Mechanisms of Fatigability in People with Type 2 Diabetes and Prediabetes

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MECHANISMS OF FATIGABILITY IN PEOPLE WITH TYPE 2 DIABETES AND PREDIABETES

by

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ABSTRACT
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Dynamic fatiguing exercise of limb muscles is the basis of exercise training and a cornerstone of management of type 2 diabetes mellitus (T2D) and prediabetes. Little is known however, about the fatigability of limb muscles (the acute exercise induced reduction in force or power) and the involved mechanisms in people with T2D and prediabetes. Current evidence suggests that people with T2D have reduced muscle strength and power, are more fatigable after static contractions, and have physical impairments affecting activities of daily living. However, impaired function in people with T2D compared with controls is larger for dynamic than static tasks. The purpose of this dissertation was to determine the magnitude and mechanisms of fatigability in people with T2D and prediabetes after a dynamic exercise task with the knee extensor muscles. Importantly, these studies matched people with T2D and prediabetes to controls based on age, sex, physical activity and body size.

The first studies determined the magnitude of fatigability and the neural and muscular mechanisms in people with T2D and controls (Study 1) and in prediabetes (Study 2). People with T2D had approximately twice the decline in both power (fatigability) and electrically-evoked muscle contractile properties than controls after the six-minute dynamic task with the knee extensor muscles. People with prediabetes also had greater fatigability (~50%) and reductions in contractile properties than controls, but less than people with T2D. The reduction in voluntary activation (neural drive to the muscle) after fatiguing exercise was not different between people with T2D, prediabetes and controls. Thus, the greater fatigability in people with T2D was due to mechanisms within the skeletal muscle rather than neural drive.

Study 3 determined whether skeletal muscle blood flow could explain the greater fatigability in people with T2D. People with T2D had greater fatigability and lower blood flow after exercise than controls, and there was an association between fatigability and the exercise-induced increase in muscle blood flow after exercise. Collectively, these data suggest that people with T2D and prediabetes have greater fatigability during dynamic exercise with knee extensor muscles due to mechanisms effecting muscle contractile properties, including impaired skeletal muscle blood flow.

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CHAPTER 1: INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 Epidemiology and Etiology of Diabetes and Prediabetes

Type 2 diabetes mellitus (T2D) is characterized by chronically elevated blood glucose and is the costliest chronic disease in the United States (Trogdon *et al.*, 2015). T2D affects approximately 13% of the US population (Menke *et al.*, 2015), and is rapidly growing in prevalence with an expected increase in incidence by 70% in the next 20 years (Shaw *et al.*, 2010). The leading cause of disability among people with T2D is diabetic polyneuropathy, which affects ~50% of people with T2D and causes gait instability, muscle atrophy, weakness, and/or increased susceptibility to fatigue (Allen *et al.*, 2013; Allen *et al.*, 2014a; Allen *et al.*, 2014b; Allen *et al.*, 2015a; Allen *et al.*, 2015b; Senefeld & Hunter, 2016; Stino & Smith, 2017). The most effective intervention to delay onset of T2D and impede the progression of T2D is lifestyle intervention including regular exercise training (Knowler *et al.*, 2002; Diabetes Prevention Program Research *et al.*, 2009; Herman *et al.*, 2017). However, there is little scientific evidence to characterize physical performance and the factors that might limit physical performance (neural and muscular factors) during a single bout of exercise. To address this gap in scientific knowledge, the aim of this dissertation is to compare performance of dynamic exercise and determine the contribution of neural and muscular factors that potentially limit exercise performance in people with T2D and prediabetes compared to people without T2D (i.e. controls). The following provides an in-depth review of the epidemiology and etiology of T2D and a pre-cursor condition known as prediabetes, the clinical manifestations of T2D, a brief review of the benefits of exercise training, and a review of

the scientific literature examining the limitations to a single bout of exercise in people with diabetes mellitus.

Diabetes mellitus is a group of metabolic disorders resulting in chronically elevated blood glucose levels (American Diabetes, 2015a) because the islet cells of the pancreas produce inadequate insulin and or tissues in the body develop resistance to the action of insulin. T2D is a clinical distinction of diabetes mellitus. People with T2D have impairments in glucose disposal that require clinical treatment and have considerable risk of developing clinical-symptoms (e.g. sensory loss) associated with chronically high glucose (American Diabetes, 2015a). The pancreas in people with T2D typically retains the ability to produce insulin but to a reduced capacity, which is in stark contrast to type 1 diabetes in which the pancreas can no longer produce insulin (American Diabetes, 2015a). Approximately 95% of cases of diagnosed diabetes mellitus are T2D (American Diabetes, 2018). Additionally, beginning in 1997, the Expert Committee on Diagnosis and Classification of Diabetes Mellitus (1997) officially recognized people who did not meet clinical criteria for diagnosis of diabetes, but had elevated clinical markers that indicated increased risk of developing T2D—i.e. increased fasting blood glucose and HbA_{1c}. This patient population is now referred to as people with *prediabetes* (American Diabetes, 2015a).

T2D and prediabetes have become global pandemics (Ogurtsova *et al.*, 2017), estimated to currently affect 13% and 37% of Americans, respectively (Menke *et al.*, 2015). However, a considerable portion of these people with T2D (~25%) and prediabetes (~90%) are undiagnosed (American Diabetes, 2018). Diabetes mellitus

(including prediabetes) is the most common chronic condition in the United States (Buttorff *et al.*, 2017).

The primary diagnostic criteria for diabetes mellitus are glucose concentration and glucose regulation. The gold standard for diagnosis is the two-hour oral glucose tolerance test (OGTT) whereby endogenous action of insulin is estimated by measuring plasma glucose levels two hours after a bolus of glucose (e.g. 75 g). This test (OGTT) can demonstrate insulin resistance and β-cell dysfunction that are the pathophysiological basis of diabetes (Bartoli *et al.*, 2011).

Another diagnostic criterion for diabetes mellitus is glycated hemoglobin, commonly known as HbA_{1c}. HbA_{1c} is an assessment of the proportion (%) of hemoglobin molecules that have bound glucose (Roszyk *et al.*, 2007), which is often considered an indication of average blood glucose levels over the previous 120-day period because 120 days is the average lifespan of an erythrocyte (Bode *et al.*, 2007). Assessment of HbA_{1c} is relatively inexpensive (Bode *et al.*, 2007), is predictive of diabetes risk factors and prognosis of diabetes (Diabetes Prevention Program Research Group *et al.*, 2014; Vijayakumar *et al.*, 2017), and has high concordance with the oral glucose tolerance test (Kim *et al.*, 2016). Therefore, HbA_{1c} assessment is often used to determine T2D or prediabetes for research purposes, but the OGTT remains the clinical gold standard for diagnosis (Kim *et al.*, 2016).

Another diagnostic criteria for diabetes is the assessment of plasma glucose after an 8-hour fast (Kim *et al.*, 2016). The American Diabetes Association has suggested values for the diagnostic criteria of T2D and prediabetes, as demonstrated in Table 1.1 (American Diabetes, 2015a). For diagnostic purposes, the HbA_{1c} and fasting glucose

must be determined by lab measurement and not by point of care testing (American Diabetes, 2018).

	T2D	Prediabetes	Control
Oral Glucose Tolerance Test ($mmol \cdot L^{-1}$)	≥ 11.1	7.8 – 11.0	≤ 7.7
HbA_{1c} (%)	≥ 6.5	5.7 – 6.4	≤ 5.6
Fasting Plasma Glucose ($mg \cdot dL^{-1}$)	≥ 126	100 – 125	≤ 99

Table 1.1. Classification criteria for type 2 diabetes and prediabetes. Oral glucose tolerance test, glycated hemoglobin (HbA_{1c}) and fasting plasma glucose are used to demarcate type 2 diabetes (T2D) and prediabetes; however, the oral glucose tolerance test is the clinical gold standard for diagnosis (Kim *et al.*, 2016).

1.2 Development and Complications of Type 2 Diabetes

The factors leading to a predisposition of T2D are multifactorial including environmental factors (e.g. obesity, non-adherence to diet, smoking and lack of physical activity) (Hu *et al.*, 2001) and genetic variants (Vassy *et al.*, 2014). In the largest known cohort study (Nurses' Health Study), risk of developing T2D was prospectively evaluated in ~85,000 women after a 16-year observation period (Hu *et al.*, 2001). Approximately 3,300 women developed T2D (~4% incidence) and the two risk factors most predictive of developing T2D were physical activity and body mass index (BMI). Relative to the most physically active (7 or more hours of exercise per week), women with the least amount of physical activity ($\leq$ 30 minutes of exercise per week) were twice as likely to develop T2D (Hu *et al.*, 2001). Similarly, women with the highest BMI ($\geq 35.0\ kg \cdot m^{-2}$) were 2.5 times more likely to develop T2D compared to women with the lowest BMI ($<23.0\ kg \cdot m^{-2}$) (Hu *et al.*, 2001).

Additionally, there are several genetic variants that have been identified to be associated with T2D and models based on genetic risk scores can predict relative risk for

development of T2D (Vassy *et al.*, 2014). However, compared to simple demographic and clinical prediction models (BMI, systolic blood pressure, fasting plasma glucose and family history of T2D) *without* genetic information, the addition of genetic information has a small impact on relative risk prediction for T2D (Vassy *et al.*, 2014). Collectively, these data highlight the importance of modifiable risk factors—particularly physical activity and body mass— in the development of T2D. As discussed in this introduction, physical activity may also be a panacea for many of the complications that develop as a result of T2D and prediabetes.

There are three primary pathophysiological complications of T2D, 1) diabetic polyneuropathy, 2) metabolic dysfunction and 3) vascular dysfunction. Each of these pathophysiological complications results in multiple clinical manifestations and marked variability between people; however, one commonality is that greater insulin resistance is associated with worsening of each complication—diabetic polyneuropathy (Lee *et al.*, 2016), metabolic dysfunction (Jelenik & Roden, 2013) and vascular dysfunction (Montero *et al.*, 2013; Mahmoud *et al.*, 2016).

Diabetic Polyneuropathy

Diabetic polyneuropathy can manifest in nerve dysfunction among motor, sensory and autonomic nerves (Senefeld & Hunter, 2016). Diabetic polyneuropathy often manifests in a symmetrical and length-dependent pattern affecting distal portions of long peripheral nervous of people with DM (Said, 2007), and can contribute to the pathogenesis of foot ulcers (Papanas *et al.*, 2007) and axonal loss and demyelination (Severinsen & Andersen, 2007). Excessive glucose in the blood, a hallmark feature of T2D, contributes to hyperglycemic milieu in the soma of cells in tissue of the nervous

system (Hasselbalch *et al.*, 1999; Chung *et al.*, 2003), resulting in oxidative stress in the nerve axons (Souayah *et al.*, 2009). This oxidative stress causes withdrawal of the longest (typically most distal) nerve axons from the innervated tissue *without* cellular death (Souayah *et al.*, 2009), providing the potential for recovery from structural damage of the axon prior to death of the nerve soma (Kennedy & Zochodne, 2005). Although in some cases small fiber injury (autonomic and sensory) is the sole pathology of diabetic polyneuropathy (Moghtaderi *et al.*, 2006; Papanas & Ziegler, 2011), there can be significant damage to motor nerve function in people with diabetic polyneuropathy. Diabetic polyneuropathy can manifest as motor nerve axon degeneration (Souayah *et al.*, 2009) and result in as muscle atrophy, weakness, and/or increased susceptibility to fatigue (Allen *et al.*, 2013; Allen *et al.*, 2014a; Allen *et al.*, 2014b; Allen *et al.*, 2015a; Allen *et al.*, 2015b). Motor dysfunction occurs as a result of alterations to the motor nerve axon, motor nerve soma, and motor unit neuromuscular junctions (Fahim *et al.*, 2000; Kaji, 2003). In human diabetes and experimental diabetes in rodent models, diabetic polyneuropathy is associated with slowing of motor nerve conduction velocity (Hansen & Ballantyne, 1977; Sima *et al.*, 1993; Kaji, 2003; Kikkawa *et al.*, 2005; Almeida *et al.*, 2008), reduced muscle contractile function (Fahim *et al.*, 1998; Allen *et al.*, 2014b), increased neuromuscular transmission failure (Miglietta, 1973; Chang & Chuang, 1996; Fahim *et al.*, 2000; Marques & Santo Neto, 2002; Allen *et al.*, 2015a), muscle atrophy (Andersen *et al.*, 1997; Severinsen *et al.*, 2007) and muscle weakness (Andersen *et al.*, 1997; Andreassen *et al.*, 2006; Allen *et al.*, 2013). Importantly, people with T2D and no clinical signs of diabetic polyneuropathy also demonstrate reduced maximal strength of limb muscles, impaired mobility, and quality of life compared to controls (Ijzerman *et al.*,

2011; IJzerman *et al.*, 2012); and diabetic polyneuropathy has a moderate additional effect to cause greater reductions in strength, mobility and quality of life in these people (Ijzerman *et al.*, 2011; IJzerman *et al.*, 2012).

In addition to direct effects on motor function, T2D and diabetic polyneuropathy may affect commands from the motor cortex. It has been demonstrated that people with T2D and diabetic polyneuropathy have lower estimated number of motor units and reduced mean motor unit firing rates in the tibialis anterior and first dorsal interosseous (Allen *et al.*, 2014a) compared with age-matched control participants. In a group of people with diabetes mellitus (type 1 or type 2), people with symptomatic diabetic polyneuropathy demonstrated reduced compound muscle action potential, reduced motor nerve conduction velocity and reduced muscle strength of the ankle dorsiflexors and knee extensors compared to people with diabetes and absent or asymptomatic diabetic polyneuropathy (Andersen *et al.*, 1998). Additionally, both groups (people with diabetes with or without diabetic polyneuropathy) demonstrated these impairments (reduced compound muscle action potential, conduction velocity and strength) relative to healthy controls without diabetes (Andersen *et al.*, 1998). Similarly, people with type 1 diabetes mellitus demonstrated reduced contractile force, slower conduction velocity and lower mean motor unit discharge rate (Almeida *et al.*, 2008).

There is more recent evidence examining motor unit firing patterns of the vastus lateralis in men with T2D but *without* diabetic polyneuropathy compared with age-matched control men (n = 8 vs 8), demonstrating that men with T2D and no diabetic polyneuropathy had lower and more variable motor unit instantaneous firing rates (Watanabe *et al.*, 2013). Using high-density surface electromyography (EMG) of the

vastus lateralis during sustained (2-minute) submaximal (10% maximum), isometric exercise with the knee extensors, it has been demonstrated that men with T2D have a reduced area of activation within the knee extensors and fewer changes (less heterogeneity) in the area of muscle activation during the 2-minute exercise (Watanabe *et al.*, 2012). These data may suggest that people with T2D may activate limited numbers of motor units continuously during sustained exercise (Watanabe *et al.*, 2012). Collectively, these data suggest that people with T2D may have reduced and more variable motor unit firing rates and reduced number of active motor units during sustained contractions (which are exacerbated in people with T2D and diabetic polyneuropathy) and may contribute to increased fatigability for people with T2D.

Metabolic Dysfunction

Skeletal muscle plays a major role in energy metabolism, and this metabolically active tissue is responsible for at least 80% of insulin-stimulated glucose disposal (Szendroedi & Roden, 2008). Mitochondria, the organelle responsible for oxidative phosphorylation in skeletal muscle, plays such a prominent role in glucose metabolism that some researchers insist that insulin resistance is a proxy for reduced mitochondrial quality or quantity (Porter & Wall, 2012). There are three primary molecular pathways thought to be impaired in people with T2D, which impair the metabolism of glucose in mitochondria and skeletal muscle causing slowed glucose disposal.

The first pathway that can impair glucose metabolism in people with T2D is reduced efficacy of the translocation of GLUT4 glucose transporter from intracellular compartments to the extracellular membrane to allow insulin-stimulated glucose uptake (Goodyear & Kahn, 1998). This translocation of GLUT4 occurs via insulin receptor

substrate 1, at a specific domain referred to as IRS1-P13K and subsequent phosphorylation of TBC1D4 (Goodyear & Kahn, 1998; Maarbjerg *et al.*, 2011; Cartee, 2015). Although the pathway is debated (Petersen & Shulman, 2002) and more intricate than originally proposed (Hue & Taegtmeyer, 2009), a second pathway thought to impair glucose metabolism in the mitochondria among people with T2D is increased concentrations of acetyl-CoA, which inhibits pyruvate dehydrogenase and therefore reduces glucose oxidation via reduced activity of the tricarboxylic acid (TCA) cycle—in a process called the 'Randle cycle' (Randle *et al.*, 1963). The third pathway which impairs glucose metabolism in people with T2D is reduced expression of the PGC1 protein (PPARγ coactivator-1), which is a master regulator of mitochondrial biogenesis (Montgomery & Turner, 2015). Reduced expression of PGC1 will reduce the generation of mitochondrial via reduced transcription of genes responsible for mitochondrial biogenesis. These three pathways are highlighted in the Figure 1.1, reprinted from (Szendroedi & Roden, 2008).

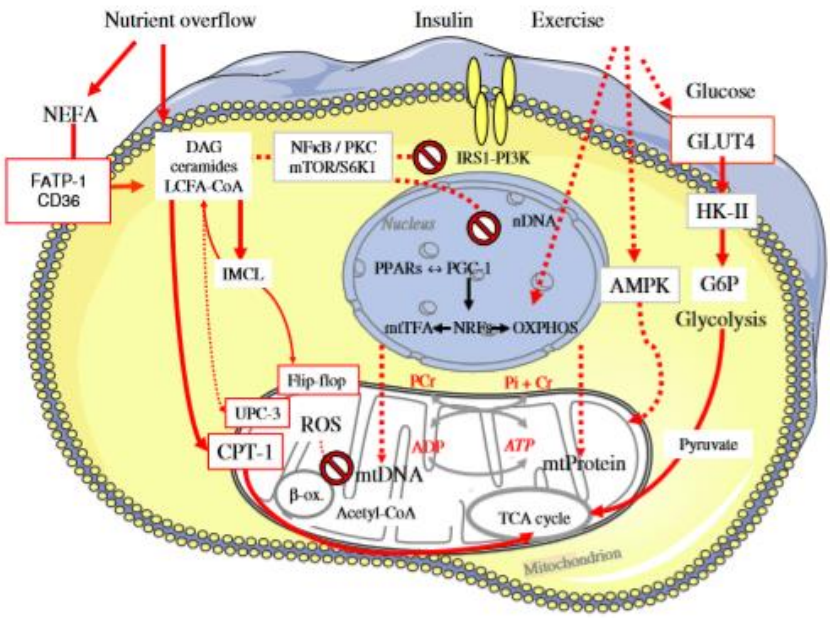

Figure 1.1. Schematic of the potential sites of metabolic dysfunction in people with type 2 diabetes. Skeletal muscle is responsible for an enormous amount of metabolism, responsible for *at least* 80% of insulin-stimulated glucose disposal. However, people with T2D often present with metabolic dysfunction within the myocyte and mitochondria within the myocyte. The metabolic dysfunction in people with T2D is often seen at the sites of glucose transport into the cell (e.g. myocyte) via GLUT4 at the IRS1-P13K domain, reduced genetic transcription and protein expression of PGC1, and increased concentrations of Acteyl-CoA, each of which reduce the magnitude and rate of glucose oxidation. The figure was reprinted from (Szendroedi & Roden, 2008).

These impairments in metabolic function would directly shift metabolic processes during exercise, resulting in a net shift toward increased anaerobic processes (phosphocreatine/creatine kinase system and anaerobic glycolysis) and reduced aerobic metabolic processes (TCA cycle and oxidative phosphorylation) (Kelley *et al.*, 2002). Importantly, this potential shift in metabolic processes for people with T2D could result in greater concentrations of anaerobic metabolic by-products (e.g. hydrogen ion and inorganic phosphate) which are known to impair skeletal muscle contractile function and increase fatigability in healthy muscle (Fitts, 1994; Kent-Braun *et al.*, 2012; Debold *et al.*, 2016), and stimulate Group III and IV afferents which reduce voluntary activation

from the motor cortex (Taylor *et al.*, 2016; Hunter, 2017). However, there is substantial research to support that these three pathways (GLUT4 pathway, acetyl-CoA pathway and PGC1 pathway) can be altered during sustained, acute bouts of exercise and prolonged exercise training, which creates a stimulus for enhanced action of insulin (Goodyear & Kahn, 1998; Holloszy, 2005; Stanford & Goodyear, 2014; Kim *et al.*, 2015), and although weight loss can accompany exercise training, these observations are likely not due to weight loss alone (Weiss *et al.*, 2007).

Vascular Dysfunction

Although there is no definitive conclusion that muscle oxygen delivery is impaired in people with T2D, there is strong evidence to support abnormal skeletal muscle blood flow regulation in people with T2D at rest and during exercise (for review, see (Poitras *et al.*, 2018)). As a broad concept, blood flow is required to support sustained exercise to deliver airborne oxygen to the exercising skeletal muscle for substrate metabolism (Saltin *et al.*, 1998) and clear exercise-stimulated metabolic by-products from the muscle tissue that inhibit contractile function of the exercising muscle in high concentrations (Fitts, 1994). The increase in blood flow during exercise, termed exercise-induced hyperemia, closely matches the metabolic demands of the contracting muscle (Andersen & Saltin, 1985). The blood flow redistribution that occurs during maximal exercise may represent the largest physiological stress on the cardiovascular system, resulting in 8× greater blood flow during exercise compared to rest (Joyner & Casey, 2015). The regulation of blood flow distribution is mediated systemically by sympathetic nervous system activity (Joyner & Casey, 2015); however, blood flow to the exercising

muscle is primarily mediated by reactivity of the vasculature supplying blood to the exercise muscles (Casey & Joyner, 2011).

Peripheral blood flow is primarily regulated by the diameter of the vasculature, and much of the dynamic control of blood flow during exercise occurs in smaller vasculature (termed resistance vessels) (Sarelius & Pohl, 2010). The exercise-induced hyperemia is circulated and gas exchange occurs within the exercising muscle(s) via capillaries in small terminal arteriole trees to replenish substrates for metabolic processes, and upstream of these terminal arteriole trees are four to six orders of artery branch sizes that are perfused from one major feed artery to the muscle (Joyner & Casey, 2015). Thus, although these large-conduit feed arteries to muscles are typically measured to determine vascular health or function (as discussed below) (Thijssen *et al.*, 2011), the vasoreactivity of these conduit arteries is not functionally significant for the regulation of blood flow to the exercising muscle (Joyner & Casey, 2015).

From a bottom-up perspective, the vasodilator response to skeletal muscle contraction is conducted upstream after being initiated within the terminal arteriole trees (a.k.a. microcirculation) (Segal *et al.*, 1989), and this vasodilatory response begins within ~1s of initiation of exercise (VanTeeffelen & Segal, 2006). The signaling process mediating the vasodilatory response to exercise is still actively debated; however, there is convincing evidence that vasodilation is primarily mediated by chemical factors released from the contracting muscles or endothelium with minimal contributions of neutrally-mediated or muscle pump-mediated vasodilation (for review, see (Joyner & Casey, 2015)). The chemically-mediated vasodilation during exercise is highly redundant and most vasoactive chemicals also stimulate the release of nitric oxide, thereby making it

difficult to determine the contribution of nitric oxide or another vasoactive chemical (Joyner & Casey, 2015). The primary vasoactive chemicals released during skeletal muscle contraction include: potassium ion (K^+), nitric oxide (NO), hydrogen ion (H^+), and adenosine (Joyner & Casey, 2015). In addition to these vasodilatory substances, two other underlying vasoactive processes occur during exercise: 1) the brief compression of arterioles due to skeletal muscle contraction initiates a reactive hyperemia response (Laughlin, 1987) causing vasodilation and 2) increased sympathetic nerve activity due to exercise leads to global vasoconstriction and can blunt the exercise-induced vasodilation in the large conduit arteries but likely not small arterioles (Segal *et al.*, 1999).

Although the vasoreactivity of large conduit arteries is not functionally significant for the regulation of blood flow to the exercising muscle (Joyner & Casey, 2015), the vasoreactivity of these arteries is commonly used as a proxy of vascular health (Thijssen *et al.*, 2011). This common clinical indication of vascular health is a flow-mediated dilation study consisting of measuring conduit artery diameter before and after a 5-minute bout of complete arterial occlusion downstream of the conduit artery. Flow-mediation dilation studies may reflect the bioavailability of nitric oxide, and these studies provide important prognostic information for endothelial health and cardiovascular disease risk (Thijssen *et al.*, 2011). A recent systematic review and meta-analysis (Montero *et al.*, 2013) demonstrated impaired endothelial function in non-exercising vasculature in people with T2D compared to age matched controls in 28 out of 29 studies—see Figure 1.2. Despite these robust differences in a proxy for vascular health, there is sparse data to examine blood flow (to infer endothelial function) *during or after* exercise in people with T2D. Moreover, there is no data to examine blood flow during or after exercise in people

with T2D compared to controls matched for age, sex, BMI and physical activity, so the independent effects of T2D and BMI or physical activity on blood flow during exercise are unknown. Our understanding of the potential association between blood flow regulation and fatigability is paramount in people with T2D, because this potential relationship may highlight a clinical target for improvement in people with T2D which could ameliorate increased exercise intolerance and reduced exercise training adherence in people with T2D (Poitras *et al.*, 2018).

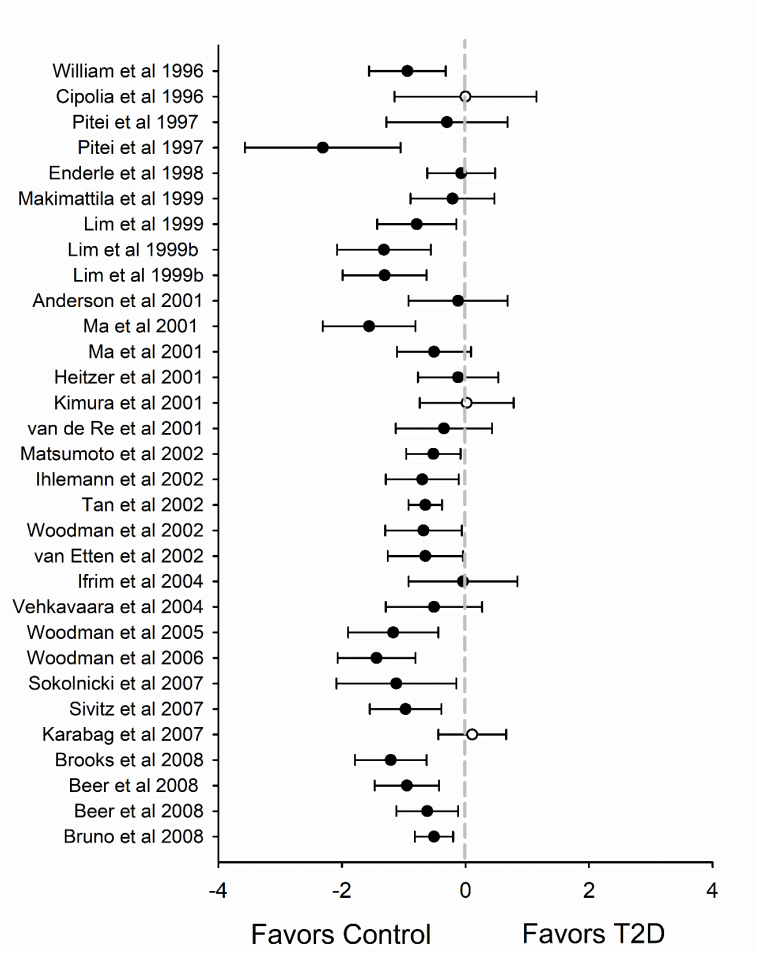

Figure 1.2. Forest Plot of standard mean differences in resting endothelial function (assessed as dilation mediated by flow, acetylcholine, methacholine or serotonin) between controls and people with type 2 diabetes. Each data point represents the mean difference of endothelial function between people with T2D and controls, with the vertical grey line denoting no difference between the groups. The filled circles denote data in favor of better endothelial function in controls and the unfilled circles denote data in favor of better endothelial function in people or animals with T2D. Collectively, these data provide strong evidence that people with T2D have impaired endothelial function compared to the controls. This figure was adapted from (Montero *et al.*, 2013), and includes data from the following studies: (Cipolla *et al.*, 1996; Williams *et al.*, 1996; Pitei *et al.*, 1997; Enderle *et al.*, 1998; Lim *et al.*, 1999a; Lim *et al.*, 1999b; Makimattila *et al.*, 1999; Anderson *et al.*, 2001a; Heitzer *et al.*, 2001; Kimura *et al.*, 2001; Ma *et al.*, 2001; van de Ree *et al.*, 2001; Ihlemann *et al.*, 2002; Matsumoto *et al.*, 2002; Tan *et al.*, 2002; van Etten *et al.*, 2002; Woodman *et al.*, 2002; Ifrim & Vasilescu, 2004; Vehkavaara & Yki-Jarvinen, 2004; Woodman *et al.*, 2005; Woodman *et al.*, 2006; Karabag *et al.*, 2007; Sivitz *et al.*, 2007; Sokolnicki *et al.*, 2007; Beer *et al.*, 2008; Brooks *et al.*, 2008; Bruno *et al.*, 2012).

1.3 Treatment of Type 2 Diabetes

The primary clinical goals of T2D treatment are to reduce HbA_{1c} and systolic and diastolic blood pressures; improve lipid profile; and limit complications of T2D (including diabetic polyneuropathy, metabolic dysfunction, and vascular dysfunction) (American Diabetes, 2015b). Due to public interest and research funding, vast improvements in diabetes management and quality of care have led to reductions in diabetes-related complications and mortality (Narayan, 2016). Better management of T2D, however, is overshadowed by alarming and rapidly increasing numbers of people with T2D— providing rationale to focus on the interventions that can prevent T2D as opposed to treat complications (Narayan, 2016). According to US and international treatment guidelines (Czupryniak, 2009; Garber *et al.*, 2018), metformin is the first prescribed medication to treat T2D. However, as T2D progresses, insulin sensitivity and secretion are reduced, and it is commonly accepted that people with T2D will eventually need insulin secretagogues and exogenous insulin therapy (Home *et al.*, 2014). Although insulin secretagogues and basal insulin are effective interventions for management of T2D symptoms (Sasali & Leahy, 2003), the reversion rate of T2D among people that are prescribed insulin secretagogues or basal insulin is very low (<2%) (Karter *et al.*, 2014). Therefore, studies that examine the potential remission of T2D or prevention of T2D among people with prediabetes focus on the potential utility of metformin.

In 1996, a randomized, controlled clinical trial involving 27 clinical centers across the United States (called the Diabetes Prevention Program or DPP) enrolled 3,149 participants who had prediabetes and were at high risk of progression to T2D and

randomly assigned participants to one of the following groups for a 4-year period (Knowler *et al.*, 2002):

Lifestyle Group: Participants exercised at least 150 minutes per week, attempted to lose 7% body weight, maintained a healthy diet, and had regular meetings with nurses and counselors.

Metformin Group: Participants took 850 mg of metformin twice daily and were provided standard advice about diet and physical activity.

Placebo Group: Participants took a placebo pill twice daily and were provided standard advice about diet and physical activity.

This on-going longitudinal study sponsored by the National Institutes of Diabetes and Digestive Kidney Diseases continues to monitor the participants enrolled in the initial trial in 1996 and represents the most comprehensive dataset to determine the efficacy of metformin on the delay or prevention of T2D. The initial studies demonstrated that after a 3-year period, participants in the metformin group had a 31% reduction in risk of developing T2D compared to participants in the placebo group (Knowler *et al.*, 2002). After 15 years, of those participants that continued to take metformin there was a 18% reduction in the incidence of T2D compared to placebo, and it was estimated that daily metformin allowed for two additional healthy years before development of T2D compared to placebo (Herman *et al.*, 2017). Although metformin was successful in the management and prevention of T2D, the lifestyle intervention group had a 58% and 27% reduction in risk of developing T2D at 3- and 15-year follow up and an additional four healthy years before development of T2D compared to placebo—providing evidence that lifestyle interventions are nearly twice as effective as metformin for prevention of T2D

(Knowler *et al.*, 2002; Herman *et al.*, 2017). The intensive lifestyle treatment worked best in older individuals and those without marked obesity.

Exercise is Medicine for People with T2D and Prediabetes

In light of the results of the Diabetes Prevention Program described above, and similar research, exercise training has become a cornerstone of T2D management and T2D prevention for those with prediabetes (Sigal *et al.*, 2006). The American Diabetes Association, International Diabetes Federation, and nearly all other organizations associated with diabetes and endocrinology suggest lifestyle therapy (diet, weight loss and exercise training) as the first intervention for the prevention and management of T2D (Czupryniak, 2009; Garber *et al.*, 2018). Regular exercise training can ameliorate pathophysiological biomarkers of T2D (e.g. fasting blood glucose) and can accomplish the primary goals of management of T2D: reduce HbA_{1c} (Umpierre *et al.*, 2011); reduce systolic and diastolic blood pressures (Chen *et al.*, 2015); improve lipid profile (Chen *et al.*, 2015); and lower BMI (Chen *et al.*, 2015). Additionally, regular exercise training is beneficial for acute glycemic control (Reynolds *et al.*, 2015), insulin sensitivity (Holloszy, 2003, 2005), has a protective influence on cardiovascular health (Padilla *et al.*, 2015), and is the only intervention that can improve aerobic fitness which is one of the best predictors of all-cause mortality in older adults (Trappe *et al.*, 2013) and people with T2D (Church *et al.*, 2004). However, people with T2D (and possibly prediabetes) have disproportionately high rates of exercise intolerance compared to controls (Reusch *et al.*, 2013; Poitras *et al.*, 2018). The potential exercise intolerance in people with T2D and prediabetes is primarily suggested to be due to: metabolic dysfunction (Stanford & Goodyear, 2014), cardiovascular and endothelial dysfunction (Poitras *et al.*, 2018),

clinical symptoms of depression/fatigue (Fritschi & Quinn, 2010), and possibly impairments in contractile properties of muscle (IJzerman *et al.*, 2012; Allen *et al.*, 2016). These four symptoms can contribute to a decline in a decline in muscle force or power during exercise—often termed 'fatigability' (Kluger *et al.*, 2013; Enoka & Duchateau, 2016). Fatigability can be more generally defined as any decline in an objective measure of muscle performance over a discrete period, which we commonly measure in the research laboratory as a reduction in muscle force or power (Kluger *et al.*, 2013; Enoka & Duchateau, 2016).

Currently, no data exist that examine fatigability in humans with prediabetes; however, there are studies determining fatigability in people with T2D. General findings from these studies suggest that people with T2D have greater or equivalent fatigability compared to lean, healthy controls after isometric and isokinetic fatiguing tasks, and these studies are described in the next section. However, there is a dearth of conclusive data on fatigability during dynamic contractions in people with T2D nor comparing fatigability in people with T2D with controls matched for anthropometry and physical activity. This line of investigation has scientific merit because exercise training is the first-line of prevention and treatment of T2D, yet fatigability and the contributing mechanisms are not well understood.

1.4 Fatigability in People with Type 2 Diabetes

The symptoms contributing to reduced exercise performance are globally termed fatigue and can be categorized into two domains: perceived fatigability and performance fatigability (Kluger *et al.*, 2013; Enoka & Duchateau, 2016). Perceived fatigability is defined as self-reported symptoms of exertion, exhaustion, and enervation, can be

assessed at rest or during exercise performance (Kluger *et al.*, 2013). In the absence of exercise (at rest), perceived fatigability is a common symptom in people with T2D, and has been associated with limitations in physical exercise and a lack of motivation to perform exercise (Fritschi & Quinn, 2010). The mechanisms for perceived fatigability in people with T2D in the rested state may be related to poor glucose control (hypo- or hyper-glycemia or glucose fluctuations) (Sommerfield *et al.*, 2004), diabetic polyneuropathy (Rijken *et al.*, 1998), impaired sleep quality (Cuellar & Ratcliffe, 2008), emotional distress from daily disease management (de Sonnaville *et al.*, 1998), depression (Anderson *et al.*, 2001b), and obesity (Pickup, 2004). Although increased perceptions of fatigability may contribute to impaired physical function (Singh *et al.*, 2016), the association between perceived and performance fatigability in people with T2D and prediabetes is unknown.

Performance fatigability is the decline in an objective measure of performance over a discrete period that limits human performance (Kluger *et al.*, 2013; Enoka & Duchateau, 2016)—which is often measured as the decline in expected force or power of limb muscles during a single bout of limb exercise (Kluger *et al.*, 2013; Hunter, 2017) in a controlled laboratory setting. The mechanisms contributing to performance fatigability, also known as fatigability or muscle fatigue, are dependent on the demands of the task being performed (i.e. task dependent) (Enoka & Stuart, 1992)—and thus may differ with type of muscle contractions. For example, there is substantial evidence to demonstrate that healthy women have less fatigability than men during submaximal, isometric fatiguing exercises (Hunter, 2009, 2014); however, women may have *greater* fatigability than men during submaximal, *dynamic* fatiguing exercises (Hunter, 2016a, b). This

paradoxical example, in combination with data presented below, provide the primary rationale for examining fatigability in people with T2D during *dynamic* fatiguing exercise and including cohorts of participants large enough to quantify sex-related differences in fatigability in the studies contributing to this dissertation. As discussed below, there is little evidence to examine fatigability in people with T2D or prediabetes during high velocity dynamic contractions or potential sex-related differences in fatigability among people with T2D or prediabetes.

In general, the mechanisms that contribute to fatigability can originate from many different tissues along the motor pathway, and are generally categorized into neural (mechanisms upstream of the neuromuscular junction) and muscular (mechanisms within the muscle) (Gandevia, 2001; Debold *et al.*, 2016), see Figure 1.3. Current scientific evidence (as discussed below) demonstrates that neural mechanisms (supraspinal and spinal; observed as a loss in voluntary activation and altered motor unit behavior) and muscular mechanisms (e.g. mitochondrial and endothelial dysfunction) could contribute to greater fatigability in people with T2D. Although these mechanisms may be exacerbated by diabetic polyneuropathy (Allen *et al.*, 2016; Senefeld & Hunter, 2016), this work in this dissertation focuses on the any impairments that may occur prior to clinically-detectable and symptomatic diabetic polyneuropathy. Additionally, these neural and muscular mechanisms are exacerbated in the presence of other co-morbidities (e.g. sedentary lifestyle and obesity), thus these co-morbidities were accounted for in the design of each study in this dissertation.

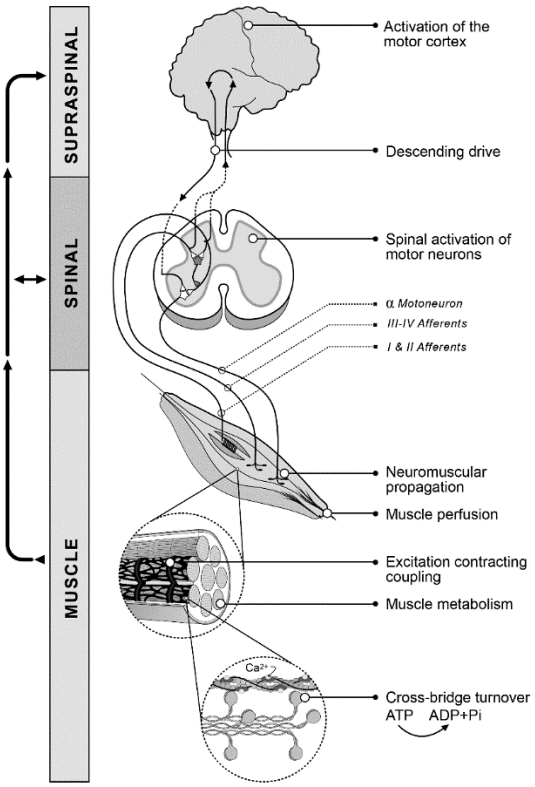

Figure 1.3. Schematic of the potential sites of fatigability in healthy people (reprinted from Hunter (Hunter, 2017)), and that may be relevant to people with type 2 diabetes During muscle contractions, activation of skeletal muscle is governed by complex interactions between activation of the motor cortex, descending drive through the ventral horn of the spinal cord, activation of motor neurons, propagation of the action potential across the neuromuscular junction, and cross-bridge cycling of the myofilaments. However, activation of skeletal muscle is regulated by interactions of excitatory and inhibitory inputs at each level of activation. During volitional exercise, it is estimated that reductions in contractile function of skeletal muscle are responsible for approximately 75% of the observed fatigability with up to 25% of the observed fatigability due to neural (spinal and supraspinal) mechanisms (Gandevia *et al.*, 1996; Taylor *et al.*, 2016).

There is growing evidence that people with T2D have greater fatigability compared with healthy controls; however, the effects of T2D *per se* (excluding the effects of insulin, diabetic polyneuropathy, physical activity and body size) are unknown. A summary of the current studies that encapsulate this body of literature are discussed below.

Recently, Allen and colleagues (Allen *et al.*, 2015a) demonstrated that people with T2D and diabetic polyneuropathy have greater fatigability than controls after isometric contractions. Ten people with T2D (6 men; 64 ± 11 years) and diabetic polyneuropathy and 10 controls matched for age and sex (6 men; 62 ± 12 years) performed a sustained maximal voluntary isometric contraction (MVIC) until the isometric torque dropped by 40% with the ankle dorsiflexor muscles, with peroneal nerve stimulations performed before and after the fatiguing task. The time to task failure was ~20% shorter (56.4 ± 14.2s vs. 71.1 ± 11.7s) in people with T2D and diabetic polyneuropathy compared with controls (i.e. greater fatigability in T2D) and there was evidence of failure in neuromuscular transmission (reduced maximal compound muscle action potential, M_{max}) in people with T2D and diabetic polyneuropathy. Because controls were not matched for physical activity or BMI—both of which may be associated with fatigability (Bogdanis, 2012; Mehta & Cavuoto, 2017)— these results may not reflect greater fatigability due to T2D *per se*, these data demonstrate that people with T2D may have greater fatigability and neuromuscular transmission or motor unit properties may play a role.

Similarly, Almeida and colleagues (Almeida *et al.*, 2008) examined motor unit properties and fatigability in people with type 1 diabetes (T1D) and diabetic polyneuropathy. Intramuscular EMG was inserted into the belly of the vastus lateralis and participants performed an intermittent (6s contraction, 4s rest) at 50% MVIC until the 50% MVIC could not be held for 3 s. The time to task failure was ~twice as long (786.4 ± 654 s vs. 358.6 ± 124.5 s) and motor conduction velocities were ~40% faster (66.7 ± 1.68 $m \cdot s^{-1}$ vs. 48.2 ± 2.8 $m \cdot s^{-1}$) in controls compared to people with T1D and diabetic

polyneuropathy. Additionally, people with T1D and diabetic polyneuropathy had a reduction in motor unit discharge frequencies during the fatiguing task whereas controls had maintained motor unit discharge frequencies. These data suggest that people with T1D and diabetic polyneuropathy have greater fatigability, which may be associated with reductions in activation of the motor neuron pool for the knee extensor muscles. Collectively, these two studies (Almeida *et al.*, 2008; Allen *et al.*, 2015a) suggest that people with diabetes and diabetic polyneuropathy have greater fatigability due to impairments in activation of motor units; however, the effects of diabetes *itself* are unknown because controls were not matched for physical activity or BMI and people with diabetes also had polyneuropathy.

Fatigability has also been assessed among cohorts of people with T2D and no signs of diabetic polyneuropathy. In a study from Bazzucchi and colleagues (Bazzucchi *et al.*, 2015), eight sedentary men with T2D and eight sedentary, age-matched men performed an isometric fatiguing task with the knee extensor muscles and (separately) with the elbow flexors, holding 80% MVIC until torque dropped below 72% MVIC for 3 s. People with T2D had a shorter time to task failure (i.e. more fatigable; 20.1 ± 0.7s vs. 26.9 ± 1.3s) compared to controls for the knee extensors, but there was no group-difference in fatigability for the elbow flexors. Thus, group-differences (T2D vs. control) in fatigability may be specific to the limb used to perform the fatiguing exercise (upper vs. lower extremity)—a commonly observed phenomenon referred to as task specificity (Hunter, 2017). However, the "controls" that participated in this study had a mean HbA_{1c} value in the prediabetes range (5.7 ± 0.1%), and the prediabetes condition may predispose participants to susceptibility to fatigability.

Another study of fatigability in people with T2D and no clinical signs of neuropathy from Petrofsky and colleagues (Petrofsky *et al.*, 2005) enrolled 10 people (five men, five women; 38 ± 9.7 years) with T2D and matched these people with 10 controls (five men, five women; 33.8 ± 3.5 years) based on age and sex. Each group performed two isometric fatiguing contractions (40% MVIC with 5 minutes between each fatiguing contraction) with the finger flexors of their right hand. Each group performed the first fatiguing contraction for a similar time (~130 s); however, the second fatiguing contraction was shorter for people with T2D (i.e. greater fatigability) compared to controls (62.1 ± 13.7 s vs. 89 ± 14.5 s). Limb blood flow was estimated using venous plethysmography, and the exercise-induced increase in blood flow was blunted (less than half) in people with T2D compared to controls. Thus, the greater fatigability for people with T2D may have been due to a blunted increase in blood flow during the isometric exercise, indicative of possible impairments in vascular function. Collectively, the results from these studies suggest that people with diabetes have greater fatigability after isometric contractions. However, these studies are limited due to inclusion of people with diabetes and diabetic polyneuropathy or "controls" with elevated HbA_{1c}. Additionally, for each study there was no specific assessment/matching of physical activity so that the activity levels which are likely to be lower in people with diabetes, were not controlled.

There are two studies that examined group-related differences in fatigability during moderate-velocity dynamic (isokinetic) contractions. As part of an intensive insulin treatment study, Halvatsiotis and colleagues (Halvatsiotis *et al.*, 2002) enrolled three cohorts of people— eight people with T2D and no diabetic polyneuropathy, eight

weight-matched controls, and eight lean healthy controls, with the groups matched for age. Each cohort performed 30 maximal voluntary concentric contractions (180 °·s^{-1}) with the knee extensors. People with T2D had greater reductions in knee extensor muscle power (52.5 ± 7%) compared with weight-matched (43 ± 3%) and lean controls (41 ± 7%), with no differences between the control groups. Thus, obesity may not be the factor mediating greater fatigability in people with T2D, although this study did not examine or control for physical activity levels between groups which may explain the lack of difference between lean and obese controls.

In another study examining fatigability during dynamic contractions (IJzerman *et al.*, 2012), 39 people (20 men) with T2D and no diabetic polyneuropathy, 98 people (80 men) with T2D and diabetic polyneuropathy, and 19 "controls" (15 men)— each group matched for age but not BMI, sex or physical activity— performed 20 maximal concentric and eccentric isokinetic contractions (120 °·s^{-1}) with the plantar- and dorsiflexors and the knee extensors and flexors. The "controls" had an average HbA_{1c} within the prediabetes range (6.0 ± 0.5%). For three muscle groups (ankle dorsi- and plantar flexors and knee extensors), fatigability was similar between the three groups (T2D without diabetic polyneuropathy, T2D with diabetic polyneuropathy, control); however, for the knee flexor muscles, "controls" were less fatigable (22 ± 9% reduction in power) than both T2D groups i.e. those with diabetic polyneuropathy (29 ± 10% reduction in power) and those without diabetic polyneuropathy (30 ± 11% reduction in power). Additionally, there was no difference in fatigability between people with T2D with or without diabetic polyneuropathy, thus, suggesting that diabetic polyneuropathy may not exacerbate fatigability when performing dynamic contractions.

Collectively, the results from both isometric and isokinetic contraction literature do not demonstrate a clear finding regarding differences in fatigability between people with T2D and healthy controls. As denoted in Table 1.1, six of the 10 datasets demonstrated that people with T2D had greater fatigability compared to controls, but 40% of the datasets demonstrated no group-related differences.

			Fatigability			
Study	Task		LHC	Other	DM	
(Allen *et al.*, 2015a)	ISOM, 100%; DF		71.1 (11.7s)	--	56.4 (14.2s)	*
(Almeida *et al.*, 2008)	ISOM, 50%; KE		786.4 (654s)	--	358.6 (124.5)	*
(Bazzucchi *et al.*, 2015)	ISOM, 80%	KE	26.9 (1.3s)	--	20.1 (0.7s)	*
		EF	N/A	--	N/A	
(Halvatsiotis *et al.*, 2002)	ISOK, 30×180 $^{o}\cdot s^{-1}$; KE		41 (7%)	43 (3%)	52.5 (7%)	*
(IJzerman *et al.*, 2012)	ISOK, 30×120 $^{o}\cdot s^{-1}$	PF	35 (14%)	43 (14%)	39 (20%)	
		DF	53 (14%)	61 (14%)	58 (16%)	
		KE	30 (8%)	34 (13%)	37 (13%)	
		KF	22 (9%)	30 (11%)	29 (10%)	*
(Petrofsky *et al.*, 2005)	ISOM, 40%; FF		89.3 |(14.5s)	--	62.1 (13.7s)	*

Table 1.2 Summary of fatigability data between people with diabetes mellitus and healthy controls. These data summarize the fatiguing task— including agonist muscle, type of contraction, intensity of contraction and number of repetitions when applicable— as well as the fatigability data between lean, healthy controls (LHC) and people with diabetes mellitus (DM). In two studies more than 2 experimental groups were included: Halvatsiotis and colleagues (Halvatsiotis *et al.*, 2002) included people with T2D and diabetic polyneuropathy, weight-matched controls ('Other') and LHC and Ijzerman and colleagues (IJzerman *et al.*, 2012) included people with T2D and *without* diabetic polyneuropathy ('Other'), people with T2D and *with* diabetic polyneuropathy, and 19 LHC. ISOM, isometric; ISOK, isokinetic; DF, dorsiflexor; KE, knee extensor; EF, elbow flexor; PF, plantar flexor; KF, knee flexor; FF, finger flexor. *, people with DM have greater fatigability compared to LHC.

To critically appraise and formally synthesize the data from these studies, the data were transformed into standard mean differences (Table 1.3) to create a Forest Plot of standard mean differences in fatigability (Figure 1.4). Although there is variability between the study designs which varied for experimental groups, fatiguing task, muscle group, and age, each study provided evidence that people with T2D had greater

fatigability than controls when the data were transformed. Thus, when the current body of literature is considered in a new light, there is general evidence that people with diabetes mellitus have greater fatigability than lean healthy controls for isometric and isokinetic fatiguing contractions in velocities up to 180 $^{o}\cdot s^{-1}$. However, there is no understanding of whether people with T2D are more fatigable for high velocities contractions (for limb velocities that commonly occur during walking), when the load does not vary (isotonic contractions) which most closely mimic activities of daily living (Senefeld *et al.*, 2017). Furthermore, it is not clear whether the greater fatigability of limb muscles is merely due to difference in activity levels between controls and people with T2D.

	T2D			Control			SMD
Study	$\overline{x}$	SD	n	$\overline{x}$	SD	n	$\overline{x}$ (SE)
(Allen *et al.*, 2015a)	56.4s	14.2	10	71.1s	11.7	10	26.1 (5.8)
(Almeida *et al.*, 2008)	358.6s	124.5	10	786.4s	654	10	119.3 (210.6)
(Bazzucchi *et al.*, 2015)	20.1s	0.7	8	26.9s	1.3	8	33.8 (0.5)
(Petrofsky *et al.*, 2005)	62.1s	13.7	10	89.3s	14.5	10	43.8 (6.3)
(Halvatsiotis *et al.*, 2002)	47.5%	7	8	59%	7	8	24.2 (3.5)
(IJzerman *et al.*, 2012)	61%	20	39	65%	14	19	6.6 (5.1)
	42%	16	39	47%	14	19	11.9 (15.4)
	63%	13	39	70%	8	19	11.1 (3.3)
	61%	10	39	78%	9	19	27.9 (9.9)
Total			202			128	33.8 (26.9)

Table 1.3. Summary of standard mean differences (SMD) in fatigability between people with diabetes mellitus and healthy controls. The standard mean differences in fatigability between people with diabetes mellitus compared to controls for each muscle group within each study was generated based on the data presented in Table 1.2. The equations listed below (*equations 1.1, 1.2 and 1.3*) were used for calculations, where $\overline{x}$ is the mean, n is the sample population, s is the standard deviation, and subscript Arabic numerals denote the referenced sample population. The positive mean values for each dataset demonstrates that people with diabetes mellitus have greater fatigability compared to lean healthy controls.

$$\overline{x}_{difference} = (\overline{x}_{Control} - \overline{x}_{T2D}) \cdot (\overline{x}_{Control})^{-1} \qquad \textit{equation 1.1}$$

$$s = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}} \qquad \textit{equation 1.2}$$

$$se(\bar{x}_1 - \bar{x}_2) = s \cdot \sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \qquad \textit{equation 1.3}$$

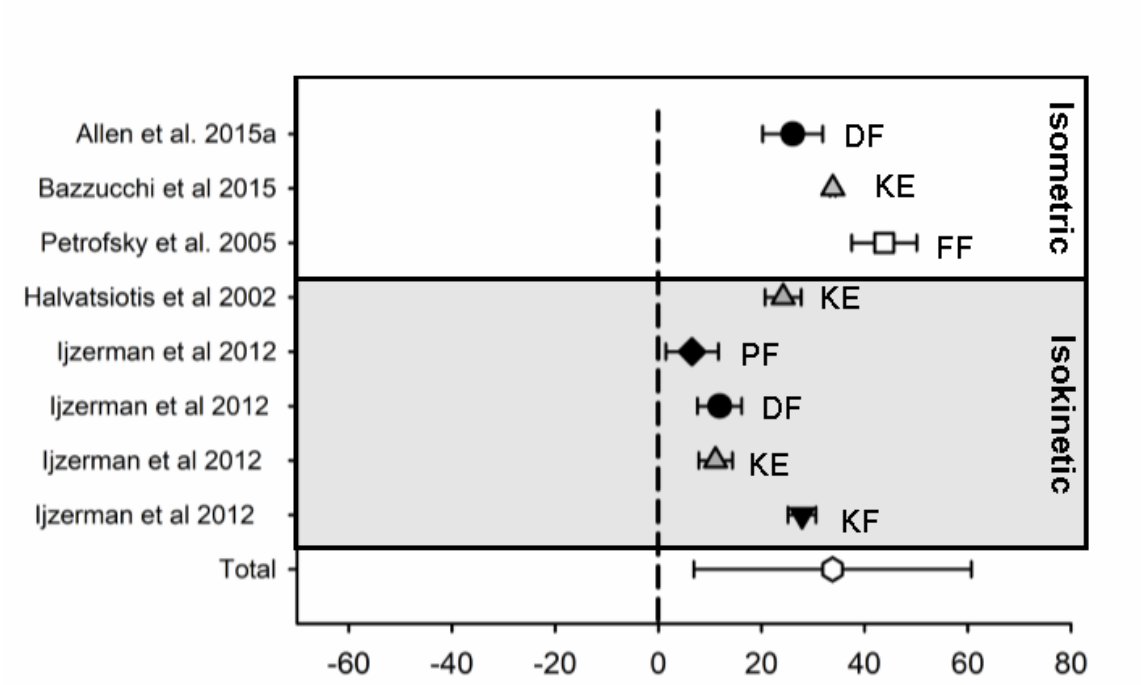

Figure 1.4. Forest Plot of standard mean differences in fatigability between people with diabetes mellitus and controls. Represented are mean data from isometric contraction studies and isokinetic studies (120 & 180°·s^{-1}). The group difference in fatigue (% controls) is plotted, calculated as the mean difference in fatigability (*equation 1.1*). Each data point is positive, indicating that controls were less fatigability than people with diabetes mellitus for these studies. Data from Almeida and colleagues (Almeida *et al.*, 2008) is not shown because the mean difference (786 ± 654%) was an outlier from this group of studies.

Thus, the purpose of this dissertation was to quantify fatigability of the knee extensor muscles for a dynamic fatiguing contraction task and identify the contributing mechanisms in healthy controls, people with prediabetes (Study 2; Chapter 4) and people with T2D (Study 1 - 3; Chapters 3 - 5). The purpose of Study 3 (Chapter 5) was to examine the potential contribution of vascular kinetics (flow-mediated dilation and exercise-induced dilation) to fatigability among healthy controls and people with T2D.

1.5 Specific Aims

Aim 1: Compare fatigability of both men and women with T2D (without clinically-evident neuropathy) with age-, BMI- and physical activity-matched controls in response to a high-velocity dynamic fatiguing task with the knee extensor muscles and determine the contribution of neural and muscular mechanisms.

Hypothesis 1.1: Fatigability of the knee extensor muscles would be greater in people with T2D compared with healthy controls.

Hypothesis 1.2: Both neural and contractile mechanisms would contribute to the greater fatigability in people with T2D compared with healthy control participants.

Aim 2: Determine the group-related difference (T2D, prediabetes, control) in and associations between [performance] fatigability and perceived fatigability.

Aim 2.1: Compare [performance] fatigability and the contributing neural and muscular mechanisms in men and women with prediabetes compared to those with T2D and healthy controls matched for age, BMI and physical activity after a high-velocity dynamic fatiguing task with the knee extensor muscles.

Hypothesis 2.1: Fatigability of the knee extensor muscles would be similar between people with T2D and prediabetes but greater compared with healthy controls.

Aim 2.2: Compare perceived fatigability, baseline and in response to dynamic fatiguing task, between people with T2D and prediabetes with controls.

Hypothesis 2.2: People with T2D and prediabetes would have greater perceived fatigability at baseline and larger increases in perceived fatigability compared with controls.

Aim 2.3: Determine the association between indices of perceived fatigability and performance fatigability.

Hypothesis 2.3: Greater perceived fatigability would be associated with greater performance fatigability among people with T2D and prediabetes

Aim 3: Determine the contributions of impaired exercise-induced hyperemia and flow-mediated dilation to greater fatigability among people with T2D compared to age-, BMI- and physical activity-matched controls.

Hypothesis 3.1: Impaired exercise-induced hyperemia and flow-mediated dilation would contribute to the greater fatigability in people with T2D compared with healthy controls.

CHAPTER 2: METHODOLOGICAL CONSIDERATIONS

2.1 Exclusion Criteria

HbA_{1c} and plasma glucose are two diagnostic criteria for people with T2D and prediabetes (Kim *et al.*, 2016), and were used to inform participant allotment into experimental groups (T2D, prediabetes and control). Participants included in the T2D group were physician-diagnosed, had no clinically-diagnosed diabetic polyneuropathy, were *not* prescribed insulin or an insulin secretagogue, and had stable glycemic control (HbA_{1c} < 10%). T2D was managed by diet, exercise and oral hypoglycemic medications for all participants included in the studies of this dissertation. HbA_{1c} and fasting plasma glucose measures were used to confirm diagnosis of T2D and assure HbA_{1c} < 10%.

However, because approximately 90% of people with prediabetes are unaware of their undiagnosed, underlying disorder (CDC, 2014), HbA_{1c} and plasma glucose tests were used to classify "controls" into the control or prediabetes groups. Importantly, we informed all participants of the results of the assays to measure HbA_{1c} and fasting plasma glucose and provided American Diabetes Association classification criteria (American Diabetes, 2015a); however, no diagnoses of T2D or prediabetes were made in the research laboratory. Participants were provided their results written and orally and participants were group based on these results, but participants were encouraged to visit their respective physicians for clinical diagnoses.

Blood samples were taken and assessed after a 4-hour fast, using standard point of care assessment tools for HbA_{1c} (Siemens Healthcare Diagnostics, DCA 2000+) and plasma glucose (Alere Cholestech LDX System). The Siemens DCA 2000+ HbA_{1c} point

of care instrument is an easy to use technology and is accepted by the National Glycated Hemoglobin Standardization Program (NGSP) (Bode *et al.*, 2007).

Screening for Diabetic Polyneuropathy

Participants, regardless of group allotment, were carefully screened for the presence of nerve dysfunction or diabetic polyneuropathy, and participants with nerve dysfunction or diabetic polyneuropathy were excluded. There is substantial evidence to suggest that sensory and autonomic nerve function is affected prior to motor nerve involvement (Allen *et al.*, 2016), therefore, diabetic polyneuropathy screening was focused on sensory and autonomic nerve function. Participants were informed (oral and written) of the results of the diabetic polyneuropathy screening and were encouraged to seek a clinical opinion or diagnosis from their physician if participants were excluded due to suspected diabetic polyneuropathy.

Sensory Dysfunction Screening

Screening for sensory neuropathy included a monofilament test and a vibratory sensation test. A neuropathy questionnaire (Michigan Neuropathy Screening Instrument) (Moghtaderi *et al.*, 2006) was administered and a standard physical evaluation was performed according to standards suggested by the Health Resources and Services Administration in the Lower Extremity Amputation Prevention (LEAP) resources (1998). The monofilament screening examination entailed sensation testing at 12 sites on each foot of the participant using 10-g monofilament with sufficient pressure applied to bend the monofilament. Participants were excluded if the monofilament could not be sensed on two or more sites. Six participants were excluded due to poor sensation of 10-g monofilament and each participant missed ~ 50% of the sites on each foot.

Detection of vibration was assessed using a 128 Hz tuning fork, applied after a standardized perturbation, over the right and left medial malleoli and the head of the 1st metatarsal of each foot. Participants were required to advise the tester whether they could feel the instrument and the vibration and when they could no longer detect vibration. Participants were excluded if vibrations could be sensed by the examiner for more than 10 s longer than the participant. No participants were excluded based on vibration sensation.

Achilles tendon reflex was tested in each leg in the seated position with the leg resting over the edge of a plinth, and participants were excluded if tendon jerk was absent. No participants were excluded based on Achilles tendon reflex.

Autonomic Dysfunction Screening

Autonomic nerve function was assessed via assessment of blood pressure in upright vs. supine posture and electrocardiogram (ECG) during a submaximal exercise test. Blood pressure was manually auscultated in supine vs. upright posture, and participants were excluded if there was a sustained decrease in systolic pressure (≥ 30 mmHg) and diastolic pressure (≥ 30 mmHg) (Barbato, 1990). ECG was used to assess arrhythmias during the aerobic fitness test (as described below), and participants were excluded if arrhythmias were consistently present (5 or more occurrences in the 30 minutes of ECG), if potentially dangerous arrhythmias were observed, or if an appropriate heart rate response was not achieved during the test. Two people were excluded due to potentially dangerous arrhythmias— atrial flutter and first degree atrioventricular block. One person was excluded due to bradycardia during exercise, the participant achieved a maximal heart rate of 100 beats per minute during exercise that

resulted in shortness of breath and rating of perceived exertion of 18 out of 20— the participant did not report a prescription to β-blocker medication; however, this test was suggestive of β-blocker use.

2.2 Recruitment

Recruitment for this dissertation encompassed five years. Recruitment was extensive due to the specific inclusion and exclusion criteria. Over the course of the five-year period, approximately 500 people were phoned from the laboratory. Nearly 200 of those 500 people were immediately excluded due to blunt reasons, for example, previous stroke or transient ischemic attack, current medical treatment (cancer, depression, etc.), or a lack of interest after hearing a description of the study. However, 304 people were fully screened via telephone, and of those people, 166 people were excluded—52 people due to physician diagnosis of diabetic polyneuropathy, 54 people due to treatment with insulin or an insulin secretagogue, 56 people due to overt medical conditions undisclosed previously (e.g. osteoarthritis in both knees), and 4 people chose to not be included during the phone screening.

After enrolling and completing a portion of a study, an additional 14 people were excluded: 1 person was intoxicated upon arrival to the laboratory, 9 people were suspected of having diabetic polyneuropathy (6 people scored poorly on LEAP screening and 3 people had suspected autonomic neuropathy), 2 people were excluded due to non-compliance with study guidelines (e.g. overnight fasting), and 2 people were excluded due to poor glycemic control (HbA_{1c} > 10.0%). Thus, although 138 people were consented, only 124 people completed one or more of the studies incorporated in this

dissertation, including 60 people with T2D, 20 people with prediabetes and 44 healthy controls. See Figure 2.1 and Figure 2.2.

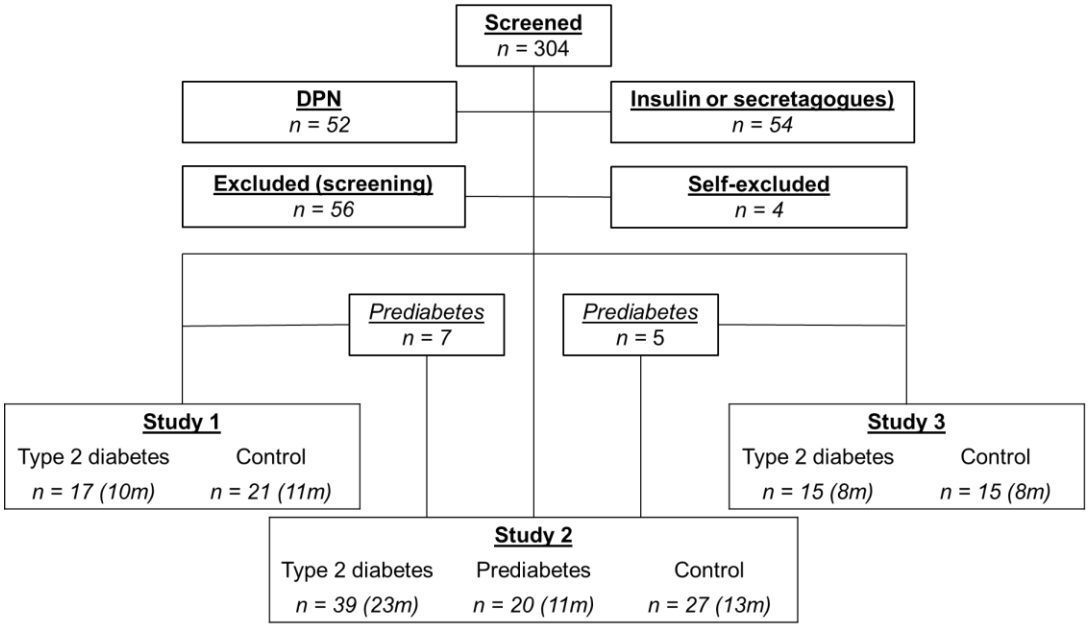

Figure 2.1. Diagram of participant recruitment. This schematic denotes the participant recruitment into each study of the dissertation from one collective cohort of potential participants.

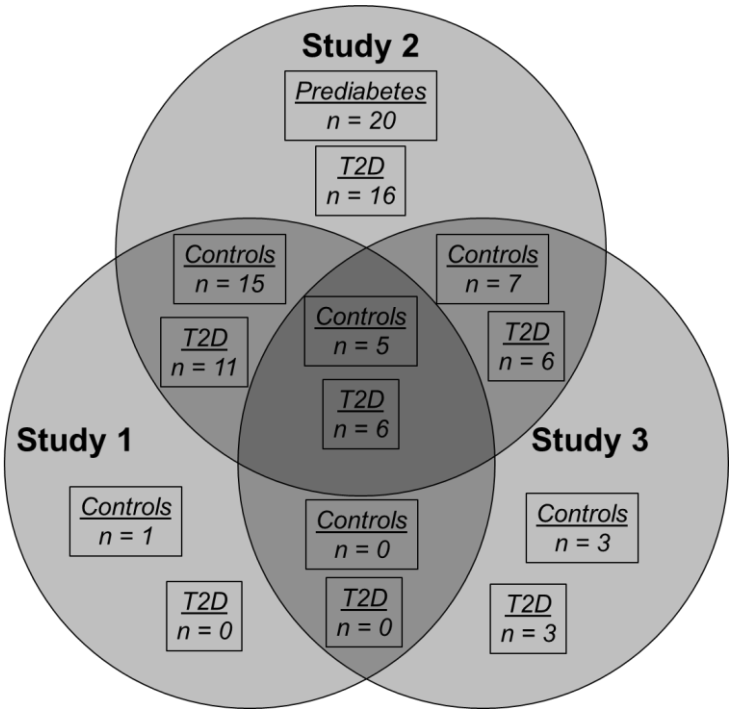

Figure 2.2. Diagram of participation within each study. This Venn diagram demonstrates the allotment of participants with each study and denotes which participants completed one or more studies that are included in this dissertation. Eleven people completed all three studies.

2.3 Matching Criteria

For each study, groups of participants were matched according to age, sex, body mass index (BMI) and physical activity (steps·day^{-1}). These matching criteria in combination with inclusion/exclusion criteria resulted in homogenous groups across many different baseline assessments, including: baseline muscle function (voluntary and electrically-evoked), body size, and physical function. This control population is a unique aspect of these studies, and a novel approach to examine fatigability in people with T2D and prediabetes. This experimental approach and control group differs to the typical studies of fatigability in people with T2D where control groups are typically lean, healthy controls (Petrofsky *et al.*, 2005; IJzerman *et al.*, 2012; Allen *et al.*, 2015a; Bazzucchi *et al.*, 2015) or people with diabetes that have diabetic polyneuropathy or insulin

prescription (Almeida *et al.*, 2008; Allen *et al.*, 2015a). This is a robust experimental approach to examine the effects of T2D *per se*.

Age

People age 40 and older were recruited for these studies. The onset of T2D at young ages is typically accompanied by increased risk for vascular complications at an earlier stage and require a more aggressive and supportive disease management (Wilmot & Idris, 2014; Al-Saeed *et al.*, 2016), and age 40 is common to delineate young-onset T2D (Song, 2015). Additionally, because fatigability can differ across the lifespan (Hunter *et al.*, 2016), age was used as a matching criterion between people with T2D and healthy controls.

Sex

There are known sex-related differences in fatigability (Hunter, 2016a, b), thus near equivalent numbers of men and women were tested within each group and sex was used as a matching criterion between people with T2D and healthy controls. Additionally, the potential sex-related differences among people with T2D are unknown, therefore, a tertiary aim of each study included in this dissertation was to quantify sex-related difference in fatigability among people with T2D. Also, the National Institutes of Health recommends testing sufficient numbers of men and women to explore potential sex-related differences in all clinical research (NOT-OD-15-102).

Anthropometry

Body anthropometry included measurements of height, body mass and waist circumference. Lean tissue mass was determined using a dual-energy X-ray absorptiometry (DEXA) scan (Lunar Prodigy full-body scanner, Madison, WI, USA) (Smith-Ryan *et al.*, 2017). The scanner was calibrated prior to each scan. The analyzed data was recorded offline (Encore 2008 software by GE Health care). In the case of participants with artificial joints, the artificial joint was excluded via encore software. Participants were matched according to body anthropometry, see Table 2.2.

			Control n = 44	Prediabetes n = 20	Type 2 Diabetes n = 60
Whole Body	Fat Mass	kg	26.8 ± 8.6	26.6 ± 7.0	31.0 ± 13.6
	Lean Mass	kg	51.3 ± 12.1	46.7 ± 9.1	52.6 ± 10.9
	Fat Mass	%	33.0 ± 8.6	34.9 ± 7.0	34.8 ± 10.1
Dom. Leg	Fat Mass	kg	4.1 ± 1.7	4.1 ± 1.4	4.3 ± 2.4
	Lean Mass	kg	8.5 ± 2.3	8.0 ± 1.7	8.7 ± 2.1
	Fat Mass	%	31.2 ± 11.9	32.3 ± 10.0	30.7 ± 11.9

Table 2.1. Anthropometrics of controls and people with prediabetes and type 2 diabetes. Data for all participants included in the studies in this dissertation are included. Fat mass (kg and %) and lean mass for the whole body and dominant leg (dom. leg) were measured via dual X-ray absorptiometry. There were no group-related differences in these measures. Values are reported as mean ± standard deviation of the mean.

Physical Activity

Accelerometry data were collected using the Actigraph GT3X+ (ActiGraph, Pensacola, FL, USA) that was worn on the hip by each participant for 4 days (2 weekdays and 2 weekend days). Sixty-second epochs of data were collected and analyzed. Wear-time authentication was performed on each participant's dataset to determine whether data were to be included in the analysis. Acceptable wear-time was set *a priori* and defined as ≥ 3 days of ≥ 9 hours (540 minutes) per day. To determine if each participant's

dataset met these criteria, data were filtered. Data were excluded if there were 60 *or more* consecutive minutes of zeros (indicating non-use), or if counts exceeded 15,000 per minute (indicating malfunction). The daily accelerometry data and the data recorded by the participant in the activity log were compared for obvious inconsistencies (e.g. equipment failure) and data considered erroneous were not included in the analysis. Wear time was recorded to be all data that did not meet exclusion criterion. No data was excluded due to malfunction or obvious inconsistencies.

Data were grouped by kcounts indicated by ActiLife v4 software. The cut-points to delineate each level of physical activity were defined according to (Freedson *et al.*, 1998; Crouter *et al.*, 2006; Hart *et al.*, 2011a). Data were analyzed as a percent of wear time (540+ minutes of wear time). Delineations were made as follows: sedentary time was considered 50 kcounts or less per minute, light lifestyle time was considered 51-759 kcounts, moderate lifestyle time was considered 760-1951 kcounts, light physical activity time was considered 50-1951 kcounts (this encompassed light lifestyle and moderate lifestyle times), moderate physical activity time was considered 1952- 5724 kcounts, and vigorous physical activity time was considered 5725-14999 kcounts. Step count was also recorded (ActiLife Software v4) and analyzed.

Acceptable data were analyzed to determine daily activity count and step counts, and were analyzed using cut points to determine period of time and percentage of wear-time spent in various intensities of physical activity. Moderate and vigorous activity data were conflated to generate data for moderate-to-vigorous activity; and bouts of moderate to vigorous physical activity were defined as lasting at least 10 minutes with allowance

for 2 minutes of below moderate intensity activity. Participants were matched according to daily physical activity, see Tables 2.2 and 2.3.

		Control n = 36	Prediabetes n = 20	Type 2 Diabetes n = 57
Daily PA	steps·day^{-1}	7,994 ± 2,956	8,283 ± 3,311	8,195 ± 4,090
Sedentary	% WT	65.2 ± 10.7	63.7 ± 6.3	64.4 ± 12.9
Light PA	% WT	31.0 ± 10.3	31.8 ± 5.6	31.7 ± 11.8
Moderate PA	% WT	3.5 ± 2.9	4.0 ± 2.2	3.8 ± 3.2
Vigorous PA	% WT	0.3 ± 0.8	0.5 ± 1.3	0.1 ± 0.2
MVPA	% WT	3.8 ± 3.4	4.5 ± 2.5	3.9 ± 3.3

Table 2.2. Physical activity assessed with accelerometry of controls and people with prediabetes and type 2 diabetes. Data for most participants included in the studies in this dissertation are included; however, some data were excluded due to *a priori* exclusion criteria. Data were measured via triaxial accelerometry (GT3X). There were no group-related differences in these measures. Values are reported as mean ± standard deviation of the mean. PA, physical activity; MVPA, moderate-to-vigorous physical activity; WT, wear time.

In addition to triaxial accelerometry, physical activity was estimated using two validated questionnaires: the Physical Activity Questionnaire (Kriska *et al.*, 1990) and the Physical Activity Scale for the Elderly (Washburn *et al.*, 1993).

		Control n = 44	Prediabetes n = 20	Type 2 Diabetes n = 60
PAQ	MET·hr·week^{-1}	34.1 ± 29.4	42.6 ± 42.2	47.9 ± 49.4
PASE	AU	176 ± 99	189 ± 97	187 ± 94

Table 2.3. Physical activity estimated with questionnaires of controls and people with prediabetes and type 2 diabetes. Data for all participants included in the studies in this dissertation are included. There were no group-related differences in estimated physical activity. Values are reported as mean ± standard deviation of the mean. PAQ, Physical Activity Questionnaire; PASE, Physical Activity Scale for the Elderly; MET·hr·week^{-1}, metabolic equivalent hours per week; AU, arbitrary units.

2.4 Perceptions and Medications

The most common perceptions among people with T2D and prediabetes that may affect assessments of performance fatigability include perceived fatigability, symptoms of depression and impaired sleep quality (Fritschi & Quinn, 2010; Fritschi *et al.*, 2012)—which were assessed with the Fatigue Impact Scale (Fisk *et al.*, 1994), Short Form Geriatric Depression Scale (Parmelee & Katz, 1990; Snowdon, 1990; Burke *et al.*, 1991), and Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989), respectively. Participants were matched according to perceptions that may contribute to perceived fatigability, see Table 2.4.

	Control n = 44	Prediabetes n = 20	Type 2 Diabetes n = 60	
FIS Cognitive	3.5 ± 4.7	3.2 ± 4.2	4.9 ± 5.7	
FIS Physical	2.9 ± 4.3	3.9 ± 5.5	6.6 ± 6.9	*†
FIS Psychological	4.5 ± 6.3	5.5 ± 10.0	8.4 ± 6.1	
FIS Total	10.9 ± 13.9	12.6 ± 18.6	19.5 ± 22.8	
GDS	0.61 ± 0.93	0.92 ± 1.09	1.18 ± 1.70	
PSQI	3.84 ± 2.24	4.67 ± 2.60	4.80 ± 2.35	

Table 2.4. Reports of clinical symptoms of fatigue, depression and sleep quality in people with prediabetes and type 2 diabetes. Data for all participants included in the studies in this dissertation are included. The physical domain of the Fatigue Impact Scale was greater for people with T2D compared to prediabetes and controls; however, there were no other group-related differences. Values are reported as mean ± standard deviation of the mean. FIS, Fatigue Impact Scale; GDS, Geriatric Depression Scale; PSQI, Pittsburgh Sleep Quality Index. Tukey's HSD post-hoc: *, T2D vs. control, $P < 0.05$; †, T2D vs. prediabetes, $P < 0.05$.

The standard treatment algorithm for people with T2D, as put forth by the International Diabetes Federation (IDF) (Czupryniak, 2009), includes metformin as second line treatment after lifestyle interventions (exercise, diet and weight management). In addition to metformin, statins and angiotensin converting enzyme

(ACE) inhibitors are commonly prescribed medications among people with T2D. Thus, the prescriptions of these medications were quantified, see Table 2.5.

			Control	Prediabetes	Type 2 Diabetes
Diabetes Duration		years	--	--	7.7 ± 6.9
Metformin		n	0	1	60
	Daily Dose	mg	--	1,000	1,155 ± 586
	Duration	years	--	7.0	4.6 ± 5.1
Statin*		n	8	5	34
	Daily Dose	mg	20.6 ± 24.6	13.0 ± 6.7	19.8 ± 21.0
	Duration	years	5.5 ± 6.0	7.2 ± 4.3	4.8 ± 4.9
ACE inhibitor#		n	4	3	21
	Daily Dose	mg	11.3 ± 7.5	10.0 ± 5.0	25.2 ± 24.7
	Duration	years	5.8 ± 7.6	9.0 ± 5.8	6.5 ± 7.1

Table 2.5. Prescriptions of daily doses of metformin, statins, and angiotensin converting enzyme (ACE) inhibitors. Data for all participants included in the studies in this dissertation are included. These data are descriptive and were not analyzed statistically. Values are reported as mean ± standard deviation of the mean. *Statin data are reported using an Atorvastatin equivalent dose per published drug information accessed via: http://www.globalrph.com/statins_comparisons.htm. #ACE inhibitor data are reported using a Lisinopril equivalent dose per published drug information accessed via: http://www.globalrph.com/aceinh.cgi.

CHAPTER 3: MECHANISMS FOR THE INCREASED FATIGABILITY OF THE LOWER LIMB IN PEOPLE WITH TYPE 2 DIABETES

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Senefeld J, Magill SB, Harkins A, Harmer AR, Hunter SK. Mechanisms for the Increased Fatigability of the Lower Limb in People with Type 2 Diabetes Journal of applied physiology. 2018: (in press).

3.1 Introduction

Type 2 diabetes mellitus (T2D) has become a global pandemic and is estimated to currently affect 8% of the world's population (Ogurtsova *et al.*, 2017). Physical activity is a cornerstone of T2D management and along with diet is the first intervention used to treat T2D (Sigal *et al.*, 2006). Incidence of T2D can be reduced by 58% with lifestyle interventions (diet, weight loss and exercise), and these lifestyle interventions were almost twice as effective as pharmacological treatment (metformin), which reduced the incidence of diabetes by 31% (Knowler *et al.*, 2002). Fatiguing contractions of limb muscles are the foundation of exercise training and the neuromuscular adaptations that accompany regular exercise (Kraemer & Ratamess, 2004). However, there is minimal understanding of the mechanisms that limit a single bout of fatiguing exercise in people with T2D.

Fatigability of limb muscles is a reversible, short-term and activity-induced reduction in muscle strength or power (Gandevia, 2001; Hunter, 2017), and can limit performance of daily tasks that require repeated or sustained contractions (Enoka & Duchateau, 2016; Senefeld *et al.*, 2017). Mechanisms that contribute to limb fatigability

in healthy adults include deficits in neural drive to the muscle, impairments in neuromuscular propagation, reduced force capacity of skeletal muscle fibers, and impaired blood flow to the muscle (Gandevia, 2001; Debold *et al.*, 2016; Hunter, 2017). Few studies have examined the mechanisms of fatigability among people with diabetes. Several studies have shown that for isometric contractions with lower limb muscles (ankle dorsiflexor and knee extensor muscles), people with type 1 diabetes and diabetic polyneuropathy and people with T2D, were more fatigable than controls (Almeida *et al.*, 2008; Allen *et al.*, 2015a; Bazzucchi *et al.*, 2015). The mechanisms contributing to greater fatigability in the people with type 1 diabetes who had diabetic polyneuropathy included disruption of neuromuscular transmission indicated by a concomitant decrease in the maximal compound muscle action potential (Allen *et al.*, 2015a) and slowed motor unit conduction velocities and discharge frequencies (Almeida *et al.*, 2008). The mechanisms for the greater fatigability in the lower limb muscles of people with T2D are not known.

Decrements in power during repeated dynamic fatiguing contractions are probably of greater functional significance than decrements in torque during isometric tasks in people with T2D. First, at baseline (without fatigue) the difference (reduction) in muscle power for people with T2D compared with controls is greater than for maximal isometric torque (Hilton *et al.*, 2008; Allen *et al.*, 2016). Second, low power and maximal velocity of limb muscles at baseline were the primary variables associated with impaired balance and gait in people with T2D (Orr *et al.*, 2006). Whether people with T2D are more fatigable during dynamic contractions, which can further exacerbate power differences between controls and people with T2D, is relatively unexplored. One study

demonstrated that after 20 moderate-velocity (120 deg·s^{-1}) isokinetic contractions performed separately with four lower limb muscle groups, people with T2D (with and without diabetic polyneuropathy) were more fatigable than age-matched controls for the knee flexor muscles, but not the ankle plantar flexor or dorsiflexor, or knee extensor muscles (IJzerman *et al.*, 2012). Another study, showed that people with T2D tended to have greater reductions in knee extensor torque over 30 isokinetic contractions at 180 deg·s^{-1} than healthy age-matched controls (both lean and weight-matched), although these differences in torque reductions did not reach statistical significance, possibly due to low subject numbers (n = 8) (Halvatsiotis *et al.*, 2002). There are no other known studies determining the fatigability during dynamic fatiguing tasks in people with T2D, and furthermore, the mechanisms are unknown. Lastly, despite potential differences in fatigability between men and women (Hunter, 2016a), studies of fatigability in people with T2D have been underpowered to determine whether there are sex-related differences among people with T2D (e.g. (Halvatsiotis *et al.*, 2002; Petrofsky *et al.*, 2005; IJzerman *et al.*, 2012; Bazzucchi *et al.*, 2015).

The mechanisms for any potential increased fatigability of limb muscles in men and women with T2D may originate from both neural (supraspinal and spinal) and muscular sites. People with T2D may have impaired skeletal muscle energetics (i.e. increased inorganic phosphate and hydrogen ion within intracellular milieu) and reduced skeletal muscle blood flow during exercise compared with healthy controls (Scheuermann-Freestone *et al.*, 2003; Lalande *et al.*, 2008), potentially eliciting greater stimulation of afferent feedback (Group III and IV afferents) to supraspinal and spinal centers during fatiguing exercise, further exacerbating any exercise-related reductions in

neural drive to the muscle (Taylor *et al.*, 2016; Hunter, 2017). Furthermore, because people with T2D are at risk of neuropathy, neuromuscular transmission may contribute to differences in fatigability between people with and without T2D (Allen *et al.*, 2015a; Allen *et al.*, 2016). In this current study, we used non-invasive stimulation at the motor cortex and muscle to determine the contribution of neural (supraspinal and spinal) and muscular mechanisms (Todd *et al.*, 2003, 2016) to any differences in fatigability between people with T2D and controls.

The *purpose* of the study was to: 1) compare fatigability of both men and women with T2D (without clinically-evident neuropathy) with age-, BMI- and physical activity-matched controls in response to a high-velocity dynamic fatiguing task with the knee extensor muscles, and 2) determine the contribution of neural and muscular mechanisms. Our *hypotheses* were that: 1) fatigability of the knee extensor muscles would be greater in people with T2D compared with healthy controls, and 2) both neural and contractile mechanisms would contribute to the greater fatigability in people with T2D compared with healthy control participants. Because the age of onset of T2D is inversely related to disease complication risk and mortality, we enrolled participants >50 years. Additionally, because there is limited understanding of sex differences in fatigability of people with T2D, a third aim was to determine whether there were sex-related differences in fatigability and mechanisms among people with T2D. Our *hypothesis* was that there would be no sex-related differences in fatigability, as we have observed in a young and older adult population previously (Senefeld *et al.*, 2017).

3.2 Materials and Methods

Seventeen people with T2D (10 men: age, 59.7 ± 9.5 years; HbA1c, 6.92 ± 1.19%; 7 women: age, 59.6 ± 9.0 years; HbA1c, 7.20 ± 1.06%) and twenty-one healthy controls (11 men: age, 58.2 ± 10.3 years; HbA1c, 5.42 ± 0.25%; 10 women: age, 61.2 ± 8.8 years; HbA1c, 5.40 ± 0.21%) participated in the study. Prior to involvement in the study, each participant provided written informed consent and the protocol was approved by the Marquette University Institutional Review Board (HR-2402) for ethical approval in accordance with the Declaration of Helsinki for human experimentation.

Aside from glycemic control, all participants were healthy. Type 2 diabetes was physician-diagnosed and confirmed at study enrolment via fasting glucose and HbA_{1c}. Exclusion criteria included: unstable diabetes, prescribed insulin or insulin secretagogue, poor glycemic control (glycosylated hemoglobin (HbA_{1c}) >10%), diabetic neuropathy (assessed via clinical diagnosis, monofilament and tuning fork sensation tests, and sensory questionnaires), peripheral edema, severe obesity (body mass index, BMI, >45 kg/m^2), untreated hypothyroidism, epilepsy, medications that affect cortical excitability, possibility of pregnancy and any neurological, cardiovascular or musculoskeletal disease that precluded exercise testing. Any potential participants who presented with HbA_{1c} >5.7% and <6.5% (and were not diagnosed with T2D) were classified as having pre-diabetes and not included in the study; thus, all controls had an HbA_{1c} ≤5.6%.

Participants completed three sessions of testing that included a screening session to determine eligibility for the study followed by two experimental sessions. The aim of the first experimental session was to familiarize participants with experimental procedures and complete a fasting blood draw and questionnaires. The aim of the second

experimental session was to complete the fatiguing task. Each session was separated by 2-7 days.

Screening Session

During the screening session, the following tests were performed: 1) lower limb sensation was assessed using a 10-gram monofilament and 128-Hz vibration sensation test, 2) autonomic nerve function was assessed using a heart rate variability test and blood pressure response to upright posture, and 3) glycemic control was assessed using a point-of-care HbA_{1c} instrument. Skeletal muscle mass of the dominant leg and whole-body fat mass were assessed utilizing DEXA and participants were assigned a triaxial accelerometer. Then, peak aerobic capacity was estimated from a submaximal graded bicycle ergometer exercise test.

Diabetic neuropathy screening: Each participant was screened for the presence of diabetic polyneuropathy. To assess symptoms and signs of sensory neuropathy monofilament screening of the feet, vibration sensation testing (bilateral malleoli and heads of the 1^{st} metatarsals) and Achilles tendon reflex testing were performed. Participants were excluded if impaired sensation was observed i.e., if the monofilament could not be sensed on any site on the foot; if vibrations could be sensed by the examiner for more than 10 s longer than the participant; or if the tendon jerk was absent. Participants who were suspected of having diabetic polyneuropathy (sensory or autonomic) were excluded from the study.

HbA_{1c}: HbA_{1c} was determined using blood from a fingerstick, analyzed using a point-of-care instrument assay (Siemens Healthcare Diagnostics, DCA 2000+).

Anthropometry and DEXA: Body anthropometry included measurements of height, body mass and waist circumference. Skeletal muscle mass of the dominant leg and whole-body fat mass (% body weight), were assessed utilizing DEXA (Lunar Prodigy full-body scanner, Madison, WI, USA). The scanner was calibrated prior to each scan. The analyzed data was recorded offline (Encore 2008 software by GE Health care). In the case of participants with artificial joints (n = 4), the artificial joint was excluded via encore software.

Physical Activity Monitor: Accelerometry data were collected using the Actigraph GT3X (ActiGraph, Pensacola, FL, USA) that was worn on the hip by each participant for 4 days (2 weekdays and 2 weekend days). Sixty-second epochs of data were collected and analyzed. Wear-time authentication was performed on each participant's dataset to determine whether data were to be included in the analysis. Acceptable wear-time was set *a priori* and defined as ≥ 3 days of ≥ 9 hours (540 minutes) per day. Step count was recorded (ActiLife Software v4) and analyzed.

Submaximal, Graded Bicycle Test: Participants performed a submaximal graded exercise test (Beekley *et al.*, 2004) on a bicycle ergometer (VIAsprint 150P, CareFusion, San Diego, CA, USA) to determine estimated oxygen consumption and to screen for exercise-induced cardiac arrhythmia. Participants were required to maintain cadence of 60 revolutions per minute that was monitored via LED screen by the participant and a researcher, and the cycle load was manipulated to attain three submaximal loads that elicited incremental heart rate responses between 40% and 70% of heart rate reserve. The participant cycled at each submaximal load for four minutes to attain steady-state. During this test, a 12-lead electrocardiogram (CASE, General Electrics, Madison, WI, USA) was

monitored to determine if arrhythmias were present. Participants were excluded if arrhythmia was detected, even if asymptomatic.

Experimental Session One

Participants fasted for *at least* 8 hours prior to experimental session one. Venous blood was obtained via venous draw, after which participants consumed a standardized breakfast (8 oz. fruit juice, one cereal bar, and one serving of fruit) prior to undertaking the remaining activities in the session. In conjunction with fasting, participants with T2D delayed administration of medications until after the venous draw.

Participants completed a questionnaire to determine handedness/footedness (Oldfield, 1971) to assess which leg which would be used for testing. Participants first practiced submaximal muscle contractions, maximal voluntary isometric contractions (MVICs) and maximal voluntary concentric contractions (MVCCs) of the knee extensor muscles while seated in a Biodex System 4 dynamometer (Biodex Medical, Shirley, NY). They were also habituated with electrical stimulation of the femoral nerve, percutaneous electrical stimulation of the knee extensor muscles and transcranial magnetic stimulation (TMS) of the motor cortex.

Blood Measures: Fasting blood glucose was determined using a point of care instrument (Alere Cholestech LDX System, Alere Inc. Waltham, MA, USA). Hemoglobin concentration was determined using a point of care instrument (StatSiteM Hemoglobin Photometer, Stanbio, Boerne, TX, USA) and hematocrit was determined manually (International Micro-capillary Reader, International Equipment Company, Boston, MA, USA) per standard instruction of each instrument. Plasma insulin and thyroid-stimulating hormone concentrations were quantitatively assayed in duplicate per manufacturer

instructions using enzyme-linked immunoassay kits (Quantikine Human Insulin Immunoassay (R&D Systems, Minneapolis, MN) and Human TSH (CGA) ELISA Kit (Thermo Scientific Pierce (Waltham, MA), respectively).

Questionnaires: All participants completed questionnaires to assess: clinical symptoms of fatigue using the Fatigue Impact Scale (Fisk *et al.*, 1994); sleep quality with the Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989); and depression with the short form Geriatric Depression Scale (Snowdon, 1990).

Experimental Session Two

Participants consumed the same standardized breakfast as during the first experimental session; after which participants with T2D administered their diabetes medications. In this second experimental session, each participant performed baseline MVICs and MVCCs followed by a maximal-velocity fatiguing task and recovery contractions with the dominant knee extensor muscles.

Measurement of Torque, Velocity and Power

Participants performed isometric and isotonic contractions with the knee extensors muscles while seated in a dynamometer. Participants performed all contractions on their dominant leg, unless there was any form of disease (e.g. osteoarthritis) or injury (e.g. knee reconstruction), in which case the non-dominant leg was tested (n = 2 controls, 2 people with T2D). Participants were seated with 90º of hip flexion. Padded straps mounted on the seat were securely tightened across the shoulders, the waist, and the non-dominant leg to minimize synergistic movements. The dominant leg was positioned such that the axis of rotation of the knee joint was aligned with the axis of rotation of the dynamometer. The internal goniometer of the Biodex dynamometer was calibrated using

a level to measure 90º flexion of the knee joint. The analog signals corresponding to joint angle, torque, and velocity were digitized and recorded through a Power 1401 analog-to-digital (A-D) converter and Spike2 software (Cambridge Electronics Design, Cambridge, UK).

Electromyography

Electromyography (EMG) electrodes (Ag–AgCl, 8-mm diameter; 20 mm intra-electrode distance) were placed on three agonist muscles (rectus femoris, vastus lateralis and vastus medialis) in a bipolar arrangement according to recommendations (Hermens *et al.*, 2000) with reference electrodes placed over the patella of the dominant knee. The EMG signals were amplified (100×) and filtered between 13 - 1000 Hz (Coulbourn Instruments, Allentown, PA) and digitized at 2,000 Hz. Mechanical recordings from the dynamometer corresponding to torque, velocity and position were recorded online at 2,000 Hz. All analog signals were digitized using a 1401 A–D converter and Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK].

Transcranial Magnetic Stimulation (TMS)

TMS was delivered via a concave double cone coil (Magstim 200, Magstim, Whitland, UK, 11.0-cm outside diameter) over the motor cortex area to elicit motor-evoked potentials (MEPs) and torque during voluntary contractions of the dominant knee extensor muscles as described before (Senefeld *et al.*, 2018d). The vertex of the motor cortex was identified, and the scalp was marked 1.0 cm lateral to the vertex (over the motor area corresponding to the dominant knee extensors) to ensure repeatability of coil placement during the experimental protocol. The optimal coil position of the TMS was determined during brief contractions of the knee extensor muscles at 20% MVIC. TMS

was elicited during the contractions and fine adjustments in the TMS coil position (~0.5 cm) were made to determine which site evoked the largest superimposed twitch (SIT) torque and MEP of the rectus femoris muscle. Optimal stimulator intensity was also determined with brief contractions (2-3 s) of knee extensor muscles (50% MVIC), which is the intensity that is known to elicit maximal MEPs (Todd *et al.*, 2003). The intensity of the stimulation (% maximal of stimulator intensity) was increased by 5% increments until maximal twitch torque of the quadriceps and maximal MEP of the rectus femoris muscle were elicited. The brief contractions at 50% MVIC were separated by 30-s rest periods to avoid fatigue when establishing the intensity of TMS.

Electrical Stimulation

Single-pulse (200 μs duration, 400 V) electrical stimulation was used for femoral nerve and percutaneous muscle stimulation (DS7AH; Digitimer, Ltd., Welwyn Garden City, UK) to elicit maximal compound muscle action potentials (M_{max}) and twitch contractions at rest and during MVICs of the knee extensor muscles.

Femoral Nerve Stimulation: The femoral nerve innervating the knee extensor muscles was stimulated supramaximally (120 – 600 mA) with a single pulse to elicit the maximal compound muscle action potential (M_{max}). The cathode electrode (Ambu Neuroline electrodes, Denmark; 1.5 cm diameter) was placed over the femoral nerve within the femoral triangle and the anode was placed over the greater trochanter of the femur. The intensity of the nerve stimulation was determined by increasing the current until the twitch amplitude plateaued. The stimulation intensity was then increased further by 20% to ensure a maximal activation of the muscles within the area of stimulation.

Percutaneous Muscle Stimulation: To assess voluntary muscle activation and twitch properties, the knee extensor muscles were stimulated with a single pulse (150 – 750 mA) via custom-made pad electrodes (6 cm × ~15 cm) placed over the quadriceps muscles. The cathode was placed near (within 10 cm) the area of the femoral triangle and the anode was placed proximal to the patella without hindering knee flexion/extension of the participant. The stimulator intensity was determined by increasing the current until the twitch amplitude plateaued, then the stimulation intensity was increased further by 20% to ensure a maximal activation of the muscles in the area of stimulation. This stimulation intensity was used for the remainder of the session. The twitch amplitude elicited via percutaneous and femoral nerve stimulation were linearly correlated ($r^2 = 0.653$, $P < 0.001$). Percutaneous muscle stimulation was used throughout the experimental protocol for assessment of voluntary activation and twitch properties, because percutaneous stimulation was more tolerable than nerve stimulation. Using the supramaximal intensity, three muscle stimulations were applied, each separated by ~15 s to assess electrically-evoked twitch contractile properties in a non-potentiated state.

Experimental Protocol

The experimental protocol entailed:

(1) *Baseline MVICs:* Participants completed at least three MVICs for ~4 seconds each with the knee extensor muscles, positioned in 90° of hip and knee flexion. Participants then performed four additional MVICs during which TMS and electrical stimulation were superimposed to estimate voluntary activation (see the 'data analysis' section for calculations). Electrically-evoked, potentiated twitch contractions were also elicited at rest immediately after each MVIC to determine contractile properties and

voluntary activation of the knee extensor muscles. Each baseline MVIC was separated by 2.5 minutes, to minimize the effect of fatigue prior to beginning the dynamic fatiguing task.

(2) *Baseline Maximal Voluntary Concentric Contractions (MVCCs):* Participants warmed-up with 10 MVCCs with a load equivalent to 20% of MVIC. These isotonic contractions were performed through an ~85º range of motion, from 90°of knee flexion until 5°of knee flexion. Participants then rested for 2.5 minutes, before initiating the dynamic fatiguing task.

(3) *Dynamic fatiguing task:* The fatiguing protocol involved 120 isotonic MVCCs of the knee extensor muscles through an ~85º range of motion (as above) with 1 MVCC performed every 3 seconds (6-minute task). Participants actively extended the knee, then the dynamometer passively returned the leg to the starting position at 90º of knee flexion after each MVCC.

(4) *Recovery Contractions:* The recovery protocol involved sets of brief contractions immediately after the fatiguing task, and then at 5 and 20 minutes of recovery. Each set of contractions involved an MVIC (with a superimposed TMS and percutaneous muscle stimulation) followed by an additional electrically-evoked twitch contraction and then five successive MVCCs.

Participants received strong verbal encouragement throughout the maximal effort contractions. During all MVCCs, participants were instructed to “kick as hard and as fast as possible” and each MVCC was initiated via strong verbal command from the authors: “KICK”. The authors provided the verbal cue each 3-s based on a visual cue from a custom-designed data collection program, and participants were encouraged to maintain

maximal effort throughout the dynamic fatiguing task using several standard statements of encouragement.

Data Analysis

The torque during the MVICs was quantified as the average value over a 0.1 s interval prior to the onset of the TMS pulse. The maximum angular velocity, power and resistance torque during MVCCs were quantified during the concentric phase of the contraction. The average resistance torque during MVCCs was calculated as the average torque during the concentric phase of the knee extension contraction. The duty cycle was calculated as: (active contraction time) $\cdot$ (active contraction time + relaxation time)$^{-1}$. The variables from the dynamic fatiguing task are presented as the average from five consecutive contractions, at baseline (contractions 1-5) or the end of the fatiguing task (contractions 116-120).

Voluntary activation was assessed with both TMS and electrical stimulation. Voluntary activation with TMS was estimated with the SIT expressed as a percentage of the total torque i.e. [SIT $\cdot$ (MVIC + SIT)$^{-1}$ $\cdot$ 100%] (Gandevia, 2001). For electrically evoked contractions, voluntary activation was calculated using the following equation: voluntary activation = (1 – SIT $\cdot$ Potentiated Twitch^{-1}) $\times$ 100% (Gandevia, 2001; Todd *et al.*, 2016). Contractile properties of the knee extensor muscles were quantified from the potentiated twitch elicited with percutaneous electrical stimulation. Variables included the peak amplitude of the potentiated twitch, contraction time, and half relaxation time. Half relaxation time was determined as the time interval in milliseconds (ms) elapsed from the peak twitch amplitude until the torque reached 50% of the peak twitch amplitude. Post-activation potentiation (PAP) from electrically-evoked twitch

contractions was calculated as: (potentiated twitch amplitude - non-potentiated twitch amplitude) · non-potentiated twitch amplitude^{-1} · 100%.

Electrophysiological properties of the knee extensors were also assessed with peak-to-peak amplitude of the MEPs for the agonist muscles (rectus femoris, vastus lateralis and vastus medialis) elicited via TMS during MVICs. Similar results were observed for the MEP amplitude and area, thus, only MEP amplitude results are presented. The duration of the silent period was determined as the interval from the time of the TMS to the return of continuous EMG after the MEP (Taylor *et al.*, 1996). Reduction in variables (MVIC torque, MVCC velocity, power, duty cycle, range of motion, peak resistance torque, and average resistance torque, voluntary activation, twitch amplitude, contraction time, half relaxation time, peak rate of relaxation, EMG silent period and MEP (%M_{max})) for before and after the fatiguing task, were calculated as [1 – (end value · baseline value^{-1})] × 100%. Representative traces of raw data are presented in Figure 1, for dynamic contractions (Fig. 1A) and MVCs with stimulations (Fig. 1 B-F).

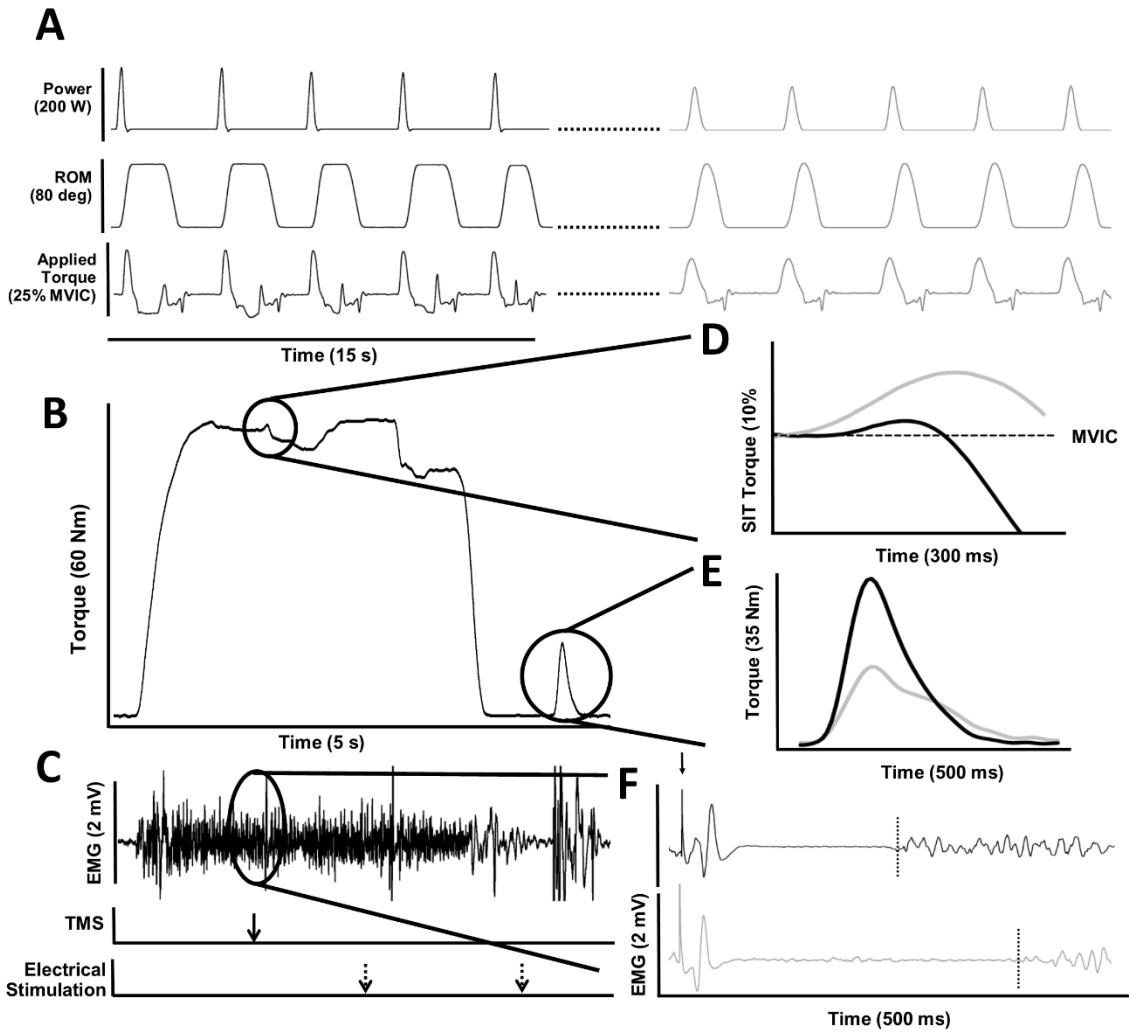

Figure 3.1. Representative data for maximal voluntary concentric contraction (MVCC) power, range of motion and applied torque, maximal voluntary isometric contraction (MVIC) torque, superimposed twitch (SIT) torque, potentiated twitch, motor evoked potential (MEP) and EMG silent period. ***A.*** Calculated power (applied torque × half-wave rectified velocity), range of motion and applied torque signals of a 62-year old control woman performing five MVCCs at the start (black lines) and end (grey lines) of the fatiguing task. The torque (***B***) and EMG (***C***) signal of the woman performing an MVIC with TMS-elicited SIT during the MVIC and electrical stimulation evoked twitches during the MVIC and at rest. The TMS-elicited SIT (***D***), electrically-evoked potentiated twitch (***E***) torque, and vastus lateralis EMG (***F***) signal displaying the MEP and EMG silent period from before (black line) and after the fatiguing task (grey line).

Homeostatic model assessment for assessing insulin resistance (HOMA-IR) was calculated using the fasting plasma insulin concentration (FPI, $mU \cdot L^{-1}$) and fasting plasma glucose (FPG, $mmol \cdot L^{-1}$): HOMA-IR = $(FPI \times FPG) \cdot 22.5^{-1}$.

Statistics

Values are reported as mean ± SD in the text and displayed as mean ± SE in the figures. Participant characteristics and baseline muscle function (Tables 1 and 2) were compared across groups using a univariate analysis of variance (ANOVA) with two between subject factors (group: T2D vs controls, and sex: male vs. female).

To determine changes over time during the dynamic fatiguing contraction or during the 20-minute recovery period (task end, 5 mins and 20 mins post the dynamic contraction), mixed model analysis of variance with group and sex as between subject factors and repeated measures over time was used for the various dependent variables (MVIC torque, MVCC velocity, power, duty cycle, range of motion, peak applied torque, and average applied torque, voluntary activation, twitch amplitude, contraction time, half relaxation time, peak rate of relaxation, EMG silent period and MEP ($\%M_{max}$)). For each ANOVA, the sphericity of data was determined, and technical corrections were performed when necessary. If needed, post hoc analysis with Bonferroni corrections were applied when an *F* test was significant. Pearson correlation coefficients (*r*) were used to determine associations between variables including fatigability (reductions in MVIC and MVCC), participant characteristics (fasting plasma glucose, HbA1c, estimated VO2 peak, skeletal muscle mass, daily step count, and questionnaire scores), baseline muscle characteristics (MVIC strength, MVCC power, voluntary activation, and potentiated twitch amplitude), and measurements of fatigue-related changes in the potentiated twitch

and voluntary activation. Linearity of bivariate correlations was verified with visual inspection, to confirm there were no violations of the assumptions of normality, linearity, and homoscedasticity.

Significance was determined at $P < 0.05$ and all analyses were performed using IBM Statistical Package for Social Sciences (SPSS, V24).

3.3 Results

Baseline Measurements

Participant and baseline characteristics are presented in Table 1. The T2D and control groups were similar in age (group effect, $P = 0.985$), BMI (group effect, $P = 0.172$), and daily physical activity (step count; group effect, $P = 0.895$). The control and T2D groups had similar body fat (group effect, $P = 0.310$), estimated VO_2 peak (group effect, $P = 0.231$) and skeletal muscle mass in the dominant leg (group effect, $P = 0.724$).

As expected, people with T2D had higher HbA_{1c} (group effect, $P < 0.001$), fasting plasma glucose (group effect, $P < 0.001$), fasting plasma insulin (group effect, $P = 0.001$) and HOMA-IR (group effect, $P < 0.001$) compared with controls (Table 1). People with T2D and controls had similar plasma thyroid-stimulating hormone concentrations (1.86 ± 0.89 vs. 1.58 ± 0.89 $mU \cdot L^{-1}$, respectively; group effect, $P = 0.306$). People with T2D and controls demonstrated no signs of anemia, hemoglobin (14.2 ± 1.8 vs. 14.6 ± 1.7 $g \cdot dL^{-1}$, respectively; group effect, $P = 0.428$) and hematocrit (42.4 ± 3.3 vs. 42.3 ± 4.0%, respectively; group effect, $P = 0.974$) concentrations were similar between the groups. Among the people with T2D, 14 people were prescribed metformin and 11 people were prescribed a statin medication. Among controls, 0 people were prescribed metformin and 4 people were prescribed a statin medication. Although not a primary aim of the study, it

is noteworthy that people with T2D prescribed to a statin medication had similar reductions in MVCC power (time × statin effect, $P = 0.458$; statin effect, $P = 0.729$) and MVIC torque (time × statin effect, $P = 0.742$; statin effect, $P = 0.571$) compared to people with T2D *not* prescribed to a statin medication. See Table 1.

	Units	Type 2 Diabetes *(n=17; 10 men)*	Control *(n = 21, 11 men)*
Age	years	59.6 ± 9.0	59.5 ± 9.6
BMI	$kg \cdot m^{-2}$	29.4 ± 7.0	27.2 ± 4.3
Body Fat	%	36.2 ± 13.8	32.4 ± 7.2
Duration of Diabetes	years	6.83 ± 6.45	0 *
HbA1c	%	7.04 ± 1.11	5.41 ± 0.23 *
Fasting Plasma Glucose	$mg \cdot dL^{-1}$	126.1 ± 32.1	87.1 ± 6.6 *
Fasting Plasma Insulin	pMol	59.1 ± 25.7	35.1 ± 17.5 *
HOMA-IR	AU	3.08 ± 1.71	1.28 ± 0.63 *
Estimated VO_2 Peak	mL/kg/min	27.9 ± 8.3	30.1 ± 7.8
Leg Muscle Mass	kg	8.22 ± 1.75	8.52 ± 2.36
Daily Step Count	n	8334 ± 3446	8295 ± 3218
Questionnaires			
PSQI	AU	4.19 ± 2.61	4.90 ± 2.41
FIS total	AU	24.18 ± 29.65	7.19 ± 14.91
FIS Cognitive	AU	6.38 ± 7.43	3.62 ± 4.42
FIS Physical	AU	7.63 ± 8.02	4.62 ± 5.84
FIS Psychological	AU	11.69 ± 15.53	6.67 ± 11.03
GDS	AU	2.00 ± 0	1.95 ± 0.22

Table 3.1. Participant characteristics and questionnaire scores. Values are displayed as mean ± SD. BMI, body mass index; HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; AU, arbitrary unit; PSQI, Pittsburgh Sleep Quality Index; FIS, Fatigue Impact Scale; GDS, Geriatric Depression Scale. (* denotes group difference between controls and T2D, $P < 0.05$).

People with T2D and controls had similar knee extensor MVIC torque (group effect, $P = 0.421$), peak angular velocities (group effect, $P = 0.949$), peak knee extensor power (group effect, $P = 0.627$), electrically-evoked potentiated twitch amplitudes (group effect, $P = 0.667$), and post-activation potentiation (group effect, $P = 0.368$). See Table 2.

Baseline levels of voluntary activation during MVICs were similar between controls and people with T2D, quantified with TMS (group effect, $P = 0.232$) and with electrical stimulation (group effect, $P = 0.715$; Table 2).

		Type 2 Diabetes (*n = 17*)			Control (*n = 21*)		
		Baseline	Task End	Δ (%)	Baseline	Task End	Δ (%)
MVCC Power	Watts	291 ± 139	163 ± 96	-42.8 ± 24.2†	261 ± 85	199 ± 90	-26.4 ± 15.0†*
MVCC Velocity	deg·s^{-1}	330 ± 62	209 ± 74	-36.2 ± 4.5†	333 ± 37	273 ± 61	-18.9 ± 4.6†*
Duty Cycle	%	13.7 ± 1.2	18.0 ± 2.0	+29.5 ± 20.5†	14.5 ± 2.1	18.1 ± 2.8	+25.8 ± 19.7†
MVIC Torque	Nm	176 ± 80	105 ± 46	-37.6 ± 18.2†	160 ± 59	115 ± 45	-26.4 ± 12.1†*
Twitch Amplitude	Nm	41.1 ± 16.8	21.2 ± 10.1	-44.0 ± 20.4†	43.4 ± 20.1	31.1 ± 12.0	-35.4 ± 20.0†*
Contraction Time	ms	83.1 ± 7.0	80.5 ± 14.0	NS	83.7 ± 10.0	82.4 ± 11.5	NS
Half-relaxation time	ms	70.2 ± 20.7	87.8 ± 30.0	+28.0 ± 12.7†	64.2 ± 18.4	74.1 ± 31.2	+27.4 ± 9.5†
VA (ES)	%	93.4 ± 3.4	84.2 ± 9.3	-12.1 ± 2.6†	92.8 ± 5.0	86.4 ± 7.3	-12.4 ± 4.4†
SIT	% MVIC	2.71 ± 1.72	4.76 ± 2.79	+55.8 ± 41.1†	3.19 ± 1.72	5.04 ± 5.73	+47.7 ± 47.2†
PAP	%	63.2 ± 51.8			74.1 ± 47.3		
M_{max} Amplitude							
RF	mV	4.88 ± 1.35	5.13 ± 1.32	NS	4.67 ± 0.78	4.47 ± 0.63	NS
VL	mV	6.40 ± 2.40	5.59 ± 1.27	NS	5.88 ± 2.30	5.70 ± 2.18	NS
VM	mV	7.16 ± 3.40	7.52 ± 3.86	NS	6.82 ± 2.20	6.77 ± 1.20	NS
MEP Amplitude							
RF	%M_{max}	39.5 ± 24.0	54.0 ± 33.2	+50.0 ± 71.0†	49.3 ± 38.6	70.9 ± 44.5	+36.5 ± 59.6†
VL	%M_{max}	38.2 ± 24.1	42.8 ± 24.7	+11.3 ± 34.3†	29.0 ± 12.6	40.9 ± 24.5	+27.5 ± 37.1†
VM	%M_{max}	47.1 ± 28.9	53.6 ± 37.1	NS	47.7 ± 29.8	52.2 ± 37.4	NS
EMG Silent Period							
RF	ms	149 ± 116	233 ± 167	+59.5 ± 71.2†	134 ± 41	203 ± 96	+61.0 ± 91.4†
VL	ms	150 ± 112	252 ± 169	+70.5 ± 74.7†	138 ± 58	189 ± 94	+47.3 ± 77.0†
VM	ms	151 ± 112	249 ± 169	+66.5 ± 62.3†	134 ± 36	166 ± 94	+47.1 ± 67.3†

Table 3.2. Baseline muscle function before and after the dynamic fatiguing contraction in people with T2D and age-and physical activity-matched healthy controls without T2D. Values are displayed as mean ± SD. The relative reduction (%) shown is from baseline to immediately after the fatiguing tasks (Task End). People with T2D demonstrated greater reductions in MVCC power, MVIC torque, and potentiated twitch amplitude compared with healthy controls. (* denotes group difference between controls and T2D, $P < 0.05$; † denotes difference between baseline and task end, $P < 0.05$).
Abbreviations: MVCC, maximal voluntary concentric contraction; MVIC, maximal voluntary isometric contraction; VA, voluntary activation; ES, electrical stimulation; SIT, superimposed twitch; PAP, post-activation potentiation; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; M_{max}, maximal compound muscle action potential; NS, not statistically significant.

For both groups, men and women were similar in age (58.9 ± 9.8 vs. 60.5 ± 8.7 years, respectively; sex effect, $P = 0.646$; group × sex, $P = 0.617$), BMI (28.9 ± 5.3 vs. 27.2 ± 6.2 kg·m^{-2}, sex effect, $p = 0.447$; group × sex, $P = 0.205$), daily physical activity

(step count: 8,690 ± 3,220 vs. 7,830 ± 3,400 steps·day^{-1}, respectively; sex effect, P = 0.499; group × sex, P = 0.608), HbA_{1c} (6.10 ± 1.11 vs. 6.19 ± 1.15%, respectively; sex effect, P = 0.612; group × sex, P = 0.568), fasting plasma glucose (106.5 ± 25.7 vs. 102.9 ± 35.2 mg·dL^{-1}, respectively; sex effect, P = 0.614; group × sex, P = 0.786), fasting plasma insulin (46.1 ± 21.2 vs. 45.4 ± 29.0 pMol, respectively; sex effect, P = 0.891; group × sex, P = 0.118), HOMA-IR (2.06 ± 1.14 vs. 2.17 ± 2.01 AU, respectively; sex effect, P = 0.762; group × sex, P = 0.191) and thyroid-stimulating hormone (1.75 ± 1.03 vs. 1.64 ± 0.68 mU·L^{-1}, respectively; sex effect, P = 0.753; group × sex, P = 0.520).

Men however, had less body fat than women (28.0 ± 6.6 vs. 38.3 ± 9.6%, respectively; sex effect, P < 0.001, group × sex, P = 0.142) and greater skeletal muscle mass of the leg (9.81 ± 1.37 vs. 6.54 ± 1.24 kg, respectively; sex effect, P < 0.001; group × sex, P = 0.116). For both groups men also had a larger MVIC torque (204.6 ± 63.1 vs. 116.9 ± 34.7 Nm, respectively; sex effect, P < 0.001, group × sex, P = 0.905), similar MVCC peak angular velocity (342.3 ± 56.6 vs. 318.3 ± 41.2 deg·s^{-1}, respectively; sex effect, P = 0.184; group × sex, P = 0.620), greater MVCC peak power (329.0 ± 120.4 vs. 213.1 ± 71.1 Watts, respectively; sex effect, P = 0.004, group × sex, P = 0.453) and a larger electrically-evoked twitch amplitude (50.3 ± 21.0 vs. 31.7 ± 4.8 Nm, respectively; sex effect, P = 0.004, group × sex, P = 0.670). Baseline voluntary activation measured during the MVICs (92.6 ± 5.2 vs. 93.6 ± 2.7%, respectively; sex effect, P = 0.529, group × sex, P = 0.955) and post-activation potentiation (60.0 ± 26.7 vs. 84.6 ± 69.9%, respectively; sex effect, P = 0.258, group × sex, P = 0.185) was similar for men and women.

Men and women did not differ in estimated VO_2 peak (31.0 ± 7.2 vs. 26.0 ± 8.6 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively; sex effect, $P = 0.060$; group × sex, $P = 0.063$), although there was a trend toward significance. Closer examination showed that the control men and women had similar estimated VO_2 peak (30.1 ± 7.0 vs. 30.1 ± 9.7 $mL \cdot kg^{-1} \cdot min^{-1}$; sex effect, $P = 0.99$); however, among people with T2D, men had greater estimated VO_2 peak compared to women (32.0 ± 7.7 vs. 22.0 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$; sex effect, $P = 0.010$).

Perception of Fatigue, Depression, and Sleep Quality: People with T2D had similar reports of perceptions of daily fatigue on cognitive function (FIS cognitive; group effect, $P = 0.216$), physical function (FIS physical; group effect, $P = 0.302$), and psychological function (FIS psychological; group effect, $P = 0.328$) compared with controls. See Table 1.

People with T2D and controls reported low but similar scores on the depression scale (group effect, $P = 0.301$), with no one reporting a clinically significant level of depression (GDS score > 5). Sleep quality was similar in people with T2D and controls (group effect, $P = 0.415$). See Table 1. The mean scores were consistent with assessments of 'healthy control' sleepers; however, some individuals reported 'poor' sleep quality (PSQI score > 5) (Buysse *et al.*, 1989).

Fatigability and Recovery

MVCC angular power and velocity: Both the control group and people with T2D had reductions in MVCC power during the dynamic fatiguing task (time effect, $P < 0.001$), but this reduction was greater in people with T2D (time × group, $P < 0.001$; Figure 2A). Recovery, however, was similar for both groups (time effect, $P < 0.001$; group effect, $P = 0.291$; time × group, $P = 0.548$).

People with T2D demonstrated greater reductions in MVCC peak angular velocity compared with controls during the dynamic fatiguing task (time effect, $P < 0.001$; group effect, $P = 0.688$; time × group, $P = 0.03$). During recovery, both groups demonstrated increases in MVCC angular velocity after the dynamic fatiguing task (time effect; $P < 0.001$), however, people with T2D had lower MVCC angular velocity than controls throughout the recovery period (R05 & R20: group effect, $P = 0.012$) with no interaction (time × group, $P = 0.865$).

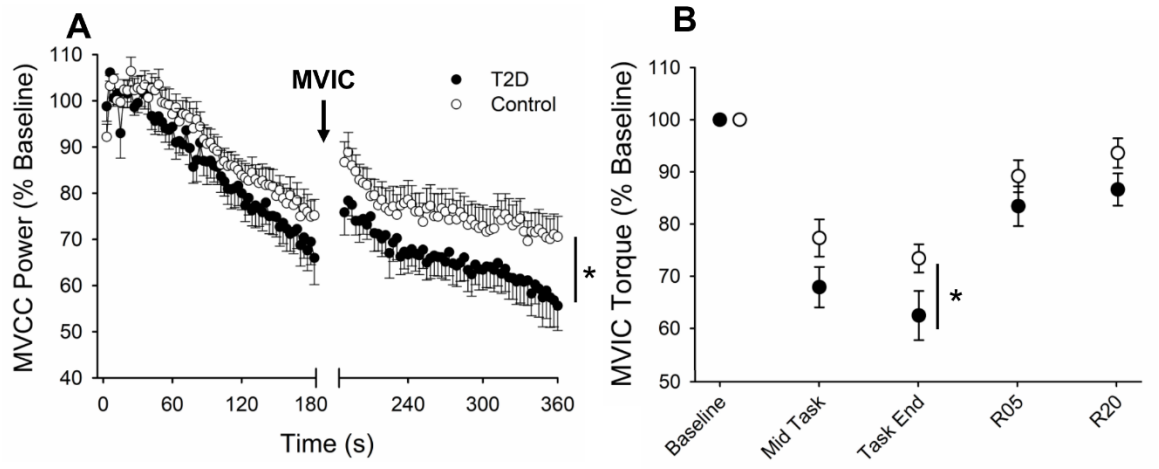

Figure 3.2. Fatigability of the maximal voluntary concentric contraction (MVCC) power (% baseline) (*A*) and maximal voluntary isometric contraction (MVIC) torque (% baseline) (*B*) in response to a dynamic fatiguing task. Values are displayed as mean ± SEM. ***A*.** The T2D group had greater reductions in the mean MVCC power (% baseline power of the mean of first 5 contractions) than controls by the last five contractions of the dynamic fatiguing task. Recovery of power during the MVCCs at 5 min (R05) and 20 mins (R20) was less for the T2D than control group. ***B*.** MVIC torque (% baseline) declined more for the T2D than the control group by the end of the dynamic fatiguing task (Task End). Recovery of MVIC was similar between people with T2D and controls for MVIC torque up to 20 mins after the fatiguing task (R20). (* group differences at $P < 0.05$).

The reduction of MVCC power during the fatiguing task was not different between men and women (last 5 contractions: 29.2 ± 20.1% vs. 38.7 ± 16.8% reduction, respectively; time × sex, $P = 0.524$) for either group (time × group × sex, $P = 0.762$; sex

effect, $P = 0.104$). During recovery (R05 & R20), the increase in MVCC power (time effect, $P < 0.001$) was similar for men and women (sex effect, $P = 0.634$; time × sex, $P = 0.473$; time × group × sex, $P = 0.276$).

Men and women demonstrated a similar reduction in MVCC velocity during the fatiguing task (last 5 contractions: 23.6 ± 18.8% vs. 31.9 ± 21.2% reduction, respectively; time × sex, $P = 0.542$; time × group × sex, $P = 0.621$) and similar recovery after the fatiguing task (time × sex, $P = 0.268$; time × group × sex, $P = 0.669$).

Duty Cycle: The duty cycle (work:rest ratio) was similar between people with T2D and controls during the first five dynamic contractions (group effect, $P = 0.146$). The duty cycle increased during the fatiguing task (due to slower contraction velocity), but this increase was similar between people with T2D and controls (time effect, $P = 0.031$; time × group, $P = 0.663$). The duty cycle was similar for men and women at the start of the fatiguing task (13.9 ± 1.8% vs. 14.6 ± 1.7%, respectively; sex effect, $P = 0.419$; group × sex, $P = 0.601$), and the increase in duty cycle at the end of the fatiguing task was similar (27.1 ± 19.5% vs. 28.4 ± 21.3% increase, respectively; time effect, $P < 0.001$; time × sex, $P = 0.903$).

Range of Motion: People with T2D and controls performed the concentric knee extension through a similar range of motion (baseline: 79.9 ± 9.0 vs. 80.2 ± 10.9 deg; group effect, $P = 0.898$) at the start of the fatiguing task, and the range of motion decreased similarly for both groups at the end of the fatiguing task (last 5 contractions: 74.1 ± 8.6 vs. 78.4 ± 10.8 deg; time effect, $P = 0.006$; time × group, $P = 0.137$). Men and women performed the concentric knee extension through a similar range of motion at the start of the fatiguing task (80.9 ± 8.3 vs. 79.0 ± 11.6 deg; sex effect, $P = 0.587$) and had

similar reductions in range of motion (last 5 contractions: 76.5 ± 7.1 vs. 75.9 ± 12.5 deg; time × sex, $P = 0.711$; time × group × sex, $P = 0.974$).

Applied Torque: The peak applied torque during the concentric knee extension was similar for people with T2D compared with controls at the start of the fatiguing task (66.9 ± 24.8 vs. 62.7 ± 16.5 Nm, respectively; group effect, $P = 0.772$). Similarly, the average applied torque did not differ between the T2D and control groups (47.9 ± 19.0 vs. 43.4 ± 13.3 Nm, respectively; group effect, $P = 0.563$). The applied torque decreased during the dynamic fatiguing task more for people with T2D compared with healthy controls, for both the peak torque (19.5 ± 8.6% vs. 13.4 ± 10.3% reduction, respectively; time effect, $P < 0.001$; time × group, $P < 0.001$) and the average torque (17.3 ± 11.6% vs. 12.0 ± 8.9% reduction, respectively; time effect, $P < 0.001$; time × group, $P < 0.001$).

Because men were stronger than women, the peak applied torque (75.6 ± 21.6 vs. 62.7 ± 16.5 Nm, respectively; sex effect, $P = 0.001$) and the average applied torque (53.9 ± 16.9 Nm vs. 35.9 ± 8.7 Nm, respectively; sex effect, $P = 0.001$) during the concentric phase of the dynamic knee extension was greater for men at the start of the fatiguing task. Men and women had a similar reduction in both peak (14.5 ± 11.0% vs. 18.9 ± 7.8% reduction; time effect, $P < 0.001$; time × sex, $P = 0.136$) and average torque (12.9 ± 11.4% vs. 16.8 ± 9.3% reduction; time effect, $P < 0.001$; time × sex, $P = 0.236$) at the end of the fatiguing task.

MVIC Torque: The reduction in MVIC torque after the dynamic fatiguing contraction (time effect, $P < 0.001$) was greater in the T2D group than controls (time × group, $P = 0.04$; Figure 2B). MVIC torque increased during the 20 minutes of recovery

(time effect, $P < 0.001$), and the increase was similar between the T2D and control groups (R05 & R20: group effect, $P = 0.120$; time × group, $P = 0.186$).

Men and women had similar reductions in MVIC torque after the dynamic fatiguing contraction (End Task: 31.5 ± 20.1% vs. 31.4 ± 9.6% reduction, respectively; time effect, $P < 0.001$; sex effect, $P = 0.917$; time × sex, $P = 0.995$; time × group × sex, $P = 0.725$). Men and women also had similar increases in MVIC torque during recovery (R05 & R20: time effect, $P < 0.001$; sex effect, $P = 0.774$; time × sex, $P = 0.951$; time × group × sex, $P = 0.110$). See Figure 2B.

Contractile Properties for the Electrically-Evoked Potentiated Twitch

Twitch Amplitude: The electrically-evoked potentiated twitch amplitude was reduced for all participants during and immediately after the fatiguing contraction (time effect, $P < 0.001$); however, people with T2D had greater reductions than controls (time × group, $P = 0.010$). Similarly, the twitch amplitude increased during recovery (time effect, $P < 0.001$) but people with T2D recovered more slowly and the twitch was more depressed, even at 20 mins post exercise, compared with controls (R05 & R20: group effect, $P = 0.027$). See Figure 3A.

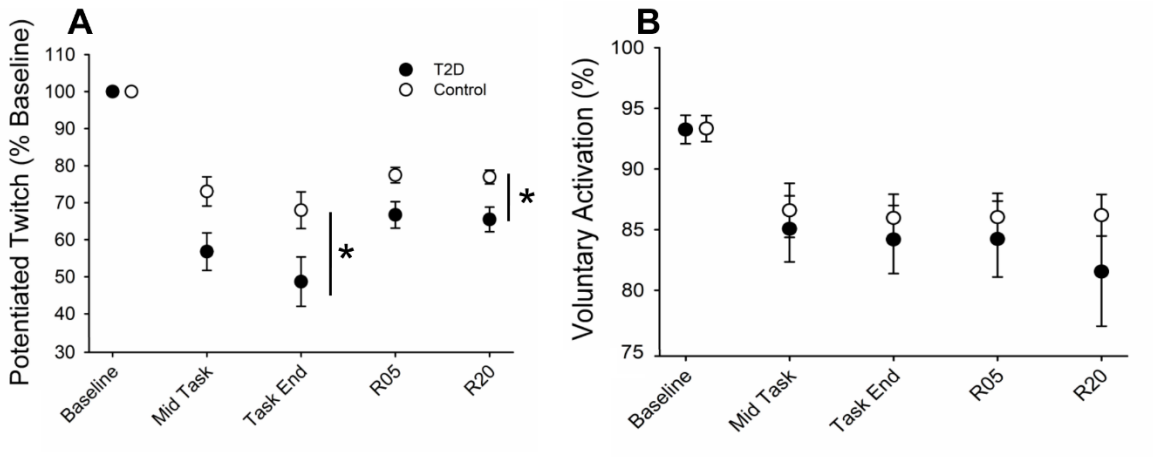

Figure 3.3. Electrically-evoked potentiated twitch amplitude (A) and voluntary activation (B) during and after the dynamic fatiguing task. Values are displayed as mean ± SEM. ***A.*** The electrically-evoked potentiated twitch amplitude (% baseline) was reduced more for the T2D group than controls and remained depressed during the 20 mins recovery ($P < 0.05$). ***B.*** *V*oluntary activation (assessed with electrical stimulation) declined in both people with T2D and controls ($P < 0.05$) but did not differ between groups ($P > 0.05$).

Men and women had similar reductions in potentiated twitch amplitude by the end of the fatiguing task for both the T2D and control groups (40.3 ± 27.6% vs. 39.8 ± 18.0% reduction; time effect, $P < 0.001$; sex effect, $P = 0.267$; time × sex, $P = 0.702$; time × group × sex, $P = 0.337$). During recovery, men and women demonstrated similar relative increases in potentiated twitch amplitude (R05 & R20: time effect, $P < 0.001$; sex effect, $P = 0.233$; time × sex, $P = 0.555$; time × group × sex, $P = 0.487$).

Half Relaxation Time: People with T2D and controls, both men and women, had similar increases in half relaxation time of the potentiated twitch after the fatiguing contraction (time effect, $P = 0.001$; sex effect, $P = 0.568$; time × group, $P = 0.511$; time × sex, $P = 0.368$; time × group × sex, $P = 0.982$; group effect, $P = 0.321$). During the 20-minutes of recovery (task end, and at 5 and 20 minutes post exercise), the half relaxation time decreased in all groups (time effect, $P = 0.002$; group effect, $P = 0.115$; sex effect, $P = 0.696$; time × group, $P = 0.458$; time × sex, $P = 0.440$; time × group × sex, $P = 0.747$).

Contraction Time: People with T2D and controls, both men and women, demonstrated no change in contraction time of the electrically-evoked potentiated twitch during the fatiguing task (time effect, $P = 0.377$; group effect, $P = 0.792$; sex effect, $P = 0.110$; time × group, $P = 0.564$; time × sex, $P = 0.212$; time × group × sex, $P = 0.717$), or during the 20-minute recovery (task end and at 5 and 20 minutes post exercise) (time effect, $P = 0.532$; group effect, $P = 0.717$; sex effect, $P = 0.126$; time × group, $P = 0.732$; time × sex, $P = 0.158$; time × group × sex, $P = 0.996$). See Table 2.

Voluntary Activation

Voluntary Activation (Electrical Stimulation): Voluntary activation decreased in people with T2D and controls during the fatiguing contraction (End Task: 84.2 ± 9.3% vs. 86.4 ± 7.3%, respectively; time effect, $P < 0.001$), but this decrease did not differ between groups (time × group, $P = 0.840$; Figure 3B). Men and women showed similar reductions in voluntary activation by the end of the fatiguing contraction (87.5 ± 7.6% vs. 81.8 ± 8.1%, respectively; sex effect, $P = 0.456$; time × sex, $P = 0.247$; time × group × sex, $P = 0.506$). Voluntary activation remained depressed during the recovery period after the fatiguing task for all groups (time effect, $P = 0.408$; time × group, $P = 0.420$; time × sex, $P = 0.260$; time × group × sex, $P = 0.348$; sex effect, $P = 0.792$).

Superimposed Twitch Amplitude (TMS): The SIT increased (i.e. voluntary activation decreased) in both people with T2D and controls (time effect, $P = 0.015$) and this effect was similar for both groups (time × group, $P = 0.995$, Table 2) and for men and women across the groups (sex effect, $P = 0.490$, time × sex, $P = 0.625$; time × group × sex, $P = 0.717$). During the 20-minute recovery, the superimposed twitch amplitude decreased (voluntary activation increased) (time effect, $P = 0.039$), similarly for people

with T2D and controls (time × group, $P = 0.600$, Table 2) and similarly for men and women (sex effect, $P = 0.944$; time × sex, $P = 0.146$; time × group × sex, $P = 0.443$).

EMG Response to Stimulation: M_{max}, MEP, Silent Period

Maximal compound muscle action potential (M_{max}): The M_{max} did not change during the fatiguing task for participants with T2D or controls for the rectus femoris (time effect, $P = 0.212$; time × group, $P = 0.176$; group effect, $P = 0.392$; group × sex, $P = 0.805$; time × sex, $P = 0.357$; time × group × sex, $P = 0.741$), vastus lateralis (time effect, $P = 0.697$; time × group, $P = 0.688$; group effect, $P = 0.825$; group × sex, $P = 0.804$; time × sex, $P = 0.294$; time × group × sex, $P = 0.989$), or vastus medialis (time effect, $P = 0.403$; time × group, $P = 0.449$; group effect, $P = 0.885$; group × sex, $P = 0.278$; time × sex, $P = 0.187$; time × group × sex, $P = 0.503$). See Table 2.

The M_{max} did not change during the 20-minute recovery period for participants with T2D or controls for the rectus femoris (time effect, $P = 0.588$; time × group, $P = 0.628$; group effect, $P = 0.880$; group × sex, $P = 0.906$; time × sex, $P = 0.623$; time × group × sex, $P = 0.901$), vastus lateralis (time effect, $P = 0.653$; time × group, $P = 0.763$; group effect, $P = 0.727$; group × sex, $P = 0.803$; time × sex, $P = 0.830$; time × group × sex, $P = 0.973$), or vastus medialis (time effect, $P = 0620$; time × group, $P = 0.736$; group effect, $P = 0.997$; group × sex, $P = 0.254$; time × sex, $P = 0.940$ time × group × sex, $P = 0.157$). See Table 2.

Motor evoked potential (MEP): The MEP amplitude (%M_{max}) evoked during the MVC increased after the fatiguing task for the men and women with T2D and controls for the rectus femoris (time effect, $P = 0.001$; time × group, $P = 0.876$; group effect, $P = 0.422$; group × sex, $P = 0.910$; time × sex, $P = 0.955$; time × group × sex, $P = 0.142$) and

vastus lateralis (time effect, $P = 0.037$; time × group, $P = 0.260$; group effect, $P = 0.949$; group × sex, $P = 0.252$; time × sex, $P = 0.324$; time × group × sex, $P = 0.231$), but not for the vastus medialis (time effect, $P = 0.139$; time × group, $P = 0.796$; group effect, $P = 0.777$; group × sex, $P = 0.747$; time × sex, $P = 0.144$; time × group × sex, $P = 0.728$). See Table 2.

The MEP amplitude (%M_{max}) reduced during recovery for men and women with T2D and controls for the rectus femoris (time effect, $P < 0.001$; time × group, $P = 0.156$; group effect, $P = 0.176$; group × sex, $P = 0.986$; time × sex, $P = 0.588$; time × group × sex, $P = 0.965$) and vastus lateralis (time effect, $P = 0.042$; time × group, $P = 0.521$; group effect, $P = 0.494$; group × sex, $P = 0.266$; time × sex, $P = 0.153$; time × group × sex, $P = 0.305$), but not for the vastus medialis (time effect, $P = 0.126$; time × group, $P = 0.958$; group effect, $P = 0.726$; group × sex, $P = 0.859$; time × sex, $P = 0.678$; time × group × sex, $P = 0.952$).

Silent Period: The EMG silent period, assessed during the MVIC, increased during the fatiguing task for the rectus femoris (time effect, $P < 0.001$; time × group, $P = 0.615$; group effect, $P = 0.632$; group × sex, $P = 0.731$; time × sex, $P = 0.502$; time × group × sex, $P = 0.133$), vastus lateralis (time effect, $P = 0.001$; time × group, $P = 0.187$; group effect, $P = 0.393$; group × sex, $P = 0.803$; time × sex, $P = 0.406$; time × group × sex, $P = 0.245$) and vastus medialis (time effect, $P = 0.002$; time × group, $P = 0.103$; group effect, $P = 0.189$; group × sex, $P = 0.516$; time × sex, $P = 0.406$; time × group × sex, $P = 0.278$). See Table 2.

The EMG silent period decreased during recovery from the fatiguing task for men and women with T2D and controls for the rectus femoris (time effect, $P < 0.001$; time ×

group, $P = 0.800$; group effect, $P = 0.722$; group × sex, $P = 0.893$; time × sex, $P = 0.453$; time × group × sex, $P = 0.585$), vastus lateralis (time effect, $P = 0.002$; time × group, $P = 0.391$; group effect, $P = 0.447$; group × sex, $P = 0.660$; time × sex, $P = 0.275$; time × group × sex, $P = 0.368$), and vastus medialis (time effect, $P = 0.042$; time × group, $P = 0.249$; group effect, $P = 0.799$; group × sex, $P = 0.922$; time × sex, $P = 0.644$; time × group × sex, $P = 0.409$).

Associations

The following variables were associated with reductions in MVIC performed after the fatiguing task: the relative reduction in potentiated twitch amplitude ($r^2 = 0.364$, $P = 0.002$; Figure 4A), baseline MVIC torque ($r^2 = 0.140$, $P = 0.032$), HbA_{1c} ($r^2 = 0.145$, $P = 0.029$), fasting glucose ($r^2 = 0.130$, $P = 0.042$), and HOMA-IR ($r^2 = 0.126$, $P = 0.046$).

The following variables were associated with reductions in MVCC power at the end of the fatiguing task: estimated VO_2peak ($r^2 = 0.494$, $P < 0.001$; Figure 4B), reduction in potentiated twitch amplitude ($r^2 = 0.345$, $P = 0.002$), HOMA-IR ($r^2 = 0.130$, $P = 0.042$), and HbA_{1c} ($r^2 = 0.154$, $P = 0.024$).

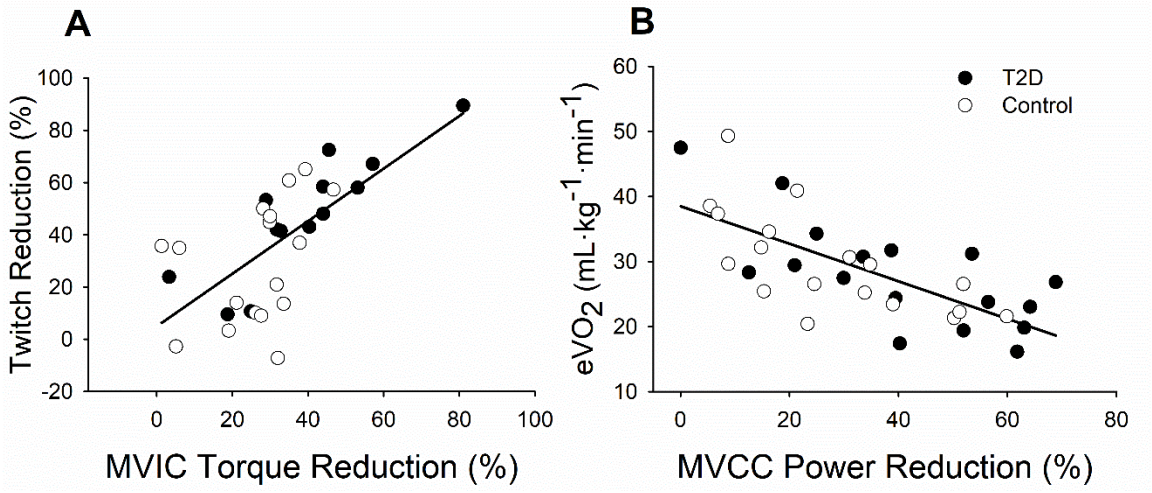

Figure 3.4. Associations with fatigability. *A*. The reduction in MVIC torque (%) was associated with the reduction in potentiated twitch amplitude (%) (***A***; r = 0.603, r^2 = 0.364, P = 0.002). ***B***. The reduction in MVCC power (%) was associated with estimated peak aerobic capacity (eVO_2) (***B***; r = -0.703, r^2 = 0.494, P < 0.001).

3.4 Discussion

The novel findings of this study were that people with T2D were more fatigable for a high-velocity dynamic fatiguing task with the knee extensor muscles than healthy controls who were matched for age, BMI and physical activity, with no differences between men and women. People with T2D demonstrated greater reductions in MVCC power, MVIC torque and twitch amplitude after the dynamic fatiguing contraction compared with the healthy controls, indicating fatigability and impairments in muscle contractile properties were greater for people with T2D. Voluntary activation was reduced, and the superimposed twitch amplitude and EMG silent period increased after the dynamic fatiguing task, demonstrating reduced neural drive and possibly increased intracortical and spinal inhibition; however, these changes were similar for people with T2D and controls of both sexes. Thus, both muscular and neural mechanisms (including supraspinal fatigue) contributed to knee extensor fatigability of men and women after

single limb dynamic exercise, however, contractile mechanisms were responsible for the greater fatigability of people with T2D compared with controls. Accordingly, the primary measures of fatigability, both the reduction in MVCC power and in the MVIC torque, were correlated with the reduction in potentiated twitch amplitude. Estimated maximal oxygen consumption (VO_2) at baseline and metabolic factors (HbA_{1c}, fasting plasma glucose and insulin) were also associated with reduction in MVCC power during the dynamic fatiguing task.

A strength of this study was that we designed it to understand the effects of T2D on fatigability of lower limb muscles, while controlling for confounding effects of age, diabetic polyneuropathy, daily physical activity levels, and participant anthropometrics, by excluding any people with clinical signs of diabetic polyneuropathy and by matching groups based on age, physical activity, estimated aerobic fitness, and BMI. Additionally, people with T2D reported similar daily levels of perceived fatigability, sleep quality and depression as the controls, indicating there was minimal influence of perceptions of fatigue that is often associated with advanced diabetes (Fritschi & Quinn, 2010) and that may confound exercise-induced fatigue of the lower limb. These findings however may underestimate the group-related differences in fatigability and the contributing mechanisms may have been different if people with T2D who have diabetic polyneuropathy were included in the study. For example, after a 20-repetition, moderate-velocity (120 $deg \cdot s^{-1}$) isokinetic fatiguing task with the knee extensors (IJzerman *et al.*, 2012), there was a progressive, albeit not significant, increase in fatigability in people with T2D and diabetic polyneuropathy (37 ± 13% reduction of muscle work) compared with people with T2D and no signs of polyneuropathy (34 ± 13%) and healthy controls

(30 ± 8%). Additionally, people with T2D and diabetic polyneuropathy demonstrated reduced motor unit number estimates, mean motor unit firing rates, and impaired neuromuscular propagation in upper and lower limb muscles compared to controls (Allen *et al.*, 2014a), which indicates impairments along the motor pathway from corticospinal centers to the interface of the nerve and muscle. These data provide a rationale for an increased role of central mechanisms contributing to fatigability of limb muscles in people with T2D and polyneuropathy, although this has not been examined.

Greater Fatigability in People with T2D

The greater fatigability of the knee extensors in people with T2D than controls was evidenced by markedly greater reductions in MVCC power (42.8% vs. 26.4% reduction) and MVIC torque at the end of the dynamic tasks (37.6% vs. 26.4% reduction) (Fig. 2). During the dynamic fatiguing task, there was a reduction in range of motion and rest time between contractions (increased duty cycle) but this was similar for both groups. However, the average applied torque declined more during the fatiguing task for people with T2D than the controls (17.3% vs. 12.0% reduction), thus, each MVCC required relatively less torque for participants with T2D compared to controls at the end of the fatiguing task. Despite this, the participants with T2D showed larger losses in power than controls. Thus, our study may have underestimated the magnitude of the difference in loss of power between the groups by up to ~5%. These results are consistent with previous research demonstrating greater fatigability for isometric contractions of people with diabetes mellitus (Type 1 or Type 2) of the handgrip (Petrofsky *et al.*, 2005), dorsiflexor (Allen *et al.*, 2015a), and knee extensor muscles (Almeida *et al.*, 2008). Importantly, our results clearly indicate that the knee extensor muscles are more fatigable

for dynamic contractions in people with T2D, although these results are not consistent with that seen for low repetition (20 – 30 repetitions), moderate velocity (120 – 180 $deg \cdot s^{-1}$) isokinetic contractions for this muscle group (Halvatsiotis *et al.*, 2002; IJzerman *et al.*, 2012). The greater fatigability of people with T2D in our study, but not others, could be due to faster contraction velocities or more repetitions in our protocol. Close examination of the muscle power during the fatiguing task (Fig. 2A) demonstrates that the differences in fatigability between people with T2D and controls did not become apparent until after ~60 repetitions. Thus, greater fatigability of people with T2D may only occur with more repetitions or faster contraction velocities, and the magnitude of the difference in fatigability between people with T2D and controls likely increases as a function of exercise time.

A unique aspect of our study was that our cohort of T2D participants did not have advanced stages of the disease, yet lower limb fatigue was greater than in controls matched for age, BMI and physical activity. Many of the processes associated with advancing severity of T2D will exacerbate fatigability of the lower limb even further, including diabetic polyneuropathy (Allen *et al.*, 2016) and loss of muscle mass (Allen *et al.*, 2014b), impaired microcirculation (Petrofsky *et al.*, 2005) and cardiovascular disease. We showed however, that even prior to detectable clinical signs of polyneuropathy and loss of muscle mass, people with T2D display greater fatigability of the knee extensor

muscles that are important for daily function, and as discussed below, was due to contractile mechanisms.

Neural Mechanisms of Fatigability

After the fatiguing task, there was a reduction in voluntary activation (assessed via electrical stimulation) (Fig. 3B), an increase in superimposed twitch amplitude (assessed via TMS), an increase in EMG silent period and a modest increase in MEP amplitude, each of which were similar between the people with T2D and control (Table 2). The reduction in voluntary activation elicited with electrical stimulation after the fatiguing task demonstrated a suboptimal output from the motor pathway, between activation of the motor cortex and excitation of the α-motor neuron (Gandevia, 2001). Because there was an increase in superimposed twitch amplitude elicited with TMS during the MVIC, the reduced neural drive was in part due to a failure to generate output from the motor cortex (Todd *et al.*, 2003). However, this failure was similar across all groups, and thus did not explain the difference in fatigability in the people with T2D (either the increased reduction in the MVIC or power).

The increase in silent period reflects intracortical inhibition evoked by the TMS during the maximal volitional effort which temporarily halts voluntary descending drive (Todd *et al.*, 2007) and recent data suggests the silent period may also reflect spinal inhibitory circuitry up to at least 150 ms after the stimulation (Yacyshyn *et al.*, 2016). Thus, among our participants, there was an increase in intracortical inhibition, which involves the γ-aminobutyric acid ($GABA_B$) receptors (Taylor *et al.*, 1996), and possibly greater spinal inhibition (Yacyshyn *et al.*, 2016), but this increase in inhibition was similar across the groups. Although there was a reduction in voluntary activation, there

was a modest increase in the MEP amplitude elicited by TMS observed in the rectus femoris and vastus lateralis muscles, indicating a net increase in corticomotor excitability (Todd *et al.*, 2006), in part due to an increase in cortical excitability, increased spinal excitability or reduced corticospinal inhibition (Kennedy *et al.*, 2016). An increase in MEP amplitude is often observed with fatiguing exercise (e.g. (Hunter *et al.*, 2006)) and may reflect increased descending drive despite a failure to increase the motor output. Despite the concomitant increases in excitability and inhibition of the motor pathway during the fatiguing task, these neural adjustments did not directly explain the greater fatigability in the men and women with T2D compared with controls.

Contractile Mechanisms Primarily Explain Fatigability in People with T2D

The reduction in MVCC power and MVIC torque were associated with the decline of the electrically-evoked potentiated twitch amplitude, indicating muscle contractile mechanisms largely explain (~35%) the greater fatigability of people with T2D. In both groups, a reduction in twitch amplitude reflected fatigue in the muscle that may be due to disturbances in excitation-contraction coupling, accumulation of metabolites, and/or impaired calcium handling (Fitts, 2008; Debold *et al.*, 2016), that ultimately reduce the torque that is able to be produced by the muscle fibers. Volitional and electrically-evoked contractile function, and lean mass of the knee extensor muscles was not different between the groups (T2D, control) at baseline, thus, baseline skeletal muscle morphology and function likely did not contribute to greater fatigability in people with T2D. However, there is evidence of contractile slowing and reduced muscle strength in people with diabetes who have polyneuropathy (Allen *et al.*, 2014b).

There are several factors thought to affect the exercising muscle specifically in people with diabetes which may contribute to the larger fatigue-related reductions in the twitch amplitude, including: i) impaired neuromuscular transmission (Allen *et al.*, 2015a), ii) impaired calcium kinetics and cross-bridge detachment, iii) impaired phosphorylation of myosin regulatory light chains (Allen *et al.*, 2014b), and iv) motor unit loss (Allen *et al.*, 2013; Allen *et al.*, 2014a). Among this cohort of people with T2D who had no signs of diabetic polyneuropathy, there was no reduction in M_{max} amplitude, providing evidence of preserved integrity of the sarcolemma and neuromuscular junction propagation properties in our cohort of men and women with T2D. There are relatively few examples of decreased M_{max} amplitude after a fatiguing contraction; however, reduced M_{max} has been observed during sustained isometric contractions of healthy young adults (first dorsal interosseous) (Fuglevand *et al.*, 1993) and in people with type 1 diabetes and diabetic polyneuropathy (ankle dorsiflexors) (Allen *et al.*, 2015a). Additionally, there was a similar increase in half-relaxation time between groups in our study, indicating similar slowing of calcium reuptake into the sarcoplasmic reticulum and slowing of cross-bridge detachment in the skeletal muscle fibers. In addition, our data indicates that post-activation potentiation, assessed by comparing electrically-evoked twitches during a non-potentiated (no muscular effort within 30 s of the stimulation) and a potentiated state (MVIC performed within 2-s prior to evoked twitch), was similar between groups. Thus, there was probably similar phosphorylation of myosin regulatory light chains (Baudry & Duchateau, 2004) between people with T2D and controls at baseline. Motor unit loss at baseline or impairments of active motor units in people with T2D may also underlie the greater impairments in contractile properties compared with

controls. However, the people with T2D had no clinical signs of diabetic polyneuropathy and similar characteristics (age, strength, muscle mass and contractile properties) compared with controls, indicating no strong rationale for differences in motor unit numbers between the groups.

The greater reduction in knee extensor power across both groups, was associated with estimated fitness level, the gold standard indicator of glycemic control over the preceding two-to-three months (HbA_{1c}) and a proxy of insulin resistance (HOMA-IR). Although the T2D and controls groups were matched for fitness, participants with lower estimated fitness had greater fatigability during the dynamic fatiguing task, indicating that a lower capacity of the cardiovascular system (systemic blood flow and skeletal muscle oxygen delivery) may contribute to greater fatigability across both groups. The association of fatigability with HbA_{1c} and the HOMA-IR indicate that fatigability was greater in people with poorer glycemic control and greater insulin resistance. The greater insulin resistance (particularly in those with advanced T2D), may be associated with greater vascular constriction due to increased expression of endothelin-1 and reduced nitric oxide phosphorylation (Reynolds *et al.*, 2017), resulting in reduced skeletal muscle blood flow during exercise and more perturbed metabolic milieu during exercise in people with T2D compared to controls. For example, there is evidence of impaired potassium handling and calcium regulation (Harmer *et al.*, 2014), and increased lactate concentrations (Metz *et al.*, 2005; Harmer *et al.*, 2008) in people with T1D and T2D after exercise compared with controls. It is therefore probable that people with T2D and diabetic polyneuropathy or other complications of advanced T2D may have even more

severe fatigability of lower limb muscles than evidenced among our cohort, and these associations warrant further investigation.

No Sex Differences in Fatigability with T2D

A unique finding of this study was there were no sex-related differences in fatigability of the knee extensor muscles in a middle-to-older aged cohort of healthy controls or people with T2D for a high velocity dynamic fatiguing task. Typically, there are sex differences in fatigability for isometric and slow-to-moderate velocity fatiguing tasks, particularly in the upper limb in young healthy and older adults (Hunter, 2016a, b). However, the magnitude of the sex differences in fatigability of young and old adults was diminished for high-velocity fatiguing contraction tasks with both the elbow flexor and knee extensor muscles (Senefeld *et al.*, 2017), and we found this to be the case in the middle-to-older aged adults in this study. We also observed no sex difference in the reduction in the MVIC measured immediately after the dynamic tasks and during recovery. However, in several other studies, men showed greater reductions than women in the MVIC immediately after the dynamic fatiguing contraction (Senefeld *et al.*, 2013; Senefeld *et al.*, 2017; Senefeld *et al.*, 2018d). The mechanism for the sex difference in the slower recovery of the men than the women in that study was due to contractile mechanisms with no sex difference in reductions in voluntary activation (Senefeld *et al.*, 2018d). The sex difference in fatigability can diminish for older adults for both isometric tasks and dynamic tasks (Hunter, 2016b, a; Sundberg *et al.*, (in review)). The lack of sex difference in fatigability between our current cohorts could be due to the age of our participants, whose average age was 60 years, which is older compared to previous reports demonstrating sex differences (Senefeld *et al.*, 2013; Senefeld *et al.*, 2018d). The

lack of sex differences in fatigability within our cohort could be secondary to our *a priori* participant matching criteria, including similar estimated maximal aerobic capacity (VO_2 peak). Women are expected to have a lower maximal aerobic capacity than men due to a number of physiological factors including smaller hearts, less haemoglobin, greater body fat (Hunter *et al.*, 2015; Hunter, 2016a); thus, the women in our cohort could be relatively more fit than the men.

Conclusion

Men and women with T2D who exhibited no clinical signs of diabetic polyneuropathy, were more fatigable during and in recovery from a high-velocity dynamic fatiguing task with the knee extensors muscles than controls without diabetes who were matched for age, body mass index, and physical activity. This difference in fatigability occurred when measured as a loss of power and the reduction of MVIC torque. Furthermore, there was no sex-based differences in fatigability for the people with T2D and controls. The greater fatigability was associated with glycemic control and contractile mechanisms, with no observed impairments in neuromuscular transmission. Although neural mechanisms of fatigability contributed to reductions in knee extensor power, the lower neural drive was moderate relative to the larger contribution of contractile mechanisms that explained the greater fatigability of the lower limb in the men and women with T2D.

CHAPTER 4: PERFORMANCE FATIGABILITY IN PEOPLE WITH TYPE 2 DIABETES AND PREDIABETES IS ASSOCIATED WITH CONTRACTILE FUNCTION AND GLYCEMIC CONTROL

This manuscript is currently in review.

Senefeld J, Harmer AR, Hunter SK. Performance Fatigability in People with Type 2 Diabetes and Prediabetes is Associated with Contractile Function and Glycemic Control.

4.1 Introduction

Type 2 diabetes mellitus (T2D) and prediabetes have become global pandemics (Ogurtsova *et al.*, 2017) and are estimated to currently affect 13% and 37% of Americans, respectively (Menke *et al.*, 2015). Exercise training is a cornerstone of T2D management, and T2D prevention for those with prediabetes, and along with diet, is the first intervention used to treat or delay the onset of T2D. Among a cohort of people with prediabetes, the incidence of T2D was reduced 58% with lifestyle interventions (diet, weight loss and exercise) over a three-year period, and these lifestyle interventions were almost twice as effective as pharmacological treatment with metformin (Knowler *et al.*, 2002). Although exercise training is important in the management and prevention of T2D, reduced exercise capacity in people with T2D and prediabetes may be associated with numerous signs and symptoms, such as metabolic dysregulation, depression and impaired muscle performance (Fritschi & Quinn, 2010; Poitras *et al.*, 2018). However, there is minimal knowledge on the mechanisms for the reduced exercise capacity in a single bout of exercise in people with prediabetes, despite the significant impact that repeated bouts of exercise can have in delaying or preventing progression of prediabetes to T2D.

The disabling symptoms contributing to reduced exercise capacity are globally termed fatigue and can be categorized into two domains: perceived fatigability and performance fatigability (Kluger *et al.*, 2013; Enoka & Duchateau, 2016). Performance fatigability is the decline in an objective measure of performance over a discrete period that limits human performance (Kluger *et al.*, 2013; Enoka & Duchateau, 2016). In the controlled laboratory setting, it is often measured as the decline in expected force or power of limb muscles during a single bout of limb exercise such as repeated contractions (Kluger *et al.*, 2013; Hunter, 2017).

People with diabetes (types 1 and 2) and diabetic polyneuropathy have greater performance fatigability compared with controls (Almeida *et al.*, 2008; IJzerman *et al.*, 2012; Allen *et al.*, 2015a). Similarly, we recently found that people with T2D and no clinical signs of neuropathy had greater reductions in knee extensor power and maximal voluntary isometric contraction (MVIC) torque after a six-minute dynamic exercise test than healthy controls matched for age, sex, body fat and physical activity (Senefeld *et al.*, 2018c). Greater fatigability of the knee extensor muscles was directly associated with reductions in contractile function and glycemic control (HbA_{1c}), providing evidence that those individuals with poorer glycemic control (higher HbA_{1c}) exhibit greater performance fatigability, partly due to greater fatigue originating within the muscle (Senefeld *et al.*, 2018c). HbA_{1c} is also elevated in prediabetes (although not to the extent of type 2 diabetes); therefore, it may be anticipated that people with prediabetes will exhibit greater fatigability than controls but less than people with T2D. However, no studies have evaluated performance fatigability of the limb muscles in people with prediabetes.

Performance fatigability can be exacerbated by perceived fatigability, particularly in clinical populations (e.g. prediabetes and T2D) (Kluger *et al.*, 2013). Perceived fatigability, defined as self-reported symptoms of exertion, exhaustion, and enervation, can be assessed at rest or during exercise performance, and may exacerbate the changes in sensations that regulate exercise performance. Perceived fatigability, even at rest, is a common symptom in people with T2D, and has been associated with limitations in physical exercise and a lack of motivation to perform exercise (Fritschi & Quinn, 2010). The mechanisms for perceived fatigability in people with T2D in the rested state are unknown but may be related to several factors including poor glucose control (hypo- or hyper-glycemia or glucose fluctuations) (Sommerfield *et al.*, 2004), diabetic polyneuropathy (Rijken *et al.*, 1998), impaired sleep quality (Cuellar & Ratcliffe, 2008), emotional distress from daily disease management (de Sonnaville *et al.*, 1998), depression (Anderson *et al.*, 2001b), and obesity (Pickup, 2004). Perceived fatigability in people with T2D can be ameliorated with improvements in disease-management and glycemic control (Fritschi & Quinn, 2010; Park *et al.*, 2015), suggesting an association between perceived fatigability and glycemic control. Although increased perceptions of fatigability may contribute to impaired physical function (Singh *et al.*, 2016), the association between perceived and performance fatigability in people with T2D and prediabetes is unknown.

The *purpose* of the study was to: 1) compare performance fatigability and the contribution of neural and contractile mechanisms for a high-velocity dynamic fatiguing task with the knee extensor muscles in people with T2D, prediabetes, and healthy controls matched for age, BMI and physical activity, 2) compare perceived fatigability in

people with T2D, prediabetes and controls, and 3) determine the association between perceived fatigability and performance fatigability. Our *hypotheses* were that: 1) performance fatigability of the knee extensor muscles would be greater in people with prediabetes compared with healthy controls, but less than in people with T2D, 2) greater performance fatigability would be associated with greater HbA1c and greater reductions in contractile function; 3) perceived fatigability in the rested condition would be greater in people with prediabetes compared with healthy controls, but less than in people with T2D, and associated with HbA_{1c}; and 4) higher perceived fatigability would be associated with greater performance fatigability in people with prediabetes and T2D. Although we have previously demonstrated no sex-related differences in fatigability in people with T2D (Senefeld *et al.*, 2018c), we tested sufficient men and women in each cohort, as recommended by the National Institutes of Health for all clinical research (NOT-OD-15-102), to determine whether there were any sex differences in perceived or performance fatigability.

4.2 Materials and Methods

Thirty-nine people with T2D (61.2 ± 8.5 years, 23 men and 16 women), 20 people with prediabetes (63.1 ± 6.0 years, 11 men and 9 women), and 27 healthy controls (58.1 ± 9.4 years, 13 men and 14 women) participated in the study. Data from a subset of the people with T2D (n = 17) and controls (n = 20) have been reported previously (Senefeld *et al.*, 2018c). Prior to involvement in the study, each participant provided written informed consent and the protocol was approved by the Marquette University Institutional Review Board (HR-2402) in accordance with the Declaration of Helsinki for human experimentation.

Presence of T2D was physician-diagnosed and was known prior to study enrolment. Potential control participants who presented with an HbA_{1c} 5.7%-6.5% without a diagnosis of T2D were classified as having prediabetes, and all controls had an HbA_{1c} ≤5.6%. Exclusion criteria included HbA_{1c} >10%, prescribed insulin or insulin secretagogue, diabetic neuropathy (assessed via clinical diagnosis, monofilament and tuning fork sensation tests, sensory questionnaires, and electrocardiogram during bicycle ergometer exercise test), peripheral edema, severe obesity (body mass index, BMI >45 $kg \cdot m^{-2}$), untreated hypothyroidism, epilepsy, medications that affect cortical excitability, current smoking, possibility of pregnancy and any neurological, cardiovascular or musculoskeletal disease that precluded exercise testing.

Participants completed three sessions of testing that included a screening session to determine eligibility for the study, followed by two experimental sessions. In the first experimental session, after overnight fasting (and delay of any diabetes medications), venipuncture and subsequent blood analyses were performed, participants then consumed a standard breakfast and any diabetes medications, completed assessments of perceived fatigability and were familiarized with the experimental procedures (stimulations, static contractions and dynamic exercise). Prior to the second experimental session, participants consumed a standard breakfast and then completed the dynamic fatiguing exercise with the knee extensor muscles upon arrival to the laboratory. Testing was conducted in the fed state (post-prandial) in order to simulate conditions encountered in daily life, and thus enable assessment of the impact of fatigue under these conditions. Each session was separated by 2-7 days.

Screening Session

During the screening session the following tests were performed: 1) lower limb sensation was assessed using a monofilament and vibration sensation test, 2) autonomic nerve function was assessed during bicycle ergometer exercise test, 3) HbA_{1c} was assayed, 4) peak aerobic capacity was estimated from a submaximal graded bicycle ergometer exercise test, 5) anthropometry (lean muscle mass, fat mass and bone density) was assessed using a dual-energy x-ray absorptiometry (DEXA) scan, and 6) daily physical activity was assessed using a triaxial accelerometer.

Diabetic neuropathy screening: Each participant was carefully screened for the presence of diabetic polyneuropathy. To assess signs of sensory neuropathy, a questionnaire and physical evaluation were performed according to standards (Lower Extremity Amputation Prevention program) (Anon, 1998). Physical evaluations included monofilament screening per standards. Participants were also screened utilizing vibration sensation testing. Vibration sensation was assessed using a 128 Hz tuning fork placed on the right and left medial malleoli and the head of the 1st metatarsal. Participants were excluded if impaired sensation was observed, i.e., if the monofilament could not be sensed on any site on the foot or if vibrations could be sensed by the examiner for more than 10 seconds longer than the participant. Achilles tendon reflex testing was performed, and participants were excluded if the tendon jerk was absent. Participants who were suspected of having diabetic polyneuropathy were excluded from the study.

Submaximal, Graded Bicycle Test: Participants performed a submaximal graded exercise test (Beekley *et al.*, 2004) on a bicycle ergometer (VIAsprint 150P, CareFusion, San Diego, CA, USA) to determine exercise tolerance and screen for cardiac arrhythmias.

Participants were required to maintain cadence at 60 revolutions per minute (monitored via LED screen by the participant and a researcher), and the resistance was manipulated to achieve three different submaximal loads that elicited heart rate responses between 40% and 70% of heart rate reserve. The participant cycled at each submaximal load for four minutes to attain a stable heart rate response. During the test, a 12-lead electrocardiogram (CASE, General Electrics, Madison, WI, USA) was monitored for presence of arrhythmias. Participants were excluded if arrhythmia was present.

Physical Activity Monitor: Accelerometry data were collected using the Actigraph GT3X (ActiGraph, Pensacola, FL, USA) that was worn on the waist by each participant for 4 days (2 weekdays and 2 weekend days) (Senefeld *et al.*, 2018c). Sixty-second epochs of data were collected and analyzed. Wear-time authentication was performed on each participant's dataset to determine whether data were to be included in the analysis. Acceptable wear-time was set *a priori* as ≥ 3 days of ≥ 9 hours (540 minutes) per day. Step count was recorded (ActiLife Software v4) and analyzed.

Anthropometry and DEXA: Height and body mass were assessed with a standard stadiometer and scale. Whole body and "tested" leg (leg used for fatiguing task as explained for 'Experimental Session 2') anthropometrics (total, fat and muscle mass) were assessed using DEXA (Lunar Prodigy full-body scanner, Madison, WI, USA). The scanner was calibrated prior to each scan. The analyzed data were recorded offline (Encore 2008 software by GE Health care). In the case of participants with artificial joints (n =3 controls, 0 people with prediabetes and 5 people with T2D), the artificial joint was excluded via encore software. In two of the patients with artificial joints, the artificial joint was present in the dominant leg.

HbA_{1c}: HbA_{1c} was determined using a point-of-care instrument assay (Siemens Healthcare Diagnostics, DCA 2000+), certified by the National Glycohemoglobin Standardization Program (Bode *et al.*, 2007).

Experimental Session One

Participants fasted for *at least* 8 hours prior to experimental session one, then consumed a standardized breakfast (8 oz. fruit juice, one cereal bar, and one serving of fruit) prior to undertaking the remaining activities in the session. In conjunction with fasting, participants delayed administration of any diabetes medications until after the standardized breakfast. Participants then completed a questionnaire to estimate handedness/footedness (Oldfield, 1971) to assess which leg which would be used for testing, and a familiarization with the protocols used in experimental session two (electrical stimulation and practice of MVICs and maximal-effort, high-velocity concentric contractions (MVCCs). Participants were also administered a set of questionnaires to assess fatigue, sleep quality, and depression.

Clinical Symptoms of Fatigue: Clinical symptoms of fatigue in the cognitive, physical and psychological domains over the previous month were self-reported via the Fatigue Impact Scale (Fisk *et al.*, 1994).

Sleep Quality: Sleep quality over the previous month was self-reported via the Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989).

Depression: Symptoms of depression over the previous month were self-reported via the short-form Geriatric Depression Scale (Snowdon, 1990).

Blood Measures: Fasting plasma glucose and cholesterol (total cholesterol, and high- and low-density lipoprotein concentrations) were determined using a point of care

instrument (Alere Cholestech LDX System, Alere Inc. Waltham, MA, USA). Hemoglobin concentration was determined using a point of care instrument (StatSiteM Hemoglobin Photometer, Stanbio, Boerne, TX, USA) and hematocrit was determined manually (International Micro-Capillary Reader, International Equipment Company, Boston, MA, USA) per standard instruction of each instrument. Plasma insulin and thyroid-stimulating hormone concentrations were quantitatively assayed in duplicate per manufacturer instructions using enzyme-linked immunoassay kits (Quantikine Human Insulin Immunoassay (R&D Systems, Minneapolis, MN) and Human TSH (CGA) ELISA Kit (Thermo Scientific Pierce (Waltham, MA), respectively). Homeostatic model assessment for assessing insulin resistance (HOMA-IR) was calculated using the fasting plasma insulin concentration (FPI, $mU \cdot L^{-1}$) and fasting plasma glucose (FPG, $mmol \cdot L^{-1}$): $HOMA\text{-}IR = (FPI \times FPG) \cdot 22.5^{-1}$.

Experimental Session Two

Approximately one hour prior to the second (last) experimental session, each participant consumed a standard breakfast. Upon arrival to the laboratory, each participant performed a single limb exercise protocol with the knee extensor muscles that included baseline MVICs and MVCCs followed by a high-velocity fatiguing task and recovery contractions. Following is a description of the experimental procedures and the protocol.

Measurement of Torque, Velocity and Power

Participants performed single-limb isometric and isotonic contractions using a Biodex System 4 dynamometer (Biodex Medical, Shirley, NY). Participants were asked to perform all contractions using their dominant leg, unless there was any form of

osteoarthritis (or other joint disease or presence of joint implant) present in the dominant limb. In those cases (*n*=2), the non-dominant leg was tested. Participants were positioned on the dynamometer seat with 90º of hip flexion. Padded straps mounted on the seat were securely tightened across the shoulders, the waist, and the leg not tested during the fatiguing task to prevent synergistic movements by the participants. The tested leg was positioned such that the axis of rotation of the knee joint was aligned with the axis of rotation of the dynamometer. The internal goniometer of the Biodex dynamometer was calibrated using a spirit level. The analog signals corresponding to joint angle, torque, and velocity were extracted from the Biodex system using custom-built cables digitized at 2,000 Hz and recorded through a Power 1401 analog-to-digital (A-D) converter and Spike2 software (Cambridge Electronics Design, Cambridge, UK).

Electrical Stimulation

Single-pulse (200 μs duration, 400 V) electrical stimulation was used for femoral nerve (100 – 700 mA) and percutaneous muscle stimulation over the quadriceps (150 – 750 mA; DS7AH, Digitimer, Ltd., Welwyn Garden City, UK). A single stimulation of the femoral nerve was used to elicit a compound muscle action potential (M wave) of three agonist muscles and a single pulse of percutaneous stimulation of the muscle belly was used to elicit twitch torque to quantify contractile function and to estimate voluntary activation of the knee extensor muscles.

Peripheral Nerve (Femoral) Stimulation: Nerve stimulation was performed to elicit the maximal M wave (M_{max}) of the three agonist muscles (rectus femoris, vastus lateralis and vastus medialis). After baseline MVICs, the femoral nerve was stimulated using supramaximal intensities, with the cathode electrode (Ambu Neuroline electrodes,

Denmark; 1.5 cm diameter) placed over the femoral nerve within the femoral triangle and the anode placed over the greater trochanter of the femur. The intensity of the nerve stimulation was determined by increasing the current until the amplitude of the M wave of all three knee extensor muscles plateaued. The stimulation intensity (80 – 500 mA) was then increased further by 20% to ensure maximal excitation of the femoral nerve. The amplitude of the bi-phasic wave elicited with the supramaximal femoral nerve stimulation (M_{max}) was used to estimate the integrity of neuromuscular propagation (Fuglevand *et al.*, 1993).

Percutaneous Muscle Stimulation: The knee extensor muscles were stimulated percutaneously with custom-made pad electrodes (6 cm × ~15 cm). The cathode was placed near (within 10 cm) the femoral triangle and the anode was placed proximal to the patella without hindering knee flexion/extension of the participant. The stimulator intensity used to elicit the twitch amplitude was determined by increasing the current in 20-50 mA increments until the resting twitch amplitude plateaued. The stimulation intensity (100 – 650 mA) was then increased further by 20% to ensure the greatest activation possible with the pad electrodes over the knee extensor muscles.

State Perceptions: Participants reported ratings of perceived exertion (RPE) of the exercising leg using a modified 11-point Likert scale (Borg, 1982). Verbal and written anchors denoted that a rating of 0 indicated no exertion and a rating of 10 indicated maximal possible exertion. Participants were asked to report RPE at baseline, and if participants did not indicate 0, the scale was explained again. Participants reported state perceptions of muscle cramps, pain, weakness, soreness and fatigue of the exercising leg using separate 10-cm visual analog scales (VAS).

Blood glucose concentration: Blood glucose concentration was determined using a point-of-care glucometer (OneTouch Ultra 2, LifeScan, Inc. Chesterbrook, PA) immediately before, and after the five-minute and 20-minute recovery muscle contractions. Blood sampling was commenced approximately 90 minutes after the standard breakfast and administration of diabetes medication— breakfast was completed 60 minutes before arrival of the participant and 30 minutes was allotted for preparation of each participant.

Experimental Protocol

The experimental protocol involved the following procedures:

(5) *Baseline MVICs:* Participants completed at least three MVICs of knee extensor muscles for ~4 seconds each with 90 s between trials. Participants then performed four additional MVICs. Percutaneous muscle stimulation was superimposed before (to elicit a twitch contraction at rest) and immediately after each MVIC (to determine contractile function and voluntary activation of the knee extensor muscles). Electrical stimulation of the femoral nerve was also performed within 10 s after each MVIC to determine neuromuscular junction propagation.

(6) After baseline MVICs, state perceptions and blood glucose were assessed.

(7) *Baseline MVCCs:* Participants performed 10 MVCCs with a load equivalent to 20% of MVIC as a warm-up. These isotonic contractions were performed through an 80º range of motion, from 90°of knee flexion to 10°of knee flexion. The participant then rested for 2.5 minutes, before initiating the dynamic fatiguing task.

(8) *Dynamic fatiguing task:* The fatiguing exercise task involved 120 isotonic MVCCs of the knee extensor muscles at a rate of 1 MVCC every 3 seconds (6-minute task). Participants only performed the contractions for knee extension and the

dynamometer motor passively returned the limb to the starting position at 90º of knee flexion. After 60 and 120 (task completion) MVCCs, participants performed one MVIC.

(9) *Recovery Contractions:* The recovery protocol involved sets of brief contractions immediately after the fatiguing task, and then at 5 and 20 minutes of recovery. Each set of contractions involved one MVIC (with superimposed percutaneous muscle stimulation) followed by an electrically-evoked twitch contraction and then five successive MVCCs.

(10) After the first recovery protocol immediately after the dynamic fatiguing task and within three minutes of completion of the dynamic fatiguing task, state perceptions and blood glucose were assessed.

Each participant received strong verbal encouragement throughout the maximal effort contractions. During all MVCCs, participants were instructed to "kick as hard and as fast as possible" and each MVCC was initiated via strong verbal command from the authors, "KICK". The authors provided the verbal cue each 3 s, based on a visual cue from a custom-designed data collection program, and participants were encouraged to maintain maximal effort throughout the dynamic exercise task with several standard statements of encouragement.

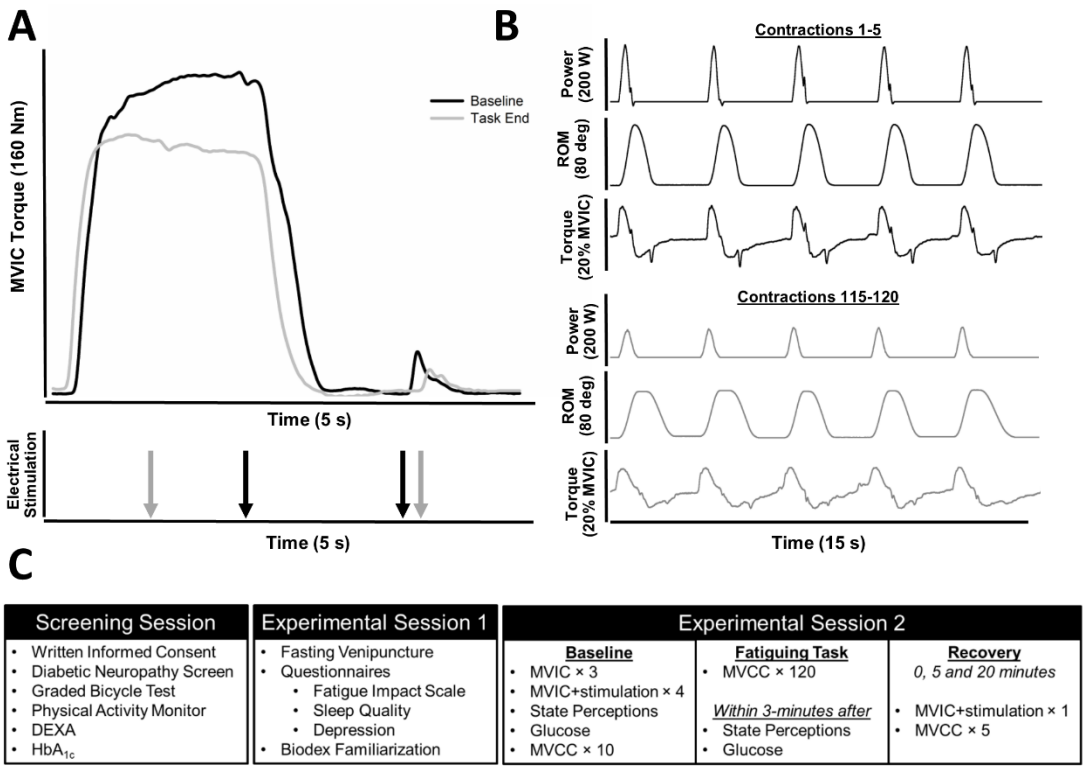

Figure 4.1. Representative data for a maximal voluntary isometric contraction (MVIC) with electrical stimulation and two sets of five maximal-effort, high-velocity concentric contractions (MVCCs) and study diagram. *A.* Torque signal from a 62-year old woman with prediabetes who performed an MVIC before (Baseline; black line) and immediately after (Task End; grey) the dynamic fatiguing task, with electrical stimulation superimposed to assess voluntary activation and contractile function. ***B.*** Calculated power (applied torque × half-wave rectified velocity), range of motion and applied torque signals from the 62-year old woman with prediabetes who performed five MVCCs at the start (black lines) and end (grey lines) of the fatiguing task. ***C.*** A diagram of the study protocol as described in Materials and Methods.

Data Analysis

Performance Fatigability: MVIC torque, MVCC power, and MVCC speed were the primary metrics of performance fatigability, although duty cycle, range of motion, and MVCC average and peak applied torque directly contribute to these metrics. The MVIC was quantified as the average torque over a 0.1 s interval around the maximal torque prior to eliciting the electrically-evoked superimposed twitch. The maximum angular velocity, power and torque during MVCCs were quantified as the maximum value during the

concentric phase of the contraction. The average torque during MVCCs was calculated during the concentric phase of the knee extension contraction. In addition, the duty cycle was calculated as: (active contraction time) · (active contraction time + relaxation time)$^{-1}$ (Sundberg & Bundle, 2015).

Contractile Function: Contractile function of the knee extensor muscles was quantified from the resting twitch elicited with electrical stimulation of the quadriceps muscles. Variables included the peak amplitude of the resting twitch, contraction time, and half relaxation time. Half relaxation time was determined as the time interval in milliseconds (ms) elapsed from the peak twitch amplitude until the torque reached 50% of the peak twitch amplitude.

Voluntary Activation: Voluntary activation was assessed with electrical stimulation and calculated using the following equation: voluntary activation = (1 – SIT · Resting Twitch^{-1}) × 100% (Gandevia, 2001; Todd *et al.*, 2016).

Fatigue and Recovery Calculations: The changes in variables during and immediately after the fatiguing task (fatigue) were calculated as: $(x_{task\ end} – x_{baseline}) \cdot (x_{baseline})^{-1} \cdot 100\%$. Task end was considered the average of the last 5 contractions (contractions 116-120) of the dynamic fatiguing task for MVCC variables (power, speed, duty cycle, range of motion, peak torque and average torque) but task end values for all other variables. The changes in variables during the 20-minute recovery were calculated (relative to baseline) as: $(x_{Recovery\ 20} - x_{task\ end}) \cdot (x_{baseline})^{-1} \cdot 100\%$.

Statistics

Values are reported as mean ± SD in the text and displayed as mean ± SE in the figures. Participant characteristics, baseline muscle function, questionnaire scores and

baseline dynamic contraction characteristics were compared across groups using a two-way univariate analysis of variance (ANOVA) with two between-subject factors (group: T2D vs prediabetes vs. controls; sex: men vs. women). To verify changes across time for the dynamic fatiguing task with the knee extensor muscles or recovery after the dynamic fatiguing task, two-way repeated measures ANOVAs with group and sex as between-subject factors were used to compare dependent variables of MVIC torque, MVCC velocity, power, duty cycle, range of motion, peak applied torque and average applied torque, electrically-evoked twitch amplitude, half relaxation time, contraction time, and voluntary activation. To determine the time effect of fatigue, variables were compared between baseline and immediately after the fatiguing task (Task End), while recovery was the time effect from immediately after the fatiguing contraction (Task End) until the end of the 20-minute recovery period (R20). Thus, two repeated measures ANOVAs were performed: one including baseline through the end of the fatiguing task (task end; to assess fatigue) and the other including task through recovery (R20; to assess recovery). *Post hoc* analysis (Tukey's Honest Significant Difference (HSD)) was used to test for differences among groups (T2D vs prediabetes vs controls) when significant main effects of group or interactions across time were identified, with Bonferroni corrected *P*-values for multiple comparisons ($P < 0.025$). Separate Pearson correlation analyses were used to determine associations between primary measures of performance fatigability (reduction in MVCC power and MVIC torque) and participant characteristics (BMI, body fat, estimated VO_2, physical activity, HbA_{1c}, fasting plasma glucose, HOMA-IR, TSH, HDL, LDL and total cholesterol), clinical symptoms of fatigue (Fatigue Impact Scale), sleep quality index, depression scale, state perceptions (muscle cramps, pain, weakness,

soreness, and fatigue, and rating of perceived exercise), depression, electrically-evoked contractile function (amplitude, contraction time, and half-relaxation time), and activation of the knee extensors (voluntary activation and neuromuscular junction propagation). Three separate linear correlation analyses between performance fatigability and characteristics expected to be different between the groups (HbA_{1c}, fasting plasma glucose, insulin, and HOMA-IR) were performed: separate regressions for each group, one regression including people with T2D and prediabetes, and one regression for the entire cohort. Linearity of bivariate correlations was verified with visual inspection. Preliminary and correlation analyses confirmed there were no violations of the assumptions of normality, linearity, or homoscedasticity. Significance was determined at $P \leq 0.05$ except *post hoc* testing ($P < 0.025$), and all the analyses were performed in IBM Statistical Package for Social Sciences (SPSS) version 24.

4.3 Results

Participant Characteristics

The three groups (T2D, prediabetes, controls) were similar in age ($P = 0.165$), height ($P = 0.329$), weight ($P = 0.085$), BMI ($P = 0.129$), relative body fat ($P = 0.398$), estimated maximal aerobic capacity ($P = 0.224$), daily physical activity ($P = 0.868$) and leg lean mass of the leg used for the dynamic fatiguing exercise ($P = 0.514$; Table 1).

Medications: Among people with T2D, all ($n = 39$) participants were prescribed Metformin and 34 participants were prescribed a statin medication. Among people with prediabetes and controls, no participants were prescribed Metformin, but 5 people with prediabetes and 8 controls were prescribed a statin (Table 1). Although not an aim of the study, it is noteworthy that people prescribed a statin medication had similar reductions in

MVCC power compared to people *not* prescribed a statin among people with T2D (time × statin, $P = 0.444$), prediabetes (time × statin, $P = 0.190$) and controls (time × statin, $P = 0.142$).

Blood Measures: Compared to controls, people with T2D had higher HbA_{1c} ($P < 0.001$), fasting plasma glucose ($P < 0.001$), insulin ($P = 0.006$) and HOMA-IR ($P < 0.001$). Compared to people with prediabetes, people with T2D had higher HbA_{1c} ($P < 0.001$) and fasting plasma glucose ($P = 0.004$); however, fasting plasma insulin ($P = 0.514$) and HOMA-IR ($P = 0.325$) were not different between the groups. Controls had lower HbA_{1c} ($P = 0.023$) and HOMA-IR ($P = 0.040$) compared to people with prediabetes; however, fasting plasma glucose ($P = 0.107$) and insulin ($P = 0.419$) were not significantly different between the control and prediabetes groups. People with T2D, prediabetes and controls had similar fasting plasma thyroid stimulating hormone (TSH; $P = 0.672$), high density lipoprotein (HDL; $P = 0.522$), low density lipoprotein (LDL; $P = 0.305$) and total cholesterol ($P = 0.156$) concentrations. See Table 1.

		Control (*n = 27*)	Prediabetes (*n = 20*)	Type 2 Diabetes (*n = 39*)	
Age	years	58.1 ± 9.4	63.1 ± 6.0	61.2 ± 8.5	
Height	m	1.72 ± 0.10	1.69 ± 0.08	1.73 ± 0.08	‡
Weight	kg	80.9 ± 17.4	77.5 ± 15.3	88.2 ± 22.5	‡
BMI	$kg \cdot m^2$	27.3 ± 4.3	26.9 ± 4.2	29.4 ± 6.4	
Body Fat	%	31.5 ± 9.4	32.8 ± 6.0	33.8 ± 9.5	‡
estimated VO_2	$mLO_2 \cdot kg^{-1} \cdot min^{-1}$	27.5 ± 8.7	36.9 ± 16.0	30.3 ± 10.2	
Physical Activity	$steps \cdot day^{-1}$	8,400 ± 3,000	8,030 ± 3,110	8,440 ± 4,220	
Leg Lean mass	kg	8.51 ± 2.37	8.25 ± 1.88	8.87 ± 2.40	‡
Duration of Diabetes	years	--	--	7.72 ± 6.87	
Metformin	n	0	0	39	
Metformin Dosage	$mg \cdot day^{-1}$	--	--	1,155 ± 586	
Duration of Prescription	years	--	--	4.6 ± 5.1	
Statin	n	8	5	34	
Duration of Prescription	years	5.5 ± 6.0	7.2 ± 4.3	4.8 ± 4.9	
HbA1c	%	5.40 ± 0.19	5.89 ± 0.29	7.15 ± 1.18	*#†
Fasting Plasma Glucose	$mg \cdot dL^{-1}$	85.9 ± 6.5	98.8 ± 18.2	123.5 ± 31.7	*†
Insulin	pMol	32.8 ± 20.3	47.2 ± 27.4	61.8 ± 42.0	*
HOMA-IR	AU	22.9 ± 13.9	42.8 ± 25.4	52.3 ± 30.4	*#
TSH	$\mu IU \cdot mL^{-1}$	1.58 ± 0.82	1.83 ± 1.05	1.75 ± 0.83	
HDL	$mg \cdot dL^{-1}$	53.6 ± 17.2	52.7 ± 16.1	47.8 ± 16.0	
LDL	$mg \cdot dL^{-1}$	103.5 ± 30.6	102.8 ± 38.9	89.1 ± 25.9	
Total Cholesterol	$mg \cdot dL^{-1}$	180.4 ± 31.2	182.5 ± 30.3	165.0 ± 26.3	

Table 4.1. Participant characteristics and matching criteria. Each group (type 2 diabetes, prediabetes and control) were similar in age, height, weight, BMI, body fat, estimated VO_2, physical activity and leg lean mass (in the leg used for the dynamic fatiguing task). Values are displayed as mean ± standard deviation. BMI, body mass index; VO_2, estimated maximal aerobic capacity; O_2, oxygen. Tukey's HSD post-hoc: *, T2D vs. control, $P < 0.05$; #, prediabetes vs. control, $P < 0.05$; †, T2D vs. prediabetes; ‡, sex difference, $P < 0.05$.

Clinical Symptoms of Fatigue, Sleep Quality and Depression

FIS: Total score ($P = 0.509$) and scores for each component of the FIS (cognitive ($P = 0.537$), physical ($P = 0.254$), and psychological ($P = 0.586$)) did not differ significantly between groups (T2D, prediabetes, control).

Sleep and Depression: Sleep quality ($P = 0.483$) and depression scale scores ($P = 0.628$) were not different between the three groups (T2D, prediabetes, control).

State Perceptions

VAS reports of muscle cramps, pain, weakness, soreness and fatigue at baseline were 0 among all people with T2D, prediabetes and controls. People with T2D, prediabetes and controls did not report an increase in muscle cramps (time, $P = 0.054$) after the dynamic fatiguing exercise; and the reported increase in muscle pain (time, $P = 0.008$; time × group, $P = 0.349$), weakness (time, $P < 0.001$; time × group, $P = 0.541$), soreness (time, $P = 0.028$; time × group, $P = 0.342$) and fatigue (time, $P < 0.001$; time × group, $P = 0.264$) was not different between the three groups. Similarly, all participants reported 0 RPE at baseline and the increase in RPE after the dynamic fatiguing exercise (time, $P < 0.001$; time × group, $P = 0.169$) was not different between the three groups (T2D, prediabetes, control) (Table 2).

	Control (*n = 27*)	Pre-Diabetes (*n = 20*)	Type 2 Diabetes (*n = 39*)	
FIS Cognitive	3.19 ± 4.15	4.25 ± 5.09	4.74 ± 5.90	‡
FIS Physical	3.89 ± 5.45	3.35 ± 4.79	6.08 ± 6.40	‡
FIS Psychological	5.48 ± 9.99	4.90 ± 6.87	8.00 ± 11.49	‡
FIS Total	12.6 ± 18.6	12.5 ± 15.1	18.3 ± 22.7	‡
Sleep Quality Index	4.67 ± 2.60	3.90 ± 2.13	4.74 ± 2.30	
Depression Scale	0.92 ± 1.09	0.65 ± 0.93	1.10 ± 1.74	
State after Fatiguing Task				
Muscle Cramps	0 ± 0.1	0.3 ± 1.2	0.4 ± 1.4	
Muscle Pain	0.2 ± 0.6	0.3 ± 0.8	0.5 ± 1.3	
Muscle Weakness	0.9 ± 1.2	1.5 ± 2.2	1.2 ± 2.4	
Muscle Soreness	0.4 ± 0.9	0 ± 0.1	0.6 ± 1.5	
Muscle "Fatigue"	2.0 ± 1.8	3.2 ± 2.6	2.2 ± 2.2	
Rating of Perceived Exertion	5.8 ± 2.0	6.3 ± 2.0	6.7 ± 2.2	

Table 4.2. Metrics contributing to perceived fatigability. Clinial symptoms of fatigue (Fatigue Impact Scale; cognitive, physical, psychological and total), sleep quality index and depression scale score were not different between groups (T2D, prediabetes, control). Reports of muscle cramps, pain, weakness, soreness and fatigue and rating of perceived exertion were 0 at baseline among all participants. After the dynamic exercise, muscle cramps did not increase; however, the increase in muscle pain, weakness, soreness, fatigue and rating of perceived exertion (RPE) was not different between the three groups (type 2 diabetes vs. prediabetes vs. controls). Values are displayed as mean ± standard deviation. ‡, sex difference, $P < 0.05$.

Performance Fatigability

At baseline, people with T2D, prediabetes and controls had similar values for MVIC torque ($P = 0.465$), MVCC power ($P = 0.649$), MVCC speed ($P = 0.623$), duty cycle ($P = 0.228$), range of motion ($P = 0.322$), peak applied torque ($P = 0.538$) and average applied torque ($P = 0.728$) (Table 3).

During the dynamic fatiguing task, the increase in duty cycle (time, $P < 0.001$; time × group, $P = 0.175$) and reduction in average applied torque (time, $P < 0.001$; time × group, $P = 0.361$) did not differ between groups (T2D, prediabetes, controls). People with T2D however, had greater reductions in MVCC power (time, $P < 0.001$; time × group, $P < 0.001$), MVCC speed (time, $P < 0.001$; time × group, $P < 0.001$), and range of motion

(time, $P < 0.001$; time × group, $P < 0.001$) than people with prediabetes ($P < 0.001$), and both groups had greater reductions in MVCC power than controls ($P < 0.001$). The reduction in peak applied torque was greater for people with T2D compared to healthy controls (T2D vs control, $P = 0.014$), however, both groups had similar reductions compared to prediabetes (T2D vs prediabetes, $P = 0.185$; Tukey's post hoc, prediabetes vs control, $P = 0.346$). After the dynamic fatiguing task, the reductions in MVIC torque were not different between groups (time, $P < 0.001$; time × group, $P = 0.203$) (Figure 2; Table 3).

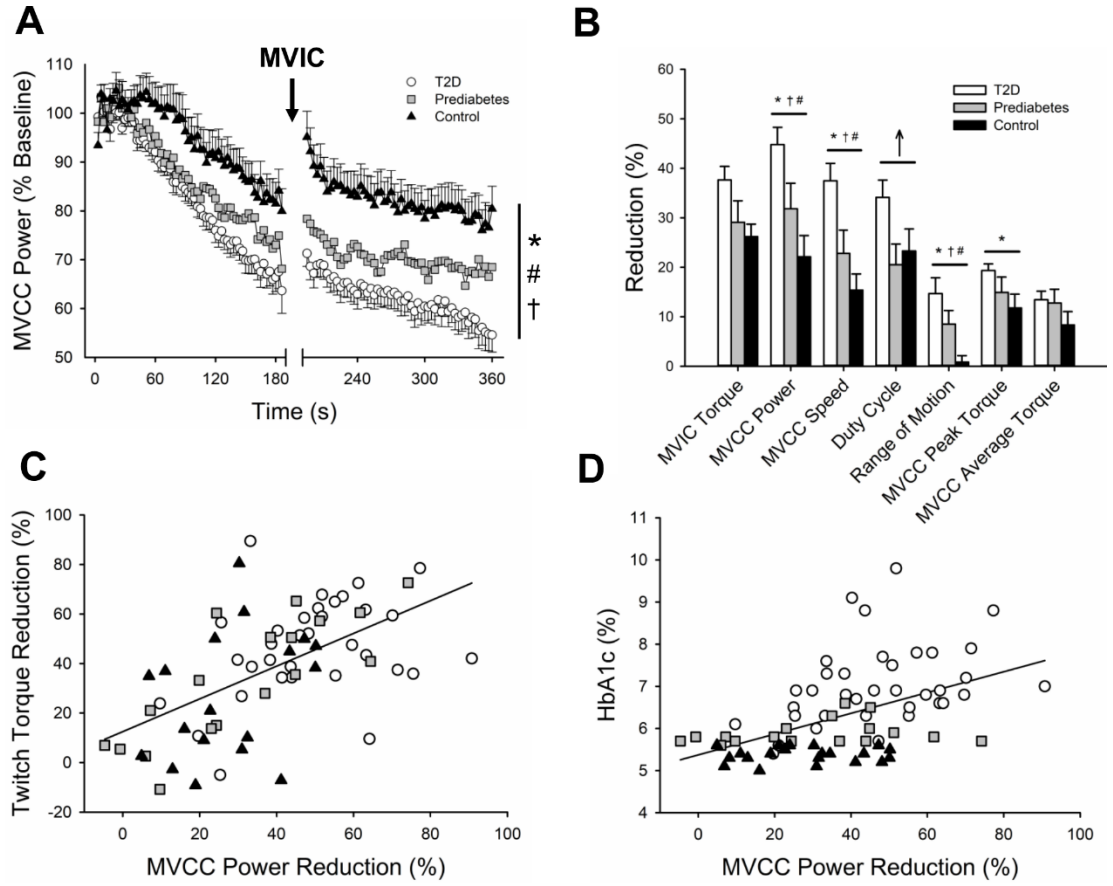

Figure 4.2. Maximal-effort, high-velocity concentric contractions (MVCC) power (% Baseline) (A), reductions (%) in measures of performance fatigability (B), and associations between MVCC power reduction (%) and electrically-evoked twitch torque reduction (C) and HbA_{1c} (D). A. People with T2D had greater reductions in MVCC power than people with prediabetes, and people with T2D and prediabetes had greater reductions in MVCC power than controls during the dynamic exercise task. **B.** People with T2D had greater performance fatigability (reductions in MVCC power, speed, range of motion and peak applied torque) compared to controls. The reduction in MVCC power was associated with the reduction in electrically-evoked twitch amplitude (**C**; $r = 0.592$, $r^2 = 0.350$, $P < 0.001$) and HbA_{1c} (**D**; $r = 0.477$, $r^2 = 0.228$, $P < 0.001$).

All the metrics of performance fatigability recovered during the 20-minute recovery (time, $P < 0.001$ for each metric). There was no difference between groups in recovery of MVIC torque (time × group, $P = 0.189$), MVCC power (time × group, $P = 0.954$), MVCC speed (time × group, $P = 0.304$), duty cycle (time × group, $P = 0.872$), range of motion (time × group, $P = 0.751$), peak applied torque (time × group, $P = 0.863$) and average applied torque (time × group, $P = 0.763$).

Baseline		Control (*n = 27)*	Prediabetes (*n = 20)*	Type 2 Diabetes (*n = 39)*	
MVIC Torque	Nm	151.3 ± 59.1	139.8 ± 58.9	164.7 ± 70.0	
MVCC Power	Watts	275.2 ± 86.1	254.0 ± 98.4	293.7 ± 132.0	
MVCC Speed	$degs \cdot s^{-1}$	336.2 ± 44.0	336.3 ± 40.0	330.1 ± 56.1	
Duty Cycle	%	14.1 ± 1.9	15.1 ± 2.0	14.8 ± 2.5	
Range of Motion	deg	74.6 ± 8.7	78.7 ± 7.2	77.1 ± 10.2	
MVCC peak Torque	Nm	65.5 ± 15.8	60.1 ± 20.1	67.4 ± 22.6	
MVCC average Torque	Nm	44.3 ± 12.6	42.0 ± 15.0	47.0 ± 18.0	
Fatigue at Task End					
MVIC Torque	%	-26.2 ± 12.6	-29.1 ± 18.9	-37.7 ± 16.4	
MVCC Power	%	-22.1 ± 21.1	-31.8 ± 22.6	-44.8 ± 21.9	*†#
MVCC Speed	%	-15.4 ± 16.0	-22.8 ± 21.1	-37.5 ± 22.0	*†#
Duty Cycle	%	+23.3 ± 20.9	+20.5 ± 18.6	+35.0 ± 20.9	
Range of Motion	%	-0.8 ± 6.3	-8.5 ± 12.3	-14.7 ± 19.9	*†
MVCC peak Torque	%	-11.8 ± 13.6	-14.9 ± 13.9	-19.4 ± 8.4	*
MVCC average Torque	%	-8.3 ± 13.2	-12.8 ± 12.4	-13.4 ± 10.8	
Recovery at 20 minutes					
MVIC Torque	%	+18.4 ± 12.2	+20.1 ± 13.9	+23.2 ± 12.7	
MVCC Power	%	+38.9 ± 19.5	+36.8 ± 21.4	+42.0 ± 25.4	
MVCC Speed	%	+22.8 ± 14.5	+26.3 ± 18.6	+33.9 ± 24.9	
Duty Cycle	%	-18.5 ± 14.1	-19.7 ± 15.4	-22.5 ± 23.0	
Range of Motion	%	+4.9 ± 5.4	+12.0 ± 11.3	+14.0 ± 11.2	
MVCC Peak Torque	%	+24.7 ± 15.1	+22.4 ± 22.8	+23.2 ± 13.5	
MVCC Average Torque	%	+13.4 ± 9.9	+11.8 ± 11.1	+12.1 ± 10.3	

Table 4.3. Performance fatigability. People with type 2 diabetes (T2D), prediabetes and healthy controls had similar volitional muscle performance at baseline. People with T2D had greater performance fatigability - greater reductions in MVCC power, MVCC speed, and range of motion - compared to people with prediabetes, and both groups (T2D, prediabetes) had greater performance fatigability compared to controls. Values are displayed as mean ± SD. MVIC, maximal voluntary isometric contraction; MVCC, maximal voluntary concentric contraction. Tukey's HSD post-hoc: *, T2D vs. control, $P < 0.05$; #, prediabetes vs. control, $P < 0.05$; †, T2D vs. prediabetes, $P < 0.05$.

Blood Glucose

At baseline, and as anticipated, post-prandial blood glucose was higher for people with T2D compared to people with prediabetes ($P = 0.001$) and healthy controls ($P < 0.001$); and there was no difference between people with prediabetes and controls ($P = 0.475$). The absolute reduction in blood glucose after the dynamic exercise (time, $P <$

0.001; time × group, $P = 0.033$) was greater for people with T2D compared to prediabetes (Tukey's HSD, $P = 0.002$) and controls (Tukey's HSD, $P < 0.001$), but not different for prediabetes and controls (Tukey's HSD, $P = 0.359$). However, the relative reduction in blood glucose (($glucose_{baseline} - glucose_{recovery5minutes}) \cdot glucose_{baseline}^{-1} \cdot 100\%$) was not different between the groups (group, $P = 0.137$). Blood glucose did not change during the 20-minute recovery period for any participant (time, $P = 0.718$). See Table 4.

Baseline		Control (*n = 27)*	Prediabetes (*n = 20)*	Type 2 Diabetes (*n = 39)*	
Potentiated Twitch	Nm	41.6 ± 18.5	43.9 ± 15.8	44.1 ± 18.5	
Contraction Time	ms	85.9 ± 9.7	92.7 ± 15.5	89.5 ± 10.8	
Half-Relaxation Time	ms	84.8 ± 24.2	84.8 ± 49.0	85.1 ± 25.3	
Blood glucose	$mg \cdot dL^{-1}$	106.1 ± 17.3	114.3 ± 28.5	158.0 ± 46.7	*†
Voluntary Activation	%	92.8 ± 5.8	93.4 ± 11.6	94.5 ± 4.8	
M_{max} Amplitude	mV	12.04 ± 8.61	12.63 ± 1.76	10.61 ± 4.65	
Fatigue at Task End					
Potentiated Twitch	%	-21.3 ± 33.0	-32.5 ± 24.9	-44.0 ± 23.4	*†
Contraction Time	%	-2.8 ± 10.4	+5.8 ± 14.1	+2.0 ± 14.2	
Half-Relaxation Time	%	+20.4 ± 37.8	+28.5 ± 46.5	+45.4 ± 46.7	
Blood glucose ¥	$mg \cdot dL^{-1}$	96.5 ± 11.1	105.0 ± 11.5	131.1 ± 37.4	*†
Voluntary Activation	%	-6.17 ± 8.13	-6.63 ± 9.60	-5.48 ± 8.75	
M_{max} Amplitude	%	+16.01 ± 23.12	+16.53 ± 18.96	+13.58 ± 33.49	
Recovery at 20 minutes					
Potentiated Twitch	%	+21.5 ± 39.8	+31.7 ± 45.0	+45.8 ± 44.3	
Contraction Time	%	-3.7 ± 11.6	+2.4 ± 11.6	-3.1 ± 9.0	
Half-Relaxation Time	%	-38.4 ± 35.8	-42.3 ± 56.1	-53.0 ± 49.7	
Blood glucose	$mg \cdot dL^{-1}$	95.6 ± 11.9	104.8 ± 10.9	139.5 ± 39.3	
Voluntary Activation	%	+1.8 ± 5.5	-3.3 ± 11.9	+2.0 ± 14.2	

Table 4.4. Electrically-evoked contractile function and activation of knee extensors. At baseline, contractile function was similar between people with type 2 diabetes (T2D), prediabetes and healthy controls. At fatigue, people with T2D had greater reductions in potentiated twitch amplitude and blood glucose compared to people with prediabetes and controls; however, impairments in contraction time and half-relaxation time were similar between groups (T2D, prediabetes, control). People with type 2 diabetes (T2D) and prediabetes and controls had a similar reduction in voluntary activation and no significant change in M_{max} amplitude of the vastus lateralis. Values are displayed as mean ± SD. ¥, task end blood glucose levels were determined after the five-minute recovery contractions. Tukey's HSD post-hoc: *, T2D vs. control, $P < 0.05$; †, T2D vs. prediabetes, $P < 0.05$.

Electrically-Evoked Twitch Contractile Function

Before the dynamic fatiguing task, the electrically-evoked twitch amplitude (group, $P = 0.908$), contraction time (group, $P = 0.162$), and half-relaxation time (group, $P = 0.821$) were not different between groups (T2D, prediabetes, control). After the dynamic fatiguing exercise (task end), the reduction in electrically-evoked twitch amplitude (time, $P < 0.001$; time × group, $P = 0.027$) was greater for people with T2D

compared to controls, but not different between people with prediabetes and T2D or prediabetes and controls (control vs. T2D, $P = 0.006$; prediabetes vs. T2D, $P = 0.297$; control vs. prediabetes, $P = 0.370$). The increase in half relaxation time of the electrically-evoked twitch was not different between the three groups (T2D, prediabetes, control) (time, $P < 0.001$; time × group, $P = 0.413$), and the contraction time of the electrically-evoked twitch did not change after the dynamic fatiguing exercise (time, $P = 0.208$).

Electrically-evoked twitch amplitude (time, $P < 0.001$; time × group, $P = 0.271$) and half-relaxation time (time, $P < 0.001$; time × group, $P = 0.457$) recovered similarly for the three groups (T2D, prediabetes, controls) during the 20 minutes of recovery. There were no changes in electrically-evoked twitch contraction time during recovery (time, $P = 0.906$). See Table 4.

Voluntary Activation

Voluntary Activation: At baseline, voluntary activation did not differ between groups (group, $P = 0.587$). Similarly, the reduction in voluntary activation after the dynamic fatiguing exercise was not different between groups (time, $P = 0.001$; time × group, $P = 0.931$). Voluntary activation did not increase (remained depressed) during the 20-minute recovery period for any participant (time, $P = 0.452$).

Neuromuscular Junction Propagation

M_{max}: The M_{max} amplitude and area for the vastus lateralis did not differ between groups at baseline and did not change after the fatiguing task. Similar results (no group differences and no change after the fatiguing task) were observed for the M_{max} amplitude and area of each agonist muscle (rectus femoris, vastus lateralis, and vastus medialis).

Given the similar results between measurements (M_{max} amplitude and area) and muscles, only the M_{max} amplitude results for the vastus lateralis are presented (Table 4).

Associations

The reduction in MVCC power during the fatiguing exercise was associated with several baseline measurements, including HbA_{1c} (r = 0.477, r^2 = 0.228, P < 0.001), estimated VO_2peak (r = -0.394, r^2 = 0.155, P = 0.001), body fat (r = 0.293, r^2 = 0.086, P = 0.008), HOMA-IR (r = 0.289, r^2 = 0.084, P = 0.050), and diabetes duration for people with T2D (r = 0.247, r^2 = 0.061, P = 0.038). Reductions in MVCC power were also associated with the declines in the range of motion (r = 0.685, r^2 = 0.469, P < 0.001), duty cycle (r = 0.630, r^2 = 0.397, P < 0.001), resting twitch amplitude (r = 0.592, r^2 = 0.350, P < 0.001), and MVIC torque (r = 0.505, r^2 = 0.256, P < 0.001).

Reductions in MVIC torque after the fatiguing exercise were associated with: range of motion reduction (r = 0.578, r^2 = 0.334, P < 0.001), resting twitch amplitude reduction (r = 0.542, r^2 = 0.294, P < 0.001), HbA_{1c} (r = 0.361, r^2 = 0.130, P = 0.001), HOMA-IR (r = 0.294, r^2 = 0.086, P = 0.050), voluntary activation reduction (r = 0.258, r^2 = 0.067, P = 0.045), and fatigue impact scale physical (r = 0.244, r^2 = 0.060, P = 0.030).

In analyses separated by group (T2D, prediabetes, control), HbA_{1c} was associated with reductions in MVCC power (r = 0.379, r^2 = 0.144, P = 0.017) but not reductions in MVIC torque (P = 0.191) for people with T2D. In analyses of data from people with prediabetes, HbA_{1c} was associated with reductions in MVIC torque (r = 0.554, r^2 = 0.307, P = 0.021) but not reductions in MVCC power; (P = 0.319). For controls, HbA_{1c} was not associated with reductions in MVIC torque (P = 0.863) or reductions in MVCC

power ($P = 0.646$). In analyses pooled for people with prediabetes and T2D, HbA_{1c} was associated with reductions in MVCC power ($r = 0.424$, $r^2 = 0.180$, $P < 0.001$) and reductions in MVIC torque ($r = 0.310$, $r^2 = 0.100$, $P = 0.024$).

Sex Differences

There were anticipated sex-related differences in several baseline measurements. Men were taller ($P < 0.001$), had greater body mass ($P < 0.001$), less percentage body fat ($P < 0.001$), and greater lean mass ($P < 0.001$). Perceived fatigability differed between the sexes because men reported higher values on FIS for the cognitive ($P = 0.033$), physical ($P = 0.025$), psychological ($P = 0.029$) and total score of the FIS ($P = 0.017$). Men were also stronger and more powerful than women ($P < 0.001$) and had greater electrically-evoked twitch amplitude than women ($P < 0.001$) for the knee extensor muscles. However, there were no sex-related differences (main effects or interactions) in any measurements of performance fatigability or perceived fatigability ($P > 0.05$).

4.4 Discussion

The novel finding of this study was that people with prediabetes had greater performance fatigability during and after a dynamic task with the knee extensor muscles compared with controls who were matched for physical activity, age, sex, and body mass index. Furthermore, performance fatigability was even greater for people with T2D than prediabetes and controls. There was no impairment in neuromuscular propagation at the neuromuscular junction and only modest reductions in neural drive from the motor cortex (~6%) which were similar for all groups. Thus, the group-related differences in performance fatigability were not due to muscle activation or neural drive to the knee extensor muscles. However, there were observed reductions in electrically-evoked

contractile function of the knee extensors and greater reductions in contractile function were associated with greater performance fatigability, such that people with T2D had the greatest reduction in knee extensor power and twitch amplitude. Accordingly, people with prediabetes exhibited an intermediate decline in both knee extensor power during the dynamic fatiguing exercise and electrically-evoked contractile function after the dynamic fatiguing task. Collectively, these results suggest that although there were (similar) reductions in voluntary activation, greater fatigability for people with T2D and prediabetes was primarily due to mechanisms impairing contractile function of the skeletal muscle.

Although there were expected and observed sex-related differences in body mass, body size and baseline muscle function, there were no observed sex-related differences in fatigability or recovery among people with T2D, prediabetes or controls. These data confirm our previous findings in male and female controls and people with T2D (Senefeld *et al.*, 2018c). Maximal knee extensor power (MVCC) and assessments of contractile function demonstrated substantial recovery during the 20 minutes after the dynamic fatiguing exercise for each group, providing evidence that the exercise did not induce muscle damage which typically lasts for up to 72 hours post exercise (Cheung *et al.*, 2003). Although each group (T2D, prediabetes and control) demonstrated recovery of MVIC torque, there were lasting reductions in MVIC torque (~10%) and voluntary activation (~6%) relative to baseline measures 20 minutes after the dynamic fatiguing exercise.

Prediabetes

The *average* participant in this cohort was ~60 years, overweight (BMI, ~28 $kg \cdot m^{-2}$; body fat ~32%) and relatively active with ~8,200 $steps \cdot day^{-1}$ (Tudor-Locke *et al.*, 2011). Despite the relatively high physical activity, the prevalence of prediabetes among the potential control participants who enrolled in the study was ~42%, which is similar to the estimated proportion of older adults with prediabetes in the US - 48% (Prevention, 2017). People with prediabetes had greater HbA_{1c}, estimated insulin resistance (HOMA-IR) and performance fatigability compared with controls, but lower HbA_{1c} and performance fatigability than people with T2D (Table 1). The reductions in MVCC power were approximately 100% greater for people with T2D than controls (42% vs. 22%) and 50% greater for people with prediabetes than controls (32% vs. 22%). These data demonstrate clear and incremental differences in performance fatigability (reduction in MVCC power and MVIC torque) between the groups; and these differences were associated with HbA_{1c} and HOMA-IR. We speculate that the association between HbA_{1c} and HOMA-IR and greater fatigability could be due to the associated impairments in metabolic function (Abdul-Ghani & DeFronzo, 2010) (e.g. reduced glucose-uptake, fat oxidation, and oxidative phosphorylation) or vascular function (Montero *et al.*, 2013; Poitras *et al.*, 2018) (e.g. decreased endothelial-dependent vasodilation, capillary rarefaction, and reduced cardiac output) secondary to insulin resistance.

Performance Fatigability

The fatigue-related reduction of knee extensor power during the high-velocity dynamic exercise was progressively greater according to group - controls (22%), people with prediabetes (32%), and people with T2D (45%). Despite these differences, the

results likely underestimate the magnitude of greater performance fatigability for people with prediabetes and T2D, because there were greater reductions in the range of motion during the dynamic fatiguing task for people with T2D (14%) and prediabetes (9%) compared with the controls (1%). The reduction in range of motion demonstrates reduced knee extensor work during the dynamic fatiguing task and was associated with performance fatigability (r^2 = 0.469). Our findings of greater fatigability are consistent with previous research demonstrating greater fatigability of people with type 1 diabetes mellitus and diabetic polyneuropathy compared to controls for isometric contractions of the dorsiflexors (Allen *et al.*, 2015a) and knee extensors (Almeida *et al.*, 2008), and greater fatigability of people with T2D and no clinically-evident diabetic polyneuropathy compared to controls for isometric contractions of the finger flexors (Petrofsky *et al.*, 2005). However, our data are the first to demonstrate greater limb fatigability in people with prediabetes compared with healthy controls matched for age, body size and physical activity; and people with T2D (and no clinically-evident diabetic polyneuropathy) had even greater limb fatigability than people with prediabetes.

Mechanisms of Performance Fatigability

The mechanisms contributing to both the reductions in knee extensor power and MVIC torque with the dynamic fatiguing task included a loss in voluntary activation within the motor cortex (~6%) and reduction in contractile function (twitch amplitude and half-relaxation time) of the knee extensor muscles (~20% for controls, ~30% for people with prediabetes and ~40% for people with T2D). However, there was no observed decline in the neuromuscular transmission of the action potentials from the alpha motor neuron to the sarcolemma (M wave).

The exercise-induced reduction in voluntary activation was not different between the groups (control, prediabetes, T2D), was weakly associated with the reduction in MVIC torque (r^2 = 0.067) and was not associated with the reduction in MVCC power, suggesting that neural mechanisms played a minor role in the difference between groups in the reduction in MVIC torque and probably the reduction in MVCC power. It is notable, however, that assessment of voluntary activation *during* these high-velocity (>350 deg·s^{-1}) dynamic contractions was not performed in this study because of the methodological limitations in assessment of voluntary activation during high-velocity contractions (Rozand *et al.*, 2017). Voluntary activation was assessed during maximal static contractions immediately after the dynamic fatiguing task and given the moderate correlation between the reduction in MVIC torque and MVCC power (r^2 = 0.256), the reduction in voluntary activation during static contractions may not accurately reflect the reduction in voluntary activation during dynamic contractions.

There is evidence that diabetes is associated with changes in the activation of the motor unit, including reduced motor nerve conduction velocities (Almeida *et al.*, 2008), reduced and more variable motor unit discharge rates (Almeida *et al.*, 2008; Watanabe *et al.*, 2013), and disrupted neuromuscular transmission (Allen *et al.*, 2015a). Additionally, greater impairments in neuromuscular transmission were associated with greater fatigability of maximal strength contractions after an isometric fatiguing task among people with T1D and diabetic polyneuropathy (Allen *et al.*, 2015a). Neuromuscular transmission was quantified in our cohort with amplitude and area of the M wave (M_{max}) of the vastus lateralis, vastus medialis and rectus femoris; however, there were no group-related differences nor any reductions of neuromuscular transmission. The difference

between studies probably is due to the absence of clinically-evident diabetic polyneuropathy in our cohort.

The greater performance fatigability of people with prediabetes and T2D was associated with larger impairments in muscle contractile properties, quantified with the electrically-evoked twitch amplitude. The magnitude of the reduction of MVCC power and electrically-evoked twitch amplitude were remarkably similar for people with T2D (44.8 vs. 44.0%), people with prediabetes (31.8 vs. 32.5%) and healthy controls (22.1 vs. 21.3%), providing evidence of the close relationship between performance fatigability and muscle contractile properties. The reductions in twitch amplitude reflect a decline in the number of high-force cross-bridges formed and the amount of force per cross-bridge (Debold *et al.*, 2016). However, the kinetics of the electrically-evoked twitch - the contraction time and half-relaxation time - were not different between the groups after the fatiguing task. The kinetics of the twitch are likely reflective of intracellular calcium and cross-bridge cycling (Kent-Braun *et al.*, 2012). Considered together, the primary mechanisms contributing to greater fatigability of the knee extensor muscles in people with prediabetes and T2D were likely due to impaired force production from the cross-bridge formation of actin and myosin, secondary to a lower number of cross-bridges or less force per cross-bridge. Accumulation of metabolic by-products (particularly hydrogen ion and inorganic phosphate) could be responsible for inhibition of cross-bridge force production (Fitts, 2008, 2016), suggesting that people with prediabetes and T2D may have experienced greater intracellular accumulation of or greater sensitivity to myocellular metabolites.

The association between larger HOMA-IR and impairments in contractile function after the fatiguing task may suggest that the downstream manifestations of insulin resistance may result in greater impairments in contractile function of people with prediabetes and T2D. Insulin resistance affects many tissues (e.g. liver, coronary arteries, brain) (Rask-Madsen & Kahn, 2012), and the effects of insulin resistance on muscle and peripheral vasculature would most likely contribute to greater impairments in contractile function during exercise. Insulin resistance can induce impairments in peripheral vasculature including impaired vasodilation (Montero *et al.*, 2013; Phillips *et al.*, 2015; Mahmoud *et al.*, 2016), capillary density or recruitment (Serne *et al.*, 1999), endothelial function and skeletal muscle blood flow (Reynolds *et al.*, 2017; Poitras *et al.*, 2018; Senefeld *et al.*, 2018b) and impaired metabolic function in skeletal muscle including reduced insulin-stimulated glucose uptake (Holloszy, 2003; Szendroedi & Roden, 2008) and reduced mitochondrial quality or quantity (Porter & Wall, 2012; Montgomery & Turner, 2015), and these mechanisms may contribute to greater fatigability in people with insulin resistance (i.e. prediabetes and T2D). These potential mechanisms warrant future investigations.

To simulate typical life conditions, participants performed dynamic fatiguing exercise ~90 minutes after consuming a standard breakfast. A recent systematic review concluded that among people with diabetes, exercise conducted postprandially blunts hyperglycemia and improves various measures of glycaemia more than exercise conducted in the fasted state (Borror *et al.*, 2018). As anticipated, postprandial blood glucose levels were greater for people with T2D compared to the other groups (prediabetes, control); however, each group demonstrated a similar relative decline in

blood glucose (~10%) after the dynamic fatiguing exercise. Postprandial (insulin-stimulated) glucose transport is impaired among people with insulin resistance (DeFronzo & Tripathy, 2009), largely accounting for the greater postprandial blood glucose in people with T2D, however, use of metformin after breakfast would have facilitated muscle glucose uptake. Exercise stimulates glucose flux into skeletal muscle via a separate pool of intracellular glucose transporters (GLUT4) and this process is not impaired in diabetes (Goodyear & Kahn, 1998). Hence it is reasonable to assume that the reductions in blood glucose concentration during and after exercise in all groups were due to the combination of exercise- and insulin-stimulated glucose disposal; the latter being facilitated by metformin among those with T2D. In terms of potential influences on fatigue, we cannot partition relative contributions of the longer-term effects of prevailing glycaemia (indicated by HbA_{1c}) and acutely elevated blood glucose concentration.

Metrics Contributing to Perceived Fatigability

Although perceived fatigability is common among people with T2D and could perhaps contribute to increased rates of depression, anxiety and fatigue in people with T2D compared to controls (Fritschi & Quinn, 2010), our cohort had minimal evidence of perceived fatigability. Our metrics of clinical symptoms of fatigue (Fatigue Impact Scale), sleep quality, depression, and state perceptions (rating of perceived exertion and muscle cramps, pain, weakness, soreness and fatigue) that can contribute to perceived fatigability were similar across the groups (T2D, prediabetes and control). Thus, among a cohort with homogeneous and low reports of perceived fatigability, there were no associations between perceived fatigability and performance fatigability. We speculate that the negligible reports of perceived fatigability are due to minimal levels of co-

morbidities in our cohort— participants had relatively low rates of obesity (30% participants), relatively good glycemic control ($HbA_{1c} \leq 7.0\%$; 75% of people with T2D), and there were high numbers of participants with physical activity greater than 7000 steps·day^{-1} (70% participants). Even so, this "healthy" cohort of people with T2D or prediabetes who volunteered for this exercise study (and hence are likely to be more active and well-disposed towards exercise) had poorer glycemic control, greater performance fatigability and greater impairments in knee extensor contractile function compared to controls, and our results likely underestimate the magnitude of these issues in the "average" person with T2D or prediabetes.

Conclusion

For the first time, we demonstrated that the knee extensor muscles of people with prediabetes were 50% more fatigable than controls for a dynamic fatiguing task even when matched for physical activity levels, body mass index, age and sex. We also showed that people with T2D were more fatigable than people with prediabetes, and this was evidenced as a greater reduction in knee extensor power and maximum isometric strength. Perceived fatigability did not differ between the groups and appeared to have minimal influence on performance fatigability. Reductions in voluntary activation contributed to the reduction in maximal force for each group; however, the greater fatigability of people with prediabetes and diabetes compared to controls was primarily due to muscle contractile mechanisms. Although exercise is a cornerstone of T2D management and prevention, the greater performance fatigability among people with prediabetes and T2D (with minimal reports of perceived fatigability and co-morbidities) may be a significant physiological barrier to exercise tolerance in this clinical population.

However, exercise training can improve glycemic control, enhance skeletal muscle blood flow and capillary density, improve insulin sensitivity and insulin-stimulated glucose uptake in skeletal muscle and increase mitochondrial quantity (Marwick *et al.*, 2009; Stanford & Goodyear, 2014). Such adaptations could reduce or offset the greater performance fatigability and reductions in contractile function in people with prediabetes and T2D; however, this remains to be tested.

CHAPTER 5: EXERCISE-INDUCED HYPEREMIA IS ASSOCIATED WITH FATIGABILITY IN HUMAN TYPE 2 DIABETES

This manuscript is currently in review.

Senefeld J, Limberg JK, Lukaszewic KM and Hunter SK. Exercise-Induced Hyperemia is Associated with Knee Extensor Fatigability in Human Type 2 Diabetes.

5.1 Introduction

The Diabetes Prevention Program demonstrated that a 3-year lifestyle intervention, including diet, weight loss and regular exercise, lowered the risk of developing type 2 diabetes mellitus by 58% among an at-risk population (Knowler *et al.*, 2002). A 10-year follow-up of this study estimated that lifestyle intervention resulted in the delay of development of type 2 diabetes by 4 years (Diabetes Prevention Program Research *et al.*, 2009). Lifestyle therapy has therefore become the first line of medical intervention for management of type 2 diabetes (Garber *et al.*, 2018), with physical exercise serving as the cornerstone of lifestyle therapy.

Physical exercise can result in fatigability of limb muscles. Fatigability of limb muscles is a reversible, short-term, activity-induced reduction in muscle strength or power (Gandevia, 2001), and when followed by adequate recovery, induces appropriate physiological adaptations of the neuromuscular system (Kraemer & Ratamess, 2004). Excessive fatigability or inadequate recovery however, may contribute to exercise intolerance and reduced adherence to exercise training. Greater fatigability has been shown to limit the performance of repeated contractions of limb muscles that are common during daily tasks (Hunter, 2017). We recently demonstrated that the knee

extensor muscles of patients with type 2 diabetes are more fatigable compared with age-, (body mass index) BMI-, and physical activity-matched controls after a six-minute, dynamic exercise due to impairments within the contractile properties of the muscles (Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c). Such deficits in contractile function could be mediated by impaired oxygen kinetics in skeletal muscle of patients with type 2 diabetes (Poitras *et al.*, 2018).

Although there is no definitive conclusion that muscle oxygen delivery is diminished in patients with type 2 diabetes (for review, see (Poitras *et al.*, 2018)), there is strong evidence to support impaired skeletal muscle blood flow regulation in this population, possibly contributing to greater fatigability than healthy controls (Montero *et al.*, 2013; Poitras *et al.*, 2018). The increase of skeletal muscle blood flow during exercise (exercise-induced hyperemia) is governed by complex interactions between cardiac and muscular pump, sympathetic nervous system control, local vasoactive metabolites and autacoids (Joyner & Casey, 2015). Ultimately, however, the control of blood flow is largely dictated by vessel diameter of skeletal muscle arterioles which is dependent on endothelial response to chemical and metabolic stimuli (Joyner & Casey, 2015). A recent meta-analysis (Montero *et al.*, 2013) demonstrated impaired endothelial function, commonly assessed with flow-mediated dilation (Thijssen *et al.*, 2011), in non-exercising vasculature in patients with type 2 diabetes compared with age-matched controls in 28 out of 29 studies. However, there is a dearth of knowledge examining patients with type 2 diabetes compared with controls when possible confounders are controlled for such as age, sex, body size and physical activity levels.

The *purpose* of the study therefore was to compare fatigability, contractile function, and exercise-induced hyperemia after dynamic exercise with the knee extensor muscles between patients with type 2 diabetes and age-, BMI- and physical activity-matched controls. Our *hypotheses* were: 1) fatigability of the knee extensor muscles would be greater in patients with type 2 diabetes compared with healthy controls, and 2) greater fatigability would be associated with greater reductions in skeletal muscle contractile function and reduced exercise-induced hyperemia.

5.2 Research Design and Methods

Fifteen patients with type 2 diabetes (7 women) and 15 healthy, age-, BMI, and physical activity-matched controls (7 women) participated in the study. Although we have previously demonstrated no sex-related differences in fatigability in patients with type 2 diabetes (Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c), biological sex was accounted for in the study design and reporting of findings as suggested by the National Institutes of Health (NOT-OD-15-102). All women enrolled were post-menopausal. No participants were taking hormone replacement drugs or vasoactive medications. All patients with type 2 diabetes were prescribed a statin and Metformin; however, control participants were prescribed no medications. Prior to involvement, each participant provided written informed consent and the protocol was approved by the Marquette University Institutional Review Board in accordance with the Declaration of Helsinki.

Inclusion and Screening Criteria

Type 2 diabetes was physician-diagnosed. Exclusion criteria included: prescribed insulin or insulin secretagogue, glycated hemoglobin (HbA_{1c})>10%, diabetic neuropathy, peripheral edema, BMI>45 $kg \cdot m^{-2}$, untreated hypothyroidism, current smoking,

cardiovascular or musculoskeletal disease that precluded exercise testing. Participants who presented with HbA_{1c} 5.7%-6.5% without a diagnosis of type 2 diabetes were classified as having pre-diabetes and not included in the study; thus, all controls had an $HbA_{1c} \leq 5.6\%$. Participants abstained from caffeine and vigorous exercise for the 24 hours prior to each session.

Screening Session

During a screening session, participants underwent diabetic neuropathy screening, HbA_{1c} testing, dual-energy x-ray absorptiometry (DEXA) scan, a graded exercise test, completed questionnaires and were issued a physical activity monitor. Participants abstained from food for 2 hours prior to the screening session.

Diabetic neuropathy screening: Participants were screened for the presence of diabetic polyneuropathy using 10-g monofilament, 128-Hz vibration, and tendon jerk, as performed previously (Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c).

HbA_{1c}: HbA_{1c} was determined using blood from a fingerstick (Siemens Healthcare Diagnostics, DCA 2000+).

DEXA: Whole body and dominant leg anthropometrics (total, fat and skeletal muscle mass) were assessed using dual x-ray absorptiometry (DEXA; Lunar Prodigy, Madison, WI).

Physical Activity: Accelerometry data were collected (Actigraph GT3X+, ActiGraph, Pensacola, FL) via waist monitor worn for 4 days (2 weekdays and weekend days) with 60s epochs. Wear-time was set *a priori* ($\geq$ 3 days of $\geq$ 9 hours per day) and was authenticated (Hart *et al.*, 2011b).

Submaximal, Graded Bicycle Test: To assess estimated aerobic capacity (eVO^2), participants performed a submaximal graded exercise test (Beekley *et al.*, 2004) on a bicycle ergometer (VIAsprint 150P, CareFusion, San Diego, CA) with 12-lead electrocardiogram (CASE, General Electrics, Madison, WI). The selected protocol was designed to elicit incremental heart rate responses between 40% and 70% of heart rate reserve during three, steady-state efforts for four-minutes each.

Questionnaires: Clinical symptoms of fatigue, depression and sleep quality were assessed with the Fatigue Impact Scale (Fisk *et al.*, 1994), short form Geriatric Depression Scale (Burke *et al.*, 1991), and Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989), respectively.

Study Protocol

Within one week of the screening session, participants completed three experimental sessions. Experimental sessions were performed on different days separated by 2 – 7 days. Experiments began in the morning (~8:00 AM) after a 12-hour fast and prior to administration of medication. During the first experimental session, participants underwent a flow-mediated dilation study of the right brachial artery and were familiarized with the lower limb dynamic fatiguing contraction. During the second experimental session, participants underwent a flow-mediated dilation study of the right superficial femoral artery and performed a six-minute dynamic fatiguing contraction with the non-dominant knee extensors. During the third experimental session, participants performed a 6-minute dynamic fatiguing contraction with the dominant knee extensors. All participants reported right limb dominance (Oldfield, 1971).

Flow-mediated Dilation

Flow-mediated dilation (FMD) studies were performed while participants were lying supine and afebrile, and the room temperature was ~70°F. After quiet rest, blood pressure was manually auscultated in the left arm of the participant every two minutes until the mean arterial pressures of two consecutive measurements were within 5% (Thijssen *et al.*, 2011).

Artery diameter and blood flow velocity were assessed simultaneously using duplex mode Doppler ultrasound (Vivid E, General Electrics, Madison, WI) with an 8-MHz linear array probe with insonation angle of 60°. For arm measurements, the ultrasound probe was placed overlying the brachial artery at the mid-point between the cubital fossa and axilla, with the arm in ~90 degrees of abduction and forearm held midway between pronation and supination. For leg measurements, the ultrasound probe was placed overlying the superficial femoral artery, 2.5 cm distal to the bifurcation of the common femoral artery; the position was recorded and replicated during exercise-induced hyperemia studies. Electrocardiogram was recorded and synced with ultrasonography (USB ECG, NORAV medical, Germany).

A rapid inflation pneumatic cuff system (Hokanson Rapid Cuff, DE Hokanson, Inc., USA) was used to apply a supra-systolic pressure (250 mmHg) to occlude blood flow for five minutes, and then immediately release the pressure. Blood flow velocities were assessed in the two-minutes before occlusion and for 30s immediately post-occlusion and arterial diameters were assessed for five minutes post-occlusion (Thijssen *et al.*, 2011). Blood flow velocity was assessed as the average maximum velocity of three consecutive cardiac cycle segment and averaged across three segments. Arterial diameter

was assessed with custom-designed software (AccessPoint Suite, Freeland Systems LLC, Georgia) by a single rater blinded to the participants' diabetes status. Arterial diameter was assessed at end diastole over a 2-cm segment. The average diameter before occlusion and the maximum diameter after occlusion were used to calculate flow-mediated dilation.

Exercise-Induced Hyperemia

Arterial diameter and blood flow velocity were assessed as described above; however, the six-minute exercise replaced the five-minute occlusion. On separate days, the dynamic fatiguing contraction was performed with the dominant and non-dominant legs. Femoral artery blood flow was assessed on the dominant leg femoral artery during both experiments. Thus, when exercise was performed with the non-dominant leg, blood flow measures in the dominant leg served as an inactive limb control and a proxy for the contribution of central mechanisms of blood flow.

Dynamic Lower Limb Exercise

Participants were seated in a Biodex dynamometer (System 4, Biodex Medical, Shirley, NY) with 90º of hip and knee flexion to perform all knee extension torques, velocity and power during isometric and dynamic contractions as described before (Senefeld *et al.*, 2018c). Padded straps were securely tightened across each shoulder and the waist.

At the beginning of each session, 3-5 brief (~4s) maximal voluntary isometric contractions (MVICs) were performed with the knee extensors until two MVIC torque values were within 5%. Four additional MVICs were performed in the same manner with stimulation within 3s after each MVIC to assess skeletal muscle contractile properties. The muscles were stimulated with a single pulse (150 – 750 mA; DS7AH, Digitimer,

Ltd., Welwyn Garden City, UK) via custom-made pad electrodes (6 cm×~15 cm). The cathode was placed within 10 cm of the femoral triangle and the anode was placed immediately proximal to the patella. Stimulator current was increased until the electrically-evoked twitch amplitude plateaued, and then the current was increased 20% (Senefeld *et al.*, 2018d).

The maximum MVIC torque value was recorded and used to calculate the 20% MVIC load for the dynamic fatiguing exercise. Participants then performed the dynamic fatiguing task involving 120 maximal voluntary concentric contractions (MVCCs) once every three seconds (six minutes total). One MVCC involved maximal velocity concentric knee extension until full extension followed by passive knee flexion ($180^{o} \cdot s^{-1}$). One MVIC was performed after the 60^{th} MVCC, and immediately after the dynamic exercise.

Mechanical recordings from the dynamometer corresponding to torque, velocity and position were digitized using a 1401 A–D converter and Spike 2 software (Cambridge Electronics Design, Cambridge, UK) with a sampling frequency of 2,000 Hz.

Data Analysis

Skeletal Muscle Function and Fatigability: The peak power during the concentric phase of each MVCC was determined. Fatigability was determined as the reduction in the average peaks of first five contractions of the fatiguing task to the last five contractions. Contractile properties that were quantified from electrically-evoked knee extensor contractions included the peak amplitude of the resting twitch, its contraction time (time elapsed from the rise in the twitch force until the peak twitch), and half relaxation time (time elapsed from the peak twitch amplitude until the torque reached 50% of the peak

twitch amplitude). The magnitude of change in outcome variables (other than power) was calculated: $(x_{taskend}-x_{baseline})/x_{baseline}\times 100\%$.

Normalization of Vasodilation and Hyperemia: Increased blood flow is a mechanical stimulus causing increased shear stress to vascular endothelium (Pyke *et al.*, 2004). Shear rate (the rate of shear stress) can increase vasodilation in a dose-dependent fashion (Thijssen *et al.*, 2011), thus FMD responses were normalized to shear rate (i.e. $FMD\times(\text{shear rate})^{-1}\times 100\%$). Similarly, because limb size can affect hyperemic responses (Limberg *et al.*, 2010), limb skeletal muscle mass was used to normalize skeletal muscle blood flow (i.e. $(\text{blood flow})\times(\text{muscle mass})^{-1}\times 100\%$. Shear rate was calculated (Gibbs *et al.*, 2011): $4\times(\text{mean blood velocity})\times(\text{artery diameter})^{-1}$. Blood flow was calculated (Padilla *et al.*, 2008): $(\text{mean blood velocity})\times\pi\times\text{radius}^2\times 60$.

Statistical Analysis

Two-way univariate analyses of variance (ANOVAs) with group and sex as between subject factors were used to compare physical characteristics and baseline measures of skeletal muscle contractile properties. To verify changes after 5-minute occlusion and the dynamic exercise, separate two-way repeated measures ANOVAs (pre vs. post) with group and sex as between subject factors and repeated measures were used to compare the dependent variables, including MVIC torque, MVCC power, peak twitch amplitude, contraction time and half relaxation time. For each ANOVA, the sphericity of data was verified with Mauchly's test. After checking for normality of data (Shapiro-Wilk test), Pearson product-moment correlation coefficient was used determine association between the measurement of fatigability (reduction in MVCC power) and participant characteristics (HbA_{1c}, estimated VO_2 peak, skeletal muscle mass, and daily

step count), vascular variables (exercise-induced hyperemia, baseline brachial and femoral diameter, and FMD responses) and changes in skeletal muscle contractile properties. Significance was determined at $P<0.05$ and analyses were performed in IBM statistical package for social sciences (SPSS) version 24.

5.3 Results

For all variables, the main effect of sex or the interactions of sex with time or group are only reported if significant, otherwise these statistics are not reported ($P>0.05$).

Physical Characteristics and Estimated Aerobic Capacity

Patients with type 2 diabetes were not different to controls for age, height, weight, BMI, body fat, daily step count, estimated aerobic capacity, and thigh lean mass ($P>0.05$, Table 1). As expected, patients with type 2 diabetes had higher HbA_{1c} compared with control subjects (group, $P<0.001$).

Men were taller (sex, $P<0.001$), had lower body fat (sex, $P<0.001$), higher estimated maximal aerobic capacity (sex, $P=0.026$) and greater thigh lean mass (sex, $P=0.001$) than women (Table 1). These effects were independent of the group.

		Type 2 Diabetes *n* = 15	Control *n* = 15	
Baseline Characteristics				
Age	years	62.4 ± 9.0	58.4 ± 6.9	
Diabetes duration	years	9.2 ± 7.1	--	*
Height	cm	173 ± 9	170 ± 7	†
Weight	kg	92 ± 29	82 ± 15	†
BMI	$kg \cdot m^{-2}$	30.4 ± 7.7	28.4 ± 4.6	
HbA_{1c}	%	7.0 ± 0.8	5.6 ± 0.3	*
Body Fat	%	34.0 ± 10.7	34.6 ± 9.9	†
Daily Steps	n	7200 ± 3200	7900 ± 2300	
eVO_2	$mL \cdot kg^{-1} \cdot min^{-1}$	36.8 ± 12.2	40.2 ± 20.4	†
Questionnaires				
Fatigue Impact Scale	AU	12.8 ± 14.7	10.9 ± 15.0	
Depression: GDS	AU	0.8 ± 1.1	0.7 ± 0.9	
Sleep Quality: PSQI	AU	5.3 ± 2.1	4.7 ± 2.4	
Muscle Function				
MVIC Torque	Nm	153 ± 67	151 ± 69	†
MVCC Power	Watts	329 ± 53	340 ± 38	†
Peak Twitch Torque	Nm	32.3 ± 12.1	40.3 ± 18.6	†
HRT	Nm	86.1 ± 21.2	74.0 ± 24.0	
CT	ms	86.9 ± 8.2	89.4 ± 6.6	†
Fatigability				
MVIC	%	32.0 ± 14.6	36.5 ± 13.9	
MVCC	%	30.0 ± 20.1	14.6 ± 19.0	*
Peak Twitch Torque	%	37.6 ± 24.8	31.6 ± 30.1	
HRT	%	+39.5 ± 46.0	+19.2 ± 29.7	
CT	%	NS	NS	

Table 5.1. Patient characteristics, questionnaire scores and skeletal muscle function. Values are displayed as mean ± standard deviation. Patients with type 2 diabetes and controls were similar in characteristics, questionnaire scores and skeletal muscle function; however, patients with type 2 diabetes had increased HbA1c compared to controls. BMI, body mass index; HbA_{1c}, glycated hemoglobin; eVO_2, estimated aerobic capacity; GDS, Geriatric Depression Scale; PSQI, Pittsburgh Sleep Quality Index; MVIC, maximal voluntary isometric contraction; MVCC, maximal voluntary concentric contraction; HRT, half-relaxation time; CT, contraction time; NS, not significant. *, group differences, $P < 0.05$; †, sex differences, $P < 0.05$.

Fatigue, Depression, and Sleep Questionnaires

Patients with type 2 diabetes and controls did not differ for scores on the Fatigue Impact Scale (group, P=0.755), depression (group, P=0.726) or sleep quality (group, P=0.579). See Table 1.

Baseline Skeletal Muscle Function

Patients with type 2 diabetes and controls were not different for baseline measures of MVIC torque (group, P=0.754), MVCC power (group, P=0.482), electrically-evoked peak twitch amplitude (group, P=0.327), half-relaxation time (group, P=0.232), and contraction time (group, P=0.339). However, men had higher MVIC torque (sex, P=0.002), MVCC power (sex, P=0.049), twitch amplitude (sex, P=0.028) and shorter contraction time (sex, P=0.038) than women, independent of group (Table 1).

Fatigability

Patients with type 2 diabetes had greater reductions in limb power than controls by the end of the fatiguing contraction (time, P<0.001; time×group, P<0.001). After the fatiguing exercise, both groups had reductions in MVIC torque (time, P<0.001; time×group, P=0.675), electrically-evoked twitch amplitude (time, P=0.002; time×group, P=0.981), and increases in half-relaxation time (time, P=0.042; time×group, P=0.358). The contraction time did not change for either group following exercise (time, P=0.710; time×group, P=0.806). See Table 1 and Figure 1.

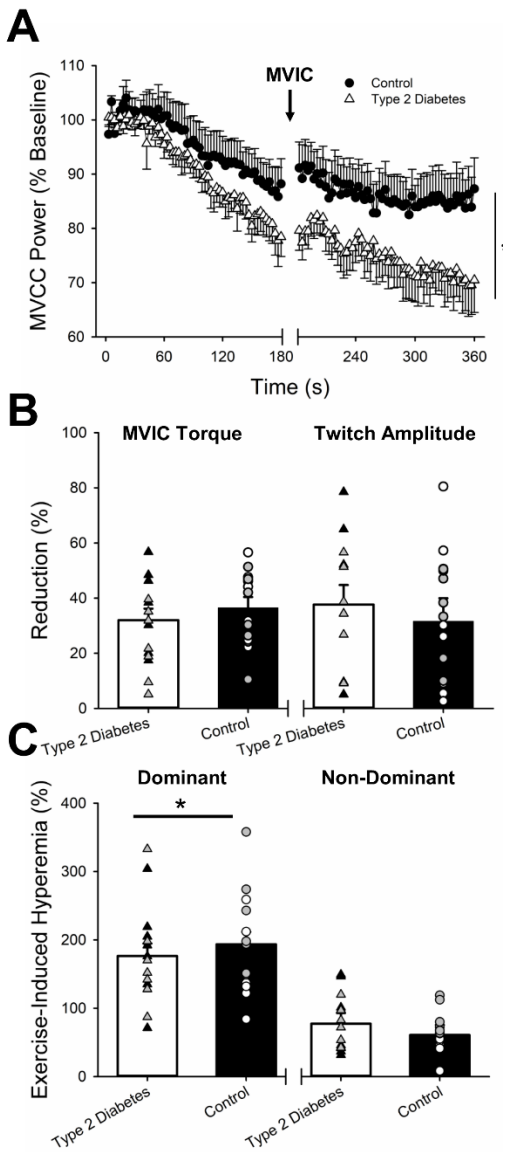

Figure 5.1. Fatigability and exercise-induced hyperemia in patients with type 2 diabetes mellitus and healthy controls. Maximal voluntary concentric contraction (MVCC) power (% Baseline) (A), reductions in maximal voluntary isometric contraction (MVIC) torque and electrically-evoked twitch amplitude (B), and exercise-induced hyperemia in the dominant artery after exercise in the dominant and non-dominant legs (C). Group data are displayed as mean ± SEM, and women are represented in grey filled symbols. **A.** Patients with type 2 diabetes had greater reductions in MVCC power than controls ($P<0.05$). **B.** The reductions in MVIC torque and electrically-evoked twitch amplitude did not differ between patients with type 2 diabetes and controls ($P>0.05$). **C.** Patients with type 2 diabetes had a lower exercise-induced hyperemia compared with controls ($P<0.05$).

Exercise-induced Hyperemia

Dominant Leg Exercise: Patients with type 2 diabetes had smaller femoral artery diameter at rest than controls (group, P=0.036) and men had greater femoral artery diameter than women (sex, P=0.012), with no interaction of sex and group. Resting femoral artery blood flow was not different between groups (group, P=0.762). However, controls had larger increases in femoral artery blood flow (exercise-induced hyperemia) than patients with type 2 diabetes after the dynamic exercise with the dominant leg when expressed relative to baseline flow (time, P<0.001; time×group, P=0.040), relative to thigh lean mass (time, P<0.001; time×group, P=0.047), and for the absolute blood flow (time, P<0.001; time×group, P=0.043). See Table 2 and Figure 1C.

Non-dominant Leg Exercise: At rest, controls and men had larger femoral artery diameter than patients with type 2 diabetes and women respectively (group, P=0.044; sex, P=0.020). Baseline femoral artery blood flow was not different between groups (group, P=0.662). After the dynamic fatiguing exercise in the non-dominant leg, both groups demonstrated similar increases in femoral artery blood flow in the resting limb when expressed relative to baseline flow (time, P<0.001; time×group, P=0.770), relative to thigh lean mass (time, P<0.001; time×group, P=0.941), and for absolute blood flow (group, P=0.683). However, the increased blood flow in the dominant leg artery was lower during the resting condition (non-dominant leg exercise; experimental session 2) than the exercising condition (dominant leg exercise; experimental session 3) (time×limb, P<0.001). See Table 2 and Figure 1C.

		Type 2 Diabetes *n* = 15	Control *n* = 15	
Brachial FMD				
Diam. Max Time	s	90.7 ± 37.8	80.2 ± 23.9	
Resting Diam.	mm	3.62 ± 0.56	3.79 ± 0.44	†
Resting Shear	s^{-1}	171.3 ± 93.1	202.5 ± 93.0	
Shear End	s^{-1}	788.4 ± 243.2	826.1 ± 232.8	
Shear Δ	%	418.2 ± 165.4	341.5 ± 135.0	
FMD	μm	32.7 ± 16.6	33.7 ± 12.6	
FMD	%	9.52 ± 6.08	9.88 ± 3.19	
FMD	$\%\cdot ks^{-1}$	14.8 ± 3.4	14.3 ± 4.1	
Femoral FMD				
Diam. Max Time	s	114.9 ± 47.8	97.3 ± 3.3	
Resting Diam.	mm	5.92 ± 0.80	6.61 ± 0.70	*†
Resting Shear	s^{-1}	132.2 ± 54.2	118.4 ± 58.9	
Shear End	s^{-1}	324.9 ± 137.2	363.3 ± 116.1	
Shear Δ	%	156.3 ± 86.8	219.3 ± 170.6	
FMD	μm	45.1 ± 24.1	36.0 ± 21.5	
FMD	%	7.31 ± 3.29	6.39 ± 2.99	
FMD	$\%\cdot ks^{-1}$	39.4 ± 18.5	32.4 ± 11.8	
EIH Dominant				
Resting Diam.	mm	5.91 ± 0.88	6.57 ± 0.83	*†
Resting Flow	$mL\cdot min^{-1}$	416 ± 137	465 ± 210	
Flow End	$mL\cdot min^{-1}$	994 ± 222	1221 ± 321	*
Flow Δ	$mL\cdot min^{-1}$	598 ± 181	778 ± 202	*
Flow Δ	%	177 ± 90	194 ± 79	*
Thigh Lean Mass	kg	8.23 ± 2.84	8.15 ± 2.05	†
Norm. Flow Δ	%	169 ± 82.4	198 ± 76.4	*
EIH Non-Dominant				
Resting Diam.	mm	5.96 ± 0.76	6.49 ± 0.68	*†
Resting Flow	$mL\cdot min^{-1}$	365 ± 141	377 ± 180	
Flow End	$mL\cdot min^{-1}$	604 ± 166	638 ± 310	
Flow Δ	$mL\cdot min^{-1}$	239 ± 104	261 ± 162	
Flow Δ	%	77.3 ± 46.9	60.9 ± 41.3	
Norm. Flow Δ	%	64.2 ± 42.4	67.9 ± 37.7	

Table 5.2: Flow-mediated dilation (FMD) and exercise-induced hyperemia (EIH) for patients with type 2 diabetes mellitus and healthy controls. Values are displayed as mean ± standard deviation. Patients with type 2 diabetes and controls did not differ in flow-mediated dilation of the brachial and femoral arteries, and exercise-induced hyperemia (EIH) of the non-dominant limb (Non). However, controls had greater EIH compared to patients with type 2 diabetes after the fatiguing contraction with the dominant (Dom) leg. FMD, flow-mediated dilation; Δ, change; ks, kilosecond; Norm., normalized. *, group differences, $P < 0.05$; †, sex differences, $P < 0.05$.

Flow-Mediated Dilation

Brachial Artery: At rest, brachial artery diameter was not different between groups (group, P=0.735); however, men had greater diameter compared to women for both groups (sex, P=0.015). After occlusion, patients with type 2 diabetes and controls had similar increases in absolute brachial artery diameter (time, P<0.001; time×group, P=0.379; group, P=0.735), relative to baseline diameter (group, P=0.651), and relative to shear rate (group, P=0.493). Both groups demonstrated similar increases in shear rate (time, P<0.001; time×group, P=0.625). See Table 2 and Figure 2E.

Femoral Artery: At rest, patients with type 2 diabetes had smaller femoral artery diameter than controls (group, P=0.004), and men had greater femoral artery diameter compared with women (sex, P<0.001). Diameter was similar across the three trials (FMD and two exercise sessions; trial, P=0.903). After occlusion, patients with type 2 diabetes and controls did not differ in the increases in absolute femoral artery diameter (time, P<0.001; time×group, P=0.203; group, P=0.689), when relative to baseline diameter (group, P=0.475), and relative to shear rate (group, P=0.798). Both groups demonstrated similar increases in shear rate (time, P<0.001; time×group, P=0.731). See Table 2 and Figure 2F.

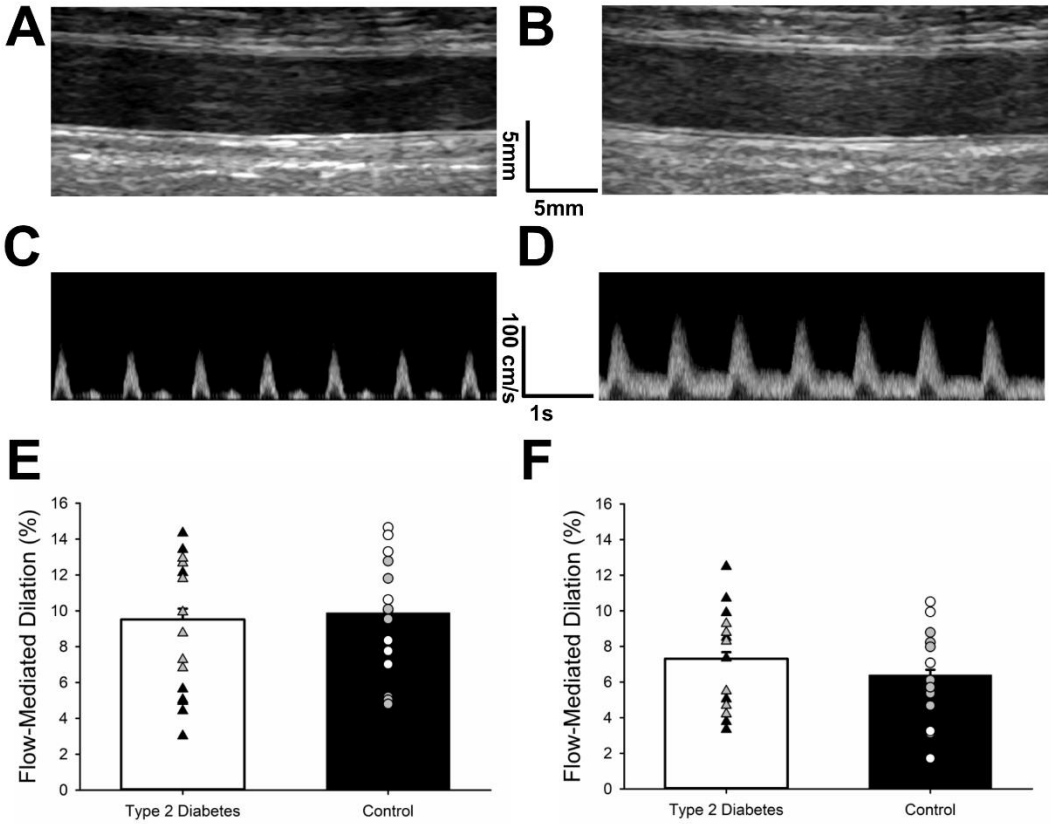

Figure 5.2: Flow-mediated dilation for patients with type 2 diabetes mellitus and healthy controls. Representative data for femoral artery diameter from before (A) and after (B) five-minute occlusion for flow-mediated dilation (FMD) studies and blood flow velocity curves before (C) and after (D) FMD, and group data for FMD studies of the brachial (E) and femoral (F) arteries. Group data are displayed as mean ± SEM, and women are represented in grey filled symbols. Femoral artery diameter and blood flow velocity of a 55-year old man with type 2 diabetes. Patients with type 2 diabetes and controls demonstrated similar FMD of the brachial artery (E) and femoral artery (F) diameter after the 5-minute bout of occlusion (P>0.05).

Correlation and Regression Analysis

The reduction in MVCC power at the end of dynamic exercise was associated with the following: the reduction in resting twitch amplitude (r=0.645, r^2=0.416, P=0.001), baseline femoral artery diameter (r=-0.631, r^2=0.398, P=0.003), change in blood flow after the dominant leg dynamic exercise (r=-0.557, r^2=0.310, P=0.009), and HbA_{1c} (r=0.544, r^2=0.296, P=0.004).

5.4 Conclusions

The novel findings of this study were that patients with type 2 diabetes had greater lower-limb fatigability and a lower hyperemic response to dynamic fatiguing exercise with the knee extensor muscles than healthy controls who were matched for age, BMI and physical activity. There were no sex differences for either variable. Greater limb fatigability was associated with a greater loss in contractile function, higher HbA_{1c}, a reduced exercise-induced hyperemic response and smaller baseline artery diameter suggesting that impairments in peripheral tissue (vascular and contractile) mediate the greater limb fatigability in people with type 2 diabetes.

There is a growing evidence indicating that patients with type 1 or type 2 diabetes have greater fatigability of skeletal muscle (Petrofsky *et al.*, 2005; Almeida *et al.*, 2008; Allen *et al.*, 2015a; Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c). We have previously shown that greater fatigability of people with type 2 diabetes is due to mechanism(s) originating in the muscle (Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c). Although neural mechanisms contribute to fatigability in patients with type 2 diabetes and controls, the reductions in neural drive were similar for both groups (Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c), and group differences were due to greater changes in contractile function in the patients with type 2 diabetes than the controls. This current investigation provides evidence that a reduced hyperemic response in skeletal muscle blood flow could be a primary contributing factor to that loss of contractile function and large lower limb fatigability in people with type 2 diabetes during repeated dynamic contractions.

Although there is substantial evidence of impaired endothelial/smooth muscle function in people with type 2 diabetes (Montero *et al.*, 2013), we demonstrated that in

the individuals studied, patients with type 2 diabetes and controls had similar flow-mediated dilation of the brachial and femoral arteries. This unique finding is likely due to study design because our participants (with and without type 2 diabetes) on average were not obese, were relatively active, had well-controlled blood glucose, relatively high aerobic fitness, minimal reports of clinical symptoms of fatigue. These data suggest that despite healthy macrovascular endothelial function and limited co-morbidities, patients with type 2 diabetes have microvascular impairments, reduced exercise-induced hyperemia and greater fatigability. Such deficits may reduce exercise training tolerance and impair performance of activities of daily living.

Exercise-Induced Hyperemia

The reduced exercise-induced hyperemia response in patients with type 2 diabetes after the dynamic fatiguing exercise are consistent with findings that patients with type 2 diabetes have reduced femoral blood flow during repeated knee extension exercise compared with controls (Lalande *et al.*, 2008). However, we also observed a small exercise-induced hyperemic response after non-dominant limb exercise that was similar between patients with type 2 diabetes and controls, providing evidence that systemic changes to the cardiovascular system did not contribute to lesser exercise-induced hyperemic response after dominant limb exercise in patients with type 2 diabetes. Instead, these data suggest that the impairments in exercise-induced hyperemia in patients with type 2 diabetes was primarily due to mechanisms within the exercising limb. The down-stream implications of reduced exercise-induced hyperemia in patients with type 2 diabetes as assessed in the present investigation may include: attenuated perfusion,

oxygenation and blood flow kinetics in skeletal muscle and microcirculation (Padilla *et al.*, 2006; Bauer *et al.*, 2007; Zheng *et al.*, 2014).

Doppler ultrasound is a measure of blood flow which is a proxy for supply of oxygen to skeletal muscle, but not an assessment of oxygen demand within the muscle. The attenuated blood flow response could be secondary to a smaller physiological stimulus to increase oxygen delivery to skeletal muscle in patients with type 2 diabetes. However, mechanisms contributing to increased oxygen demand in the skeletal muscle (e.g. increased concentrations of hydrogen ion and inorganic phosphate) (Korthuis, 2011) also cause reductions in twitch amplitude (Debold *et al.*, 2016). Because patients with type 2 diabetes and controls had similar reductions in twitch amplitude after the fatiguing exercise, the greater attenuated blood flow response after exercise was likely *not* due to reduced metabolic demand in patients with type 2 diabetes. Thus, we speculate that the reduced exercise-induced hyperemic response (possibly indicating impairments in skeletal muscle microcirculation) likely contributed to the greater reductions of contractile function and fatigability in patients with type 2 diabetes. These mechanisms however, warrant future investigations.

Flow-Mediated Dilation

Flow-mediated dilation was similar for patients with type 2 diabetes and healthy controls for both the brachial artery and femoral artery, even when normalized to shear rate. Patients with type 2 diabetes commonly have reduced FMD which may be indicative of impaired microvascular and macrovascular endothelial health and vascular smooth muscle function (for review, see (Montero *et al.*, 2013)). Our data demonstrate that attenuated FMD-response may not be an obligatory consequence of type 2 diabetes *per se*

but may occur in the presence of additional co-morbidities or worsened symptoms of type 2 diabetes (e.g. poor glycemic control or diabetic polyneuropathy).

Importantly, despite similar thigh mass and position of recording relative to common femoral artery bifurcation, the controls repeatedly demonstrated a larger resting femoral artery diameter compared to patients with type 2 diabetes for three separate measurements (femoral FMD and both dynamic fatiguing exercises). This group-related difference in artery diameter likely reflects increased vasoconstrictor tone in patients with type 2 diabetes, possibly secondary to an imbalance of vasoconstrictors (e.g. endothelin-1) and vasodilators (e.g. nitric oxide) (Phillips *et al.*, 2015; Mahmoud *et al.*, 2016). Although vascular remodeling could induce a physiologically-limited artery size (Kozakova & Palombo, 2016), our data demonstrated a robust flow-mediated dilation response in patients with type 2 diabetes, suggesting the reduced artery size is due to vascular tone versus remodeling. The increased vasoconstrictor tone has been associated with upregulation of smooth muscle endothelin receptors, and both are implicated in the pathogenesis of hypertension and atherosclerosis (Mahmoud *et al.*, 2016). Thus, although the functional measure of FMD was similar to controls, there may be an underlying impairment in vascular health in patients with type 2 diabetes.

Correlations with Fatigability

Our data suggest vascular dysfunction as a mechanism underlying greater fatigability in patients with type 2 diabetes. Vascular dysfunction was observed as increased resting vasoconstrictor tone and an attenuated hyperemic response to dynamic exercise. These impairments in vascular function may contribute to reduced O_2 delivery

and ultimately transport within the microvasculature, which may disrupt skeletal muscle contractile function.

Clinical Importance

Patients with type 2 diabetes have greater lower limb fatigability than controls during dynamic exercise, independent of the effects of obesity, diabetic polyneuropathy, poor glycemic control, and physical activity. We showed for the first time that this greater limb fatigability was associated with reduced skeletal muscle blood flow and contractile function [the latter as we have observed previously (Senefeld *et al.*, 2018a; Senefeld *et al.*, 2018c)]. Greater limb fatigability can promote exercise intolerance, possibly resulting in reduce exercise training adherence. Importantly, exercise training is primary to type 2 diabetes management and there is evidence that vascular function and fatigability can improve with exercise training in patients with type 2 diabetes (Maiorana *et al.*, 2001; Hamdy *et al.*, 2003; Green *et al.*, 2006; Laughlin, 2016). These data suggest treatments focused on improving vascularization and/or blood vessel tone may be effective in enhancing perfusion and reversing impairments in fatigability and contractile function, in-turn improving exercise tolerance and adherence.

CHAPTER 6: CONCLUSIONS & FUTURE DIRECTIONS

The purpose of this dissertation was to determine the difference in fatigability during and in response to high-velocity dynamic contractions between people with T2D compared to controls matched for age, sex, anthropometry and physical activity, and identify the contributing mechanisms. The studies in this dissertation clearly and repeatedly demonstrated greater fatigability in people with T2D compared to matched controls. For the first time, we demonstrated that people with prediabetes have less fatigability than people with T2D but greater fatigability than matched controls. These studies provide evidence that impaired muscle contractile function and skeletal muscle blood flow were associated with the greater fatigability after the dynamic contractions. This chapter provides a summary of the key findings of this dissertation and proposes future studies to continue to address limitations in knowledge. The set of descriptive studies included in this dissertation demonstrated that people with T2D (Chapter 3-5) and prediabetes (Chapter 4) have greater fatigability due to impairments in muscular mechanisms, particularly the blood flow response during exercise (Chapter 5), rather than greater impairments in neural mechanisms.

These data add to the current body of literature that demonstrate across several contraction types and tasks, people with diabetes mellitus (type 1 or type 2; with or without diabetic polyneuropathy) have greater fatigability during single limb exercise (isometric and low-to-moderate velocity and high-velocity contractions) for several muscles groups (dorsiflexors, plantar flexors, knee extensors, knee flexors, and finger flexors) compared with controls—even when matched for lean mass, body size, and physical activity levels, see Figure 6.1.

Collectively with other data from the literature (see Figure 6.1), it is clear that people with T2D have greater limb fatigability across different contraction types and contraction intensities, and this dissertation indicates that the primary mechanisms responsible for these differences are due to greater fatigue within the exercising muscle among people with T2D. The primary mechanisms could be related to diabetic polyneuropathy-related reductions in muscle contractile function, metabolic dysfunction, endothelial dysfunction and impaired motor unit properties. However, previous studies have predominantly compared fatigability in people with T2D compared to controls with low BMI (lean controls) and physical activity has not been objectively assessed to describe participants. Thus, the greater fatigability in people with T2D compared to controls may have been attributed to higher BMI or lower physical activity levels in people with T2D compared with controls. Crucially, the studies in this dissertation matched people with T2D to controls based on BMI and objectively assessed physical activity (accelerometer)—in addition to matching according to age and sex. This dissertation demonstrated that people with T2D and prediabetes have greater fatigability than matched controls, providing evidence that T2D *per se* contributes to increased fatigability outside of the detrimental effects of increased BMI and reduced physical activity.

These findings have important application to exercise training and activities of daily living. These findings highlight potential mechanisms (muscular and peripheral blood flow) for increased exercise intolerance and reduced adherence to exercise training in people with T2D (Poitras *et al.*, 2018). Future interventional studies should focus on

mitigating the impairments in metabolic build-up or sensitivity and the vascular (macro-, micro-vascular or both) response during exercise.

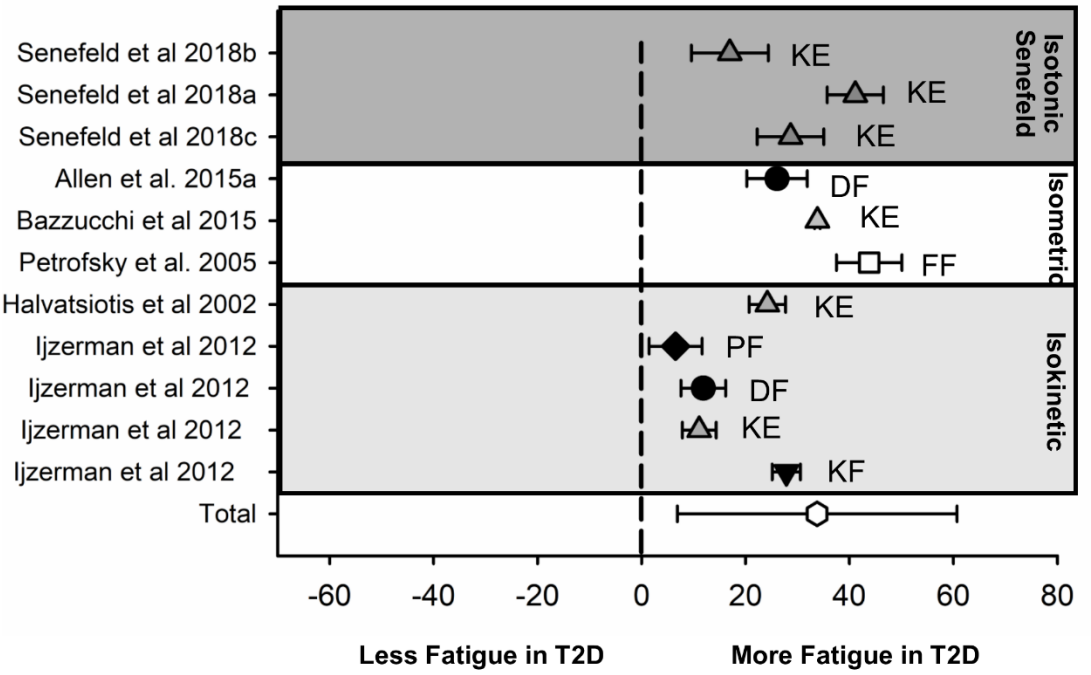

Figure 6.1. Forest Plot of standard mean differences in fatigability between controls and people with T2D. Represented are mean data from high-velocity, isotonic (*studies from this dissertation*), isometric and isokinetic (120 & 180°·s^{-1}) contraction studies. The group difference in fatigability (% controls) is plotted and calculated as the mean difference (*equation 1.1*). Collectively, across all tasks represented in these plotted studies, people with diabetes mellitus were more fatigable across several limb muscles than controls.

In Chapter 3, people with T2D and *no signs of diabetic polyneuropathy* demonstrated greater fatigability of the knee extensor muscles compared to healthy, matched controls after a dynamic contraction. In this study, transcranial magnetic stimulation and electrical stimulation of the muscles were used to determine the site along the motor pathway which contributed to the group-related differences in fatigability. This study addressed two important limitations in the current body of literature: 1) matching people with T2D and controls based on age, sex, anthropometrics

and physical activity and 2) examining fatigability during high-velocity dynamic contractions. The findings demonstrated that people with T2D have greater reductions in muscle power (i.e. greater fatigability) than controls matched for age, sex, body size and physical activity. Although there was no group-related difference in the reduction of voluntary activation nor impairments in neuromuscular propagation, people with T2D had greater reductions in electrically-evoked muscle contraction amplitude and this was positively correlated with fatigability. These data suggest that people with T2D have greater fatigability than healthy controls—even when closely matched— and that impaired contractile function was the primary mechanism. Whether people with T2D have greater fatigability for isometric and low-to-moderate velocity contractions than controls matched for age, sex and physical activity is not known and warrants future investigation.

In Chapter 4, three cohorts of people were recruited and matched according to age, sex, anthropometry and physical activity—people with T2D and *no signs of diabetic polyneuropathy*, people with HbA_{1c} levels in a prediabetes range (>5.6% and <6.5%) and healthy controls. Electrical stimulation of the knee extensors was used to determine the site along the motor pathway which contributed to fatigability after high-velocity dynamic contractions. This study determined difference in fatigability between people with prediabetes compared to people with T2D and controls, a novel investigation. The findings demonstrated a progressive increase in fatigability in people with prediabetes and T2D than controls. The findings supported Study 1 and provided repeatable evidence of greater fatigability in people with T2D compared to controls, and reductions in electrically-evoked twitch amplitude were associated with fatigability, but reductions in

voluntary activation were not. These data replicated findings from Study 1 and provided evidence that people with prediabetes have greater fatigability than controls but less fatigability than people with T2D.

In Chapter 5, Doppler ultrasonography was used to assess flow-mediated dilation of the brachial and femoral arteries (as a proxy for endothelial function) and exercise-induced hyperemia after a dynamic exercise task with the knee extensors in people with T2D and *no signs of diabetic polyneuropathy* compared to healthy, matched controls. This study was used to determine the contribution of vascular impairments to greater fatigability in people with T2D. The findings supported that people with T2D have greater reductions in muscle power (greater fatigability) and smaller increases in skeletal muscle blood flow after dynamic fatiguing contraction (exercise-induced hyperemia) compared to matched controls, as observed in Study 1 and 2. Greater knee extensor muscle fatigability was associated with lesser exercise-induced hyperemia at the end of the dynamic contractions, suggesting that an impaired ability to adequately increase blood flow in response to exercise may contribute to greater fatigability in people with T2D. Whether precise mechanisms of vascular function during exercise that may mediate greater fatigability in people with T2D during dynamic contractions is unknown and warrants future investigation.

6.1 Contractile and Metabolic Function Contribution to Fatigability

Each study in this dissertation demonstrated a strong association between greater reductions in electrically-evoked contraction amplitude and power during dynamic contractions of the knee extensor muscles, suggesting that skeletal muscle regulation during exercise may be impaired in people with T2D. Impaired contractile function of

skeletal muscle after fatiguing exercise is thought to reflect disturbances in excitation-contraction coupling, accumulation of metabolites, and/or impaired calcium handling (Fitts, 2008; Debold *et al.*, 2016), that ultimately reduce the torque that is able to be produced by the muscle fibers. As discussed in Chapter 3, people with T2D have maintained neuromuscular transmission, half-relaxation time of the electrically-evoked contraction and post-activation potentiation suggesting that impaired contractile function is due to metabolic by-products in the skeletal muscle (e.g. hydrogen ion and inorganic phosphate). These data lead to two logical hypotheses: 1) people with T2D have greater accumulation of metabolic by-products or 2) the muscle fibers of people with T2D have greater sensitivity to metabolic by-products.

6.2 Skeletal Muscle Blood Flow Contribution to Fatigability

Study 3 demonstrated an association between fatigability and blood flow at the end of the high-velocity dynamic contractions, suggesting that skeletal muscle blood flow during exercise may be blunted in people with T2D. The blunted blood flow response would be secondary to dysfunction in the micro- and/or macro-vasculature. There is evidence of impaired microvascular and macrovascular function in people with T2D, and this is one of the primary mechanisms implicated to cause poor exercise tolerance in people with T2D (Poitras *et al.*, 2018). As discussed in Chapter 5, people with T2D have maintained macrovascular function in the femoral artery, suggesting microvascular dysfunction may contribute to greater fatigability in people with T2D. These data provide rationale to closely examine microvascular function and the potential association with greater fatigability in people with T2D.

The similar flow-mediated dilation of both the femoral and brachial arteries between people with T2D compared to controls observed in Aim 3 (Chapter 5) strikingly contrasts a robust difference in flow-mediated dilation between people with T2D and controls observed in the literature (Montero *et al.*, 2013). Data were extracted from 11 different studies (black bars in Figure 6.2) assessing flow-mediated dilation of the brachial artery in people with T2D compared to controls, and each study demonstrated less flow-mediated dilation in people with T2D—see Figure 6.2 (Enderle *et al.*, 1998; Anderson *et al.*, 2001a; Ma *et al.*, 2001; Ihlemann *et al.*, 2002; Tan *et al.*, 2002; Woodman *et al.*, 2002; Ifrim & Vasilescu, 2004; Karabag *et al.*, 2007; Bruno *et al.*, 2012). Importantly, however, the group average of flow-mediated dilation from the 469 people with T2D was 5.1 ± 2.9% (grey bar in Figure 6.2), which was nearly 50% of the response of the observed cohort in Aim 3 (white bar in Figure 6.2). This marked difference between the data from Aim 3 and previous data is likely reflective of the characteristics of the participants with T2D recruited for Aim 3—these participants with T2D had well-controlled HbA_{1c} (7.0 ± 0.8%), were highly physically active (7,200 ± 3,200 steps per day), were relatively aerobically fit (36.8 ± 12.2 $mL \cdot kg^{-1} \cdot min^{-1}$), had no prescription to insulin, and had no signs of diabetic polyneuropathy. Importantly, the mean flow-mediated dilation of the controls from Aim 3 (9.88 ± 3.19%) was not different compared to the mean flow-mediated dilation of the 325 control participants from the 11 studies (7.72 ± 3.76%). The data providing evidence of convergent validity of the flow-mediated dilation experiments performed for Aim 3 and supporting evidence that the striking lack of difference in flow-mediated dilation between people with T2D and

controls observed in Aim 3 was due to a difference in the cohort of people with T2D participating in Aim 3 relative to the body of literature.

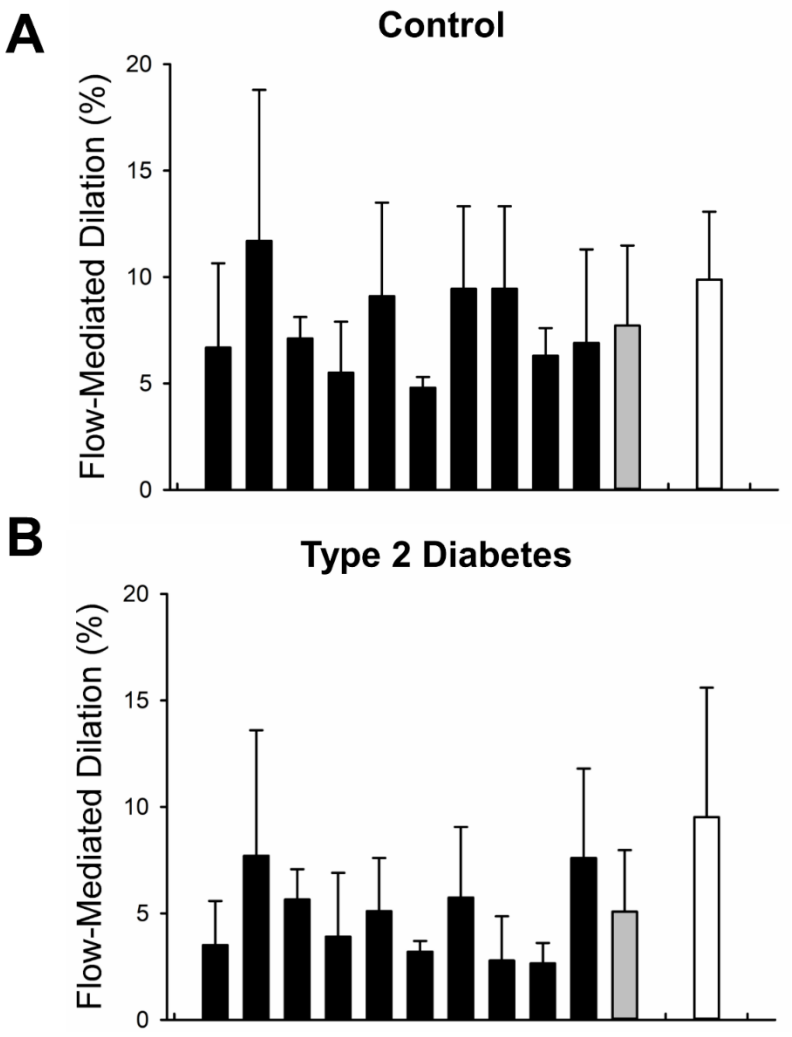

Figure 6.2. Summary of flow-mediated dilation studies in brachial artery of controls and people with type 2 diabetes. Each bar graph represents the mean and standard deviation of flow-mediated dilation in controls (panel A) and people with T2D (panel B). The bars filled with black represent 11 studies (Enderle *et al.*, 1998; Anderson *et al.*, 2001a; Ma *et al.*, 2001; Ihlemann *et al.*, 2002; Tan *et al.*, 2002; Woodman *et al.*, 2002; Ifrim & Vasilescu, 2004; Karabag *et al.*, 2007; Bruno *et al.*, 2012) identified from a recent review (Montero *et al.*, 2013), the bar filled with grey represents the mean of the 11 studies, and the bar filled with white represents data from the brachial artery included in Aim 3.

6.3 Motor Unit Properties Contribution to Fatigability

Although not assessed in this dissertation, there is evidence that impaired modulation of motor units during exercise may contribute to greater fatigability in people with diabetes mellitus (type 1 and type 2). Previous studies have demonstrated that people with diabetes mellitus have disrupted neuromuscular transmission (Allen *et al.*, 2015a), slower baseline motor nerve conduction velocities (Almeida *et al.*, 2008), decreased mean motor unit discharge rates (Almeida *et al.*, 2008; Watanabe *et al.*, 2013), and greater variability of motor unit discharge rates (Watanabe *et al.*, 2013) compared to controls. This area of study warrants future investigations.

6.4 Future Directions

Three areas of future mechanistic studies were suggested (above)— metabolic function, skeletal muscle blood flow and motor unit properties. To determine if people with T2D have greater accumulation of metabolic by-products, near-infrared spectroscopy (NIRS) and magnetic resonance spectroscopy (^{31}P-MRS) can be used to evaluate baseline mitochondrial function and accumulation of metabolic by-products *in vivo* during dynamic contractions with the knee extensors in people with T2D and controls. NIRS can be used to determine recovery kinetics of concentrations oxyhemoglobin and deoxyhemoglobin after a sustained perturbation (brief occlusion or exercise) and subsequent occlusion/perfusion sequence using a portable, superficial probe (Ryan *et al.*, 2012), and compare the time constant of a recovery curve (plotting the slope of each curve as a function of time) to quantify mitochondrial function in people with T2D and controls. The ^{31}P-MRS can be used *in vivo* during dynamic exercise to estimate

relative concentrations of metabolic by-products (e.g. hydrogen ion and inorganic phosphate) to directly compare accumulation of metabolic by-products between people with T2D and controls.

To examine skeletal muscle blood flow, NIRS could be used to determine skeletal muscle oxygenation during exercise (REF) and microvascular function could be determined in response to flow-induced dilation and vasoactive reagents to determine the reactivity of the microvasculature—which is indicative of endothelial and vascular smooth muscle function (Mahmoud *et al.*, 2016). To examine motor unit properties, high-density, surface EMG with multichannel linear array electrodes could be used in conjunction with blind source separation and decomposition algorithms to assess motor unit properties during isometric exercise (Farina *et al.*, 2014; De Luca *et al.*, 2015; Muceli *et al.*, 2015; Negro *et al.*, 2016).

6.5 Summary

In summary, this dissertation demonstrated that people with T2D have greater fatigability of the knee extensor muscles during a high-velocity exercise compared to people with prediabetes, and both groups (T2D, prediabetes) had greater fatigability than controls, with each group matched for age, sex, anthropometry and physical activity. Specifically, this dissertation provided evidence of 2-fold greater reductions in muscle power during a six-minute task for people with T2D (Chapters 3 - 5) than controls and 1.5-fold greater fatigability in people with prediabetes compared with controls (Chapter 4). The greater fatigability was associated with impairments in electrically-evoked muscle contractile properties (T2D and prediabetes, Chapter 3 - 5) and impaired blood flow response during exercise (T2D, Chapter 5). These findings may: 1) direct

mechanistic studies to examine the properties within the muscle that contribute to greater fatigability, 2) inform longitudinal studies to improve fatigability in people with T2D and prediabetes, which may reduce the incidence of T2D and prediabetes, and 3) provide a standard for control groups to examine the effects of prediabetes and T2D *per se*.

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