO-dependent function (Furchgott & Zawadzki, 1980; Limberg et al., 2020), this result
suggests an impairment in the balance of NO synthesis and degradation (i.e., decreased NO bioavailability) in the vasculature of people with prediabetes. In short, NO is a potent vasodilator that is synthesized by endothelial NO synthase (eNOS) through the oxidation of the amino acid L-arginine to form L-citrulline and NO (Lundberg & Weitzberg, 2022). Nitric oxide then diffuses into vascular smooth muscle cells where it activates soluble guanylate cyclase which in turn produces guanosine 3′,5′-cyclic monophosphate and leads to subsequent lowering of calcium concentrations in the cell, and thus smooth muscle relaxation (Lundberg & Weitzberg, 2022; Rush et al., 2005). The finding of impaired microvascular endothelial function and attenuated NO bioavailability in people with prediabetes is further supported by the attenuated heat- and ACh-mediated skin blood flow and impaired retinal artery vasodilation identified in people with prediabetes compared with healthy controls (Caballero et al., 1999; Jaap et al., 1994; Jaap et al., 1997; Sorensen et al., 2016). Additionally, an attenuated flow-mediated dilation (FMD) [an NO-dependent measure of macrovascular endothelial function (Thijssen et al., 2011)] of the brachial artery following a 5-minute bout of arterial occlusion has been identified in individuals with prediabetes compared with controls indicating macrovascular endothelial dysfunction and further suggesting impaired NO bioavailability in people with prediabetes (Caballero et al., 1999; Su et al., 2008).

The suggested mechanisms for the identified endothelial dysfunction in prediabetes include hyperglycemia and the formation of advanced glycated end products, vascular insulin resistance, and potential mitochondrial dysfunction, all leading to an increased oxidative stress and systemic inflammation (Wasserman et al., 2018; Widlansky & Hill, 2018). Reactive oxygen species are known to scavenge available NO which
reduces NO bioavailability and leads to impaired endothelial function (Goodwill & Frisbee, 2012; Rush et al., 2005; Taddei et al., 2001; Thijssen et al., 2011). Specifically, elevated levels of superoxide can rapidly react with NO to form peroxynitrite rendering NO unusable (Goodwill & Frisbee, 2012; Rush et al., 2005). Hence, increased oxidative stress as a result of metabolic dysfunction can decrease bioavailable NO and could lead to attenuated exercise vascular responses (Joyner & Casey, 2015). However, this is yet to be investigated in people with prediabetes.

A healthy vasculature is important for optimal vascular responses during exercise (Dorff et al., 2023; Hanson et al., 2020; Joyner & Casey, 2015; Kingwell et al., 2003). Hence, it is possible that vascular dysfunction impairs exercise-induced blood flow in individuals with prediabetes leading to a greater fatigability. To date, no studies have investigated the exercise hyperemic response in humans with prediabetes. However, impaired exercise hyperemia and vascular responses to exercise-like conditions in prediabetic animal models suggest impaired vascular responses to exercise may occur in humans with prediabetes. In a study conducted by Novielli and Jackson (2014), a mouse model of prediabetes exhibited up to an ~80% blunted vasodilatory and blood flow response in the arterioles of the gluteus maximus compared with control mice during electrically-induced tetanic and rhythmic contractions. A follow up study by the same group repeated these findings and linked the attenuated vasodilatory and blood flow responses in the prediabetes group to elevated sympathetic nervous system activity which is thought to impair functional sympatholysis (Novielli-Kuntz et al., 2018). Additionally, Lesniewski et al. (2008) identified elevated vasoconstrictor responses in the vasculature of a prediabetic rodent model that was associated with decreased NO bioavailability.
which suggests an impaired functional sympatholysis could also be a result of attenuated NO bioavailability. In alignment with these findings, a recent study identified a blunted functional sympatholysis in humans with T2D which was linked to an attenuate exercise-induced blood flow response compared with healthy controls (Bock et al., 2020). Hence, if present in humans with prediabetes, an attenuated functional sympatholysis could impair exercise hyperemic responses in prediabetes.

Research in a prediabetic rat model has further identified a reduced adenosine triphosphate (ATP) release from red blood cells in low $P_{O2}$ conditions, such as during exercise (Ellis et al., 2010). This finding was proposed to contribute to a decreased vascular response and oxygen supply to exercising skeletal muscle which has additionally been shown in humans with T2D (Groen et al., 2019). In short, hemoglobin oxygen desaturation during exercise leads to the release of ATP from red blood cells triggering increased NO formation (Mortensen et al., 2009) and vasodilation from activation of inward rectifying potassium channels in vascular cells (i.e., endothelial and smooth muscle cells) which both contribute to the matching of oxygen supply to demand (Crecelius et al., 2015; Joyner & Casey, 2015). However, higher glycated hemoglobin and insulin are thought to impair the release of ATP from red blood cells which could contribute to an attenuated blood flow response in people with prediabetes.

Evidence of a blunted vascular response to exercise has been shown in individuals with T2D through an attenuated exercise-induced blood flow and slowed microvascular blood flow kinetics (Bauer et al., 2007; Bock et al., 2020; Groen et al., 2019; Kingwell et al., 2003; Senefeld et al., 2019). Kingwell et al. (2003) first identified an attenuated exercise-induced blood flow during cycling exercise in individuals with T2D compared to
age-, sex-, weight-, and cardiorespiratory endurance-matched healthy controls. Furthermore, this study identified a relationship between impaired ACh-induced vasodilation and the attenuated exercise-induced blood flow suggesting the role of impaired endothelial function and NO bioavailability as the mediator of the attenuated exercise-induced blood flow in people with T2D (Kingwell et al., 2003). Indeed, in a similar study in individuals with T2D who had preserved endothelial function, the exercise-induced blood flow was not attenuated compared with controls (Thaning et al., 2011). These findings support the well understood role of the vascular endothelium as an important regulator of the exercise hyperemic response, although a number of redundant mechanisms of exercise hyperemia exist (Joyner & Casey, 2015). Thus, dysfunctional vascular endothelium and attenuated NO bioavailability in people with prediabetes could contribute to an attenuated blood flow during exercise.

In a study employing near-infrared spectroscopy to measure deoxyhemoglobin and deoxymyoglobin (deoxy[heme]) responses at the onset of moderate intensity, constant load cycling, Bauer et al. (2007) identified a deoxy[heme] overshoot and slowed whole body oxygen consumption (VO₂) kinetics in people with T2D compared to age- and BMI-matched healthy controls. These findings indicate a delayed vascular response at the onset of exercise in people with T2D. Higher physical activity levels present in the control group compared with the T2D group, although marginal, may explain the differences between groups. Further support lies in T2D rat models showing delayed vascular responses as seen in altered P₀₂ responses (i.e., fast P₀₂ kinetics and a P₀₂ undershoot) during electrically stimulated exercise (Behnke et al., 2002; Padilla et al., 2007). The delayed microvascular blood flow could lead to an increased VO₂ deficit at
the onset of exercise and a greater reliance on anaerobic metabolism and accumulation of H\(^+\) and P\(_i\) (Poole & Jones, 2012). It is important to note that a similar study conducted by Wilkerson et al. (2011) did not replicate the slowed VO\(_2\) kinetics and deoxy[heme] overshoot in an older cohort of individuals with T2D. However, impaired exercise blood flow at the onset of exercise was still indicated by an elevated \(\Delta\text{deoxy[heme]}/\Delta\text{VO}_2\) during the rest to exercise transition. In other words, deoxy[heme] increased more so at a given VO\(_2\) suggesting a greater degree of oxygen extraction was needed to compensate for the attenuated blood flow at a given VO\(_2\) (Wilkerson et al., 2011).

Most recently, our lab conducted the first study linking lower exercise hyperemia to greater fatigability in people with T2D (Senefeld et al., 2019). Senefeld et al. (2019) measured exercise-induced blood flow in response to dynamic, knee-extension exercise in individuals with T2D compared to age-, sex-, BMI-, and physical activity-matched healthy controls. Individuals with T2D were found to have an attenuated exercise-induced blood flow compared to controls. Importantly, the attenuated exercise-hyperemic response was associated with a greater lower limb fatigability (reduction in power) supporting a mechanistic link between attenuated exercise-induced blood flow and fatigability in people with T2D (Senefeld et al., 2019).

When considering the presence of endothelial dysfunction and attenuated NO bioavailability in people with prediabetes and impaired exercise-induced blood flow in prediabetic animal models and humans with T2D, it is reasonable to believe that the greater fatigability seen in individuals with prediabetes could be a consequence of impaired exercise-induced blood flow. However, to our knowledge, this mechanism for
the greater fatigability in people with prediabetes is still yet to be studied presenting a crucial knowledge gap.

1.4 Therapeutic Potential of Dietary Nitrate Supplementation for Fatigability in Individuals with Prediabetes

Increasing NO bioavailability in people with prediabetes is a plausible and attractive therapeutic target to improve fatigability because of the important physiological functions of NO that influence exercise tolerance and human performance (Jones et al., 2021). These functions not only include vasodilation and tissue perfusion as described above (Cosby et al., 2003; Joyner & Casey, 2015; Maiorana et al., 2003), but also mitochondrial respiration (Brown & Cooper, 1994; Poderoso et al., 2019) and skeletal muscle contractile function and efficiency (Bailey et al., 2010; Coggan & Peterson, 2018; Marechal & Gailly, 1999). A decreased NO bioavailability could impair each of these key functions and ultimately lead to greater fatigability. Thus, treatments to increase NO bioavailability are of particular interest for improving exercise performance.

It was previously believed that NO was exclusively produced by the vascular endothelium in the presence of oxygen through the oxidation of the amino acid L-arginine to produce NO and L-citrulline by eNOS (Ahluwalia et al., 2016). Excess NO is then rapidly oxidized to nitrite (NO$_2^-$) and NO$_3^-$ (Piknova et al., 2022). These metabolites were previously thought to be end products of the NO-synthase pathway that were later excreted by the kidneys. However, additional studies identified that during hypoxic conditions, NO$_2^-$ is reduced back to NO for physiological processes creating an alternative to the NO-synthase pathway (Lundberg et al., 2008). This NO$_3^-$-NO$_2^-$-NO pathway is now considered to be complementary to the oxygen-dependent NO-synthase
pathway, acting as an “NO reservoir” for the maintenance of NO bioavailability in hypoxic conditions such as exercise (Lundberg et al., 2008; Piknova et al., 2022; Woessner et al., 2018).

Since the discovery of the NO\textsuperscript{3-} - NO\textsuperscript{2-} - NO pathway, researchers have become interested in increasing NO bioavailability through the use of supplemental NO\textsuperscript{2-} and NO\textsuperscript{3-} (Lundberg et al., 2008). After ingestion, NO\textsuperscript{3-} is rapidly absorbed in the small intestine. After entering the bloodstream, plasma NO\textsuperscript{3-} is absorbed by the salivary glands and later reduced to NO\textsuperscript{2-} by anaerobic oral bacteria located on the dorsal surface of the tongue (Govoni et al., 2008; Jones et al., 2021; Tannenbaum et al., 1976). Salivary NO\textsuperscript{2-} is then swallowed and reduced to NO in the acidic environment of the stomach (McKnight et al., 1997) or enters the circulation. The remaining plasma NO\textsuperscript{2-} and NO\textsuperscript{3-} remains in the circulation, is stored in tissues such as the skeletal muscle and liver, or is excreted by the kidney (Kadach et al., 2023; Park et al., 2023; Piknova et al., 2022). Nitrite can later be reduced to NO in hypoxic and low pH conditions, such as the skeletal muscle during exercise, by reductases such as xanthine oxidase, proteins such as deoxy[heme], and non-enzymatic reduction by H\textsuperscript{+} (Figure 1.3) (Piknova et al., 2022; Woessner et al., 2018).
Dietary NO\textsubscript{3}\textsuperscript{-} supplementation has become a popular, natural means of increasing NO bioavailability to improve performance in athletes and diseased populations with NO deficits (e.g. CVD and T2D) (Jones et al., 2021; Woessner et al., 2018). In a comprehensive systematic review conducted by Senefeld et al. (2020), NO\textsubscript{3}\textsuperscript{-} supplementation was shown to have a \(~3\%\) performance-enhancing effect in healthy, young men. This systematic review also investigated components of NO\textsubscript{3}\textsuperscript{-} dosing and found that supplementation with a NO\textsubscript{3}\textsuperscript{-} concentration of \(\geq 5.0\) mmol for a single dose or taken daily over multiple days resulted in improvements in performance. However, it seems as though the greater the NO\textsubscript{3}\textsuperscript{-} dosing, the greater the ergogenic effect. A supplementation duration of 2-6 days was determined to be optimal for performance.
Importantly, no effect of NO₃⁻ supplementation was identified in females. This may be due to the underrepresentation of females in NO₃⁻ supplement studies leading to little available data or due to a true biological sex differences. Recent data suggests sex-differences in the ergogenic effect of NO₃⁻ supplementation in young, healthy adults may exist (Ortiz de Zevallos et al., 2023). In the only known study specifically designed to investigate the differential effect of NO₃⁻ supplementation on males and females Ortiz de Zevallos et al. (2023) identified a 15% increase in time-to-task failure in males during cycling exercise following NO₃⁻ supplementation whereas females had no improvements. Other studies have identified no ergogenic effect of NO₃⁻ supplementation in females (Lane et al., 2014; Wickham et al., 2019), although not all (Bond et al., 2014; Peeling et al., 2015). These findings show the potential role of sex on the ergogenic effect of NO₃⁻ supplementation.

The performance-enhancing effects of NO₃⁻ supplementation, and consequentially increasing NO bioavailability, are thought to be mediated by improved skeletal oxygen delivery and/or extraction (Bailey et al., 2015; Breese et al., 2017; Richards et al., 2018), mitochondrial function (Larsen et al., 2011), and skeletal muscle contractile efficiency (Bailey et al., 2010) and contractile function (Coggan, Leibowitz, Kadkhodayan, et al., 2015; Coggan, Leibowitz, Spearie, et al., 2015; Haider & Folland, 2014; Whitfield et al., 2017). The vasodilator effects of NO as well as improved functional sympatholysis have been suggested to mediate improved exercise vascular responses from NO₃⁻ supplementation (Bock, Ueda, et al., 2022; Lundberg & Weitzberg, 2022; Woessner et al., 2018). Enhanced skeletal muscle oxygen delivery and/or extraction has been observed in both healthy humans (Bailey et al., 2015; Bailey et al., 2009; Breese et al., 2017; Craig et
al., 2018; Richards et al., 2018) and rats (Ferguson et al., 2013; Ferguson et al., 2015). Ferguson et al. (2013) first identified NO₃⁻ supplementation enhanced exercise-induced hind limb blood flow, particularly in muscle groups composed of greater proportion of type II fibers, during treadmill running in healthy rats. A following study in humans identified similar improvements in forearm blood flow during higher, but not lower, intensity hand grip exercise following NO₃⁻ supplementation supporting the notion of a selective effect of NO₃⁻ supplementation on type II fiber exercise-induced blood flow responses (Richards et al., 2018). In short, the selective effect of NO₃⁻ supplementation on type II fibers may be a result of lower exercise microvascular PO₂ in muscles composed of a greater proportion of type II fibers (McDonough et al., 2005). This hypoxic environment in type II fibers would enhance NO production from the NO₃⁻-NO₂⁻-NO pathway (Piknova et al., 2022).

Although enhanced exercise-induced blood flow has been suggested in healthy individuals following NO₃⁻ supplementation, the findings have been highly variable with some studies documenting no change (Craig et al., 2018; Fenuta et al., 2024; Kim et al., 2015) and others reporting reduced exercise-induced blood flow responses (Nyberg et al., 2021; Thurston et al., 2021) following NO₃⁻ supplementation compared to placebo supplementation. A number of physiological adjustments during exercise (e.g., improved skeletal muscle contractile efficiency and diffusive oxygen delivery) and experimental considerations (e.g., exercise intensity used, fitness status of participants, NO₃⁻ supplementation dosing and duration, and exercise mode) must be taken into account when considering the inconsistent results. For example, in an eloquently designed study, Nyberg et al. (2021) identified an attenuated blood flow response to fixed load, knee
extension exercise following NO3− supplementation compared to placebo supplementation. Leg VO2, as determined through arterial and venous blood sampling, was similarly reduced; however, blood lactate levels were not altered between the placebo and NO3− conditions. These findings suggest NO3− supplementation enhanced muscle contractile efficiency which led to a concomitant reduction in leg VO2 and blood flow (Nyberg et al., 2021). Similar attenuated lower limb blood flow has been identified during cycling exercise following NO3− supplementation (Thurston et al., 2021).

However, pulmonary VO2 was unaltered suggesting an increase in diffusive oxygen delivery (i.e., enhanced oxygen extraction). Thus, the varying blood flow responses to exercise (i.e., enhanced, unaltered, or attenuated) and determinants of these blood flow responses following NO3− supplementation may be a result of studies using different modes of exercise.

Although NO3− supplementation may improve oxygen delivery and extraction during exercise, the effects of NO3− supplementation on mitochondrial function and skeletal muscle contractile efficiency have become increasingly emphasized as the key contributor to the ergogenic effect of NO3− supplementation. One of the first studies to investigate NO3− supplementation as an ergogenic aid was conducted by Larsen et al. (2007). Prior to this study, the oxygen cost of exercise was largely thought to be fixed to the absolute workload being performed. However, Larsen et al. (2007) demonstrated a reduction in the oxygen cost of submaximal exercise as a result of NO3− supplementation. A number of studies have since replicated these findings (Bailey et al., 2009; Lansley et al., 2011; Larsen et al., 2011; Nyberg et al., 2021; Pawlak-Chaouch et al., 2016; Vanhatalo et al., 2010; Whitfield et al., 2016; Wylie et al., 2016), albeit not all (Esen et
al., 2022; Thurston et al., 2021), supporting the notion that NO₃⁻ supplementation may improve mitochondrial and skeletal muscle contractile efficiency.

The first study to investigate the effect of NO₃⁻ supplementation on mitochondrial efficiency was conducted by Larson et al. (2011). In this study, participants underwent a 3-day supplementation period of sodium nitrate or a placebo (sodium chloride). Following supplementation, participants performed submaximal, constant load cycling at a workload equivalent to 50% of VO₂max. Skeletal muscle mitochondria were isolated from vastus lateralis muscle biopsies prior to exercise. The investigators found that NO₃⁻ supplementation lead to an improved mitochondrial efficiency (i.e., less oxygen consumed per ATP produced) that was associated with a reduced oxygen consumption during submaximal exercise (Larsen et al., 2011). No change was identified in the placebo condition. This improved mitochondrial efficiency was suggested to be a result of reduced proton leak that could be related to decreased expression of the protein adenine nucleotide translocase (a protein associated with mitochondrial uncoupling) (Larsen et al., 2011). Although convincing, these results have since been contradicted (Whitfield et al., 2016). Whitefield et al. (2016) similarly isolated skeletal muscle mitochondria pre and post 7-days of NO₃⁻ supplementation with beetroot juice. The investigators found mitochondrial efficiency was unaltered with NO₃⁻ supplementation, however, the oxygen cost during submaximal exercise was reduced. The conflicting results could stem from the varying NO₃⁻ supplementation (sodium nitrate vs. beetroot juice) or NO₃⁻ supplementation timeline (3 days vs. 7 days), however, this is unlikely seeing that the oxygen cost of exercise was similarly reduced by 3% in both studies.
Because the oxygen cost of exercise was reduced in the study by Whitefield et al. (2016), but mitochondrial efficiency was not improved, the most likely explanation for the reduced oxygen cost of exercise is an improvement in skeletal muscle contractile efficiency during exercise. Indeed, this hypothesis was confirmed in a study by Bailey et al. (2010) using $^{31}$P-MRS to measure in-vivo skeletal muscle metabolism during exercise. Following 6 days of NO$_3^-$ supplementation, total ATP turnover during knee-extension exercise was reduced during both low- and high-intensity exercise, primarily due to the reduction in ATP turnover as a result of phosphocreatine (PCr) hydrolysis and oxidative phosphorylation, but not glycolysis. The reduced ATP turnover from both anaerobic and aerobic energy production suggest that improvements in skeletal muscle contractile efficiency, and not solely mitochondrial efficiency, result from NO$_3^-$ supplementation. Furthermore, the researchers identified a reduced oxygen cost during exercise and improved exercise tolerance (i.e., increased time to task failure) that paralleled the improved skeletal muscle contractile efficiency following NO$_3^-$ supplementation (Bailey et al., 2010). The authors speculated that the mechanisms of an improved skeletal muscle contractile efficiency following NO$_3^-$ supplementation could be improvements in Ca$^{2+}$ handling and/or slowed cross-bridge cycling (Bailey et al., 2010). Both mechanisms could reduce ATP hydrolysis from Ca$^{2+}$-ATPase and actomyosin-ATPase. Further support for improved Ca$^{2+}$ handling has been identified in isolated mouse skeletal muscle fibers treated with NaNO$_2^-$ (Bailey et al., 2019). Altogether, these findings suggest the ATP turnover for a given power may be reduced as a result NO$_3^-$ supplementation and that this physiological adjustment can lead to an enhanced exercise performance in young, healthy men.
Considering the profound effects of NO₃⁻ supplementation in healthy, young adults with uncompromised NO bioavailability, NO₃⁻ supplementation has a profound potential to work as a therapeutic aid in populations with compromised NO bioavailability. Indeed, NO₃⁻ supplementation has been shown to increase time to claudication onset pain and peak walking time and distance in peripheral artery disease through increased vascular function and muscle oxygenation during exercise (Bock et al., 2018; Kenjale et al., 2011; Pekas et al., 2021), improve vascular and muscle contractile function in older adults (Casey et al., 2015; Coggan et al., 2020; Coggan, Leibowitz, Kadkhodayan, et al., 2015; Rammes et al., 2014; Walker et al., 2019), and increase peak oxygen consumption (Zamani et al., 2015) and time to exhaustion (Eggebeen et al., 2016; Zamani et al., 2015) in individuals with heart failure. Thus, increasing NO bioavailability through NO₃⁻ supplementation is a logical target to improve fatigability in people with prediabetes.

Although no studies have investigated the effect of NO₃⁻ supplementation in people with prediabetes, growing literature exists in rodent models of T2D and humans with T2D. Tian et al. (2020) conducted a comprehensive investigation on the effect of NaNO₃⁻ on oxidant stress, endothelial function, and glucose management in a mouse model of T2D. Supplementing with NaNO₃⁻ in drinking water led to an attenuated oxidative stress which resulted in improved endothelial function as assessed by ACh-induced vasorelaxation of isolated aortas. Additionally, the researchers identified improved glucose clearance and insulin resistance following NaNO₃⁻ supplementation. Although promising, earlier studies investigating the effect of NO₃⁻ supplementation in humans with T2D have primarily produced null results (Gilchrist et al., 2013; Shepherd
et al., 2015). Gilchrist et al. (2013) investigated the effects of a 2-week NO$_3^-$ supplementation on vascular function (i.e., FMD and ACh- and SNP-induced skin blood flow) and insulin sensitivity in individuals with T2D. Nitrate supplementation had no effect on indices of vascular function or insulin sensitivity. In a follow-up study conducted by the same group, oxygen cost of exercise and 6-minute walk performance were found to be unaltered following 4 days of NO$_3^-$ supplementation in individuals with T2D (Shepherd et al., 2015). The lack of benefits in exercise performance following NO$_3^-$ supplementation may be a result of the low-intensity mode of exercise used (i.e., walking) which may not elicit the hypoxic and low pH environments needed to reduce NO$_2^-$ to NO. Furthermore, metformin is known to impair mitochondrial respiration through the inhibition of the mitochondrial electron transport chain at complex I (Bridges et al., 2014). With >80% of the individuals with T2D in Shepherd’s study prescribed metformin, it is plausible that metformin use could have negated the ergogenic effect of NO$_3^-$ supplementation through impaired mitochondrial respiration.

Although previous studies in humans have produced null results, a recent clinical trial investigating the effects of combined NO$_3^-$ and NO$_2^-$ supplementation (similar to the NO$_3^-$ and NO$_2^-$ composition seen in beetroot and spinach) in people with T2D has produced promising effects on vascular function (Bock, Ueda, et al., 2022) and exercise performance (Bock, Hanson, et al., 2022; Turner et al., 2022). In each of these studies, individuals with T2D were assigned to supplement with either NO$_3^-$- and NO$_2^-$-rich beetroot juice or a NO$_3^-$- and NO$_2^-$-depleted placebo beetroot juice for 8 weeks. Combined NO$_3^-$ and NO$_2^-$ supplementation was found to increase both peak oxygen consumption and the workload at which the gas-exchange threshold occurred during
cycling exercise (Bock, Hanson, et al., 2022). Furthermore, forearm exercise-induced blood flow was improved in addition to improved functional sympatholysis following NO$_3^-$ and NO$_2^-$ supplementation (Bock, Ueda, et al., 2022). Lastly, skeletal muscle oxidative capacity was determined from permeabilized vastus lateralis muscle fibers pre- and post-supplementation (Turner et al., 2022). Combined NO$_3^-$ and NO$_2^-$ supplementation resulted in no increase in skeletal muscle oxidative capacity; however, subgroup analysis identified responders and non-responders to NO$_3^-$ and NO$_2^-$ supplementation with 42% of the NO$_3^-$ and NO$_2^-$ supplementation group showing improvements (>15% increase; range = 15% - 150% increase) in both carbohydrate- and fatty acid-supported respiration compared to 0% of the placebo supplementation group. These recent findings present support for the therapeutic potential of NO$_3^-$ supplementation on fatigability in individuals with prediabetes.

Thus, the purpose of this dissertation was to investigate the mechanisms of fatigability in individuals with prediabetes, specifically related to the contribution of both resting and exercise vascular function, compared with healthy controls matched for age, sex, BMI, and physical activity (Study 1, Chapter 2). The purpose of Study 2 (Chapter 3) was to determine effects of NO$_3^-$ supplementation of fatigability in individuals with prediabetes.

1.5 Specific Aims

Aim 1: Compare resting macro- and micro-vascular function in people with prediabetes compared to age-, sex-, BMI-, and physical activity-matched healthy controls and
determine the association of macro- and micro-vascular function with lower limb
fatigability and exercise vascular responses.

*Hypothesis 1.1:* People with prediabetes would have an attenuated FMD and post-
occlusive reactive hyperemia compared to matched, healthy controls which will
be related to an increased fatigability and impaired skeletal muscle blood flow and
oxygenation.

Aim 2: Compare exercise-induced skeletal muscle blood flow and oxygenation in people
with prediabetes compared to age-, sex-, BMI-, and physical activity-matched healthy
controls during fatiguing exercise.

*Hypothesis 2.1:* People with prediabetes would have attenuated exercise-induced
limb blood flow and compromised skeletal muscle oxygenation responses
compared to matched, healthy controls which will be related to an increased
fatigability.

Aim 3: Determine the therapeutic effects of NO₃⁻ supplementation on fatigability and
exercise-induced blood flow, skeletal muscle oxygenation, and resting vascular function
in people with prediabetes.

*Hypothesis 3.1:* Fatigability will be attenuated in people with prediabetes after
short-term NO₃⁻ supplementation.

*Hypothesis 3.2:* NO₃⁻ supplementation will lead to improved exercise-induced
blood flow, skeletal muscle oxygenation, and resting vascular function that will be
associated with improvements in fatigability in individuals with prediabetes.
2.1 Introduction

Prediabetes is characterized as hyperglycemia below the clinic cutoff for type 2 diabetes (T2D) (ElSayed et al., 2023a). It is estimated that over 95 million (1 in 3) American adults and 720 million individuals worldwide have prediabetes (Centers for Disease Control and Prevention, 2022; Sun et al., 2022). Prediabetes not only increases the risk for T2D, but also independently increases an individual’s risk for cardiovascular disease, as well as all-cause mortality (Cai et al., 2020; Echouffo-Tcheugui et al., 2023; Huang et al., 2016). Optimal interventions to return individuals with prediabetes to normoglycemic levels is imperative to prevent the onset of future disease.

Frontline treatment for prediabetes is lifestyle modification, specifically in the form of increased physical activity and dietary modification (Echouffo-Tcheugui et al., 2023; ElSayed et al., 2023b). Indeed, the U.S. Diabetes Prevention Program study, a lifestyle invention with the goals of 7% weight loss and 150 minutes/week of physical activity, found that incidence rates for developing T2D were reduced by 58% in the lifestyle group compared to a control group, this being superior to pharmacological intervention with metformin (31%) (Knowler et al., 2002). Although the beneficial effect of physical activity in prediabetes is well established, there is evidence of neuromuscular impairments in this population leading to a greater performance fatigability (i.e., acute reduction in muscle power and strength in response to exercise and hereon referred to as fatigability) (Hunter, 2018; Senefeld, Harmer, et al., 2020). The greater fatigability was
quantified as a larger reduction in lower limb power output during dynamic, knee extension exercise in individuals with prediabetes when compared to age-, body mass index- (BMI), and physical activity-matched healthy control individuals (Senefeld, Harmer, et al., 2020). Increased fatigability has the potential to impair physical function (Shang et al., 2021) and decrease exercise tolerance (Solomon et al., 2015), ultimately leading to reduced physical activity (Taylor et al., 2010; Xu et al., 2018; Yen & Li, 2021), decreased ability to perform activities of daily living (Tapp et al., 2006), and overall decreased quality of life (Tapp et al., 2006; Taylor et al., 2010; Xu et al., 2018) in individuals with prediabetes. Despite the potential impact, little is known about the mechanisms of the greater fatigability in individuals with prediabetes.

Fatigue of the neuromuscular system can originate from both neural (i.e., proximal to the neuromuscular junction) and muscular (i.e., at or distal to the neuromuscular junction) sites (Gandevia, 2001; Hunter, 2018). Very few studies have been conducted investigating the mechanisms of fatigability in individuals with prediabetes (Senefeld, Harmer, et al., 2020; Senefeld, Singh-Peters, et al., 2020), and of these studies, only one has compared people with prediabetes to healthy controls (Senefeld, Harmer, et al., 2020). This study showed that muscular mechanisms leading to impaired muscle contractile function (i.e., reduction in electrically evoked twitch amplitude) were the primary factors contributing to increased fatigability in people with prediabetes (Senefeld, Harmer, et al., 2020). Possible mechanisms include an attenuated exercise-induced limb blood flow and skeletal muscle perfusion (Bock et al., 2020; Groen et al., 2019; Kingwell et al., 2003; Novielli & Jackson, 2014; Novielli-Kuntz et al., 2018; Senefeld et al., 2019; Wasserman et al., 2018), reduced mitochondrial function (Meex et
al., 2010; Mogensen et al., 2007; Petersen et al., 2004; Schrauwen-Hinderling et al., 2007; Turner et al., 2022) and/or reduced skeletal muscle contractile efficiency (i.e., ATP utilized for a given amount of power) (Lewis et al., 2019), with evidence for each mechanism primarily derived from studies in individuals with T2D. Each of these proposed mechanisms would lead to an increased accumulation of fatigue inducing metabolites (i.e., H+ and P) and fatigue of the muscle (Sundberg & Fitts, 2019).

Previous studies showing both micro- and macrovascular endothelial dysfunction in people with prediabetes suggest that an attenuated exercise-induced blood flow and skeletal muscle perfusion may exist in this population (Caballero et al., 1999; Jaap et al., 1994; Jaap et al., 1997; Sorensen et al., 2016; Su et al., 2008; Vehkavaara et al., 1999; Wasserman et al., 2018). Vascular responses during exercise are largely dependent on a healthy vasculature (Dorff et al., 2023; Hanson et al., 2020; Joyner & Casey, 2015; Kingwell et al., 2003); hence, it is possible that vascular dysfunction impairs exercise-induced blood flow in individuals with prediabetes leading to a greater fatigability. To date, no studies have been conducted investigating the exercise hyperemic response in humans with prediabetes. However, impaired exercise hyperemia and vascular responses to exercise-like conditions in prediabetic rodent models suggest impaired vascular responses to exercise may exist in humans with prediabetes (Ellis et al., 2010; Novielli & Jackson, 2014; Novielli-Kuntz et al., 2018). Lastly, our lab has identified a mechanistic link between attenuated exercise-induced blood flow and greater lower limb fatigability in individuals with T2D (Senefeld et al., 2019), further supporting the potential role of attenuated exercise-induced blood flow and skeletal muscle perfusion to an increased
fatigability in people with prediabetes. However, the vascular contributions to fatigability in people with prediabetes have not been determined.

Thus, the aims of this study were to 1) investigate both resting micro- and macrovascular function of the lower limb in individuals with prediabetes and age-, sex-, body mass index (BMI) and physical activity-match healthy controls and determine their relation with lower limb fatigability and exercise vascular responses and 2) determine the exercise-induced blood flow and muscle oxygenation responses in individuals with prediabetes and matched, healthy controls and determine their relation with lower limb fatigability. We hypothesized that 1) impaired micro- and macrovascular function in individuals with prediabetes will be related to lower limb fatigability and exercise-induced blood flow and muscle oxygenation responses and 2) individuals with prediabetes will have attenuated exercise-induced induced blood flow and muscle oxygenation responses compared to healthy controls and these impaired vascular responses will be associated with lower limb fatigability.

2.2 Materials and Methods

Participants

Twenty people with prediabetes [Males = 9, Females = 11; Glycated hemoglobin (HbA1c) = 5.7 ± 0.3%; Fasting plasma glucose (FPG) = 104 ± 9 mg/dl; Age = 58 ± 16 yr] and 25 healthy control participants (Males = 9, Females = 17; HbA1c = 5.3 ± 0.2%; FPG = 90 ± 5 mg/dl; Age = 55 ± 12 yr) participated in the study. All participants provided written informed consent prior to involvement in the study. The experimental protocol was approved by the Marquette University Institutional Review Board (HR-2402) in
accordance with the Declaration of Helsinki. Participant characteristics are presented in Table 2.1.

All participants underwent screening by survey and phone call prior to study inclusion. Prediabetes was defined according to the American Diabetes Association criteria as a FPG of 100 to 125 mg/dL, and/or HbA1c of 5.7 to 6.4% (ElSayed et al., 2023a). Thus, control participants had a FPG of < 100 mg/dL and a HbA1c < 5.7%. Participants were excluded due to the following: BMI ≥ 40 kg/m², type 1 or type 2 diabetes, uncontrolled hypertension, active cancer, untreated hypothyroidism, current tobacco use, severe arthritis, and any neurological, cardiovascular, or musculoskeletal disease that precluded exercise.

Participants reported to the laboratory on two separate occasions including a screening session followed by an experimental session. The screening session was used to familiarize participants to several of the experimental techniques and screen HbA1c from a finger-prick blood sample. Primary outcome measures for the study were conducted in the experimental session. Study sessions were separated by ≥ 48 hours.

**Screening Session**

Participants underwent the following during the screening session: body composition and lower limb lean mass was assessed using dual x-ray absorptiometry (DXA), blood pressure from the brachial artery was taken following 5 minutes of quiet rest, and HbA1c was assessed using a point of care instrument. Participants were also familiarized with the muscle electrical stimulation and knee extension exercise to be conducted in the experimental session including maximal voluntary isometric contractions (MVIC), measurements of voluntary activation, and high-velocity, dynamic
contractions. Lastly, a triaxial accelerometer was administered to each participant for the measurement of physical activity.

*Anthropometry and DXA*

Height was obtained from a stadiometer and weight was obtained from a calibrated scale. Body composition consisting of total body lean mass, total body fat mass, % body fat, and lean mass of the exercising leg were obtained using DXA (Lunar Prodigy, GE, Madison, WI, USA). The scanner was calibrated for each scan according to the manufacturer’s instructions.

*HbA1c*

A finger prick blood sample was used to measure HbA1c using one of two point-of-care instruments (DCA 2000+, Siemens Healthcare Diagnostics, Malvern, PA, USA; Afinion 2, Abbott Diagnostics, Scarborough, ME, USA).

*Physical Activity*

A waist worn, triaxial accelerometer (ActiGraph wGT3X-BT, ActiGraph, Pensacola, FL, USA) was used for the assessment of physical activity. Participants were instructed to wear the accelerometer for 4 days, including 2 weekdays and 2 weekend days (Hart et al., 2011) and concurrently completed a physical activity log which was used for wear-time validation. Physical activity data were exported in 60 second epochs and analyzed for steps/day using ActiLife software (ActiLife Version 6, ActiGraph, Pensacola, FL, USA). Data is only reported if participants wore the accelerometer for ≥ 8 hours/day for at least 3 days.
Experimental Session

Participants arrived at the laboratory the morning of the experimental session following a $\geq 8$-hour fast from food and caffeine. In addition, each participant abstained from strenuous exercise, alcohol consumption, and non-steroidal anti-inflammatory drugs $\geq 24$ hours prior to the experimental session. All participants continued prescribed medication throughout the study and medications were not withheld. Medications and comorbidities of participants are included in Table 2.1. The experimental session started with a blood draw followed by the assessment of flow-mediated dilation (FMD) and post-occlusive reactive hyperemia of the superficial femoral artery. Participants were then setup in a dynamometer and performed baseline MVICs with measurements of voluntary activation and contractile properties. Following at least 5 minutes of rest, superficial femoral artery blood flow was measured using doppler ultrasonography. Participants then performed a 4-minute dynamic fatiguing exercise task. Exercise muscle oxygenation responses in the rectus femoris muscle were measured using near-infrared spectroscopy (NIRS) and immediately post-exercise blood flow through the superficial femoral artery was measured using doppler ultrasonography. At 1.5- and 2-minutes post-exercise, recovery knee extension MVICs with measurements of voluntary activation and contractile properties were performed. The experimental protocol is visually presented in Figure 2.1.
<table>
<thead>
<tr>
<th><strong>Comorbidities &amp; Conditions</strong></th>
<th>Prediabetes (n)</th>
<th>Control (n)</th>
</tr>
</thead>
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<tr>
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<tr>
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<td>Liver Disease</td>
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</tr>
<tr>
<td>Sleep Apnea</td>
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</table>

<table>
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<tr>
<td>Angiotensin II receptor blocker</td>
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<tr>
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<td>0</td>
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<tr>
<td>Calcium channel blocker</td>
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<tr>
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<td>2</td>
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<tr>
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<td>0</td>
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<td>Glucagon-like peptide 1</td>
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<td>Methimazole</td>
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<tr>
<td>NSAIDs (as needed)</td>
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<td>3</td>
</tr>
<tr>
<td>Statins</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 2.1. Participant comorbidities, conditions, and medications.** NSAIDs, non-steroidal anti-inflammatory drugs; n, number of participants with the comorbidity, condition, or prescribed medication.
Figure 2.1. Visual schematic of experimental protocol. Participants rested in a supine position for 20 minutes before the measurement of superficial femoral artery vascular function. Doppler ultrasound measurements were conducted at rest and following a 5-minute bout of lower limb ischemia to obtain measures of vascular function. Following participant setup in the dynamometer, participants performed 3-5 knee extension MVICs followed by 4-5 knee extension MVICs with electrical stimulation of the quadriceps muscles to obtain baseline measurements of voluntary activation and muscle contractile properties. After at least 5 minutes of rest, baseline superficial femoral artery blood flow was measured using Doppler ultrasound and baseline muscle oxygenation was measured using NIRS. Participants then performed a 4-minute dynamic fatiguing task of the knee extensor muscles in which participants performed one MVCC every 3 seconds against a load of 20% MVIC. Muscle oxygenation was measured during the fatiguing task using NIRS. Immediately following the fatiguing task, exercise-induced blood flow was measured. Five recovery MVCCs were conducted 1-minute post-exercise and two MVICs with electrically stimulation were performed at 1.5- and 2-minutes post-exercise to measure recovery MVIC torque, voluntary activation, and muscle contractile properties. Lastly, a 5-minute bout of lower limb ischemia was conducted to obtain a physiological calibration for muscle oxygenation data. MVIC, maximal voluntary isometric contraction; MVCC, maximal voluntary concentric contraction; VA, voluntary activation; NIRS, near-infrared spectroscopy; \( \Delta \), Doppler ultrasound; \( \downarrow \), electrical stimulation.

Blood Draw and Analysis

A fasting blood sample was obtained from a blood draw. Fasting plasma glucose was determined using a point of care instrument (Cholestech LDX, Abbott Diagnostics, Scarborough, ME, USA). Serum blood samples were analyzed in duplicate according manufacturer instructions to determine fasting insulin concentrations using an enzyme-linked immunosorbent assay kit (Human Insulin ELISA Kit, Invitrogen, Waltham, MA,
| Homeostatic model of insulin resistance (HOMA-IR) was then calculated from the following equation:

\[
\text{HOMA-IR} = \frac{\text{fasting insulin (uIU/mL) \times FPG (mg/dL)}}{405}
\]

Serum samples were not obtained from three individuals with prediabetes and four controls; hence, fasting insulin and HOMA-IR were not determined in these participants.

*Flow-Mediated Dilation and Post-Occlusive Reactive Hyperemia*

Participants rested while lying supine for ≥ 20 minutes in a dimly lit, temperature-controlled room (~21°C). Doppler ultrasound measurements of superficial femoral artery diameter and blood velocity were obtained ~2.5 cm distal to the common femoral artery bifurcation using a 9-MHz liner probe (Vivid e95, General Electrics, Madison, WI, USA). Of note, ultrasound measurements for vascular function and exercise-induced blood flow were obtained from three individuals with prediabetes and three control participants matched for age, sex, BMI, and physical activity using separate ultrasound machine with an 8-MHz liner probe (Vivid E, General Electrics, Madison, WI, USA). Artery diameter was measured in B-mode with the ultrasound probe perpendicular (90°) to the vessel. Blood flow was measured using duplex mode at an insonation angle of 60° and a frequency of 5 MHz. The sample gate size was set to 2 mm for all participants and was positioned in the center of the vessel. A 3-lead electrocardiogram recording was obtained concurrently with ultrasound measurements.

Baseline superficial femoral artery diameter and blood flow were measured for 30 seconds following ≥ 20 minutes of rest. Of note, blood flow measured at rest using the separate ultrasound machine (Vivid E, General Electrics, Madison, WI, USA) was
measured for 12 seconds for three individuals with prediabetes and three control participants. A 12 cm pneumatic cuff was then placed around the upper leg distal to the ultrasound probe. The cuff was rapidly inflated (Hokanson Rapid Cuff Inflation System, D.E. Hokanson, Bellevue, WA, USA) to a suprasystolic pressure (250 mmHg) and remained inflated for 5 minutes, occluding blood flow to the distal portion of the leg. Blood flow was measured for 15 seconds prior to cuff deflation and for 30 seconds immediately post-cuff deflation to determine peak post-occlusive reactive hyperemia (RH\text{peak}) and shear rate. Blood Flow was calculated using the following equation:

$$BF = MBV \times \left[ \pi \times \left( \frac{D}{2} \right)^2 \right] \times 60$$

where BF is blood flow in mL/min, MBV is mean blood velocity in cm/sec, D is artery diameter in cm, and 60 is used to convert mL/sec to mL/min (Limberg et al., 2020). In addition, shear rate was calculated using the following equation:

$$Shear\ rate = 4 \times (MBV) \times (D)^{-1}$$

where MBV is mean blood velocity in cm/sec and D is artery diameter in cm.

Following the 30 seconds of post-cuff deflation blood flow measures, vessel diameter was measured continuously using B-mode until 5 minutes post-cuff deflation to determine changes in vessel diameter. End-diastole vessel diameter was measured by a single rater using edge detection software (Brachial Anaylzer, Medical Imaging Applications, Coralville, IA, USA). Post-cuff deflation vessel diameter measurements were averaged into 5-second bins. The average vessel diameter at baseline and the highest 5-second average post-cuff deflation were used to calculate FMD using the following equation:

$$FMD (\%) = \left[ \frac{(Peak\ Diameter - Baseline\ Diameter)}{Baseline\ Diameter} \right] \times 100$$
The flow-mediated vasodilatory response is dependent on NO bioavailability and represents endothelial function of the conduit artery (Thijssen et al., 2011). Blood flow post-cuff deflation was quantified using the time-averaged mean for each cardiac cycle (beat-by-beat) and a 3-cardiac cycle rolling average was applied to the data. The highest 3-cardiac cycle average was used as RH_{peak}. Peak post-occlusive reactive hyperemia is presented both as absolute blood flow and blood flow normalized to thigh lean mass obtained from the DXA scan. The post-occlusive reactive hyperemic response is considered an index of microvascular function (Limberg et al., 2020). Data from three controls were not included in analysis due to poor ultrasound image quality. Additionally, one individual with prediabetes did not undergo vascular function testing due to discomfort with the cuff occlusion.

Maximal Strength, Voluntary Activation, and Contractile Properties

Participants were seated upright at 70° of hip flexor in a dynamometer (Biodex System 4, Biodex Medical, Shirely, NY, USA). The knee angle was set to 90° of flexion and the axis of rotation of the knee was align with the axis of rotation of the dynamometer motor. Padded shoulder and waist straps were used to secure the participants in the chair and the leg was strapped to the dynamometer lever arm proximal to the malleoli using a non-compliant strap. Mechanical recordings from the dynamometer including torque, velocity, and position data were digitized and sampled at 500 Hz and stored online (Power 1401 A/D Converter and Spike 2, Cambridge Electronics Design, Cambridge, UK). Custom made pad electrodes (~5 cm × 15 cm) were used for the stimulation of the knee extensor muscles. A constant current stimulator (DS7AH, Digitimer, Welwyn Garden City, Hertortshire, UK) was used to deliver two 200
μs square wave pulses at 100 Hz to electrically evoke contractions of knee extensor muscles. A ramp protocol was used to identify the stimulator current at which the electrically evoked twitch amplitude plateaued. The stimulator current was set at 50 mA and then increased incrementally by 50 mA until the twitch plateau occurred. The current was then increased 20% to ensure supramaximal stimulation.

Following the stimulation ramp protocol, participants performed a minimum of three brief (3 seconds) MVICs to assess maximal strength of the knee extensor muscles. Strong verbal encouragement and visual feedback was provided during each MVIC. Participants were given ≥ 60 seconds between each MVIC to ensure adequate rest. Maximal voluntary isometric contractions were continued until two trials were within 5% of each other. Maximal voluntary isometric contraction peak torque was quantified as the highest 500 ms mean value during the MVIC.

Next, a minimum of four MVICs were conducted with the addition of electrically evoked twitches delivered at the MVIC torque plateau (superimposed twitch) and at rest ~2 seconds after the MVIC (resting potentiated twitch). Voluntary activation was calculated using the following equation:

\[
\text{Voluntary Activation} (%) = (1 - \frac{\text{SIT}}{\text{Q}_{\text{tw}}}) \times 100
\]

where SIT is the amplitude of the superimposed twitch and \( \text{Q}_{\text{tw}} \) is the amplitude of the resting potentiated twitch (Allen et al., 1995). Contractile properties of the knee extensor muscles were determined from each resting potentiated twitch including twitch amplitude (\( \text{Q}_{\text{tw}} \)), rate of torque development (RTD), rate of torque relaxation (RTR) and half-relaxation time (HRT). The \( \text{Q}_{\text{tw}} \) was measured as an index of the force-generating capacity of the muscle. The RTD and RTR were quantified with the derivative of the
torque signal as the highest rate of torque increase (RTD) and decrease (RTR) over a 10-ms interval. Lastly, the HRT was determined as the amount of time (ms) it took for the torque signal to reach half of the peak torque amplitude during twitch relaxation. Baseline voluntary activation and contractile properties are reported as the median value of the best four trials. Post-exercise recovery MVIC, voluntary activation, and contractile properties were assessed at 1.5- and 2-minutes post-exercise. Recovery measures are reported from the highest/best trial from the two recovery measures. Muscle stimulation was not conducted for two participants prediabetes due to discomfort with the stimulations. Recovery MVIC/voluntary activation trials were not conducted in three people with prediabetes and three controls due to deviations in the recovery experimental protocol.

Dynamic Exercise Task

While still positioned in the dynamometer as described above, participants performed a 4-minute dynamic, fatiguing exercise task of the knee extensor muscles that consisted of 80 maximal voluntary concentric contractions (MVCC) (1 contraction every 3 seconds) as previously described (Sundberg, Kuplic, et al., 2018). Participants were verbally cued to kick out every 3 seconds and lifted a load equivalent to 20% of MVIC through a 95° range of motion. Following knee extension, participants were instructed to fully relax and the leg was passively returned to the starting position (90°) by the investigator to avoid mechanical work performed by the knee flexor muscles. Participants were instructed to kick “as fast as possible” through the entire range of motion with every kick and were provided strong verbal encouragement throughout the entire fatiguing exercise task. At 1-minute post-exercise, participants performed five additional MVCCs
to determine post-exercise recovery of the knee extensor power. Participants were habituated to the MVCCs followed by ~10 minutes of rest before conducting the fatiguing exercise task. Fatigability as a result of the exercise task was quantified as the percent reduction in limb power during the 4-minute exercise task. Baseline power output was determined as the highest average power over five consecutive contractions within the first ten contractions of the fatiguing exercise task. End-exercise power output was determined as the average power of the last five contractions of the fatiguing exercise task. Knee extensor mechanical power output (W) was calculated as the product of the torque (N·m) and angular velocity (rad/sec) and was quantified as the mean value during the concentric phase of the muscle contraction. Recovery (i.e., 1-min post-exercise) MVCCs were not conducted in three people with prediabetes and four controls due to deviations in the recovery experimental protocol.

**Exercise-Induced Blood Flow**

Prior to the fatiguing exercise task, resting blood flow through the superficial femoral artery was measured using Doppler ultrasonography as described above. In short, the probe was placed ~4 cm distal to the common femoral bifurcation. The probe was placed slightly more distal than for FMD measures due to limited access to the upper thigh as a result of the seated position used for exercise. Baseline blood flow prior to exercise was measured for 30 seconds after ≥ 5 minutes of rest. Of note, blood flow measured at rest using the separate ultrasound machine (Vivid E, General Electrics, Madison, WI, USA) was measured for 12 seconds for three individuals with prediabetes and three control participants. Immediately following the 4-minute fatiguing exercise task, post-exercise blood flow was measured beat-by-beat and a 3-cardiac cycling rolling
average was applied to the data. Due to difficulty obtaining clear images for the measurement of vessel diameter in Duplex mode, post-exercise diameter was measured in B-mode immediately following post-exercise recovery MVIC, voluntary activation, and contractile properties and was used to quantify blood flow. Pre- and post-blood flow is presented both as absolute blood flow and blood flow normalized to thigh lean mass obtained from the DXA scan. Change in blood flow as a result of exercise is presented as an absolute change and as a percent change.

**Near-infrared Spectroscopy**

Relative changes rectus femoris oxygenated myo/hemoglobin (oxy[heme]), deoxygenated myo/hemoglobin (deoxy[heme]), and total myo/hemoglobin (total[heme]) were obtained using multi-channel, continuous wave near-infrared spectroscopy (NIRS) (Portamon, Artinis, Amsterdam, Netherlands). Of note, myoglobin is considered to contribute minimally to NIRS oxygenation responses during exercise compared to hemoglobin (Barstow, 2019). The spatial resolved spectroscopy approach was used to determine tissue oxygen saturation (StO₂) which was quantified from oxy[heme] and total[heme]. The probe emitted near-infrared light from three optodes spaced at 30, 35, and 40 mm from the receiver. Each optode emitted 760 and 850 nm wavelengths of light which are specific to the chromophores deoxy[heme] and oxy[heme]. The site of the NIRS probe was shaved and cleaned with alcohol prior to probe placement. The NIRS probe was secured to the skin over the rectus femoris using double side tape along the edge of the NIRS probe and was wrapped with a black, opaque elastic bandage to reduce ambient light contamination. All signals were sampled at 10 Hz. Due to movement
artifact from the MVCCs, a rolling 3-second median filter was used to smooth the data prior to analysis.

Due to the impact of adipose tissue on the NIRS signal, a physiological calibration was conducted using a 5-minute bout of ischemia at the end of the experimental session. A 10 cm pneumatic cuff was rapidly inflated (Hokanson Rapid Cuff Inflation System, D.E. Hokanson, Bellevue, WA, USA) proximal to the NIRS probe to obtain maximum and minimum values (physiological range) for each signal. Near-infrared spectroscopy data obtained during exercise was normalized to the physiological range of each signal (Barstow, 2019).

Lastly, the deoxy[heme] signal can be viewed as a proxy of local oxygen extraction. The deoxy[heme] signal can be used to provide insight into the matching of muscle oxygen delivery and utilization at the onset of exercise by investigating the kinetic profile of the deoxy[heme] response during the transition from rest to exercise (Barstow, 2019; Koga et al., 2012). A mono-exponential model was used to fit deoxy[heme] signal during the transition from rest to exercise (0-60 seconds). The following equation was used:

$$\text{Deoxy[heme]}(t) = \text{Deoxy[heme]}(b) + \Delta\text{Deoxy[heme]} \times [1 - e^{-(t-\text{TD})/\tau}]$$

where \( \text{Deoxy[heme]}(t) \) is the deoxy[heme] value at time \( t \), \( \text{Deoxy[heme]}(b) \) is the resting baseline value, \( \Delta\text{Deoxy[heme]} \) is the change in the Deoxy[heme] signal from baseline to steady state, TD is the time delay or duration of time from the onset of exercise to the primary component of deoxy[heme], and \( \tau \) is the time constant which represents the amount of time it takes for the deoxy[heme] signal to reach 63% of the change in the signal from rest to exercise (Koga et al., 2012). The mean response time (MRT) was
calculated as the sum of the TD and \( \tau \) to provide a representation of the overall deoxy[heme] response. The overshoot was calculated as the maximal deoxy[heme] response subtracted by the end-exercise deoxy[heme] (Bauer et al., 2007).

*Adipose Tissue Thickness*

Adipose tissue thickness was measured at the sight of the NIRS probe with ultrasound using a 9 MHz liner probe in B-mode (Vivid e95, General Electric, Madison, WI, USA). The ultrasound probe was placed on the skin and minimum pressure was used to avoid compression of adipose tissue. The light emitted from the NIRS probe reaches an estimated depth of half the transmitter-receiver distance. With the shortest path-length of our probe being 30 mm, this depth was 15 mm. Data from any participants with greater than 15 mm of adipose tissue thickness were excluded from the NIRS analysis (Prediabetes = 8; Control = 12). NIRS data for two control participants were removed from analysis due to poor signal quality throughout exercise.

*Statistical Analysis*

Data are reported as means ± SD in the text and tables and are displayed as means ± SEM in the figures. The normality and homogeneity of variance of the data were assessed using Shapiro-Wilk and Leven’s tests, respectively. Separate two-way ANOVAs with group (prediabetes vs. control) and sex (male vs. female) as between-subject factors were used to determine differences in participant characteristics and baseline, post-occlusion/exercise, and relative changes in neuromuscular, vascular, and muscle oxygenation measurements in response to either the 5-minute occlusion and dynamic exercise task. When a significant interaction was identified, post-hoc pairwise comparisons were conducted using a Bonferroni correction. When assumptions of
normality or homogeneity of variance were violated, separate Mann-Whitney tests were conducted and Bonferroni corrections used to correct for multiple comparisons. When reporting the results, main effects of sex and the interactions between sex and group are only reported if significant. Separate Pearson correlations were used to determine the association between fatigability (% reduction in lower limb power) and participant characteristics and potential mechanistic measures of fatigability. When assumptions of normality were violated, a Spearman’s Rho correlation analysis was used. Significance was determined at $P < 0.05$ and all analyses were performed with JASP version 0.17.3.

2.3 Results

*Participant Characteristics*

Participant characteristics are reported in Table 2.1. People with prediabetes and controls did not differ in age, height, weight, BMI, % body fat, and physical activity levels ($P > 0.05$). As expected, people with prediabetes had higher FPG ($103.5 \pm 9.2$ vs. $90.2 \pm 5.2$ mg/dl; $P = 0.001, \eta^2_P = 0.475$) and HbA1c ($5.72 \pm 0.28$ vs. $5.33 \pm 0.18\%$; $P = 0.001, \eta^2_P = 0.476$) compared with controls; however, fasting serum insulin ($11.8 \pm 4.8$ vs. $10.8 \pm 2.9$ µIU/ml; $P = 0.374, \eta^2_P = 0.023$) and HOMA-IR ($3.0 \pm 1.4$ vs. $2.4 \pm 0.7$ a.u.; $P = 0.065, \eta^2_P = 0.094$) were similar between groups. In addition, males were taller ($P = 0.001, \eta^2_P = 0.221$), had less percentage body fat ($P = 0.001, \eta^2_P = 0.342$), and had greater FPG ($P = 0.009, \eta^2_P = 0.153$) and HOMA-IR ($P = 0.036, \eta^2_P = 0.119$) compared with females.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Prediabetes (n = 20)</th>
<th>Control (n = 26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>55 ± 19</td>
<td>52 ± 14</td>
<td></td>
</tr>
<tr>
<td>Height, m</td>
<td>1.72 ± 0.07</td>
<td>1.72 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85 ± 12</td>
<td>83 ± 10</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.7 ± 3.6</td>
<td>27.9 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Body fat, %</td>
<td>32.1 ± 5.1</td>
<td>29.4 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>Physical activity, steps/day</td>
<td>8644 ± 3404</td>
<td>8824 ± 2814(8)</td>
<td></td>
</tr>
<tr>
<td>Prediabetes duration, yr</td>
<td>2.3 ± 2.9(8)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>5.8 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>106 ± 7</td>
<td>94 ± 2</td>
<td></td>
</tr>
<tr>
<td>Fasting serum insulin, µIU/ml</td>
<td>13.9 ± 6.2(8)</td>
<td>10.7 ± 2.5(8)</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR, a.u.</td>
<td>3.7 ± 1.8(8)</td>
<td>2.5 ± 0.6(8)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2. Participant characteristics. People with prediabetes were similar to controls in age, height, weight, body mass index, body fat (%), and physical activity. The sample sizes of each group and certain variables are presented in parentheses. Values are presented as mean ± standard deviation. HbA₁c, glycated hemoglobin; HOMA-IR, homeostatic model of insulin resistance; *, group effect, \( P < 0.05 \); #, sex effect, \( P < 0.05 \).

Resting Vascular Function

Vascular measures at rest and following cuff occlusion are presented in Table 2.3.

Blood pressure at rest was not different between people with prediabetes and controls for systolic blood pressure (127.2 ± 15.3 vs. 119.4 ± 9.8 mmHg; \( P = 0.073, \eta_p^2 = 0.078 \)), diastolic blood pressure (77.1 ± 10.1 vs. 74.5 ± 5.8 mmHg; \( P = 0.397, \eta_p^2 = 0.018 \)), and mean arterial pressure (93.8 ± 10.9 vs. 89.5 ± 6.2 mmHg; \( P = 0.163; \eta_p^2 = 0.048 \)).

Superficial femoral artery diameter at rest was not different between people with prediabetes and controls (6.42 ± 1.30 vs. 6.21 ± 0.93 mm; \( P = 0.698, \eta_p^2 = 0.004 \)); however, there was a difference between males and females with males having a larger artery diameter than females (7.07 ± 1.23 vs. 5.83 ± 0.70 mm; \( P = 0.001, \eta_p^2 = 0.299 \)).
Peak post-occlusive reactive hyperemia was not different between people with prediabetes and controls for either absolute blood flow ($1415.0 \pm 448.2$ vs. $1,425.1 \pm 441.8$ ml·min$^{-1}$; $P = 0.653$, $\eta^2_\rho = 0.005$) and blood flow normalized to thigh lean mass ($231.1 \pm 62.1$ vs. $248.1 \pm 59.8$ ml·min$^{-1}$·kg$^{-1}$; $P = 0.370$, $\eta^2_\rho = 0.021$; Figure 2.2). Males had a greater absolute RH$_{\text{peak}}$ compared with females ($1,688.5 \pm 371.4$ vs. $1,255 \pm 399.4$ ml·min$^{-1}$; $P = 0.002$, $\eta^2_\rho = 0.234$); however, there was no sex difference in RH$_{\text{peak}}$ when normalized to thigh lean mass ($252.2 \pm 61.9$ vs. $233.3 \pm 60.0$ ml·min$^{-1}$·kg$^{-1}$; $P = 0.301$, $\eta^2_\rho = 0.028$). Peak shear rate following cuff deflation was not different between prediabetes and controls ($551.3 \pm 271.9$ vs. $546.2 \pm 184.0$ s$^{-1}$; $P = 0.833$, $\eta^2_\rho = 0.001$). Flow-mediated dilation as a percent change was not different between prediabetes and controls ($2.27 \pm 2.18$ vs. $2.26 \pm 1.47$ %; $P = 0.795$, $\eta^2_\rho = 0.002$; Figure 2.2). Similarly, FMD as an absolute change in vessel diameter was not different between prediabetes and controls ($0.15 \pm 0.15$ vs. $0.14 \pm 0.08$ mm; $P = 0.575$, $\eta^2_\rho = 0.008$). There was no association between FMD as a percent change and peak shear rate ($P = 0.436$, $r = 0.123$).
Table 2.3. Superficial femoral artery measurements at rest and vascular function for people with prediabetes and controls as well as males and females. People with prediabetes and controls did not differ for any resting vascular measures and measures of vascular function. Females had smaller arteries, lower resting absolute blood flow, and absolute RH_{peak}. The sample sizes of each group are presented in parentheses. Norm, normalized to thigh lean mass; RH_{peak}, peak post-occlusive reactive hyperemia; FMD, flow-mediated dilation. Values are presented as mean ± standard deviation. #, sex effect, $P < 0.05$.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prediabetes (n = 19)</th>
<th>Control (n = 23)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (8)</td>
<td>Females (11)</td>
<td></td>
</tr>
<tr>
<td>Resting diameter, mm</td>
<td>7.12 ± 1.52</td>
<td>5.91 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>Resting blood flow, ml-min(^{-1})</td>
<td>112.5 ± 52.6</td>
<td>94.7 ± 39.3</td>
<td></td>
</tr>
<tr>
<td>Norm resting blood flow, ml-min(^{-1})-kg(^{-1})</td>
<td>20.4 ± 14.5</td>
<td>23.0 ± 11.3</td>
<td></td>
</tr>
<tr>
<td>RH_{peak}, ml-min(^{-1})</td>
<td>1626.1 ± 400.1</td>
<td>1261.5 ± 433.6</td>
<td></td>
</tr>
<tr>
<td>Norm RH_{peak}, ml-min(^{-1})-kg(^{-1})</td>
<td>244.8 ± 68.8</td>
<td>221.2 ± 58.1</td>
<td></td>
</tr>
<tr>
<td>Peak shear rate, s(^{-1})</td>
<td>497.6 ± 259.3</td>
<td>590.3 ± 286.4</td>
<td></td>
</tr>
<tr>
<td>FMD diameter $\Delta$, mm</td>
<td>0.15 ± 0.16</td>
<td>0.15 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>FMD, %$\Delta$</td>
<td>1.93 ± 1.92</td>
<td>2.53 ± 2.41</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.2. Superficial femoral artery flow-mediated dilation and peak post-occlusive reactive hyperemia normalized to thigh lean mass for people with prediabetes and controls. People with prediabetes and controls did not differ for flow-mediated dilation (A) and normalized peak post-occlusive reactive hyperemia (B) ($P > 0.05$). Values are presented as mean ± standard error of the mean. Individual data points are presented as open circles.
Baseline Neuromuscular Function

Baseline MVIC torque and baseline mechanical outputs for MVCCs at the beginning of the fatiguing exercise task including MVCC torque, velocity, power and range of motion are presented in Table 2.4. People with prediabetes and controls did not differ in baseline MVIC torque (168.5 ± 42.1 vs. 162.3 ± 57.3 Nm; \(P = 0.691, \eta^2_p = 0.004\)), voluntary activation (93.1 ± 5.4 vs. 93.1 ± 4.0 %; \(P = 0.908, \eta^2_p = 0.000\)), and contractile properties including \(Q_{tw}\) (62.4 ± 17.0 vs. 55.3 ± 18.1 Nm; \(P = 0.428, \eta^2_p = 0.016\)), RTD (657.7 ± 190.9 vs. 568.3 ± 186.7 Nm·s\(^{-1}\); \(P = 0.248, \eta^2_p = 0.033\)), RTR (318.8 ± 161.4 vs. 263.4 ± 128.7 Nm·s\(^{-1}\); \(P = 0.409, \eta^2_p = 0.018\)), and HRT (107.4 ± 25.1 vs. 115.3 ± 27.5 ms; \(P = 0.578, \eta^2_p = 0.008\)). Similarly, people with prediabetes and controls did not differ in baseline MVCC torque (46.5 ± 11.6 vs. 46.8 ± 13.8 Nm; \(P = 0.384, \eta^2_p = 0.018\)), velocity (199.6 ± 33.3 vs. 194.1 ± 19.7 °/s; \(P = 0.583, \eta^2_p = 0.007\)), power (160.9 ± 59.6 vs. 159.0 ± 55.7 W; \(P = 0.556, \eta^2_p = 0.008\)), and range of motion (88.7 ± 5.4 vs. 89.5 ± 8.2 °; \(P = 0.662, \eta^2_p = 0.006\)) at the beginning of the fatiguing exercise task.

Males had higher baseline MVIC torque than females (201.7 ± 44.0 vs. 141.4 ± 40.2 Nm; \(P = 0.001, \eta^2_p = 0.283\)) and higher baseline MVCC torque (56.5 ± 10.3 vs. 40.3 ± 9.9 Nm; \(P = 0.001, \eta^2_p = 0.397\)), velocity (206.9 ± 31.9 vs. 189.8 ± 19.9 °/s; \(P = 0.001, \eta^2_p = 0.397\)) and power (204.6 ± 49.7 vs. 131.0 ± 40.3 W; \(P = 0.001, \eta^2_p = 0.407\)) compared with females. Additionally, compared with females, males had greater \(Q_{tw}\) (72.8 ± 14.4 vs. 48.1 ± 12.0 Nm; \(P = 0.001, \eta^2_p = 0.460\)), RTD (772.1 ± 151.7 vs. 489.0 ± 115.3 Nm·s\(^{-1}\); \(P = 0.001, \eta^2_p = 0.529\)), and RTR (390.9 ± 155.0 vs. 211.5 ± 72.7 Nm·s\(^{-1}\); \(P = 0.001, \eta^2_p = 0.460\)).
= 0.001, $\eta^2_p = 0.370$), and a faster HRT (100.6 ± 23.9 vs. 120.2 ± 25.8 ms; $P = 0.030, \eta^2_p = 0.115$) compared to females.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prediabetes (n = 20)</th>
<th>Control (n = 26)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (9)</td>
<td>Females (11)</td>
<td>Males (9)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC Torque, Nm</td>
<td>176.5 ± 35.9</td>
<td>125.7 ± 27.6</td>
<td>181.4 ± 56.4</td>
</tr>
<tr>
<td>MVCC Torque, Nm</td>
<td>53.4 ± 9.3</td>
<td>40.8 ± 10.5</td>
<td>59.6 ± 10.8</td>
</tr>
<tr>
<td>Velocity, °/s</td>
<td>210.6 ± 41.7</td>
<td>190.6 ± 22.8</td>
<td>203.2 ± 19.8</td>
</tr>
<tr>
<td>Power, W</td>
<td>194.9 ± 63.4</td>
<td>133.1 ± 40.5</td>
<td>214.4 ± 31.9</td>
</tr>
<tr>
<td>Range of motion, °</td>
<td>90.8 ± 4.6</td>
<td>87.1 ± 5.6</td>
<td>91.6 ± 4.9</td>
</tr>
<tr>
<td><strong>Fatigability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVCC Torque, %Δ</td>
<td>-11.8 ± 9.0</td>
<td>-7.8 ± 6.3</td>
<td>-11.8 ± 11.9</td>
</tr>
<tr>
<td>Velocity, %Δ</td>
<td>-30.4 ± 18.7</td>
<td>-29.9 ± 12.3</td>
<td>-27.6 ± 8.7</td>
</tr>
<tr>
<td>Power, %Δ</td>
<td>-38.0 ± 19.2</td>
<td>-35.1 ± 11.5</td>
<td>-37.0 ± 13.2</td>
</tr>
<tr>
<td>Range of motion, %Δ</td>
<td>-16.7 ± 14.4</td>
<td>-18.1 ± 13.3</td>
<td>-14.4 ± 8.8</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC Torque, %Δ</td>
<td>-27.5 ± 10.8 (8)</td>
<td>-22.7 ± 7.8 (9)</td>
<td>-25.6 ± 5.0 (8)</td>
</tr>
<tr>
<td>MVCC Torque, %Δ</td>
<td>-12.4 ± 7.6 (8)</td>
<td>-4.7 ± 3.8 (9)</td>
<td>-5.8 ± 6.1 (8)</td>
</tr>
<tr>
<td>Velocity, %Δ</td>
<td>-13.0 ± 10.5 (8)</td>
<td>-10.0 ± 7.5 (9)</td>
<td>-10.7 ± 8.4 (8)</td>
</tr>
<tr>
<td>Power, %Δ</td>
<td>-22.2 ± 14.8 (8)</td>
<td>-11.5 ± 10.3 (9)</td>
<td>-14.7 ± 9.3 (8)</td>
</tr>
<tr>
<td>Range of motion, %Δ</td>
<td>-3.8 ± 4.5 (8)</td>
<td>-2.9 ± 5.6 (9)</td>
<td>-2.8 ± 3.2 (8)</td>
</tr>
</tbody>
</table>

**Table 2.4.** Baseline, fatigability (i.e., end-exercise) as a percent change from baseline, and recovery as a percent change from baseline in MVIC torque and mechanical outputs for MVCC contractions including MVCC torque, velocity, power and range of motion in people. People with prediabetes and controls did not differ in all variables expect recovery MVCC torque as a percent change. As expected, females had lower baseline MVIC torque as well as baseline MVCC torque, velocity, and power. Females had a better recovery than males in MVCC torque and power. Recovery measures for MVCCs were conducted at 1-min post-exercise. Recovery MVICs were conducted at 1.5- and 2-min post-exercise. The highest recovery MVIC trial was used for analysis. The sample sizes of each group and certain variables are presented in parentheses. Values are presented as mean ± standard deviation. MVIC, maximal voluntary isometric contraction; MVCC, maximal voluntary concentric contraction. *, group effect, $P < 0.05$; #, sex effect, $P < 0.05$.

**Fatigability and Recovery MVCC Mechanics and MVIC Torque**

Fatigability and recovery as a percent change from baseline in mechanical outputs are presented in Table 2.4. These include MVIC torque, MVCC torque, velocity, power,
The percent change in MVCC power from power output at the beginning of the fatiguing exercise task did not differ between people with prediabetes and controls (-36.4 ± 15.0 vs. -30.7 ± 14.5 %Δ; \( P = 0.336, \eta_p^2 = 0.022 \); Figure 2.3). In addition, recovery MVCC power as a percent change from baseline at 1-min post-exercise did not differ between people with prediabetes and controls (-16.5 ± 13.4 vs. -9.3 ± 11.7 %Δ; \( P = 0.111, \eta_p^2 = 0.071 \)). However, people with prediabetes demonstrated an attenuated recovery in MVCC torque compared with controls (-8.3 ± 7.0 vs. -4.0 ± 6.4 %Δ from baseline; \( P = 0.046, \eta_p^2 = 0.109 \)). All other mechanical output measures of fatigability and recovery MVCC did not differ between prediabetes and controls (\( P > 0.05 \)). Recovery of MVIC torque following the fatiguing exercise was similar between prediabetes and controls (-25.0 ± 9.4 vs. -22.1 ± 7.6 %Δ from baseline; \( P = 0.422, \eta_p^2 = 0.018 \)). Lastly, there was a sex difference in recovery in that males had an attenuated recovery compared with females in MVCC power (-18.5 ± 12.6 vs. -8.3 ± 11.5 %Δ from baseline; \( P = 0.001, \eta_p^2 = 0.397 \)) and MVCC torque (-9.1 ± 7.5 vs. -3.6 ± 5.6 %Δ from baseline; \( P = 0.001, \eta_p^2 = 0.397 \)).
Figure 2.3. Fatigability in people with prediabetes and controls. Contraction by contraction MVCC power output as a percent of baseline for people with prediabetes (open circles) and controls (black circles) (A) and fatigability as a percent reduction from baseline with individual data points presented as open circles (B). People with prediabetes and controls did not differ in fatigability ($P = 0.336$). Values are presented as mean ± standard error of the mean.

Recovery of Voluntary Activation and Contractile Properties

The absolute change in voluntary activation from baseline to recovery did not differ between people with prediabetes and controls (-0.2 ± 4.6 vs. 0.0 ± 3.0 Δ; $P = 0.981$, $\eta_p^2 = 0.000$). The percent change from baseline to recovery for contractile properties did not differ between prediabetes and controls for $Q_{tw}$ (-9.8 ± 11.9 vs. -4.3 ± 7.9 %Δ; $P = 0.152$, $\eta_p^2 = 0.059$), RTD (-10.6 ± 14.2 vs. -5.9 ± 9.7 %Δ; $P = 0.357$, $\eta_p^2 = 0.025$), RTR (-13.8 ± 21.7 vs. -1.4 ± 18.4 %Δ; $P = 0.059$, $\eta_p^2 = 0.107$), and HRT (8.9 ± 19.7 vs. -2.7 ± 15.5 %Δ; $P = 0.382$, $\eta_p^2 = 0.122$). Recovery of contractile properties (percent change from baseline) was less in males than females because males maintained greater
reductions in recovery compared with females in Qtrw (-11.4 ± 11.2 vs. -2.9 ± 7.1 %Δ; $P = 0.009, \eta^2_p = 0.185$) and RTD (-13.0 ± 13.3 vs. -4.0 ± 9.0 %Δ; $P = 0.020, \eta^2_p = 0.149$).

*Exercise-Induced Blood Flow*

Vascular measures at rest and following exercise are presented in Table 2.5. Superficial femoral artery diameter at rest was not different between people with prediabetes and controls (6.2 ± 1.2 vs. 6.0 ± 1.1 mm; $P = 0.760, \eta^2_p = 0.002$); however, there was a sex difference with males having a larger artery diameter than females (6.7 ± 1.2 vs. 5.7 ± 0.9 mm; $P = 0.004, \eta^2_p = 0.185$).

Peak exercise-induced blood flow was not different between people with prediabetes and controls for absolute blood flow (845.6 ± 232.1 vs. 910.2 ± 233.6 ml·min$^{-1}$; $P = 0.242, \eta^2_p = 0.032$); however, people with prediabetes had an attenuated exercise-induced blood flow when normalized to thigh lean mass (138.5 ± 38.0 vs. 163.4 ± 33.2 ml·min$^{-1}$·kg$^{-1}$; $P = 0.01, \eta^2_p = 0.087$; Figure 2.4). Exercise-induced blood flow as a percent change from blood flow at rest did not differ between prediabetes and controls (817.3 ± 316.3 vs. 1000.7 ± 380.8 %Δ; $P = 0.231, \eta^2_p = 0.034$; Figure 2.4); however, there was a group X sex interaction for exercise-induced blood flow as a percent change ($P = 0.031, \eta^2_p = 0.106$). Post-hoc analysis did not reveal any significant differences between any groups. However, a trend towards females with prediabetes having an attenuated exercise-induced blood flow response compared with female controls was identified ($P = 0.057, d = 1.052$).

Males had a greater absolute exercise-induced blood flow compared with females (969.7 ± 198.5 vs. 825.8 ± 238.7 ml·min$^{-1}$; $P = 0.033, \eta^2_p = 0.104$); however, there was no
sex difference in exercise-induced blood flow when normalized to thigh lean mass (143.4 ± 27.3 vs. 158.4 ± 41.8 ml·min⁻¹·kg⁻¹; \( P = 0.288, \eta_P^2 = 0.027 \)).

<table>
<thead>
<tr>
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<th>Control (n = 26)</th>
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</tr>
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<tr>
<td></td>
<td>Males (9)</td>
<td>Females (11)</td>
<td></td>
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<td>Resting diameter, mm</td>
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<tr>
<td>Resting blood flow, ml·min⁻¹</td>
<td>112.5 ± 52.6</td>
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<tr>
<td>End-exercise blood flow, ml·min⁻¹</td>
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<tr>
<td>Norm end-exercise blood flow, ml·min⁻¹·kg⁻¹</td>
<td>138.5 ± 30.7</td>
<td>138.5 ± 44.6</td>
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</tr>
<tr>
<td>Blood flow, Δ%</td>
<td>882.3 ± 435.0</td>
<td>764.2 ± 178.2</td>
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</tr>
</tbody>
</table>

Table 2.5. Superficial femoral artery measurements at rest and following exercise for people with prediabetes and controls as well as males and females. People with prediabetes and controls did not differ in all variables except end-exercise blood flow normalized to thigh lean mass. Females had smaller arteries, lower resting and end-exercise absolute blood flow. A significant group X sex interaction was identified for blood flow as a percent change from rest to end-exercise; however, post-hoc analysis did not reveal a difference between groups. The sample sizes of each group are presented in parentheses. Norm, normalized to thigh lean mass. Values are presented as mean ± standard deviation. *, group effect, \( P < 0.05 \); #, sex effect, \( P < 0.05 \); †, group X sex interaction, \( P < 0.05 \).
Figure 2.4. Exercise-induced blood flow normalized to thigh lean mass and as a percent change from baseline in people with prediabetes and controls. People with prediabetes had an attenuated exercise-induced blood flow response compared to controls ($P = 0.01$); however, blood flow as a percent change was similar between groups ($P > 0.05$). Values are presented as mean ± standard error of the mean. Individual data points are presented as open circles. *, group effect, $P < 0.05$.

Near-infrared Spectroscopy

Data obtained from NIRS at rest and during exercise are presented in Table 2.6.

Adipose tissue thickness under the site of the NIRS probe was not different between people with prediabetes and controls (10.0 ± 2.7 vs. 9.6 ± 1.9 mm; $P = 0.761$, $\eta^2_p = 0.005$). Tissue oxygen saturation as a percent of the physiological range was not different between prediabetes and controls for rest (81.5 ± 7.7 vs. 78.2 ± 10.1 %; $P = 0.225$, $\eta^2_p = 0.073$), exercise nadir (24.3 ± 15.3 vs. 22.5 ± 12.6 %; $P = 0.387$, $\eta^2_p = 0.038$), end-exercise (49.0 ± 13.1 vs. 45.6 ± 14.5 %; $P = 0.371$, $\eta^2_p = 0.040$), or the average exercise response (43.3 ± 11.8 vs. 40.0 ± 12.3 %; $P = 0.321$, $\eta^2_p = 0.049$; Figure 2.5).
Deoxygenated myo/hemoglobin as a percent of the physiological range was not different between people with prediabetes and controls for rest (10.5 ± 8.8 vs. 13.1 ± 7.2%; \(P = 0.276, \eta^2 = 0.059\)), exercise maximum (59.1 ± 15.3 vs. 69.0 ± 17.1%; \(P = 0.394, \eta^2 = 0.037\)), end-exercise (45.7 ± 13.8 vs. 58.3 ± 16.3%; \(P = 0.167, \eta^2 = 0.093\)), or the average exercise response (48.4 ± 12.9 vs. 58.9 ± 15.4%; \(P = 0.252, \eta^2 = 0.065\); Figure 2.5).

Rest to exercise deoxy[heme] kinetic variables were not different between people with prediabetes and controls for the TD (6.2 ± 1.4 vs. 6.9 ± 1.9 s; \(P = 0.482, \eta^2 = 0.025\)), time constant (\(\tau\)) (6.8 ± 3.5 vs. 4.9 ± 1.9 s; \(P = 0.298, \eta^2 = 0.054\)), MRT (13.0 ± 3.7 vs. 11.8 ± 2.2 s; \(P = 0.595, \eta^2 = 0.014\)), \(\Delta\) deoxy[heme] (44.9 ± 16.2 vs. 52.1 ± 18.6%; \(P = 0.780, \eta^2 = 0.004\)), and overshoot (9.7 ± 6.7 vs. 6.9 ± 9.0%; \(P = 0.339, \eta^2 = 0.046\)). A sex difference was identified for the time constant (\(\tau\)) indicating that females had a faster \(\tau\) compared with males (4.0 ± 1.8 vs. 6.7 ± 2.9 s; \(P = 0.038, \eta^2 = 0.189\)).
<table>
<thead>
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<th>Prediabetes (n = 12)</th>
<th>Control (n = 12)</th>
<th>P</th>
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<td>Males (9)</td>
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</tr>
<tr>
<td>SiO₂, % Phys Range</td>
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<td></td>
</tr>
<tr>
<td>Resting</td>
<td>80.5 ± 8.6</td>
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<td>74.2 ± 7.1</td>
</tr>
<tr>
<td>Exercise nadir</td>
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<tr>
<td>End-exercise</td>
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<td>43.7 ± 18.8</td>
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<tr>
<td>Average exercise response</td>
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</tr>
<tr>
<td>Deoxy[heme], % Phys Range</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>11.4 ± 9.3</td>
<td>11.0 ± 8.1</td>
<td>16.1 ± 4.9</td>
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<tr>
<td>Exercise maximum</td>
<td>55.7 ± 12.0</td>
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<td>68.4 ± 19.0</td>
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<td>End-exercise</td>
<td>43.0 ± 13.4</td>
<td>59.1 ± 19.9</td>
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<tr>
<td>Average exercise response</td>
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<tr>
<td>TD, s</td>
<td>6.1 ± 1.4</td>
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<td>τ, s</td>
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<td>MRT, s</td>
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<td>Δ Deoxy[heme], % phys range</td>
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<td>Overshoot, % phys range</td>
<td>8.9 ± 6.1</td>
<td>6.9 ± 6.7</td>
<td>7.0 ± 12.5</td>
</tr>
</tbody>
</table>

Table 2.6. Near-infrared spectroscopy responses at rest and during exercise in people with prediabetes and controls as well as males and females. People with prediabetes and controls did not differ in all variables. Females had a faster τ than males. The sample sizes of each group are presented in parentheses. SiO₂, tissue oxygen saturation; Deoxy[heme], deoxygenated myo/hemoglobin; Phys, physiological; TD, time delay (duration of time from the onset of exercise to the change in deoxy[heme] signal from baseline); τ, time constant (amount of time it takes for the deoxy[heme] signal to reach 63% of the change in the signal from rest to exercise); MRT, mean response time. Values are presented as mean ± standard deviation. #, sex effect, P < 0.05.
Figure 2.5. Near-infrared spectroscopy responses at rest and during exercise in people with prediabetes and controls. People with prediabetes (open circles) and controls (black circles) did not differ in StO$_2$ (A) and deoxy[heme] (B) responses at rest and during exercise. The sample sizes of each group and the number of females in each group are presented in parentheses. StO$_2$, tissue oxygen saturation; Deoxy[heme], deoxygenated myo/hemoglobin; n, sample size; F, females. Values are presented as mean ± standard error of the mean.

Fatigability Correlation Analysis

Fatigability as a percent reduction in lower limb power was not associated with either FMD as a percent change ($r = -0.184$, $P = 0.242$) or RH$_{peak}$ ($r = 0.201$, $P = 0.202$). Fatigability however, was associated with the percent change (i.e., baseline to recovery)
in $Q_{tw}$ ($r = -0.670, P = 0.001$; Figure 2.6), RTD ($r = -0.628, P = 0.001$), RTR ($r = -0.562, P = 0.001$), and HRT ($r = 0.530, P = 0.001$).

Fatigability was not associated with end-exercise blood flow normalized to thigh lean mass ($r = -0.226, P = 0.131$). However, fatigability was associated with exercise-induced blood flow as a percent change from baseline ($r = -0.310, P = 0.036$; Figure 2.6). Fatigability was associated with maximum ($r = -0.407, P = 0.048$), end-exercise ($r = -0.458, P = 0.024$), and average exercise ($r = -0.479, P = 0.018$; Figure 2.6) deoxy[heme] responses from NIRS. In addition, fatigability was associated with the deoxy[heme] time constant ($\tau$) ($r = 0.429, P = 0.036$). Lastly, fatigability was not associated with FPG ($r = 0.096, P = 0.526$), HbA$_1c$ ($r = 0.124, P = 0.411$), fasting serum insulin ($r = 0.236, P = 0.148$), or HOMA-IR ($r = 0.289, P = 0.075$).

The percent change (i.e., baseline to recovery) in $Q_{tw}$ was associated with exercise-induced blood flow as a percent change from baseline ($r = 0.374, P = 0.021$) and end-exercise ($r = 0.495, P = 0.022$) and average exercise ($r = 0.475, P = 0.029$) deoxy[heme] responses.
Figure 2.6. Fatigability associations. Fatigability (% reduction in limb power from baseline) was associated with exercise-induced blood flow (% change from rest; n = 46) (A), average exercise deoxy[heme] response (n = 24) (B), and Q_{tw} (% reduction from baseline to recovery; n = 38) (C). Phys, physiological; Deoxy[heme], deoxygenated myo/hemoglobin; Q_{tw}, potentiated twitch torque.
2.4 Discussion

This study determined the resting and exercise vascular contributions to fatigability of the knee extensor muscles in individuals with prediabetes compared with age-, BMI-, and physical activity-matched healthy controls. We hypothesized that 1) individuals with prediabetes would have impaired resting micro- and macrovascular dysfunction and 2) attenuated exercise hyperemic and muscle oxygenation responses compared with healthy controls and these would contribute to a greater fatigability with prediabetes. The main findings were that people with prediabetes exhibited an attenuated exercise-induced blood flow but were not more fatigable in the knee extensor muscles than matched, healthy controls. In addition, people with prediabetes had similar resting vascular function and muscle oxygenation responses during the fatiguing exercise compared with controls. These results suggest that people with prediabetes are not more fatigable than healthy controls although exercise-induced blood flow may be impaired in people with prediabetes.

*Performance Fatigability and Muscle Contractile Properties*

A main finding of this study is that people with prediabetes had similar fatigability compared with age-, BMI-, and physical activity-matched controls and fatigability was explained by contractile mechanisms in both groups. The lack of group differences in fatigability is not consistent with previous findings showing larger lower limb fatigability (i.e., reduction in MVCC limb power) in individuals with prediabetes than controls (Senefeld, Harmer, et al., 2020). However, the individuals with prediabetes had a similar fatigability in the current and previous studies (36% vs. 32% reduction in
limb power, respectively), but in the current study the fatigability in healthy controls was markedly greater than the previous study (31% vs. 22% reduction in limb power, respectively) (Senefeld, Harmer, et al., 2020). Healthy control participants had higher fasting insulin and HOMA-IR values in the present study compared to Senefeld et al. (2020) (Fasting Insulin = 10.8 vs. 5.5 µIU/ml, respectively; HOMA-IR = 2.4 vs. 1.2 a.u., respectively) but were similar in all other characteristics (e.g. similar age, BMI, steps/day, HbA1c, FPG). Hence, higher fasting insulin and HOMA-IR values may contribute to the higher fatigability of the control group in the present study; however, fasting insulin and HOMA-IR were not associated with fatigability in the present study.

An additional explanation for the varying fatigability in the control group could be variations in the dynamic fatiguing exercise tasks used in each of the studies with Senefeld et al. (2020) using longer duration task but allowing some recovery at the midway point of the fatiguing task. Senefeld et al. (2020) used a 6-minute dynamic fatiguing task that was similar to the present study in all parameters (20% MVIC load, 1 MVCC every 3 seconds) except for the use of a MVIC midway through the fatiguing task which led to a brief rest period before resuming MVCCs. Although not quantified, control participants visually showed a marked recovery in MVCC power following the midway MVIC in that study. This recovery could have led to the attenuated fatigability in controls participants compared with the current study. Lastly, people with prediabetes in the current study had HbA1c and FPG levels on the lower end of the clinical ranges for prediabetes (i.e., HbA1c = 5.7 and FPG = 106). This HbA1c is slightly lower than Senefeld et al. (2020); however, FPG was similar, if not higher, in the current study.
Changes in voluntary activation and contractile properties following exercise did not differ between the people with prediabetes and the control group. The finding of no change in voluntary activation following exercise in people with prediabetes is similar to previous findings from our group for dynamic exercise (Senefeld, Harmer, et al., 2020). However, Senefeld et al. (2020) found that people with prediabetes had greater impairments in muscle contractile function (i.e., $Q_{tw}$) following the 6-minute dynamic fatiguing task. These differing results could be due to the delayed measure of contractile properties of the current study (1.5- and 2-minutes post-exercise) compared with the immediate-post exercise measure quantified in Senefeld et al. (2020). However, the finding of no difference in fatigability between both groups in the current study suggests that regardless of the time of assessment, contractile properties may not have differed between the groups. Despite the varying results, impairments in contractile properties following exercise were associated with fatigability for both people with prediabetes and controls indicating a muscular origin of fatigability, specifically impairments in skeletal muscle excitation-contraction coupling and/or crossbridge function.

Resting Vascular Function

In the present study, individuals with prediabetes had similar resting vascular function compared with healthy controls. To our knowledge, this study is the first to investigate FMD and reactive hyperemia of the lower limb in people with prediabetes. Of the studies investigating conduit artery endothelial function, only the brachial artery has been studied showing impaired brachial artery FMD in people with prediabetes compared with healthy controls (Caballero et al., 1999; Su et al., 2008). However, it is important to
note that these studies did not match for physical activity which could explain the contradictory findings. Indeed, our findings of similar lower limb macrovascular endothelial function compared with controls are consistent with findings in people with T2D when matching for physical activity (Senefeld et al., 2019). Furthermore, Senefeld et al. (2019) identified a similar brachial artery FMD between controls and people with T2D when matched for physical activity. These findings suggest macrovascular endothelial dysfunction may not be an obligatory consequence of metabolic dysfunction if adequate physical activity is performed.

Impairment of microvascular function in people with prediabetes is not always reported. Studies that have employed post-occlusive reactive hyperemia have identified similar microvascular function in people with prediabetes and healthy controls (Caballero et al., 1999; Su et al., 2008). Post-occlusive reactive hyperemia is thought to be minimally endothelial nitric oxide (NO) dependent and thought to be strongly dependent on the extent of local ischemia and activation of inward rectifying potassium channels and Na⁺/K⁺-ATPase of the vascular smooth muscle cells (Crecelius et al., 2013; Ferreira-Santos et al., 2024; Tagawa et al., 1994). Thus, the current findings of similar microvascular function between individuals with prediabetes and healthy controls are in agreement with previous studies using post-occlusive reactive hyperemia.

**Exercise-Induced Blood Flow**

A novel finding is that exercise-induced blood flow of the lower limb was impaired in individuals with prediabetes compared with matched, healthy controls. This is the first study to investigate exercise-induced blood flow responses in humans with
prediabetes. Previous studies in rodent models of prediabetes identified similar results of an attenuated exercise hyperemic response (Novielli & Jackson, 2014; Novielli-Kuntz et al., 2018). The mechanisms of the attenuated exercise hyperemia were investigated in prediabetic mice by Novielli-Kuntz et al. (2018). The researchers reported elevated neuropeptide Y1 and alpha-1 adrenergic receptor activation during electrically stimulated contractions suggesting impaired functional sympatholysis may be contributing to the impaired exercise hyperemic response in prediabetic mice. Additionally, research in a prediabetic rat model has identified a reduced adenosine triphosphate (ATP) release from red blood cells in low P_{O2} conditions, such as during exercise, which could additionally blunt functional sympatholysis and exercise hyperemia (Ellis et al., 2010). This finding has been replicated in humans with T2D and it is suggested that elevated glycated hemoglobin and insulin result in increased affinity of ATP in red blood cells (Groen et al., 2019). Of interest, our findings of similar NO-mediated endothelial function between people with prediabetes and controls (i.e., FMD) suggest the attenuated exercise-induced blood flow identified in people with prediabetes was not due to NO deficits. Hence, it is possible that lower exercise-induced blood flow identified in the current study could be a result of impaired functional sympatholysis that is mediated by elevated sympathetic, vasoconstrictor activity and/or impaired ATP release from red blood cells.

An association was identified between exercise-induced blood flow as a percent change from baseline and fatigability and impairments in contractile function when combining groups. This suggests that oxygen supply to the working muscle contributed to fatigability and skeletal muscle contractile function. A lower oxygen supply to the muscle during exercise would increase the muscles reliance on anaerobic metabolism and
ultimately lead to a greater accumulation of metabolites known to impair skeletal muscle function and, thus, greater fatigability (Hepple, 2002; Sundberg et al., 2019).

**Muscle Oxygenation Responses**

Muscle oxygenation responses measured by NIRS were similar between people with prediabetes and healthy controls. The StO$_2$ provides a proxy of the balance of oxygen delivery and utilization at the muscle and deoxy[heme] provides a proxy of oxygen extraction (Barstow, 2019). Hence, the results of this study suggest the subset of people with prediabetes and healthy controls with NIRS data were able to similarly match oxygen supply to demand of the working muscle, specifically in the rectus femoris. Rest to exercise deoxy[heme] kinetics were also similar between groups suggesting that the increase in oxygen delivery at the onset of exercise was not impaired in people with prediabetes. This is in contrast to what has been seen in individuals with T2D (Bauer et al., 2007; Wilkerson et al., 2011) suggesting that the degree of metabolic dysfunction in individuals with prediabetes may not be severe enough to impair the balance of oxygen demand to oxygen supply of the muscle during the rest to exercise transition.

The association identified between exercise deoxy[heme] responses and fatigability and impairments in contractile function when combining groups suggest impairments in oxygen extraction at the muscle play a role in increased skeletal muscle fatigue in the combined group. Indeed, a reduced oxygen extraction would contribute to a lower muscle oxygen consumption and greater reliance on anaerobic metabolism. However, whole body and muscle oxygen consumption was not assessed in this study limiting our ability to draw these conclusions.
Sex Differences

Expected sex differences were identified in the current study including females being shorter, having greater % body fat, lower maximal isometric strength, and lower limb power production. However, a few sex differences are worth noting. Females in the current study had a more rapid recovery in lower limb power than males following the fatiguing task (i.e., 1-minute post-exercise). Similar findings have been reported by our group in young, healthy adults (Senefeld et al., 2018) with MVIC torque recovery being greater in females compared to males following a dynamic fatiguing task. In addition, both studies identified males having less recovery of contractile properties (i.e., electrically evoked twitch properties) suggesting this sex difference in recovery is of muscular origin.

Conclusion

This study provides evidence that fatigability of the lower limb during and in response to a high-velocity dynamic exercise may not differ between people with prediabetes and matched, healthy controls. However, this study identified that exercise-induced blood flow was attenuated in people with prediabetes. Future studies are needing to confirm these results and determine the mechanisms of the attenuated exercise-induced blood flow. Resting vascular function of the lower limb did not differ between people with prediabetes and healthy controls. Muscle oxygenation was similar between groups suggesting the balance of muscle oxygen delivery and utilization is not impaired during dynamic fatiguing exercise. Lastly, this data suggests the mechanisms of fatigability in
the combined group were of a muscular origin with contributions from both muscle oxygen supply and extraction.
CHAPTER 3: THE EFFECTS OF DIETARY NITRATE SUPPLEMENTATION ON FATIGABILITY AND RESTING AND EXERCISE VASCULAR FUNCTION IN INDIVIDUALS WITH PREDIABETES

3.1 Introduction

Prediabetes is an intermediate stage of glucose dysregulation, characterized by blood glucose above normoglycemic levels, but below the clinical cutoff for type 2 diabetes (T2D) (ElSayed et al., 2023a). It is estimated that 1 in 3 American adults have prediabetes which independently increases one’s risk for T2D, cardiovascular disease, and all-cause mortality (Cai et al., 2020; Centers for Disease Control and Prevention, 2022; Echouffo-Tcheugui et al., 2023; Huang et al., 2016). Frontline treatment for prediabetes includes lifestyle modification with an emphasis on increased physical activity (Echouffo-Tcheugui et al., 2023). However, we have identified an increased performance fatigability (i.e., acute reduction in strength or power as a result of physical activity and hereon referred to as fatigability) in people with prediabetes which can lead to exercise intolerance, reduced physical function, and can act as a barrier for participation in physical activity (Hunter, 2018; Senefeld, Harmer, et al., 2020). Hence, interventions to improve fatigability in people with prediabetes are important to improve daily function and increase physical activity adherence.

Fatigability during contractile activity of the skeletal muscle can originate from both neural (i.e., proximal to the neuromuscular junction) and muscular (i.e., including and distal to the neuromuscular junction) sites within the neuromuscular system (Gandevia, 2001; Hunter, 2018). Our laboratory previously showed that the increased fatigability in individuals with prediabetes is primarily due to mechanisms within the
muscle leading to impaired skeletal muscle contractile function (Senefeld, Harmer, et al., 2020). A number of impaired physiological functions that accompany metabolic diseases may contribute to an impaired contractile function and possibly a greater reliance on anaerobic metabolism during exercise including attenuated oxygen delivery (Bock et al., 2020; Groen et al., 2019; Kingwell et al., 2003; Novielli & Jackson, 2014; Novielli-Kuntz et al., 2018; Senefeld et al., 2019), reduced mitochondrial function (Mogensen et al., 2007; Petersen et al., 2004; Turner et al., 2022), and impaired skeletal muscle contractile efficiency (Lewis et al., 2019). Nitric oxide (NO) plays a critical role in each of these physiological functions (Bailey et al., 2010; Joyner & Casey, 2015; Larsen et al., 2011). However, previous studies have identified a reduced NO bioavailability in people with prediabetes (Caballero et al., 1999; Su et al., 2008; Vehkavaara et al., 1999). Thus, increasing NO bioavailability is a logical target for improving fatigability in people with prediabetes.

Dietary nitrate (NO$_3^-$) supplementation is a promising, cost-effective intervention to increase NO bioavailability and improve fatigability in individuals with prediabetes (Woessner et al., 2018). When ingested, dietary NO$_3^-$ becomes concentrated in the salivary glands and is reduced to nitrite (NO$_2^-$) (a direct precursor to NO) by bacteria in the oral cavity and thereby enters the plasma following absorption in the small intestine (Jones et al., 2021). Additionally, dietary NO$_3^-$ increases NO$_3^-$ reserves in various tissues including the skeletal muscle following its absorption (Kadach et al., 2023; Park et al., 2023). This is advantageous as muscle NO$_3^-$ stores can be reduced to NO$_2^-$ by enzymes within the muscle such as xanthine oxidase (Piknova et al., 2022). Both plasma and muscle NO$_2^-$ can then readily undergo a one-step reduction to bioactive NO in hypoxic
and low pH conditions such as exercise by multiple sources (e.g., deoxyheme- and
deyomyoglobin, mitochondrial enzymes, and H⁺) (Piknova et al., 2022). This NO₃⁻-
NO₂⁻-NO pathway provides a complimentary pathway to the oxygen dependent NO-
synthase pathway and, when supplemented, increases NO bioavailability.

Dietary NO₃⁻ supplementation has been extensively studied as an ergogenic aid in
healthy young adults. A recent systematic review identified a ~3% performance
enhancing effect from NO₃⁻ supplementation (Senefeld, Wiggins, et al., 2020). The
performance enhancing effect of NO₃⁻ supplementation is thought to result from a
reduced oxygen cost during exercise (Bailey et al., 2009; Lansley et al., 2011; Larsen et
al., 2007; Nyberg et al., 2021; Pawlak-Chaouch et al., 2016; Vahhatalo et al., 2010; Wylie
et al., 2016), increased mitochondrial efficiency (Larsen et al., 2011), improved skeletal
muscle contractile efficiency (Bailey et al., 2010), and enhanced oxygen delivery
(Richards et al., 2018), although the effect of NO₃⁻ supplementation on exercise-induced
limb blood flow has been highly variable in young adults (Craig et al., 2018; Fenuta et
al., 2024; Kim et al., 2015; Nyberg et al., 2021; Thurston et al., 2021). These enhanced
physiological functions are profound considering the normal NO bioavailability in
healthy young adults. This highlights the large ergogenic potential of NO₃⁻ in populations
with attenuated NO bioavailability. Indeed, NO₃⁻ supplementation has been shown to
increase time to claudication onset pain and peak walking time and distance in peripheral
artery disease through increased vascular function and muscle oxygenation during
exercise (Bock et al., 2018; Kenjale et al., 2011; Pekas et al., 2021), improve vascular and
muscle contractile function in older adults (Casey et al., 2015; Coggan et al., 2020;
Coggan, Leibowitz, Kadkhodayan, et al., 2015; Rammoms et al., 2014; Walker et al.,
2019), and increase peak oxygen consumption (Zamani et al., 2015) and time to exhaustion (Eggebeen et al., 2016; Zamani et al., 2015) in individuals with heart failure. However, no studies have been conducted investigating the effects of dietary NO₃⁻ supplementation in individuals with prediabetes.

The effects of dietary NO₃⁻ supplementation have been mixed in individuals with T2D with both no effect (Gilchrist et al., 2013; Shepherd et al., 2015) and positive effects (Bock, Hanson, et al., 2022; Bock, Ueda, et al., 2022; Turner et al., 2022) of NO₃⁻ supplementation being identified. A recent clinical trial investigating the effects of long term (i.e., 8 weeks) combined NO₂⁻ and NO₃⁻ supplementation has produced promising findings in individuals with T2D such as improvements in peak oxygen consumption (Bock, Hanson, et al., 2022), improved functional sympatholysis and exercise-induced blood flow (Bock, Ueda, et al., 2022), and the potential of improved skeletal muscle oxidative capacity, although improvements in oxidative capacity were only identified in a subset of responders (Turner et al., 2022). These findings support the potential of dietary NO₃⁻ supplementation to improve fatigability in individuals with prediabetes.

Thus, the aim of this study was to investigate the effects of short-term (3-days) dietary NO₃⁻ supplementation in the form of beetroot juice on fatigability, exercise-induced blood flow, muscle oxygenation, and resting vascular function in individuals with prediabetes. We hypothesized that 1) dietary NO₃⁻ supplementation would improve fatigability in individuals with prediabetes and 2) we would see parallel improvements in exercise-induced blood flow and muscle oxygenation, as well as improved resting vascular function.
3.2 Materials and Methods

**Experimental Design and Protocol**

This was a randomized, double-blind crossover study (Figure 3.1). Participants reported to the laboratory on four separate occasions including a screening session followed by three experimental sessions. Experimental session 1 involved data collection for Aims 1 and 2 of this dissertation. Experimental sessions 2 and 3 were conducted following either NO₃⁻-rich (nitrate) or placebo NO₃⁻-depleted (placebo) beetroot juice supplementation (Beet It Sport, James White Drinks, Ipswich, UK).

**Participants**

Nine people with prediabetes [5 men, 4 women; Fasting plasma glucose (FPG) = 102 ± 8 mg/dl; Glycated hemoglobin (HbA₁c) = 5.8 ± 0.2 %; Age = 60 ± 15 yr] participated in a double-blind, placebo-controlled, cross-over designed study. All participants provided written informed consent prior to involvement in the study. The experimental protocol was approved by the Marquette University Institutional Review Board (HR-2402) in accordance with the Declaration of Helsinki. Participant characteristics are presented in Table 3.1.
Variable & Males (n = 5) & Females (n = 4) 
\hline 
Age, yr & 58 ± 20 & 64 ± 5 
Height, m & 1.71 ± 0.09 & 1.67 ± 0.07 
Weight, kg & 87 ± 16 & 78 ± 13 
Body mass index, kg/m² & 29.8 ± 4.3 & 28.2 ± 3.9 
Body fat, % & 32.8 ± 4.6 & 39.3 ± 8.7 
Physical activity, steps/day & 6948 ± 3201 & 7428 ± 1907 
Prediabetes duration, yr & 3.5 ± 3.2 & 3.7 ± 2.4 
HbA₁c, % & 5.7 ± 0.2 & 5.9 ± 0.2 
Fasting plasma glucose, mg/dl & 107 ± 3 & 96 ± 9 
Fasting serum insulin, µIU/ml & 15.7 ± 7.1 & 9.4 ± 2.7 
HOMA-IR, a.u. & 4.3 ± 2.1 & 2.3 ± 0.8 
\hline 

Table 3.1. **Participant characteristics.** The sample sizes of each sex are presented in parentheses. Values are presented as mean ± standard deviation. HbA₁c, glycated hemoglobin; HOMA-IR, homeostatic model of insulin resistance.

All participants underwent screening by survey and phone call prior to study inclusion. Prediabetes was defined according to the American Diabetes Association criteria as a FPG of 100 to 125 mg/dL, and/or HbA₁c of 5.7 to 6.4% (ElSayed et al., 2023a). Participants were excluded due to the following: BMI ≥ 40 kg/m², type 1 or type 2 diabetes, uncontrolled hypertension, active cancer, untreated hypothyroidism, current tobacco use, severe arthritis, and any neurological, cardiovascular, or musculoskeletal disease that precluded exercise. Participant comorbidities, conditions, and medications are presented in Table 3.2.
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<thead>
<tr>
<th>Comorbidities &amp; Conditions</th>
<th>n</th>
</tr>
</thead>
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<td>Metformin</td>
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<td>Methimazole</td>
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</tr>
<tr>
<td>Statins</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.2. Participant comorbidities, conditions, and medications. n, number of participants with the comorbidity, condition, or prescribed medication.

**Screening Session**

The screening session was identical to the screening session described in Chapter 2 of this dissertation. In short, the screening session was used to assess anthropometric measurements, body composition using dual x-ray absorptiometry (DXA) (Lunar Prodigy, GE, Madison, WI, USA), HbA\textsubscript{1c} from a finger prick blood sample (DCA 2000+, Siemens Healthcare Diagnostics, Malvern, PA, USA; Afinion 2, Abbott Diagnostics, Scarborough, ME, USA), blood pressure, and administer a triaxial accelerometer (ActiGraph wGT3X-BT, ActiGraph, Pensacola, FL, USA) used to quantify physical activity. In addition, participants were familiarized with the experimental techniques used in experimental sessions 1-3. This included electrical stimulation of the quadriceps muscles, knee extension maximal voluntary isometric contractions (MVIC), MVICs with electrical stimulation (i.e., voluntary activation trials), and knee extension dynamic,
maximal voluntary concentric contractions (MVCC) that were conducted during the
dynamic, fatiguing exercise task.

**Supplementation Procedures**

Supplementation order was assigned in a pseudorandomized, counterbalanced order. Participants reported to the laboratory following either 3 days of NO₃⁻-rich (~13.0 mmol NO₃⁻, 140 mL/day) or NO₃⁻-depleted placebo (0.05 mmol NO₃⁻, 140 mL/day) beetroot juice supplementation. The NO₃⁻ supplementation dose used in this study was considered the optimal dose and duration of supplementation for performance enhancement (Senefeld, Wiggins, et al., 2020; Shannon et al., 2022). Participants were instructed to ingest the supplementation each morning. On the day of testing (i.e., day 3 of supplementation), participants ingested the last 140 mL of supplementation 1.5 hours before arriving at the laboratory and otherwise remained fasted until the conclusion of the session. Following a ≥10-day washout period, participants followed the same supplementation protocol using the alternate supplementation. Participants were asked to abstain from the following prior to and during supplementation: consumption of NO₃⁻-rich foods such as beets and green leafy vegetables 7 days prior the experimental session, mouthwash and tongue scraping 7 days prior the experimental session, and toothpaste 4 hours before and after supplementation.

**Experimental Sessions**

The experimental sessions for this study were identical to the experimental session described in Chapter 2 of this dissertation. Participants arrived at the laboratory
the morning of the experimental session after abstaining from caffeine consumption ≥ 8 hours, and strenuous exercise, alcohol consumption, and non-steroidal anti-inflammatory drugs ≥ 24 hours prior to the experimental session. All participants continued prescribed medication throughout the study and medications were not withheld. Participant medications are included in Table 3.2. The following was conducted during the experimental session: exhaled NO breath analysis, a blood draw (see Blood Sampling and Analysis), flow-mediated dilation (FMD) and post-occlusive reactive hyperemia of the superficial femoral artery, knee extension MVICs with measurements of voluntary activation and muscle contractile properties using electrically evoked contractions, and a 4-minute dynamic fatiguing exercise task with measurements of muscle oxygenation using near-infrared spectroscopy (NIRS) and pre- and immediately post-exercise blood flow through the superficial femoral artery using doppler ultrasonography. Measurement sites for NIRS and ultrasound probe placement were replicated in each session. The NIRS and ultrasound probe placements were recorded by measuring the distance of the probes from the proximal border of the patella in session 2 and the placement was replicated in subsequent sessions. A visual representation of the experimental design is provided in Figure 3.1.
Figure 3.1. Visual schematic of experimental design. Participants underwent a double-blind, placebo-controlled, cross-over designed study. Each participant was randomized to start the study with either 3-days of nitrate-depleted (placebo) or nitrate-rich beetroot juice. Following the first supplementation period and experimental session 2, there was a ≥ 10-day washout period of no supplementation followed by 3-days of the alternate supplementation and subsequent testing. On the morning of experimental sessions 2 and 3, participants consumed the last dose of the assigned supplement 1.5 hours before reporting to the laboratory. At the beginning of the session, a blood draw was conducted to determine plasma nitrate and nitrite concentrations. Thereafter, participants underwent measurements of resting vascular function (i.e. flow-mediated dilation and post-occlusive reactive hyperemia), baseline measurements of knee extension MVIC torque, voluntary activation, and muscle contractile properties as well as measures blood flow and muscle oxygenation at rest. Participants then performed a 4-minute dynamic fatiguing task of the knee extensor muscles in which one MVCC was conducted every 3 seconds against a load of 20% MVIC. Muscle oxygenation was measured during the fatiguing task. Immediately following the fatiguing task, exercise-induced blood flow was measured. Five recovery MVCCs were conducted 1-minute post-exercise and two MVICs with electrically stimulation were performed at 1.5- and 2-minutes post-exercise to measure recovery MVIC torque, voluntary activation, and muscle contractile properties. MVIC, maximal voluntary isometric contraction; MVCC, maximal voluntary concentric contraction.

Exhaled Nitric Oxide

Nitric oxide in the exhaled breath was measured using a fractional exhaled NO point-of-care analyzer (NIOX VERO, Circassia, Morrisville, NC, USA). Exhaled NO was measured according to published guidelines (American Thoracic & European
Respiratory, 2005) in sessions 3 and 4 and investigators were blinded to exhaled NO results.

*Flow-Mediated Dilation and Post-Occlusive Reactive Hyperemia*

Participants rested while lying supine for ≥ 20 minutes in a dimly lit, temperature-controlled room (~21°C). Doppler ultrasound measurements of superficial femoral artery diameter and blood velocity were obtained ~2.5 cm distal to the common femoral artery bifurcation using a 9-MHz liner probe (Vivid e95, General Electrics, Madison, WI, USA). Artery diameter was measured in B-mode with the ultrasound probe perpendicular (90°) to the vessel. Blood flow was measured using duplex mode at an insonation angle of 60° and a frequency of 5 MHz. The sample gate size was set to 2 mm for all participants and was positioned in the center of the vessel. A 3-lead electrocardiogram recording was obtained concurrently with ultrasound measurements.

Baseline superficial femoral artery diameter and blood flow were measured for 30 seconds following ≥ 20 minutes of rest. A 12 cm pneumatic cuff was then placed around the upper leg distal to the ultrasound probe. The cuff was rapidly inflated (Hokanson Rapid Cuff Inflation System, D.E. Hokanson, Bellevue, WA, USA) to a suprasystolic pressure (250 mmHg) and remained inflated for 5 minutes, occluding blood flow to the distal portion of the leg. Blood flow was measured for 15 seconds prior to cuff deflation and for 30 seconds immediately post-cuff deflation to determine peak post-occlusive reactive hyperemia (RH_peak) and shear rate. Blood flow was calculated using the following equation:

\[
BF = MBV \times \left[ \frac{\pi \times (D/2)^2}{60} \right]
\]
where BF is blood flow in mL/min, MBV is mean blood velocity in cm/sec, D is artery diameter in cm, and 60 is used to convert mL/sec to mL/min (Limberg et al., 2020). In addition, shear rate was calculated using the following equation:

\[
\text{Shear rate} = 4 \times (\text{MBV}) \times (\text{D})^{-1}
\]

where MBV is mean blood velocity in cm/sec and D is artery diameter in cm.

Following the 30 seconds of post-cuff deflation blood flow measures, vessel diameter was measured continuously using B-mode until 5 minutes post-cuff deflation to determine changes in vessel diameter. End-diastole vessel diameter was measured by a single rater using edge detection software (Brachial Anaylzer, Medical Imaging Applications, Coralville, IA, USA). Post-cuff deflation vessel diameter measurements were averaged into 5-second bins. The average vessel diameter at baseline and the highest 5-second average post-cuff deflation were used to calculate FMD using the following equation:

\[
\text{FMD} (\%) = \left[ (\text{Peak Diameter} - \text{Baseline Diameter}) / \text{Baseline Diameter} \right] \times 100
\]

The flow-mediated vasodilatory response is dependent on NO bioavailability and represents endothelial function of the conduit artery (Thijssen et al., 2011). Blood flow post-cuff deflation was quantified using the time-averaged mean for each cardiac cycle (beat-by-beat) and a 3-cardiac cycle rolling average was applied to the data. The highest 3-cardiac cycle average was used as the RH\text{peak}. The post-occlusive reactive hyperemic response is considered an index of microvascular function (Limberg et al., 2020).

*Maximal Strength, Voluntary Activation, and Contractile Properties*

Participants were seated upright at 70° of hip flexor in a dynamometer (Biodex System 4, Biodex Medical, Shirely, NY, USA). The knee angle was set to 90° of flexion
and the axis of rotation of the knee was align with the axis of rotation of the
dynamometer motor. Padded shoulder and waist straps were used to secure the
participants in the chair and the leg was strapped to the dynamometer lever arm proximal
to the malleoli using a non-compliant strap. Mechanical recordings from the
dynamometer including torque, velocity, and position data were digitized and sampled at
500 Hz and stored online (Power 1401 A/D Converter and Spike 2, Cambridge
Electronics Design, Cambridge, UK).

Custom made pad electrodes (~5 cm × 15 cm) were used for the stimulation of the
knee extensor muscles. A constant current stimulator (DS7AH, Digitimer, Welwyn
Garden City, Hertortshire, UK) was used to deliver two 200 µs square wave pulses at 100
Hz to electrically evoke contractions of knee extensor muscles. A ramp protocol was used
to identify the stimulator current at which the electrically evoked twitch amplitude
plateaued. The stimulator current started at 50 mA and increased incrementally by 50 mA
until the twitch plateau occurred. The current was then increased 20% to ensure
supramaximal stimulation.

Following the stimulation ramp protocol, participants performed a minimum of
three brief (3 seconds) MVICs to assess maximal strength of the knee extensor muscles.
Visual feedback on the computer monitor and strong verbal encouragement were
provided during each MVIC. Participants were given ≥ 60 seconds between each MVIC
to ensure adequate rest between maximal efforts. MVICs were repeated until two trials
were within 5% of each other. Maximal voluntary isometric contraction peak torque was
quantified as the highest 500 ms mean value during the MVIC.
Next, a minimum of four MVICs were conducted with the addition of electrically
evoked twitches delivered at the MVIC torque plateau (superimposed twitch) and at rest
~2 seconds after the MVIC (resting potentiated twitch). Voluntary activation was
calculated using the following equation:

\[
\text{Voluntary Activation (\%)} = (1 - \frac{SIT}{Q_{tw}}) \times 100
\]

where SIT is the amplitude of the superimposed twitch and \(Q_{tw}\) is the amplitude of the
resting potentiated twitch (Allen et al., 1995). Contractile properties of the knee extensor
muscles were determined from each resting potentiated twitch including twitch amplitude
\(Q_{tw}\), rate of torque development (RTD), rate of torque relaxation (RTR) and half-
relaxation time (HRT). The \(Q_{tw}\) was measured as an index of the force-generating
capacity of the muscle. The RTD and RTR were quantified with the derivative of the
torque signal as the highest rate of torque increase (RTD) and decrease (RTR) over a 10-
ms interval. Lastly, the HRT was determined as the amount of time (ms) it took for the
torque signal to reach half of the peak torque amplitude during twitch relaxation. Baseline
voluntary activation and contractile properties are reported as the median value of the
best four trials. Post-exercise recovery MVIC, voluntary activation, and contractile
properties were assessed at 1.5- and 2-minutes post-exercise.

**Dynamic Exercise Task**

While still positioned in the dynamometer as described above, participants
performed a 4-minute dynamic, fatiguing exercise task of the knee extensor muscles that
consisted of 80 maximal-velocity concentric contractions (1 contraction every 3 seconds).
Participants were verbally cued to kick out every 3 seconds and lifted a load equivalent to
20% of MVIC through a 95° range of motion. Following knee extension, participants
were instructed to fully relax and the leg was passively returned to the starting position (90°) by the investigator to avoid mechanical work performed by the knee flexor muscles. Participants were instructed to kick “as fast as possible” through the entire range of motion with every kick and were provided strong verbal encouragement throughout the entire fatiguing exercise task. At 1-minute post-exercise, participants performed five additional maximal velocity contractions to determine post-exercise recovery of the knee extensor power. Participants were habituated to the maximal-velocity contractions followed by ~10 minutes of rest before conducting the fatiguing exercise task. Fatigability as a result of the exercise task was quantified as the percent reduction in limb power during the 4-minute exercise task. Baseline power output was determined as the highest average power over five consecutive contractions within the first ten contractions of the fatiguing exercise task. End-exercise power output was determined as the average power of the last five contractions of the fatiguing exercise task. Knee extensor mechanical power output (W) was calculated as the product of the torque (N·m) and angular velocity (rad/sec) and was quantified as the mean value during the concentric phase of the muscle contraction.

*Exercise-Induced Blood Flow*

Prior to the fatiguing exercise task, resting blood flow through the superficial femoral artery was measured using Doppler ultrasonography as previously described. In short, the probe was placed ~4 cm distal to the common femoral bifurcation. The probe was placed slightly more distal than for FMD measures due to limited access to the upper thigh as a result of the seated position used for exercise. Baseline blood flow prior to exercise was measured for 30 seconds after ≥ 5 minutes of rest. Immediately following
the 4-minute fatiguing exercise task, post-exercise blood flow was measured beat-by-beat and a 3-cardiac cycling rolling average was applied to the data. Due to difficulty obtaining clear images for the measurement of vessel diameter in Duplex mode, post-exercise diameter was measured in B-mode immediately following post-exercise recovery MVIC, voluntary activation, and contractile properties and was used to quantify blood flow. Change in blood flow as a result of exercise is presented as an absolute change and as a percent change.

*Near-infrared Spectroscopy*

Relative changes rectus femoris oxygenated myo/hemoglobin (oxy[heme]), deoxygenated myo/hemoglobin (deoxy[heme]), and total myo/hemoglobin (total[heme]) were obtained using multi-channel, continuous wave near-infrared spectroscopy (NIRS) (Portamon, Artinis, Amsterdam, Netherlands). Of note, myoglobin is considered to contribute minimally to NIRS oxygenation responses during exercise compared to hemoglobin (Barstow, 2019). The spatial resolved spectroscopy approach was used to determine muscle oxygenation (StO₂) which was quantified from oxy[heme] and total[heme]. The probe emitted near-infrared light from three optodes spaced at 30, 35, and 40 mm from the receiver. Each optode emitted 760 and 850 nm wavelengths of light which are specific to the chromophores deoxy[heme] and oxy[heme]. The site of the NIRS probe was shaved and cleaned with alcohol prior to probe placement. The NIRS probe was secured to the skin over the rectus femoris using double side tape along the edge of the NIRS probe and was wrapped with an opaque elastic bandage to reduce ambient light contamination. All signals were sampled at 10 Hz. Due to movement artifact from the maximal velocity contractions, a rolling 3-second median filter was used
to smooth the data prior to analysis. Signals measured as relative changes (e.g. 
deoxy[heme]) were analyzed as the change in the signal during exercise from rest (i.e., 30 
second rest period prior to the initiation of exercise).

*Adipose Tissue Thickness*

Adipose tissue thickness was measured at the sight of the NIRS probe with 
ultrasound using a 9 MHz liner probe in B-mode (Vivid e95, General Electric, Madison, 
WI, USA). The ultrasound probe was placed on the skin and minimum pressure was used 
to avoid compression of adipose tissue. The light emitted from the NIRS probe reaches 
an estimated depth of half the transmitter-receiver distance. With the shortest path-length 
of our probe being 30 mm, this depth was 15 mm. Data from any participants with greater 
than 15 mm of adipose tissue thickness were excluded from the NIRS analysis (n = 2).

*Blood Sampling and Analysis*

A fasting blood sample was obtained at the beginning of experimental session 1 to 
assess fasting plasma glucose using a point of care instrument (Cholestech LDX, Abbott 
Diagnostics, Scarborough, ME, USA) and fasting insulin. Serum blood samples were 
analyzed in duplicate according manufacturer instructions to determine fasting insulin 
centations using an enzyme-linked immunosorbent assay kit (Human Insulin ELISA 
Kit, Invitrogen, Waltham, MA, USA). Homostatic model of insulin resistance (HOMA-
IR) was then calculated from the following equation:

\[
\text{HOMA-IR} = \frac{[\text{fasting insulin (uIU/mL)} \times \text{FPG (mg/dL)}]}{405}
\]

A blood sample was obtained in a tube containing K2-EDTA at the beginning of 
experimental sessions 2 and 3 to determine plasma NO\textsubscript{3} and NO\textsubscript{2} following
supplementation. Blood samples were immediately centrifuged at 1500 g for 10 min and plasma was aliquoted in 300 µL samples and stored at -80°C. For plasma NO$_3^-$ and NO$_2^-$ analysis, plasma samples were thawed to room temperature and analyzed within 10 min. Plasma NO$_3^-$ and NO$_2^-$ were assessed using an ozone-based chemiluminescence nitric oxide analyzer (Sievers NOA model 280i, General Electric Analytical Instruments, Boulder, CO, USA). Plasma NO$_2^-$ was determined using the reductant potassium iodide in acetic acid at room temperature and plasma NO$_3^-$ was determined using the reductant vanadium (III) chloride in hydrochloric acid at 90°C. Plasma NO$_3^-$ and NO$_2^-$ concentrations were determined using a standard curve derived from known concentrations of sodium NO$_3^-$ or sodium NO$_2^-$. The area under the curve of the voltage signal was determined using R statistical software. Due to equipment issues, plasma NO$_3^-$ analysis was only conducted for two participants. We were unable to obtain blood samples in two participants during experimental sessions 2 and 3. Thus, plasma NO$_2^-$ is only reported for seven participants.

**Statistical Analysis**

Data are reported as means ± SD in the text and tables and are displayed as means ± SEM in the figures. The normality and homogeneity of variance of the data were assessed using Shapiro-Wilk and Leven’s tests, respectively. Separate mixed model two-way ANOVAs with condition (placebo vs. nitrate) as a within-subject factor and sex (male vs. female) as a between-subject factor were used to determine the sex-dependent effects of NO$_3^-$ supplementation on baseline, post-occlusion/exercise, and relative changes in neuromuscular, vascular, and muscle oxygenation measurements in response
to either the 5-minute occlusion and dynamic exercise task. Preliminary analysis revealed no sex-differences in the effects of nitrate supplementation on any of the primary outcome measures. Thus, males and females were pooled to increase statistical power. Separate paired samples t-tests were used to determine differences between placebo and NO\textsubscript{3} conditions for baseline, post-occlusion/exercise, and relative changes in neuromuscular, vascular, and muscle oxygenation measurements in response to either the 5-minute occlusion and dynamic exercise task. Effect sizes are present as Cohen’s \(d\). The direction of the effect is reported so that a negative Cohen’s \(d\) reflects a higher placebo value compared to NO\textsubscript{3}. Significance was determined at \(P < 0.05\) and all analyses were performed with JASP version 0.17.3.

3.3 Results

*Exhaled Nitric Oxide and Plasma Nitrate and Nitrite*

Exhaled nitric oxide (NO) was greater in the nitrate condition compared to the placebo \((46.2 \pm 30.6\) vs. \(35.9 \pm 39.7\) ppb; \(P = 0.044, d = 0.797\)). Plasma nitrate (NO\textsubscript{3}) was greater in the nitrate condition compared to the placebo condition for the 2 participants with analyzed data \((903.3 \pm 557.9\) vs. \(41.6 \pm 14.2\) \(\mu\)mol; Figure 3.2). Plasma nitrite (NO\textsubscript{2}) was also greater in the nitrate condition compared to the placebo condition \((132.4 \pm 86.3\) vs. \(24.3 \pm 12.3\) nmol; \(P = 0.010, d = 1.392\); Figure 3.1).
Figure 3.2. Plasma nitrate and nitrite following placebo and nitrate supplementation. Plasma nitrate (n = 2) (A) and nitrite (n = 7) (B) increased as a result of nitrate supplementation. Statistical analysis was not conducted on plasma nitrate due to the small sample size. Values are presented as mean ± standard error of the mean. Individual data points are presented as open circles. *, P < 0.05.

Resting Vascular Function

Vascular measures at rest and following cuff occlusion are presented in Table 3.3. Resting blood pressure did not differ between placebo and NO$_3^-$ supplementation for systolic blood pressure (126.3 ± 19.3 vs. 131.2 ± 14.3 mmHg; $P = 0.305$, $d = 0.365$), diastolic blood pressure (75.2 ± 9.6 vs. 76.9 ± 7.8 mmHg; $P = 0.502$, $d = 0.235$), and mean arterial pressure (92.2 ± 12.4 vs. 95.0 ± 9.1 mmHg; $P = 0.388$, $d = 0.304$). Placebo and NO$_3^-$ conditions did not differ in superficial femoral artery diameter ($P = 0.282$, $d = 0.412$) and blood flow at rest ($P = 0.378$, $d = 0.332$). Following cuff occlusion, placebo and NO$_3^-$ conditions did not differ in FMD ($P = 0.724$, $d = -0.130$; Figure 3.3), RH$_{peak}$ ($P = 0.326$, $d = -0.373$; Figure 3.3), and peak shear rate ($P = 0.281$, $d = -0.413$). There was
no association between FMD as a percent change and peak shear rate ($P = 0.841$, $r = 0.06$).

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<th>Nitrate (n = 8)</th>
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<td>Resting diameter, mm</td>
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<td>6.42 ± 0.73</td>
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<tr>
<td>Resting blood flow, ml·min$^{-1}$</td>
<td>95.6 ± 17.6</td>
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<td>RH$_{peak}$, ml·min$^{-1}$</td>
<td>1,602.2 ± 447.5</td>
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<tr>
<td>Peak shear rate, s$^{-1}$</td>
<td>546.9 ± 138.6</td>
<td>495.0 ± 163.7</td>
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<td>FMD diameter Δ, mm</td>
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<td>0.14 ± 0.01</td>
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<tr>
<td>FMD, %Δ</td>
<td>2.35 ± 2.20</td>
<td>2.05 ± 2.05</td>
</tr>
</tbody>
</table>

Table 3.3. Superficial femoral artery measurements at rest and after cuff occlusion following placebo and nitrate supplementation. Placebo and nitrate conditions did not differ for any vascular measurements at rest and following the occlusion ($P > 0.05$). The sample sizes are presented in parentheses. RH$_{peak}$, peak post-occlusive reactive hyperemia; FMD, flow-mediated dilation. Values are presented as mean ± standard deviation.

Figure 3.3. Superficial femoral artery flow-mediated dilation and peak post-occlusive reactive hyperemia following placebo and nitrate supplementation. Placebo and nitrate conditions did not differ for flow-mediated dilation (A) and peak post-occlusive reactive hyperemia (B) ($n = 8$; $P > 0.05$). Values are presented as mean ± standard error of the mean. Individual data points are presented as open circles.
Baseline Neuromuscular Function

Baseline MVIC torque and baseline mechanical outputs for MVCCs at the beginning of the fatiguing exercise task including MVCC torque, velocity, power, and range of motion following placebo and NO₃⁻ supplementation are presented in Table 3.4. Placebo and NO₃⁻ conditions did not differ in baseline MVIC torque ($P = 0.649, d = -0.158$) and in baseline MVCC mechanical outputs including torque ($P = 0.931, d = -0.030$), velocity ($P = 0.942, d = -0.025$), and power ($P = 0.531, d = 0.218$). However, NO₃⁻ supplementation resulted in a higher range of motion at the beginning of the fatiguing exercise task compared to placebo supplementation ($P = 0.037, d = 0.830$).

Voluntary activation at baseline did not differ between placebo and NO₃⁻ conditions (95.3 ± 6.5 vs. 94.8 ± 4.0 %; $P = 0.621, d = -0.183$). Placebo and NO₃⁻ conditions did not differ in all baseline measurements of contractile properties including $Q_{tw}$ (70.7 ± 20.8 vs. 70.4 ± 18.6 Nm; $P = 0.882, d = -0.051$), RTD (721.5 ± 221.8 vs. 764.8 ± 269.5 Nm·s⁻¹; $P = 0.247, d = 0.416$), RTR (393.4 ± 209.7 vs. 362.0 ± 158.0 Nm·s⁻¹; $P = 0.215, d = -0.449$), and HRT (101.0 ± 27.2 vs. 105.4 ± 27.2 ms; $P = 0.290, d = 0.378$).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC Torque, Nm</td>
<td>180.8 ± 44.1</td>
<td>179.3 ± 46.6</td>
</tr>
<tr>
<td>MVCC Torque, Nm</td>
<td>47.6 ± 10.9</td>
<td>47.5 ± 11.5</td>
</tr>
<tr>
<td>Velocity, °/s</td>
<td>199.6 ± 24.6</td>
<td>199.4 ± 24.5</td>
</tr>
<tr>
<td>Power, W</td>
<td>162.4 ± 54.3</td>
<td>165.0 ± 57.0</td>
</tr>
<tr>
<td>Range of motion, °</td>
<td>89.7 ± 4.4</td>
<td>90.8 ± 4.2*</td>
</tr>
<tr>
<td>Fatigability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVCC Torque, %Δ</td>
<td>-6.7 ± 4.7</td>
<td>-8.8 ± 5.4</td>
</tr>
<tr>
<td>Velocity, %Δ</td>
<td>-27.8 ± 20.5</td>
<td>-26.2 ± 20.8</td>
</tr>
<tr>
<td>Power, %Δ</td>
<td>-32.3 ± 18.6</td>
<td>-31.9 ± 20.7</td>
</tr>
<tr>
<td>Range of motion, %Δ</td>
<td>-18.2 ± 17.9</td>
<td>-16.8 ± 5.6</td>
</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVIC Torque, %Δ</td>
<td>-20.7 ± 6.1 (8)</td>
<td>-22.4 ± 9.2 (8)</td>
</tr>
<tr>
<td>MVCC Torque, %Δ</td>
<td>-5.2 ± 4.8</td>
<td>-6.2 ± 5.5</td>
</tr>
<tr>
<td>Velocity, %Δ</td>
<td>-10.2 ± 13.8</td>
<td>-10.2 ± 15.4</td>
</tr>
<tr>
<td>Power, %Δ</td>
<td>-13.0 ± 15.5</td>
<td>-14.3 ± 18.0</td>
</tr>
<tr>
<td>Range of motion, %Δ</td>
<td>-7.3 ± 13.2</td>
<td>-5.7 ± 10.3</td>
</tr>
</tbody>
</table>

Table 3.4. Baseline, fatigability (i.e., end-exercise) as a percent change from baseline, and recovery as a percent change from baseline in MVIC torque and mechanical outputs for MVCC contractions including MVCC torque, velocity, power and range of motion following. Placebo and nitrate conditions did not differ for any of the above variables except baseline range of motion. Recovery measures for MVCCs were conducted at 1-min post-exercise. Recovery MVICs were conducted at 1.5- and 2-min post-exercise. The highest recovery MVIC trial was used for analysis. The sample sizes of each condition for certain variables are presented in parentheses. Values are presented as mean ± standard deviation. MVIC, maximal voluntary isometric contraction; MVCC, maximal voluntary concentric contraction. *, P < 0.05.

Fatigability and Recovery of MVCC Mechanics and MVIC Torque

Fatigability and recovery as a percent change from baseline in mechanical outputs including MVCC torque, velocity, power, and range of motion as well as MVIC torque are presented in Table 3.4. Placebo and NO₃⁻ conditions did not differ in fatigability (% reduction from baseline) in MVCC power (P = 0.835, \(d = -0.072\); Figure 3.4). Placebo and NO₃⁻ conditions also did not differ in percent reductions of MVCC torque (P = 0.293, \(d = 0.375\)), velocity (P = 0.388, \(d = -0.304\)), and range of motion (P = 0.393, \(d = -0.301\)).
For recovery MVCCs, placebo and NO₃⁻ conditions did not differ in recovery (% change from baseline) MVCC power ($P = 0.651$, $d = 0.152$), torque ($P = 0.670$, $d = 0.147$), velocity ($P = 0.970$, $d = 0.013$), and range of motion ($P = 0.542$, $d = -0.212$). Lastly, the reduction in MVIC torque from baseline (%) did not differ between placebo and nitrate conditions ($P = 0.276$, $d = 0.418$).

**Figure 3.4. Fatigability following placebo and nitrate supplementation.** Contraction by contraction MVCC power output as a percent of baseline for people with prediabetes (open circles) and controls (black circles) (A) and fatigability as a percent reduction from baseline with individual data points presented as open circles (B). Placebo and nitrate conditions did not differ in fatigability ($P > 0.05$). Values are presented as mean ± standard error of the mean.

**Recovery Voluntary Activation and Contractile Properties**

The change in voluntary activation from pre- to post-exercise did not differ between the placebo and NO₃⁻ conditions (6.4 ± 5.1 vs. 6.0 ± 5.9 %; $P = 0.850$, $d = -0.069$). In addition, placebo and NO₃⁻ conditions did not differ in the percent change of contractile from baseline to post exercise including $Q_{tw}$ (-6.3 ± 12.8 vs. -5.9 ± 10.4 %; $P = 0.861$, $d = 0.060$), RTD (-6.5 ± 15 vs. -7.7 ± 13.4 %; $P = 0.656$, $d = 0.154$), RTR (-10.6
± 26.8 vs. -4.1 ± 35.0 %; $P = 0.369, d = -0.318$), and HRT (12.0 ± 33.3 vs. 8.0 ± 30.7 %; $P = 0.514, d = 0.228$).

**Exercise-Induced Blood Flow**

Vascular measures at rest and following exercise are presented in Table 3.5. Superficial femoral artery diameter at rest was similar between the placebo and NO$_3^-$ conditions ($P = 0.755, d = 0.108$). Blood flow at rest also did not differ between the placebo and NO$_3^-$ conditions ($P = 0.939, d = 0.026$). The exercise-induced blood flow response did not differ between the placebo and NO$_3^-$ conditions for post-exercise blood flow ($P = 0.863, d = -0.059$; Figure 3.5) and blood flow as a percent change from rest ($P = 0.706, d = -0.130$; Figure 3.5).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting diameter, mm</td>
<td>6.08 ± 0.83</td>
<td>6.10 ± 0.85</td>
</tr>
<tr>
<td>Resting blood flow, ml·min$^{-1}$</td>
<td>90.8 ± 34.2</td>
<td>91.6 ± 26.6</td>
</tr>
<tr>
<td>End-exercise blood flow, ml·min$^{-1}$</td>
<td>873.1 ± 187.8</td>
<td>868.6 ± 200.9</td>
</tr>
<tr>
<td>Blood flow, Δ%</td>
<td>991.4 ± 464.7</td>
<td>927.6 ± 398.3</td>
</tr>
</tbody>
</table>

**Table 3.5. Superficial femoral artery measurements at rest and after exercise following placebo and nitrate supplementation.** Placebo and nitrate conditions did not differ in all resting and exercise blood flow measures ($P > 0.05$). Values are presented as mean ± standard deviation.
Figure 3.5. Exercise-induced blood flow immediately post-exercise and as a percent change from baseline following placebo and nitrate supplementation. Placebo and nitrate conditions did not differ in end-exercise blood flow (A) and blood flow as a percent change (B) \( (P > 0.05) \). Values are presented as mean ± standard error of the mean. Individual data points are presented as open circles.

Near-Infrared Spectroscopy

Data obtained from NIRS at rest and during exercise are presented in Table 2.6. The StO2 did not differ between placebo and NO3⁻ conditions for rest \( (P = 0.185, d = 0.566) \), exercise nadir \( (P = 0.262, d = 0.468) \), end-exercise \( (P = 0.670, d = 0.169) \), and the average exercise response \( (P = 0.402, d = 0.341; \text{Figure 3.6}) \). Deoxygenated myo/hemoglobin as a change from baseline also did not differ between placebo and NO3⁻ conditions for exercise maximum \( (P = 0.458, d = 0.300) \), end-exercise \( (P = 0.723, d = -0.140) \), and the average exercise response \( (P = 0.871, d = 0.064; \text{Figure 3.6}) \).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n = 7)</th>
<th>Nitrate (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>StO$_2$, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>60.8 ± 2.4</td>
<td>63.8 ± 6.5</td>
</tr>
<tr>
<td>Exercise nadir</td>
<td>41.9 ± 9.8</td>
<td>45.6 ± 8.6</td>
</tr>
<tr>
<td>End-exercise</td>
<td>51.1 ± 6.9</td>
<td>52.2 ± 6.9</td>
</tr>
<tr>
<td>Average exercise response</td>
<td>48.4 ± 7.3</td>
<td>50.5 ± 7.6</td>
</tr>
<tr>
<td><strong>Deoxy[heme], Δ a.u.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise maximum</td>
<td>6.3 ± 5.9</td>
<td>6.9 ± 4.8</td>
</tr>
<tr>
<td>End-exercise</td>
<td>4.9 ± 5.6</td>
<td>4.6 ± 4.7</td>
</tr>
<tr>
<td>Average exercise response</td>
<td>4.8 ± 4.7</td>
<td>4.9 ± 3.9</td>
</tr>
</tbody>
</table>

**Table 3.6. Near-infrared spectroscopy responses at rest and during exercise following placebo and nitrate supplementation.** Placebo and nitrate conditions did not differ in all variables. The sample sizes are presented in parentheses. StO$_2$, tissue oxygen saturation; Deoxy[heme], deoxygenated myo/hemoglobin. Values are presented as mean ± standard deviation.
Figure 3.6. Near-infrared spectroscopy responses at rest and during exercise following placebo and nitrate supplementation. Placebo (open circles) and nitrate conditions (black circles) did not differ in StO\textsubscript{2} (A) and deoxy[heme] (B) responses at rest and during exercise ($P > 0.05$). StO\textsubscript{2}, tissue oxygen saturation; Deoxy[heme], deoxygenated myo/hemoglobin; a.u., arbitrary units. Values are presented as mean ± standard error of the mean.

3.4 Discussion

The purpose of this study was to determine the effects of short term (3-days) dietary NO\textsubscript{3}⁻ supplementation on fatigability, exercise-induced blood flow, muscle oxygenation, and resting vascular function in people with prediabetes. We hypothesized
that dietary NO$_3^-$ supplementation would improve fatigability, as well as exercise-induced blood flow, muscle oxygenation, and resting vascular function. The main finding of this study is that dietary NO$_3^-$ supplementation did not improve fatigability in individuals with prediabetes. In addition, exercise-induced blood flow and muscle oxygenation, as well as resting vascular function as assessed with FMD and post-occlusive reactive hyperemia did not improve with short-term dietary NO$_3^-$ supplementation. These results suggest three days of NO$_3^-$ supplementation is not an effective intervention to improve lower limb fatigability and vascular function in individuals with prediabetes.

**Performance Fatigability**

Short-term dietary NO$_3^-$ supplementation did not improve lower limb fatigability in individuals with prediabetes. Results from previous studies investigating the effect of NO$_3^-$ supplementation on knee extensor muscle fatigability are mixed. For example, Bailey et al. (2010) found that 6 days of NO$_3^-$ supplementation in young males resulted in a 25% increase in time to task failure for dynamic knee extension exercise compared with the placebo condition. However, other studies using both dynamic and isometric fatiguing tasks of the knee extensor muscles have shown no effect of a single dose (~12 mmol the day of testing) of NO$_3^-$ supplementation on knee extension fatigability in young males (Kadach et al., 2023; Le Roux-Mallouf et al., 2019). Similar findings were identified in older women with no effect of a single dose (~13 mmol the day of testing) of NO$_3^-$ supplementation on knee extensor fatigability (Zoughaib et al., 2023). One potential reason for the discrepancies in findings could be the duration of NO$_3^-$ supplementation (i.e., 6-days vs. single dose). This suggests that the use of a 3-day NO$_3^-$ supplementation
period in the present study may have resulted in a lack of effects. Lastly, the low sample size of the present study could have resulted in type II statistical error; however, this is unlikely with NO₃⁻ supplementation showing little to no magnitude of effect ($d = -0.072$).

**Exercise-Induced Blood Flow and Muscle Oxygenation**

Nitric oxide is understood to play a crucial role in vasomotor regulation during exercise (Joyner & Casey, 2015). However, studies investigating the effect of dietary NO₃⁻ supplementation on exercise hyperemic responses have been highly variable with NO₃⁻ supplementation resulting in increased (Richards et al., 2018), decreased (Nyberg et al., 2021; Thurston et al., 2021), and similar (Craig et al., 2018; Fenuta et al., 2024; Hughes et al., 2020; Kim et al., 2015) blood flow responses compared with placebo supplementation. Our current results demonstrate that NO₃⁻ supplementation had no effect on exercise-induced blood flow in people with prediabetes. Contrary to our findings, Bock et al. (2022) found that NO₃⁻/NO₂⁻ supplementation improved exercise-induced blood flow in the forearm in people with T2D which was mediated by an improved functional sympatholysis (Bock, Ueda, et al., 2022). However, this study used a 2-month supplementation period, far greater than the 3-day supplementation in the present study. Both the prolonged supplementation period and the use of NO₂⁻ within the supplementation (i.e., NO₃⁻/NO₂⁻ vs. NO₃⁻ alone) could have contributed to the discrepancies with our current findings.

Fractional oxygen extraction is thought to be reflected in deoxy[heme] responses during exercise (Barstow, 2019). Similar to conduit artery exercise-induced blood flow responses, previous studies using NIRS in young adults have identified variable
responses in deoxy[heme] during exercise following NO$_3^-$ supplementation (Bailey et al., 2009; Breese et al., 2017; Craig et al., 2018). Specifically in the knee extensor muscles, NO$_3^-$ supplementation was shown to attenuate maximal exercise deoxy[heme] of the vastus lateralis during moderate-intensity cycling exercise (Bailey et al., 2009); however, NO$_3^-$ supplementation had no impact on deoxy[heme] responses in the vastus lateralis during severe-intensity cycling exercise (Bailey et al., 2009; Breese et al., 2017). Interestingly, when measuring deoxy[heme] responses in the vastus lateralis, vastus medialis, and rectus femoris independently, NO$_3^-$ supplementation showed no effect on deoxy[heme] responses in each individual muscle (Breese et al., 2017). However, when averaging the deoxy[heme] response across the three muscles, NO$_3^-$ supplementation resulted in greater deoxy[heme] responses suggesting increased oxygen extraction in the quadriceps muscle group during severe-intensity cycling exercise (Breese et al., 2017). Reductions in muscle oxygenation were also shown to be attenuated during cycling exercise as a result of NO$_3^-$ supplementation when averaging responses from the vastus lateralis and rectus femoris (Cocksedge et al., 2020). Thus, the use of a single measurement site (i.e., rectus femoris) could explain the lack of effect from NO$_3^-$ supplementation on deoxy[heme] and StO$_2$ responses in the current study.

Resting Vascular Function

Recent studies in older adults and individuals with peripheral artery disease have shown promising results from NO$_3^-$ supplementation by improving both macrovascular and microvascular function in the lower limb (Bock et al., 2018; Pekas et al., 2023; Pekas et al., 2021; Walker et al., 2019). However, the findings of the present study do not
suggest a beneficial effect of a 3-day NO$_3^-$ supplementation on resting vascular function in people with prediabetes. Studies conducted in groups with metabolic dysfunction (i.e., T2D and obesity) have identified no nitrate-mediated improvements in vascular function in both the brachial and femoral arteries (Bock, Ueda, et al., 2022; Gilchrist et al., 2013; Smeets et al., 2022). Elevated oxidative stress in metabolic dysfunction could attenuate improvements in vascular function from NO$_3^-$ supplementation due to degradation of NO (Rush et al., 2005). However, this seems unlikely due to the observed improvements in vascular function as a result of NO$_3^-$ supplementation in other populations with elevated oxidative stress such as peripheral artery disease and older adults (Bock et al., 2018; Pekas et al., 2023; Pekas et al., 2021; Walker et al., 2019). An additional explanation for the lack of improvements in vascular function could be an impaired gastric reduction of NO$_2^-$ to NO in individuals with metabolic dysfunction due to an elevated gastric pH (Hasler et al., 2008) and reduced gastric ascorbic acid (Wilson et al., 2017). Gastric acidity and gastric ascorbic acid levels have been suggested to play a key role in NO$_2^-$ to NO conversion in the stomach (Bahadoran et al., 2021). Consequently, an impaired gastric NO production would reduce increases in basal NO from NO$_3^-$ supplementation and, thus, attenuate improvements in resting vascular function.

**Conclusion**

In summary, the results of this study suggest short-term (3-day) NO$_3^-$ supplementation does not improve fatigability as well as exercise-induced blood flow, muscle oxygenation, and resting vascular function in individuals with prediabetes. Future research should investigate the effect of prolonged NO$_3^-$ supplementation in individuals
with prediabetes due to the demonstrated improvements in exercise-induced blood flow and cardiorespiratory fitness in individuals with T2D (Bock, Hanson, et al., 2022; Bock, Ueda, et al., 2022).
CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

The purpose of this dissertation was to determine the mechanisms, specifically vascular contributions, of fatigability in people with prediabetes compared to healthy age-, sex-, body mass index-, and physical activity-matched controls, and determine the potential effects of dietary nitrate (NO$_3^-$) supplementation on fatigability in people with prediabetes. This dissertation provides novel insights into exercise-induced vascular responses in people with prediabetes and, contrary to previous findings, suggests increased fatigability may not exist in all people with prediabetes. Additionally, the integrative methodology used in chapter 3 provides a multifaceted assessment of NO$_3^-$ supplementation in people with prediabetes and suggests NO$_3^-$ supplementation does not improve fatigability in people with prediabetes. This chapter provides a summary of the key findings of this dissertation, discusses the clinical relevance of these findings, and concludes by discussing future studies needed to increase our understanding of exercise vascular regulation and functional consequences of prediabetes.

4.1 Main Findings

Through the use of methodological approaches to provide insights at both macro- and microvascular levels of the vascular tree, chapter 2 provided insights into vascular responses during exercise in people with prediabetes. A novel finding of chapter 2 is that people with prediabetes had an attenuated exercise-induced blood flow compared with matched controls, and this was independent of physical activity. This is the first study to investigate exercise blood flow responses in people with prediabetes and the finding of an attenuated exercise-induced blood flow supports previous data from prediabetic rodent
models (Ellis et al., 2010; Novielli & Jackson, 2014; Novielli-Kuntz et al., 2018). Interestingly, NO-dependent macrovascular endothelial function did not differ between people with prediabetes and healthy controls suggesting that NO-independent mechanisms may play a role in the attenuated exercise-induced blood flow in people with prediabetes. Indeed, previous studies using prediabetic rodent models suggest the release of the potent vasodilator adenosine triphosphate from red blood cells is impaired in exercise-like conditions (i.e., hypoxia) in prediabetes (Ellis et al., 2010), a finding that has since been confirmed in humans with T2D during exercise (Groen et al., 2019). Additionally, a reduced functional sympatholysis due to increased sympathetic activity has been shown to attenuate exercise hyperemic responses in prediabetic mice models (Novielli-Kuntz et al., 2018). Lastly, previous data also suggests the occurrence of capillary rarefaction in early stages of metabolic dysfunction (Frisbee et al., 2014; Wasserman et al., 2018). It is possible that decreased capillary density in the skeletal muscle could contribute to an attenuated exercise hyperemia in people with prediabetes. We are currently studying this mechanism by investigating the relationship between exercise-induced blood flow and the capillary density of skeletal muscle biopsies from the vastus lateralis in people with prediabetes. Altogether, the present findings of an attenuated exercise-induced blood in humans with prediabetes in concert with findings from previous studies warrant future studies to confirm our findings of an attenuated exercise-induced blood flow and determine the contributing mechanisms.

An additional main finding of chapter 2 is that people with prediabetes presented with similar fatigability compared with matched controls. These data demonstrate a similar degree of fatigability in people with prediabetes compared to previous data (36%
vs. 32% reduction in lower limb power, respectively); however, healthy controls in this dissertation had markedly greater fatigability than that reported in previous data (31% vs. 22% reduction in lower limb power, respectively) (Senefeld, Harmer, et al., 2020). This difference between studies may be due to the elevated fasting insulin/HOMA-IR levels in the current study compared to Senefeld et al. (2020) and/or variations in the fatiguing exercise protocols. With such little data available, future studies are needed to further determine the degree of fatigability in people with prediabetes. Additionally, the task demands of exercise (e.g., contraction intensity and type, duty cycle, exercise duration) largely dictate the magnitude and contributing mechanisms to fatigability (Hunter, 2018). Thus, future studies should investigate fatigability using additional fatiguing tasks to provide a more comprehensive assessment of fatigability and neuromuscular function in people with prediabetes.

Another significant finding of chapter 2 was that exercise-induced impairments in muscle contractile function as well as exercise-induced blood flow and deoxygenated myo/hemoglobin (deoxy[heme]) responses during exercise were associated with fatigability. The association identified between exercise-induced blood flow and fatigability of the lower limb elicited from the high-velocity, dynamic fatiguing task further confirm previous findings from our lab (Senefeld et al., 2019). This study contributes additional insights suggesting oxygen extraction by the working muscle may contribute to fatigability for this given fatiguing task. Moreover, impairments in muscle contractile function were associated with both exercise-induced blood flow and exercise deoxy[heme] responses suggesting impairments in oxygen delivery and extraction during exercise may impair muscle contractile function and ultimately lead to greater
fatigability. An inability to adequately match oxygen supply to oxygen demand of the working muscle could lead to a greater reliance on anaerobic metabolism resulting in greater metabolite accumulation and impaired muscle contractile function (Sundberg & Fitts, 2019). Collectively, these results suggest that contractile mechanisms involving both oxygen delivery and extraction at the muscle could be responsible for the fatigability experienced in both people with prediabetes and healthy control participants.

Chapter 3 of this dissertation was the first investigation of the effects of dietary NO$_3^-$ supplementation on neuromuscular and vascular function in people with prediabetes. We sought to determine if dietary NO$_3^-$ supplementation could improve fatigability in people with prediabetes due to the previously documented improvements in performance (Senefeld, Wiggins, et al., 2020), exercise bioenergetics (Bailey et al., 2010; Larsen et al., 2011), and vascular function (Lara et al., 2016). Our results suggest that dietary NO$_3^-$ supplementation has no effects on fatigability, exercise-induced blood flow, muscle oxygenation, and resting vascular function in people with prediabetes. Recent data investigating fatigability of the knee-extensor muscles in young males and older females are in line with the present findings of no beneficial effect of NO$_3^-$ supplementation (Kadach et al., 2023; Le Roux-Mallouf et al., 2019; Zoughaib et al., 2023), although contradictory data does exist (Bailey et al., 2010). The lack of effect of NO$_3^-$ supplementation could be a result of the short-term supplementation dose used in the current (i.e., 3-days) and previous studies (i.e., single dose). However, the supplementation dosing and duration used in the current study have been suggested as optimal for eliciting improvements in performance in a recent comprehensive systematic review of 80 studies (Senefeld, Wiggins, et al., 2020). The lack of effect of NO$_3^-$
supplementation could additionally be specific to the characteristics of the fatiguing task used (i.e., high-velocity, single limb exercise). Indeed, a majority of the studies investigating the ergogenic effect of NO₃⁻ supplementation have used large muscle mass, rhythmic exercise such as cycling and running (Senefeld, Wiggins, et al., 2020). Future studies could investigate the effect of NO₃⁻ supplementation in people with prediabetes during rhythmic, whole-body exercise.

Exercise-induced blood flow and muscle oxygenation responses following NO₃⁻ supplementation have been highly variable in previous studies. The present data from chapter 3 suggests NO₃⁻ supplementation does not enhance exercise hyperemic and muscle oxygenation responses in people with prediabetes. This is contrary to findings in individuals with T2D with NO₃⁻ supplementation resulting in improvements in exercise hyperemia (Bock, Ueda, et al., 2022). This discrepancy in findings could be related to the greater duration of supplementation used by Bock et al. (2022) (i.e., 2 months). Additionally, Bock et al. (2022) used a small muscle mass exercise paradigm (i.e., handgrip exercise) designed to study vascular regulation during exercise. The exercise task used in the current study was specifically designed to elicit neuromuscular fatigue of the knee-extensor muscles. With this, it is of interest if the current findings of no improvements in exercise-induced blood flow would hold if an exercise paradigm specifically designed to study vascular regulation (e.g., rhythmic exercise at a fixed load) were used. Lastly, NO₃⁻ supplementation did not have any effect on resting vascular function in people with prediabetes. These results are in alignment with previous data from studies investigating NO₃⁻ supplementation in other populations with metabolic dysfunction (Bock, Ueda, et al., 2022; Gilchrist et al., 2013; Smeets et al., 2022).
could be a result of the pathophysiological manifestations in metabolic dysfunction (e.g., increased oxidative stress and elevated gastric pH) that could impair the potential vascular benefits of NO$_3^-$ supplementation (Bahadoran et al., 2021). Considering the important role of increased oxidative stress in vascular dysfunction (Goodwill & Frisbee, 2012; Rush et al., 2005), future studies could investigate the combined effect of antioxidant and NO$_3^-$ supplementation on vascular function in populations with metabolic dysfunction.

4.2 Clinical Significance

The finding of similar fatigability between people with prediabetes and healthy controls is of clinical importance. An absence of increased fatigability would be one less barrier to participation in physical activity in this population. Thus, other strategies could be emphasized to increase physical activity in people with prediabetes such as self-regulatory behavior methods, motivational interviewing, use of technology, or pet ownership (Thielen et al., 2023). Nevertheless, identifying strategies to improve fatigability in people with prediabetes is still of clinical relevance to increase physical activity adherence and improve physical function. Although dietary NO$_3^-$ supplementation was found to not be effective, other interventions (e.g., exercise strategies) should be explored to offset fatigability and improve vascular function in people with prediabetes.
4.3 Future Directions

Although this study suggests people with prediabetes may not have an increased lower limb fatigability compared to healthy controls, future studies are needed to confirm these results. Future studies should additionally include participants with more severe metabolic dysfunction given that the participants in this dissertation were on the lower end of the prediabetic range (i.e., HbA₁c = 5.7%; FPG = 106 mg/dl). Future studies could also incorporate an oral glucose tolerance test to determine glucose tolerance. This test is more specific to skeletal muscle insulin resistance (Echouffo-Tcheugui et al., 2023) which may have greater relevance to exercise performance and fatigability.

This study is the first to identify an attenuated exercise-induced blood flow in people with prediabetes. Future studies are needed to confirm these findings and determine potential mechanisms of the attenuated exercise hyperemic response in people with prediabetes (i.e., attenuated functional sympatholysis). Furthermore, future studies are needed to determine the functional consequences of an attenuated exercise-induced blood flow. Current research suggests cardiorespiratory fitness may be impaired in individuals with prediabetes (Solomon et al., 2015). Whether an attenuated exercise blood flow response and attenuated oxygen delivery to the working muscle contributes to reduced cardiorespiratory fitness should be investigated.

4.4 Summary

This dissertation demonstrated that people with prediabetes have an attenuated exercise-induced blood flow compared with age-, body mass index-, and physical activity-matched, healthy controls; however, people with prediabetes and controls did not differ in fatigability. Fatigability (reduction in power) was associated with impaired...
skeletal muscle contractile properties, exercise-induced blood flow, and deoxy[heme] responses during exercise in both people with prediabetes and healthy controls. In addition, impairments in skeletal muscle contractile properties were associated with exercise-induced blood flow and deoxy[heme] responses during exercise suggesting that muscle oxygen supply and extraction contributed to the impairments in muscle contractile function. Altogether, these results suggest that the mechanisms of fatigability in the combined group are of a muscular origin with contributions from oxygen supply and extraction at the muscle. Lastly, dietary nitrate supplementation was not effective in improving fatigability, exercise-induced blood flow, muscle oxygenation, or vascular function in people with prediabetes suggesting that other interventions should be considered (e.g., exercise strategies) targeting muscle oxygen delivery and extraction.
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type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes, 59*(3), 572-579.


