MOTOR UNIT CHARACTERISTICS AND PROPRIOCEPTIVE DEFICITS OF THE UPPER LIMB IN PATIENTS WITH TYPE II DIABETES

by
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EPIGRAPH

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

- Marie Curie

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ABSTRACT

Persons with Type II Diabetes (pwT2D) are at risk of developing nerve disorders that result in sensorimotor dysfunction, particularly of the hands and feet. Neuromuscular and proprioceptive dysfunction associated with diabetes are two areas needing more research to determine their contribution to T2D related functional impairments. Most work in these two areas has been focused on deficits in the lower limb. Proprioceptive dysfunction in the upper limb associated with T2D has yet to be characterized. Similarly, evaluation of neuromuscular dysfunction in the upper limb is lacking. Knowing that peripheral neuropathy (PN) affects the hands in addition to the feet and legs, this is an area that should be explored. Thus, the purpose of this study is to examine the neuromuscular and proprioceptive effects on the upper limb in pwT2D. This will be achieved by a) evaluating the effects of T2D on kinematic performance of a reach-to-pinch task; b) characterizing T2D associated motor unit properties in five upper limb muscles; and c) determine if observed alterations to motor unit characteristics are length-dependent in the upper limb. Three groups were recruited: young, healthy controls (group 1, 18-30 years old), T2D group (group 2, 60+ years old with established T2D), and an age- and sex-matched Control group without T2D (group 3, 60+ years old without a history of diabetes). Group 1 underwent sensory evaluation (tactile and vibrotactile sensation) and neuromuscular evaluation (nerve stimulation and motor evaluation) and served as an approach validation. Groups 2 and 3 underwent blood sugar and cholesterol testing, sensory evaluation (tactile and vibrotactile sensation), neuromuscular evaluation (nerve stimulation and motor evaluation), and proprioceptive evaluation (reach-to-pinch task). Overall, sensory

thresholds did not differ between T2D and Control participants. CMAP amplitude and MUNIX were significantly reduced in Control participants compared to pwT2D. Additionally, mean pinch location error was significantly worse for pwT2D in addition to differences in wrist extension/flexion (ex/fl), wrist abduction/adduction (ab/ad), CMC1 ab/ad, MCP2 ex/fl, and MCP2 ab/ad angular joint trajectories and index finger and hand transport trajectories between the two groups. These findings suggest proprioception of the upper extremity is altered in pwT2D and they exhibit a unique aperture position and aiming strategy during a reach-to-pinch task. These findings help to understand proprioceptive and neuromuscular function of the upper limb in pwT2D, with implications to early identify patients who may need medical and/or lifestyle intervention.

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1 INTRODUCTION

1.1 Background

As of 2014, the World Health Organization estimates there to be 422 million people in the world with diabetes, about 90% of which are classified as having Type II Diabetes (T2D) (WHO, 2017). Consequences of T2D can include cardiovascular disease (CVD), peripheral neuropathy (PN), amputation, blindness, and kidney failure. It is well known T2D causes PN, resulting in sensorimotor dysfunction, particularly of the hands and feet. Previous studies have suggested that motor deficits associated with T2D can be attributed to tactile impairment caused by PN (Orlando, Balducci, Bazzucchi, Pugliese, & Sacchetti, 2016). However, these functional impairments may be due to other issues such as neuromuscular or proprioceptive dysfunction (Ochoa, Gogola, & Gorniak, 2016).

Sensorimotor deficits caused by T2D may affect proprioception. Proprioception is the perception of movement and position of body segments from sensory receptors in the joints, skin, and muscles. Proprioception is necessary for precise neuromuscular control when performing movements. Many studies have assessed the effects of T2D on postural stability, including proprioceptive ability of lower body joints and segments.

1.1.1 T2D and postural control

Postural stability studies of T2D patients and those with PN have found these individuals to have impaired balance and an increased risk of falling. Hong, Chia & Ling (1997) measured changes in center of pressure (COP) using a force platform

while standing. Compared to healthy controls, persons with non-insulin dependent diabetes (pwT2D) were overall more unstable with increased length of sway path, larger COP velocity, and a larger sway area. A similar study examined differences between pwT2D with PN and those without. PwT2D with PN were found to be more unstable than their non-PN counterparts and healthy controls, characterized by greater mean COP velocity and larger sway area and sway path (Uccioli, 1995). In more challenging situations there are differing results. In the case of Lord, Caplan, Colagiuri, Colagiuri, & Ward (1993), pwT2D had significantly worsened measures of body sway when standing on firm and compliant (foam) surfaces. Similar findings were reported by Simmons, Richardson & Pozo (1997) in which scores on postural stability measures when tested in challenging Neurocom sensory organization test protocols for pwT2D with tactile deficits in the foot revealed significant postural instability, greater risk of falling and even a shift from ankle to hip strategy during the perturbation compared to healthy controls. In the same study, pwT2D without tactile deficits in the foot did not score differently on postural stability measures as compared to controls.

The negative impact of T2D on postural performance is not strictly due to PN alone, but also by the effects of T2D on the sensorimotor system (Hewston & Deshpande, 2016; Lord et al., 1993; Mustapa, Justine, Mohd Mustafah, Jamil, & Manaf, 2016; Simoneau, Derr, Ulbrecht, Becker, & Cavanagh, 1996; R. W. M. van Deursen & Simoneau, 1999). Proprioception, a key component of the sensorimotor system, plays a role in postural stability. Because loss of tactile sensation is more easily measured during upright stance than functioning of proprioceptive receptors,

many postural stability studies are not able to assess the effects of T2D on proprioception. A common method to evaluate proprioception is by using joint position perception tasks. Studies have found impaired proprioception in the lower limb associated with T2D and related PN. For example, ankle movement perception is significantly worse in pwT2D (Lord et al., 1993; Simoneau et al., 1996).

Muscle spindles are key receptors providing proprioceptive information and they can be influenced by muscle tendon vibration. When muscle tendon vibration is applied during a joint movement matching task, the kinesthetic illusion can only occur if muscle spindles are intact. Vibration introduces bias to muscle spindle output, leading to a perception of joint movement and muscle lengthening. However, in pwT2D, the kinesthetic illusion induced by muscle tendon vibration is reduced compared to healthy controls (Simoneau et al., 1996; R. W. M. van Deursen et al., 2001; R. W. van Deursen, Sanchez, Ulbrecht, & Cavanagh, 1998). A study introduced vibration to the Achilles tendon and anterior tibialis tendon during an ankle movement matching task while minimizing plantar tactile information. Compared to age matched controls and young healthy controls, pwT2D with PN had the greatest difficulty tracking ankle movement. They also were least effected by the muscle tendon vibration (R. W. van Deursen et al., 1998). The findings from these studies suggest proprioceptive ability is degraded by T2D in the lower limbs.

1.1.2 T2D and lower extremity control

Neuromuscular dysfunction in pwT2D has been somewhat explored by previous studies; however, most work has been done in the lower limbs. Allen, Choi,

Kimpinski, Doherty, & Rice (2013) measured motor unit number and muscle strength of the tibialis anterior muscle (TA) in pwT2D compared to healthy controls. Using surface electromyography (SEMG), they reported a 60% loss in dorsiflexion strength that coincided with 60% fewer functioning motor units in the T2D group as compared to controls. Decomposition based quantitative EMG (DQEMG) analysis revealed significantly reduced motor unit number estimate (MUNE), compound muscle activation potential (CMAP), surface detected motor unit action potential (SMUAP) and mean firing rate in the TA of pwT2D with PN when compared to controls (Allen, Kimpinski, Doherty, & Rice, 2014).

Another study characterized motor unit firing patterns of the vastus lateralis muscle associated with T2D. In this study, two types of muscle contractions were performed; one sustained contraction at 10% of maximum voluntary contraction (MVC) and one ramp up contraction to 20% MVC. Findings from this study included a lower mean motor unit instantaneous firing rate during ramp up contraction along with higher variability of motor unit firing rate at later periods of the sustained contractions. These results suggest the threshold of motor unit firing rate that variability begins to increase is different in pwT2D compared to the healthy population (Watanabe et al., 2013).

Another study out of the same laboratory (Watanabe, Miyamoto, Tanaka, Fukuda, & Moritani, 2012) examined the spatial activation of motor units in the vastus lateralis muscle during a sustained isometric contraction at 10% MVC. PwT2D exhibited lower numbers of motor unit activation during the low force sustained contraction as well as reduced redistribution of activation. Overall, fewer motor units

of the VL were activated more continuously in T2D patients compared to controls (Watanabe et al., 2012). The findings from the above studies indicate motor unit loss, remodeling, and altered firing rates are associated with PN in T2D.

1.1.3 T2D and upper extremity control

Sensorimotor dysfunction associated with T2D in the lower extremities has been well documented, however there have been few studies investigating the effects on the upper extremity. We know that the upper extremity is affected by PN in T2D. Studies have reported impaired tactile sensation, decreased grip and hand strength associated with T2D (Cetinus, Buyukbese, Uzel, Ekerbicer, & Karaoguz, 2005; Ochoa et al., 2016; Ochoa & Gorniak, 2014; Ramji, Toth, Kennedy, & Zochodne, 2007; Sayer et al., 2005). The contribution of tactile dysfunction to motor dysfunction in T2D has been further examined in the upper extremity. Tactile impairment due to T2D was reported to be a main contributor to impairment to maximal force production. In contrast, in the same study it was revealed through timed clinical measures of motor function that tactile dysfunction alone cannot be responsible for T2D associated functional declines in the upper extremity (Ochoa et al., 2016).

One study that has examined T2D neuromuscular impairment in the upper limb did so in the first dorsal interosseous muscle (FDI), a small muscle in the hand. PwT2D with PN had significantly reduced MUNE, CMAP, and mean firing rate of the FDI when compared to controls (M. D. Allen et al., 2014). Because of the size and location of this muscle, it is difficult to generalize these findings to other muscles located in the upper limb. Larger, more functional muscles involved in gross motor

movements may provide more meaningful motor unit characterization. Additionally, preservation of maximum pinch force but decreased maximum grip force reported in a study by Ochoa et al. (2014) provides evidence to suggest motor unit remodeling attributed to T2D may be different across muscle groups within the upper extremity.

1.1.4 Comparable deficits in other related populations

A number of other patient populations experience neuromuscular dysfunction. Studies have reported age-related differences in muscle recruitment and reaction time performance in the upper limb of healthy older adults. Compared to younger populations, older subjects have increased muscle activation time following stimulus onset as well as an altered muscle firing sequence (Arnold et al., 2015). During isometric contractions, age associated changes in muscle activity have been reported as well. Using SEMG, Duque, Petitjean, & Swinnen (2016) reported age associated increases in motor unit density along with reduced number of motors units in the biceps muscle.

As previously mentioned, T2D is associated with an increased morbidity and mortality of CVD, suggesting T2D to be a cardiovascular disorder itself (Kannel & McGee, 1979). An explanation for the strong association between T2D and CVD is the “common soil” hypothesis. The “common soil” hypothesis suggests that T2D and CVD share common antecedents including obesity, dyslipidemia (reduced high-density lipoprotein (HDL) cholesterol levels), hypertension, and insulin resistance (Ceriello & Motz, 2004; Laakso, 1999; Stern, 1995). Similarly, stroke is a classified as a CVD. Given stroke and T2D are both considered CVDs, neuromuscular changes

in the stroke population may help inform hypotheses for pwT2D. Stroke survivors exhibit neuromuscular changes in their paretic muscles post-stroke. These changes include decreased motor unit number, lower firing rates, lower compound firing action potential amplitude, and disordered recruitment thresholds such that larger motor neurons are recruited earlier than smaller motor neurons (Dai, Suresh, Suresh, Rymer, & Hu, 2017; Dattola et al., 1993; Gemperline, Allen, Walk, & Rymer, 1995; Hu, Suresh, Li, Zev Rymer, & Suresh, 2012; Hu, Suresh, Rymer, & Suresh, 2015; Li et al., 2015; Li, Liu, Li, Wang, & Zhou, 2014; Suresh et al., 2014). Hu, Suresh, Li, Zev Rymer, & Suresh (2012) reported reduced firing rates and recruitment range of the paretic first dorsal interosseous (FDI) muscle compared to the intact side for one stroke survivor with moderate impairment. On the other hand, a stroke survivor with minor impairment did not experience such differences between paretic and intact muscles suggesting motor unit reorganization may be associated with the degree of sensorimotor impairment.

1.2 Problem Statement, Aims, and Hypotheses

The overall goal of this project is to examine the neuromuscular and proprioceptive effects on the upper limb in pwT2D. This will be achieved by: a) evaluating the effects of T2D on kinematic performance of a reach-to-pinch task; b) characterizing T2D associated motor unit properties in five upper limb muscles; and c) determine if observed alterations to motor unit characteristics in pwT2D length-dependent in the upper limb. Understanding proprioceptive and neuromuscular

dysfunction in pwT2D could help with early identification and treatment of the disease.

Aim 1: Evaluate the contribution of proprioception to motor impairment associated with T2D.

Hypothesis 1a: PwT2D will exhibit increased movement variability of the digits, hand, and arm when compared to controls.

Hypothesis 1b: PwT2D will be less accurate and less precise relative to the target location when compared to controls.

Rationale: Recent studies have provided reasons to suggest functional impairments associated with T2D are not due to sensory dysfunction alone. Evidence from postural stability and ankle joint studies provide support for impaired proprioception contributing to functional deficits. Both the upper and lower extremities of pwT2D experience PN and functional impairments. Thus, it is important to understand the role proprioception plays in upper limb sensorimotor dysfunction associated with T2D. To address this gap, pwT2D will perform a reach-to-pinch task relying on only proprioceptive feedback. Any impairment performing this task would then be directly attributed to proprioception.

Aim 2.1: Evaluate motor unit characteristics of five muscles of the upper limb (the Abductor Pollicis Brevis, the Flexor Digitorum Superficialis, the Extensor Digitorum, the Triceps Brachii, and the Biceps Brachii) in young healthy adults, healthy older adults, and pwT2D.

Hypothesis 2.1a: PwT2D will have fewer number of motor units, and increased motor unit size when compared to controls.

Hypothesis 2.1b: Motor unit impairment will correlate with impaired motor, tactile and proprioceptive function.

Rationale: While other researchers have assessed neuromuscular dysfunction in the lower limbs, characterization of motor unit size and functioning is lacking for the upper limbs. Older adults and stroke survivors alike display alterations in their upper limb motor unit characteristics, which may provide insight into T2D upper limb neuromuscular functioning. To solve this problem, we will use surface electromyography and neuromuscular stimulation techniques to characterize motor unit impairment of muscles in the upper limb of pwT2D.

Aim 2.2: Examine how motor unit characteristics change with respect to muscle location (proximal to distal) in the upper limb in pwT2D.

Hypothesis 2.2: Motor unit characteristics will differ in the muscles examined in pwT2D. Impairment will be more pronounced in muscles located more distally compared to those more proximal.

Rationale: It is commonly believed that T2D-associated PN develops as a length-dependent degeneration. Similarly, a recent study has provided evidence to suggest a length-dependent pattern of T2D associated alterations to motor unit characteristics occurs. Motor unit loss and remodeling was significantly more severe in the TA muscle compared to the FDI muscle in patients with T2D-associated PN; however,

within limb comparison of motor unit characteristics remains absent from the evidence base.

2 LITERATURE REVIEW

2.1 Type II Diabetes (T2D)

Diabetes Mellitus is a group of metabolic disorders characterized by high levels of glucose (sugar) in the blood stream over a prolonged time period. High blood sugar level, called hyperglycemia, is caused by the inability of the pancreas to produce enough insulin or by dysfunction of the body's response to insulin. Insulin is a hormone produced by β-cells in the pancreas and regulates the metabolism by promoting the absorption of glucose from the blood by muscle, fat, and liver cells to be used as energy. As of 2014, the World Health Organization (WHO) stated the global prevalence of diabetes among adults to be 8.5%, about 422 million people. In 2015 alone, 1.5 million deaths were caused by diabetes and an additional 2.2 million deaths were associated with high blood glucose (WHO, 2017). By the year 2030, WHO predicts diabetes to be the seventh leading cause of death.

Currently, there are three main types of diabetes. Type I Diabetes (T1D) is caused by the inability of the pancreas to produce enough insulin, requiring treatment of daily insulin therapy. This type is sometimes known as "insulin-dependent diabetes mellitus (IDDM)" or "'juvenile diabetes". The cause of T1D is unknown and makes up 5-10% of all diabetes diagnoses (Daneman, 2006). Type II Diabetes (T2D) is characterized by insulin resistance, where the body improperly responds or lacks response to the produced insulin. As T2D progresses, production of insulin may eventually cease. T2D is commonly referred to as "non-insulin dependent diabetes mellitus (NIDDM)" or "adult onset diabetes" because of its prevalence in adults.

Recently however, T2D has become more common in children as childhood obesity becomes more prevalent. T2D makes up about 90% of diabetes diagnoses (WHO, 2017). The causes of T2D are primarily obesity, lack of physical activity, and poor diet. The third type of diabetes is Gestational Diabetes. Gestational diabetes occurs in women when they develop high blood sugar levels while pregnant due to increased insulin resistance. Gestational diabetes only lasts throughout the pregnancy. However, there is evidence that suggests having gestational diabetes during pregnancy is a major risk factor for developing T2D later in life for both the mother and the child (Bellamy, Casas, Hingorani, & Williams, 2009; Pandey, Chawla, & Guchhait, 2015). Of the three types of diabetes, the focus of this dissertation will be on T2D.

As stated earlier, T2D is due to the development of insulin resistance. With insulin resistance, cells no longer properly respond to insulin, resulting in poor absorption of glucose from the blood. Thus, there is an increased demand for insulin, and β-cells secrete more to compensate, known as hyperinsulinemia. Eventually, increased insulin production is no longer sufficient, and glucose builds up in the blood, resulting in hyperglycemia. The commonly accepted theory suggests hyperinsulinemia is a response of β-cells to overcome insulin resistance. Another proposed theory suggests the opposite order of events such that insulin resistance develops as a result of disordered insulin secretion by the β-cells (Schofield & Sutherland, 2012). Regardless of the order of events, both result in T2D.

The exact cause of T2D is unknown, however, influencing factors for the disease include: obesity, fat distribution, physical inactivity, genetics, family history, increased age, race, and gestational diabetes (Rita Rastogi Kalyani, Corriere, &

Ferrucci, 2014; Rita Rastogi Kalyani & Egan, 2013; Mayo Clinic, 2018; WHO, 2017; Wu, Ding, Tanaka, & Zhang, 2014). Of these factors, obesity is considered the most important. According to WHO, about 90% of those with diabetes developed T2D relating to excess body weight (WHO, 2017).

Insulin resistance and diabetes are more common in older adults. In the United States, about 1/3 of the population aged over 60 has diabetes, which is double the prevalence in middle-aged adults (Cowie et al., 2009). Frailty is considered to be a condition of physiological vulnerability that is associated with increased age (Boyd, Xue, Simpson, Guralnik, & Fried, 2005; Fried, Tangen, Walston, & Al., 2001). There is evidence that insulin resistance and diabetes are associated with frailty (Blaum et al., 2009; Goulet et al., 2009; Kalyani et al., 2012; Kalyani, Varadhan, Weiss, Fried, & Cappola, 2012; Zaslavsky, Walker, Crane, Gray, & Larson, 2016). A study by Goulet et al. reported findings to suggest a link between obesity, frailty, and insulin insensitivity such that frail obese adults but not frail lean adults had reduced insulin sensitivity compared to their non-frail counterparts (Goulet et al., 2009). Conversely, another study reported an association between insulin resistance with frailty independent of obesity (Kalyani et al., 2012). Together, this suggests older adults, especially those with excess weight, are more at risk of developing T2D.

Symptoms associated with T2D include excessive urination, thirst, hunger, weight loss, vision changes, fatigue, loss of muscle mass, slow healing wounds, frequent infections, and areas of darkened skin (Kalyani et al., 2014; Kalyani & Egan, 2013; Mayo Clinic, 2018; WHO, 2017). PwT2D are at increased risk of developing complications such as macrovascular diseases, microvascular diseases, and cancers.

Macrovascular diseases associated with T2D include: cardiovascular disease, hypertension, hyperlipidemia, heart attack, coronary artery disease, and peripheral vascular disease (Kalyani & Egan, 2013; Wu et al., 2014). Microvascular diseases associated with T2D include: retinopathy, nephropathy, and neuropathy (Kalyani & Egan, 2013; WHO, 2017; Wu et al., 2014). Prevalence of T2D related macrovascular diseases is similar in patients with middle-age T2D onset and those with older-age T2D onset. When it comes to prevalence of T2D related microvascular diseases however, those with middle-age T2D onset are at greater risk of developing a microvascular disease compared to those with older-age onset (Selvin, Coresh, & Brancati, 2006; Wang, Qin, Liu, & Chang, 2010). Thus, the duration of disease may impact T2D complications.

2.1.1 T2D and Peripheral Neuropathy

A common complication of T2D is nerve dysfunction, known as Peripheral Neuropathy (PN). Approximately 50% of pwT2D will develop peripheral neuropathy (Harati, Hadaegh, Saadat, & Azizi, 2009). Risk of PN increases with age, duration of disease, uncontrolled blood sugar, and weight (NIDDK, 2018). Common symptoms of PN include numbness, insensitivity to pain or temperatures, tingling, burning sensation, prickling, sharp pains, and sensitivity to touch (NIDDK, 2018; Vinik, Nevoret, Casellini, & Parson, 2013). Numbness of the limbs, particularly the feet, can become a serious condition if injured without awareness. Small wounds, blisters and sores can become infected which could lead to gangrene and amputation.

Nerve damage develops over time with typical T2D-associated PN being chronic, symmetrical throughout the body, and is commonly assumed to be nerve length-dependent (Dyck, O'brien, & Kosanke, 1993). This type of PN is also sometimes referred to as "sensorimotor polyneuropathy" because it results in both sensory and motor dysfunction. Being length-dependent, sensory and motor neurons with longer axons are first affected by PN, proceeding in a distal to proximal direction (Dyck et al., 1993; Zochodne, 2007). Symptoms of PN usually begin in the feet, then in the legs and hands. Currently, there are a few theories as to the mechanism of PN development. One mechanism, axonal loss, consists of the "dying back" of neurons. Neuron degeneration begins with axon atrophy until there is complete loss of the axon, followed by a gradual loss of the neuronal cell body (Zochodne, 2007). A second mechanism of PN is demyelination of peripheral nerves (Valls-Canals, Povedano, Montero, & Pradas, 2002; Zochodne, 2007). There is evidence to support that it is a combination of both mechanisms, axonal loss and nerve demyelination, that leads to PN (Valls-Canals et al., 2002).

Nerve conduction velocity (NCV) testing is a tool used to diagnose neuropathies. This type of testing can be used to locate neuropathy as well as the extent of the nerve damage. Slowing of NCV has been found to be associated with T2D as well as the duration of disease (Vinik et al., 2013). Conduction slowing is believed to be a result of nerve demyelination. Another electrophysiological test, Sensory Nerve Action Potential (SNAP), is used to evaluate nerve functioning. One of the earliest signals of T2D-associated PN is a reduced or absent sural nerve SNAP amplitude at the ankle. Axon degeneration is characterized by reduced sensory and

motor action potential amplitudes with normal or slightly lower NCV (Behse, Buchthal, & Carlsen, 1977; Dunnigan et al., 2013). Symptoms of PN are primarily a result of axonal loss (Valls-Canals et al., 2002). Multiple studies have found evidence of a linear relationship of reduced compound muscle action potential (CMAP) amplitude and decreased motor nerve conduction velocity in T2D-associated PN, further supporting the two mechanisms occurring at the same time (Campfens et al., 2015; Tankisi, Pugdahl, Johnsen, & Fuglsang-Frederiksen, 2007; Wilson, Stittsworth, Kadir, & Fisher, 1998).

T2D-associated PN is also known to be associated with low-grade chronic inflammation (Zhou & Zhou, 2014). Cytokine levels, a chemical marker of inflammation, are generally higher in pwT2D, particularly in adipose tissue (Donath & Shoelson, 2011; Zhou & Zhou, 2014). Elevated levels of tumor necrosis factor alpha (TNF-α) cytokine have been found in T2D-associated PN model rodents (Chen et al., 2016). Additionally, elevated levels of other proinflammatory cytokines, IL-6 and IL-1β, have been reported in sciatic nerve of T2D rodents, indicating inflammation as a mechanism contributing to T2D-associated PN (Chen et al., 2016).

2.2 Sensory Function and T2D

2.2.1 Physiology of Proprioception

Proprioception, sometimes called the “sixth-sense”, is the perception and awareness of the position of the body and its parts in space. The term proprioception was first used by Sherrington in 1906 when he said, “In muscular receptivity we see the body itself acting as a stimulus to its own receptors – the proprioceptors”

(Sherrington, 1907). Proprioception plays an important role in motor control. It allows for the ability to perform precise and accurate movements in the absence of continuous vision, to perform movements requiring multi-limb coordination, and adjust movement patterns. There are multiple components of proprioception: limb position sense, sense of limb movement (kinesthesia), sense of tension or force, sense of effort, and sense of balance. Unlike receptors in the eyes and ears, proprioception does not have an identifiable sensation attributable to it (Proske & Gandevia, 2012). When making movements, the central nervous system (CNS) predicts or anticipates the sensory feedback the movement will create. A common theory is that what we "feel" is the difference between what was predicted compared to the sensory feedback generated by what movement actually occurred (Proske & Gandevia, 2012).

Muscle spindles are the primary proprioceptors, providing information on both movement and position (Casey, 1999). Muscle spindle primary afferent fibers are myelinated, large in diameter (12-20 μm), and conduct at velocities from 72 – 120 m/s. Muscle spindles are sensitive to change in muscle length as well as rate of change of length (Arthur Prochazka & Gorassini, 1998). Primary afferent fibers are found in both muscles and skin. When located in muscle, they are group Ia, when located in the skin they are group Aα, see Figure 2.1 (Gilman, 2002). Muscle spindle primary endings provide input for both position and movement sense. Velocity of change in muscle length detected by primary endings produces the sense of movement while position sense is signaled by the rate of background discharge created by primary and secondary endings (Proske & Gandevia, 2012). Secondary afferent fibers (group II, Aβ) within muscle spindles are myelinated, smaller (6-12 μm) and slower (36-72 m/s)

than their primary counterparts, see Figure 2.1 (Gilman, 2002). Secondary fibers provide information only about limb position sense via muscle length (Nardone et al., 2000; Arthur Prochazka & Gorassini, 1998). In addition to muscle spindles, other mechanoreceptors in joint capsules and cutaneous tactile receptors provide proprioceptive information (Gardner, Martin, & Jessell, 2000).

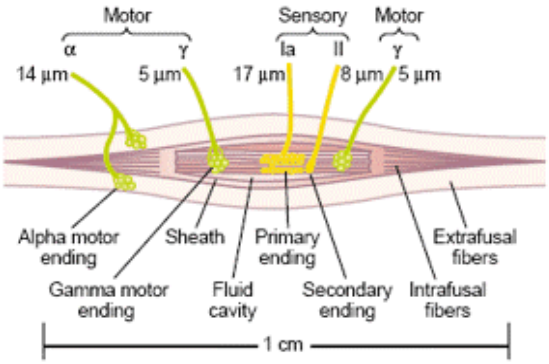

Figure 2.1 *Schematic of a skeletal muscle spindle showing sensory and motor fibers. Adapted from* Guyton, A.C., & Hall, J.E. (2006). *Textbook of medical physiology.* Philadelphia, PA: Elsevier Saunders.

Cutaneous afferent fibers provide feedback from the stretching of the skin, contributing to the sense of movement and direction. Two other types of receptors, Golgi tendon organs and joint receptors, provide limb position and movement information as well (Gardner et al., 2000; Prochazka, 1996). Golgi tendon organs are receptors located in tendons that respond to contractile force and heaviness (effort) from muscle fibers (Prochazka & Gorassini, 1998; Proske & Gandevia, 2012). These type of receptors are myelinated, large in diameter (12-20 µm), and have similar conduction speeds as muscle spindle primary afferents (Gilman, 2002). Golgi tendon organ afferent fibers are categorized as group Ib or Aα, see Figure 2.2.

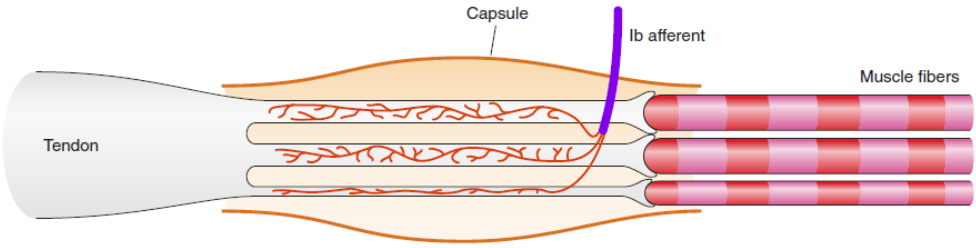

Figure 2.2 *Schematic of Golgi tendon organ showing sensory and motor fibers. Adapted from* Proske, U., & Gandevia, S. C. (2012). The Proprioceptive Senses: Their Roles in Signaling Body Shape, Body Position and Movement, and Muscle Force. *Physiological Reviews*, 92(4), 1651–1697.

Group III and Aδ joint receptor afferent fibers are small (1-6 µm) and thinly myelinated, with conduction velocities of 4-36 m/s. Group IV and C joint receptors are even smaller (0.2-1.5 µm) and unmyelinated, with conduction velocities of 0.4-2.0 m/s (Gilman, 2002). Joint receptors primarily act as limit detectors, providing feedback at extreme joint positions. When a joint is rotated, the joint capsule and skin of one side is stretched while the other is unloaded. Joint receptors signal stress in the tissue of the joint capsule and Pacinian corpuscles (deep cutaneous mechanoreceptors) signal local compression (Proske & Gandevia, 2012).

Though most study findings conclude Golgi tendon organs and joint receptors contribute very little to joint position sense compared to muscle spindles (Casey, 1999), there is evidence to suggest joint receptors contribute in the movements of the finger joints (Clark, Grigg, & Chapin, 1989; Ferrell, Gandevia, & Mccloskey, 1987). At the distal interphalangeal joint of the middle finger, detection of angular displacement was severely impaired with the absence of muscle spindle afferent information and intact skin and joint input. However, detection of angular

displacement was also impaired when the experiment was reversed; joint and skin afferent information was absent while muscle spindle input remained. These findings suggest both sources of feedback are required for the most precise proprioceptive awareness (Gandevia, Hall, McCloskey, & Potter, 1983; Gandevia & McCloskey, 1976).

Contrary to findings in finger joints, joint replacement surgery studies provide evidence that muscle spindles and not joint receptors are more important to proprioception. After full joint replacement, position and movement sense are retained in the hips and knees. (Clark, Horch, Bach, & Larson, 1979; Grigg, Finerman, & Riley, 1973). The most compelling evidence suggesting muscle spindles as the primary proprioceptors is the phenomena of kinesthetic illusion induced by muscle tendon vibration. Vibration at 100 Hz of the biceps brachii or triceps brachii results in an illusion of arm movement in the direction of muscle lengthening of the vibrated muscle, but, when applied on the elbow joint, no such illusion occurred (Eklund, 1972; Goodwin, McCloskey, & Matthews, 1972b). Vibration stimulates the Ia primary endings of muscle spindles, producing an illusion of limb movement and joint perception errors (Cordo, Gurfinkel, Bevan, & Kerr, 1995; Goodwin, McCloskey, & Matthews, 1972a; Kito, Hashimoto, Yoneda, Katamoto, & Naito, 2006; Rogers, Bendrups, & Lewis, 1985; Roll & Vedel, 1982; Seizova-Cajic & Azzi, 2011). While muscle spindle stimulation is predominantly responsible for the kinesthetic illusion, Golgi tendon organs are known to be sensitive to muscle tendon vibration as well (Cordo, Gandevia, Hales, Burke, & Laird, 1993; Fallon & Macefield, 2007).

Proprioceptive awareness is generally consistent on a day to day basis. When performing bilateral arm matching tasks, joint position matching ability is consistent over multiple trials, but not necessarily accurate. Joint matching error may be consistently biased in one direction. These findings suggest that relative arm positions are perceived, however they are not accurate and can become more pronounced with muscle fatigue (Allen & Proske, 2006). Proprioceptive sense acuity seems to vary throughout the body. Hall and McCloskey found that proprioception at the shoulder and elbow was more accurate than in the fingers (Hall & McCloskey, 1983).

2.2.2 Abnormal Sensory Function associated with T2D

2.2.2.1 Postural Control Studies

Postural control utilizes both tactile and proprioceptive feedback to maintain upright balance. Impaired balance and increased risk of falling have been associated with T2D and T2D-associated PN (Hong et al., 1997; Lord et al., 1993; Mustapa et al., 2016; Simmons et al., 1997; Uccioli, 1995). Hong , Chia, and Ling (1997) reported T2D-associated changes in center of pressure (COP) when compared to healthy, age- and sex-matched controls. PwT2D exhibited increased length of sway path, larger sway area, and faster COP velocity, indicating an overall decrease in postural stability (Hong et al., 1997).

Furthermore, differences between pwT2D without PN and those with PN has been established. When compared to pwT2D without PN, pwT2D with PN have larger COP velocity, larger sway area, and larger sway path, (Uccioli, 1995). Similarly,

Simmons and colleagues (1997) found that after being perturbed, pwT2D with tactile impairment of the feet had greater postural instability, were at greater risk of falling, and changed their balance strategy from ankle to hip compared to healthy controls and pwT2D without tactile impairment (Simmons et al., 1997). In the same study, pwT2D without tactile impairment did not perform significantly different on postural measures compared to healthy controls. The somatosensory system provides sensory information about body segment position in relation to each other and the support surface for balance control via proprioceptive and tactile feedback. T2D-associated PN degrades sensory nerve fibers which can lead to somatosensory system dysfunction. The findings from Ucciloli et al. (1995) and Simmons et al. (1997) suggest the presence of PN is a contributor to T2D-associated postural deficits.

Loss of tactile sensation is more easily measured and quantifiable during postural performance than it is to measure proprioceptor functioning. This is why most postural studies are not able to discern T2D's effect specifically on proprioception separately from loss of foot tactile sensation.

2.2.2.2 Lower Extremity Proprioception studies

A joint position perception task is a common method for researchers to evaluate proprioceptive functioning. Proprioceptive sensory function in the lower extremity in pwT2D has been somewhat examined in the literature base and findings point to proprioceptive dysfunction (Lord et al., 1993; Simoneau et al., 1996; van Deursen & Simoneau, 1999; van Deursen et al., 1998). Ankle movement perception,

which relies on proprioceptive information, is impaired in this population (Simoneau et al., 1996). Simoneau et al. (1996) reported a correlation between ankle joint movement perception threshold and cutaneous sensory tests (Semmes- Weinstein monofilaments for tactile threshold; Vibrometer for vibration threshold) such that pwT2D with tactile impairment demonstrated a significant loss of ankle movement perception (Simoneau et al., 1996). Additionally, there were no significant differences between controls and pwT2D without PN, further establishing the link between T2D-associated PN and impaired sensory function (Simoneau et al., 1996).

Vibration perception is also diminished in pwT2D (Simoneau et al., 1996; van Deursen et al., 2001; van Deursen et al., 1998). Muscle spindle primary afferents are stimulated by muscle tendon vibration, inducing a kinesthetic illusion (see section 2.2.1). Muscle tendon vibration is used by researchers during joint perception matching tasks to study proprioceptive functioning. The presence of a kinesthetic illusion and its strength can signify if muscle spindles are intact and conveying the appropriate information. PwT2D with PN are less responsive to Achilles tendon and Anterior Tibialis muscle tendon vibration compared to healthy controls (van Deursen et al., 1998). Also, ankle movement tracking performance during muscle tendon vibration is worse in pwT2D (van Deursen et al., 1998). Collectively, impaired joint tracking and diminished muscle tendon vibration response suggest muscle spindle impairment resulting in proprioceptive dysfunction in the lower extremities is a result of T2D-associated PN.

2.2.2.3 Abnormal Sensory Function in other populations

Carpal Tunnel Syndrome (CTS) is a median nerve entrapment mononeuropathy which results in sensorimotor deficits of the hand (Maeda et al., 2014; Nataraj, Evans, Seitz, & Li, 2014; Wolny, Saulicz, Linek, & Myśliwiec, 2016). Essentially, CTS is a mechanically induce neuropathy, due to increased pressure and inflammation of the nerve located within the carpal tunnel in the volar wrist. In persons with CTS (pwCTS), maximal pinch voluntary contraction (MVC) and sensory nerve conduction velocity are reduced when compared to healthy subjects (Maeda et al., 2014; Wolny et al., 2016). Furthermore, sensory discrimination accuracy is impaired in all fingers in pwCTS (Maeda et al., 2014; Wolny et al., 2016).

In the 2014 study by Nataraj et al., proprioceptive functioning was examined in pwCTS using a reach-to-pinch task with a virtual target (Nataraj et al., 2014). A reach-to-pinch task requires dexterity (precision pinch of the thumb and index finger) and the coordination of upper extremity segments during transport of the hand to the target (Nataraj et al., 2014). A virtual target eliminates tactile feedback, ensuring evaluation of proprioception alone (Nataraj et al., 2014). CTS is known to impair precision pinch movement, however during daily life vision can be used to overcome sensorimotor dysfunction (Gehrmann et al., 2008). If vision were to be occluded, kinematic dyscoordination (movement variability) of the reach-to-pinch task could be arguably attributable to poor proprioceptive information. In this study, the ability of pwCTS to accurately and precisely locate a target was 40% worse than their healthy counterparts (Nataraj et al., 2014). These findings suggest proprioceptive information of the upper extremity is impaired in pwCTS, leading to poor movement coordination. Thus, sensorimotor dysfunction associated with CTS may be similar to that associated with

T2D-associated PN. Additionally, both CTS and T2D-associated PN share an association with inflammation. Because of this similar feature, work in the CTS literature can help inform potential dysfunction in the T2D population.

Stroke is another clinical population that experiences sensorimotor impairment of the upper limb. Tactile and proprioceptive dysfunction of the upper limb have been reported in this population (Bowden, Lin, & McNulty, 2014; Simo, Ghez, Botzer, & Scheidt, 2011; Tyson, Lesley Crow, Connell, Winward, & Hillier, 2013). Cutaneous sensation is impaired in 30-55% of stroke patients, with muscle weakness and upper limb neglect influencing impairment (Bowden et al., 2014; Tyson et al., 2013). Bowden et al. (2014) used the Wolf Motor Function test and the upper limb Fugl Meyer Assessment to assess motor functioning in addition to Monofilament test of the hand. Results suggested a correlation between motor impairment and sensation at the fingertip and palm such that those with low motor function generally had poorer cutaneous sensation (Bowden et al., 2014). Proprioception of the hand is diminished in this population, signified by increased movement detection threshold (Simo et al., 2011).

In stroke, proprioceptive impairment seems to be tied to cutaneous sensation impairment. In the study by Simo et al. (2011), all stoke patients who exhibited proprioceptive impairment also exhibited tactile deficits, while one lone subject had tactile deficit without proprioceptive (Simo et al., 2011). The authors did not offer an explanation for this finding, but perhaps the presence of proprioceptive impairment is determined by stroke severity.

2.3 Motor Function and T2D

2.3.1 Physiology of Neuromuscular Control

The neuromuscular system is made up of all muscles in the body and the nerves surrounding them and serves to produce movements and locomotion. To produce a movement, there must be communication between the brain and the muscles. Alpha (α) motor neurons are large, multipolar lower motor neurons that innervate and control muscle fibers of skeletal muscle. A multipolar neuron possesses a single axon with many dendrites, allowing for integration of information from many other neurons. Alpha motor neurons are considered to be a part of both the Central Nervous System (CNS) and the Peripheral Nervous System (PNS). Their cell bodies are located in the CNS while their axons are found in the periphery, innervating skeletal muscles. Signals in the form of action potentials are transmitted from the CNS to skeletal muscle fibers by α- motor neurons, initiating contractions and thus, resulting in movement.

A motor unit is the smallest functional unit of the neuromuscular system and is comprised of a single α-motor neuron and the skeletal muscle fibers that are innervated by its axonal terminals, see Figure 2.3 (Guth, 1983; Latash, 1998). A single muscle fiber is innervated by a single neuronal axon branch. Motor neurons work according to the all-or-none law, allowing for synchronized contraction of all of the skeletal muscles fibers that a motor neuron innervates, in response to each action potential signal. This means that muscle fibers abide by the all-or-none law as well (Latash, 1998). Within each individual motor unit, all of the skeletal muscle fibers are of the same type. Contractions of a muscle are coordinated by many motor units

working together, and all of the motor units within a particular muscle are called the motor pool (Latash, 1998).

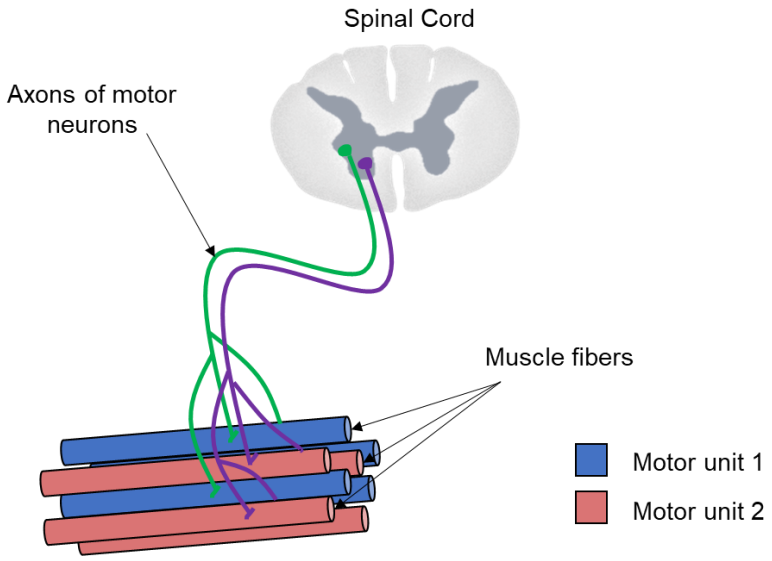

Figure 2.3 *Two motor units are shown. Motor units consist of a single α-motor neuron and the muscle fibers it innervates. Motor neuron cell bodies are located within the ventral horn of the spinal cord whereas their axons form synapses with a set of muscle fibers.*

Motor units range in size depending on the size of α-motor neuron and the number of innervated muscle fibers. Larger α-motor neurons innervate more muscle fibers than smaller α-motor neurons; a single neuron can innervate between 10-1000 muscle fibers depending on the muscle and its function (Feinstein, Lindegard, Nyman, & Wohlfart, 1955; Latash, 1998).

Muscles involved in fine movements, such as eye and hand muscles, are generally characterized as having motor units that are smaller in size but large in number (Feinstein et al., 1955; Guth, 1983; Latash, 1998). In contrast, muscles involved in gross movements, such as postural muscles, usually are characterized as

having fewer but larger motor units (Feinstein et al., 1955; Guth, 1983; Latash, 1998). With age, the number of motor neurons generally decreases, resulting in reinnervation that causes motor unit size to increase as well as an increase in innervation ratio (Latash, 1998).

There are different types of motor units that vary on contractile properties including: isometric contraction speed, rate of rise of force, and time to peak of a twitch contraction. Fibers types designated by contractile properties include: fast - fatigable (FF), fast - fatigue resistant (FR), and slow- fatigue resistant (S) (Burke, Levine, Tsairis, & Zajac, 1973; Latash, 1998). FF type have the highest conduction velocity (100 m/s), high force output, and large axon diameter but fatigue quickly (a few seconds) (Burke et al., 1973; Latash, 1998). FR type have medium diameter axons, high contraction speeds, produce intermediate force, and are resistant to fatigue (Burke et al., 1973; Latash, 1998). Lastly, S type motor units typically have fewer muscle fibers, smaller motor neurons, and small diameter axons. They also have the slowest conduction speed (40 m/s) produce low force, and are highly fatigue resistant (Burke et al., 1973; Latash, 1998). Muscles are generally made up of a mixture of all three motor unit types, with varying percentages.

Within the CNS, there is a coordinative rule used to determine the order in which the motor unit types are recruited, called the Henneman principle (also known as the size principle) (Henneman, Somjen, & Carpenter, 1965; Henneman, 1957; Latash, 1998; Milner-Brown, Stein, & Yemm, 1973). The Henneman principle sates that “recruitment of motor units within a muscle proceeds from small motor units to large ones” (Henneman et al., 1965; Henneman, 1957; Latash, 1998; Milner-Brown et

al., 1973). This means that small and slow muscle fibers (Type S) are generally recruited fist during a contraction, followed by larger and faster muscle fibers (FF, FR). In a low force contraction, most of the force is produced by S motor units. When the contraction force is increased, larger motor units are recruited. Finally, the largest motor units are recruited at maximal voluntary contraction. De-recruitment of motor units occurs in the opposite order, such that the largest motor units are deactivated first while the smallest motor units are the last to be deactivated.

The orderly recruitment of motor units based on size is called spatial summation (Adrian & Bronk, 1928, 1929; Tanji & Kato, 1973). Motor unit size and frequency of action potentials are the two factors that determine the contribution of a single motor unit to total muscle force (Latash, 1998). Motor units are able to generate more or less force with increasing or decreasing action potential frequency, referred to as temporal summation (Adrian & Bronk, 1928, 1929; Latash, 1998). This means the CNS is able to regulate muscle force using the mechanisms of spatial summation and temporal summation (Latash, 1998).

2.3.2 Motor Unit Number Estimation method and EMG Decomposition

Motor Unit Number Estimation (MUNE) is a technique that uses electromyographic measures and can be used for evaluation of nerve function (Allen et al., 2013, 2014; Arnold et al., 2015; Peng et al., 2016; Watanabe et al., 2013, 2012). MUNE measures the number of functional motor units in a particular muscle. MUNE is the ratio of the maximal compound muscle action potential (CMAP) amplitude divided by the mean single motor unit action potential (SMUAP) amplitude. CMAP is

a measure that describes the maximal electrophysiological size of the entire motor pool in a muscle, while mean SMUAP measures the size of the average single motor unit within the same muscle. There are various methods of MUNE, differing on how SMUAPs are obtained. Motor unit number index (MUNIX) is one variation of MUNE and will be discussed in this dissertation. Generally, MUNIX is considered to be faster, easier to perform and well tolerated by patients than the standard MUNE technique (Nandedkar, Nandedkar, Barkhaus, & Stalberg, 2004; Nandedkar, Barkhaus, & Stålberg, 2010; Nandedkar, Barkhaus, Stålberg, Neuwirth, & Weber, 2018). This technique determines an index value related the number of motor units, compared to actual motor unit count estimated with MUNE (Nandedkar et al., 2010). A benefit of the MUNE/MUNIX method is it is noninvasive (Nandedkar et al., 2010; Peng et al., 2016). Unlike other methods, such as spike triggered averaging, invasive intramuscular needles are not needed.

The following MUNIX method has been established and refined in healthy and patient populations (Nandedkar et al., 2004; Nandedkar et al., 2010). For the first step of MUNIX, electrical stimulation is incrementally applied to the innervating nerve of the desired muscle until maximal CMAP amplitude, area, and power are obtained, see Figure 2.4A. Following nerve stimulation, the surface electromyographic interference pattern (SIP) is recorded while the patient performs isometric contractions varying from low level to maximal effort. The area and power of each SIP is then determined. Next, the “ideal case motor unit count” (ICMUC) is calculated using equation 2.1. The ICMUC assumes that all MUs are identical. Typically, this is true at very light muscle

contraction force. Because ICMUC depends on force level, as force increases and larger MUs are recruited, ICMUC decreases.

$$ICMUC = \frac{(CMAP\ Power \times SIP\ Area)}{(CMAP\ Area \times SIP\ Power)} \quad (2.1)$$

ICMUC versus SIP Area is plotted and power regression analysis is used to determine the parameters A and α in equation 2.2. The relationship between ICMUC and SIP Area is shown in Figure 2.4B.

$$ICMUC = A(SIP\ Area)^{\alpha} \quad (2.2)$$

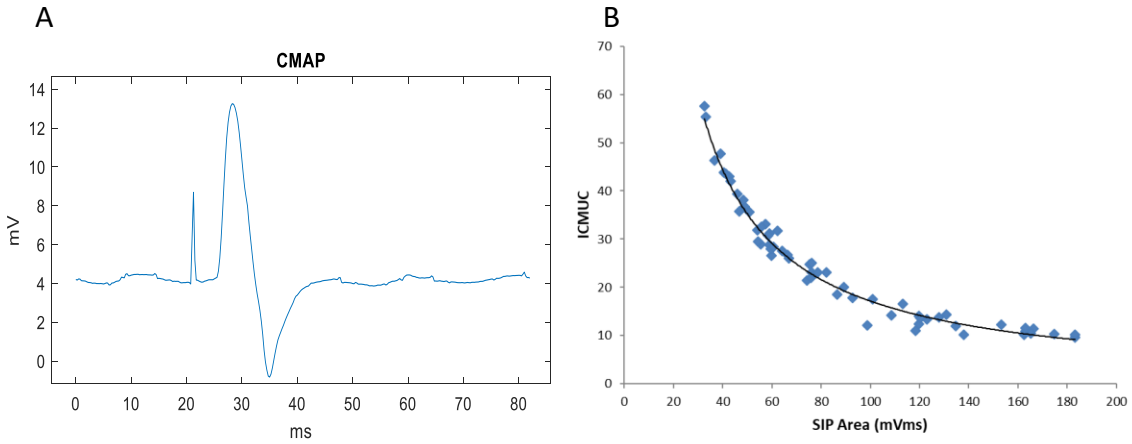

Figure 2.4 *A)Recorded CMAP in APB muscle. B) ICMUC versus SIP Area plotted in one study participant. Solid line depicts the power regression analysis.*

Next, MUNIX is determined using the parameters determined from the previous power regression and equation 2.3. MUNIX is the ICMUC value when SIP Area is equal to 20 mVms.

$$MUNIX = A(20)^{\alpha} \quad (2.3)$$

Evidence from previous studies point toward a contraction intensity effect on MUNE methods (Boe, Stashuk, Brown, & Doherty, 2005; McNeil, Doherty, Stashuk,

& Rice, 2005a, 2005b; Peng et al., 2016). For example, in the first dorsal interosseous (FDI) and tibialis anterior (TA) muscles, increasing contraction intensities from 10-50% maximal voluntary contraction (MVC) coincided with increases in mean SMUAP. Increased SMUAPs resulted in decreased MUNE values (Boe et al., 2005; McNeil et al., 2005a, 2005b; Peng et al., 2016).

MUNE/MUNIX techniques have been used in neurological and neuromuscular disease populations including ALS, spinal cord injury, Charcot Marie Tooth, stroke, and T2D-associated PN (Aggarwal & Nicholson, 2002; Allen et al., 2013, 2014; Bromberg, 2007; Hara, Akaboshi, Masakado, & Chino, 2000; Lawson, Gordon Smith, & Bromberg, 2003; Nandedkar et al., 2004; Nandedkar et al., 2010; Swoboda et al., 2005; Watanabe et al., 2012, 2013). MUNE has also been used in older adults to characterize neuromuscular changes due to natural aging (Arnold et al., 2015; Dalton, McNeil, Doherty, & Rice, 2008; Duque et al., 2016; McNeil et al., 2005a).

2.3.3 Abnormal Motor Function associated with T2D

Generally, early and mild PN symptoms point to sensory neurons being targeted first. The motor system is targeted by hyperglycemia induced PN too, leading to dysfunction of the neuromuscular system (Ramji et al., 2007). Overall, studies of neuromuscular dysfunction associated with T2D have reported reduced motor unit firing rates, increased firing rate variability, and changes in motor neuron excitability (Allen et al., 2013, 2014; Krishnan & Kiernan, 2005; Watanabe et al., 2013). Consequences of neuromuscular dysfunction include skeletal muscle atrophy and

weakness (Allen et al., 2013; Andersen, Stålberg, Gjerstad, & Jakobsen, 1998; Andreassen, Jakobsen, Ringgaard, Ejskjaer, & Andersen, 2009; Sacchetti et al., 2013). Animal studies provide evidence for alterations at the muscle level as well, characterized by disrupted t-tubules, degenerated mitochondria, and dysfunctional ion pumps and channels (Fahim, Hasan, & Alshuaib, 2014; Kjeldsen, Braengaard, Sidenius, Larsen, & Norgaard, 1987; Krishnan, Lin, & Kiernan, 2008; Lowell & Shulman, 2005; Nobe, Aomine, Arita, Ito, & Takaki, 1990).

2.3.3.1 Lower Extremity Control studies

Effects of T2D-associated PN induced neuromuscular dysfunction and its consequences has been documented in the lower limb. Ankle plantar and dorsi- flexors and knee extensors in patients with T2D-associated PN exhibited a loss of isometric strength when compared to their healthy counterparts, signifying muscle atrophy (Andersen, 1998; Andreassen et al., 2009; van Schie, Vermigli, Carrington, & Boulton, 2004). Furthermore, in addition to decreased plantar flexion and strength, reduced isokinetic power and power per unit of muscle volume have been reported, suggesting decreased muscle quality (Hilton, Tuttle, Bohnert, Mueller, & Sinacore, 2008). Increased weakness and reduced muscle quality in the lower extremities, particular those reported in the plantar and dorsi- flexors can affect daily life and health outcomes. Lower extremity muscle weakness can lead to gait abnormalities, increased risk of falling, damage to ankle joints, cause ulcers, and potentially amputations (Abbott, Vileikyte, Williamson, Carrington, & Bolton, 1998; Jeffcoate &

Harding, 2003; Martinelli et al., 2013; van Schie et al., 2004; Volpato et al., 2012). Injuries and amputations have the potential to limit daily activities, such as exercise, that are necessary to maintain a healthy lifestyle and reduce T2D-associated symptom severity.

In the lower extremities, motor system impairment occurs in a distal to proximal pattern, similar to PN. In the tibialis anterior, pwT2D exhibited 60% less dorsiflexion force as well as 60% fewer motor units when compared to healthy controls (Allen et al., 2013). Animal models of diabetes indicate that distal axonal dropout (dying back) of motor neurons occurs in T2D (Ramji et al., 2007; Souayah & Potian, 2009). Motor neuron axons gradually retract their terminals from their distal associated motor units. Results from Allen et al (2013) indicate that in humans with T2D-associated PN, denervation of muscle fibers occurs at a faster rate than compensatory reinnervation by surrounding motor neurons (called collateral reinnervation) (Allen et al., 2013). Also, larger mean surface motor unit action potentials (SMUAPs) in T2D-associated PN suggest either growth of remaining motor units via compensatory sprouting or degradation targeting smaller motor units first (Allen et al., 2013, 2014; Ramji et al., 2007). The reported decrease in muscle strength signifies the unbalance of nerve degeneration and collateral reinnervation, with the former winning out (Allen et al., 2013). This leads to a reduction in motor unit number, and ultimately muscle atrophy and loss of strength (Allen et al., 2013, 2014; Doherty, 2003).

In both the tibialis anterior and vastus lateralis muscles, T2D-associated neuromuscular dysfunction consists of fewer MUNEs, smaller CMAP amplitudes,

smaller SMUAP amplitudes, and reduced mean motor unit firing rates (Allen et al., 2013, 2014; Watanabe et al., 2013). In the vastus lateralis muscle, Watanabe et al (2013) found lower mean motor unit firing rates during a ramp up to 20% MVC contraction. They also found higher variability of motor unit firing rates during later periods of a 10% MVC sustained contraction (Watanabe et al., 2013). Alterations in motor unit firing rate indicate that the threshold at which motor unit firing rate variability begins to increase is affected by T2D-associated PN. In addition to altered motor unit firing rate variability, denervation results in motor unit remodeling in the lower extremities. During isometric contraction, motor unit activation is lower in addition to impaired redistribution of motor unit activation (Watanabe et al., 2012). This means overall, fewer motor units are activated more continuously.

2.3.3.2 Upper Extremity Control studies

Upper extremity motor dysfunction associated with T2D is beginning to be explored. Most work in the literature has examined hand functioning using manual dexterity and muscle force production tests (Andersen, 2012; Casanova, Casanova, & Young, 1991; Cetinus et al., 2005; de Freitas & Lima, 2013; Gorniak, Khan, Ochoa, Sharma, & Phan, 2014; Ochoa et al., 2016; Ochoa & Gorniak, 2014). PwT2D require an overall longer time to complete manual dexterity tests (e.g. Purdue Pegboard test, O'Connor Tweezer test, Smith Hand Function test) compared to healthy controls, with exception of the zipper and block building portions (Casanova et al., 1991). A similar study reported deficits in this population only during the building section of the

Purdue Pegboard test, with all other portions of the test being similar to healthy controls (Gorniak et al., 2014). Conversely, the Jebsen Taylor Hand Function test, another test of manual dexterity, did not reveal any differences associated with T2D (de Freitas & Lima, 2013; Gorniak et al., 2014). These findings suggest dysfunction of fine motor skills in the upper extremity associated with T2D, while gross motor skills remain.

Work exploring maximal force production in the hand has varying results (Andersen, 2012; Cederlund et al., 2009; Cetinus et al., 2005; de Freitas & Lima, 2013; Gorniak et al., 2014; Ochoa & Gorniak, 2014). Two studies, Cederlund et al. (2009) and de Freitas & Lima (2013), reported no effects of T2D on manual force production in the hand. A review by Andersen (2012) concluded that motor functioning was preserved in the upper extremity but was degraded in the lower extremities. However, recent studies suggest grip strength but not pinch strength is impaired in T2D, providing evidence that motor unit remodeling may be different across muscles groups in the upper limb (Cetinus et al., 2005; Gorniak et al., 2014; Ochoa & Gorniak, 2014). While tactile impairment and sensory dysfunction are known to contribute to functional motor deficits in T2D, evidence suggests it may not be the only cause (Gorniak et al., 2014; Ochoa et al., 2016; Ochoa & Gorniak, 2014; Orlando et al., 2016).

To date, motor unit dysfunction of the upper extremity in T2D has only been examined in the first dorsal interosseous (FDI) muscle (Allen et al., 2014). In this muscle, patients with T2D-associated PN exhibited a 30% reduction in MUNEs, 20% reduction in CMAP amplitudes, and 15% reduction in mean firing rates when

compared to healthy controls (Allen et al., 2014). SMUAPs however, were not affected in the FDI muscle while they are reduced in lower limb muscles (Allen, 2014; Allen et al., 2013). Within the same study, neuromuscular properties were compared between the FDI muscle in the upper limb and the TA muscle in the lower limb. Overall, the TA was more severely affected by T2D than the FDI, suggesting degree of neuromuscular dysfunction is dependent on the muscle and occurs is a distal to proximal progression (Allen et al., 2014).

2.3.3.3 Neuromuscular Control in other populations

Various clinical populations along with natural aging result in neuromuscular dysfunction. Motor unit loss is a key characteristic of neuromuscular diseases and motor deficits associated with aging (Bromberg, 2007; Galea, 1996; Hara et al., 2000; Hu et al., 2012; McNeil et al., 2005a). At the muscle level, aging is associated with delayed muscle activation in response to stimulus along with altered muscle recruitment, indicating neuromuscular dysfunction (Arnold et al., 2015). However, the literature on age associated neuromuscular dysfunction is varying and potentially dependent on muscle location and function (Brown, Strong, & Snow, 1988; Dalton et al., 2008; Duque et al., 2016; Galea, 1996).

A study by Dalton et al. (2008) did not find differences in MUNE of the soleus muscle when compared between old (about 75 years old) and young (about 27 years old) participants, concluding there to be no age associated reductions in MUNE in this muscle (Dalton et al., 2008). Conversely, age associated motor unit loss in the biceps

brachii (BB), thenar, and extensor digitorum brevis (EDB) muscles have been reported (Brown et al., 1988; Galea, 1996). Brown et al. (1988) found subjects over the age of 60 had one half the number of motor units in the BB compared to those under 60 years old, however Galea et al. (1996) reported the BB was unaffected by age (Brown et al., 1988; Galea, 1996). Excitable fiber mass is reduced with age in the thenar, EDB, and BB muscles while SMUAP is not affected (Galea, 1996). The ratio of SMUAP to CMAP however is increased with age in these muscles (Galea, 1996).

Taken together, these findings indicate natural aging affects the neuromuscular system in the upper and lower extremities differently. Also, muscle deterioration caused by changes to muscle fibers and motor nerves differ between distal and proximal muscles, with distal muscles being more severely affected. Increased age is a risk factor of T2D, with increased incidence in older adults. T2D-associated neuromuscular dysfunction may present similarly to natural aging neuromuscular alterations, but may be more pronounced, especially in those older than sixty years.

Many consider T2D to be considered a CVD (Kannel & McGee, 1979). The "common soil" hypothesis states that T2D and CVD share many of the same antecedents, which include obesity, dyslipidemia, hypertension, and insulin resistance (Ceriello & Motz, 2004; Laakso, 1999; Stern, 1995). Stroke is another CVD that exhibits neuromuscular dysfunction of paretic muscles. The common soil hypothesis suggests stroke-associated neuromuscular changes may be similar to those experience found in pwT2D. Neuromuscular dysfunction associated with stroke is characterized by reduced number of motor units, reduced motor unit firing rates, reduced maximal CMAP amplitudes, and disordered motor unit recruitment (Dai et al., 2017; Dattola et

al., 1993; Gemperline et al., 1995; Hara et al., 2000; Hu et al., 2012, 2015; Li et al., 2015; Suresh et al., 2014). Specifically, these findings have been reported in two upper extremity muscles, the FDI and Abductor Pollicis Brevis (APB or thenar) (Hara et al., 2000; Hu et al., 2012). Neuromuscular dysfunction in this population is dependent on sensorimotor impairment such that patients with moderate to severe hemiplegic stroke being more affected than lose with minor impairment (Hara et al., 2000; Hu et al., 2012).

3 METHODS

3.1 Participants

3.1.1 Young Healthy Control Study Cohort

Thirteen (13) young and healthy controls (YHC) participated in an equipment and methods validation study. The inclusion criteria for the YHC group included: age 18-30 years and right-handed. Handedness was assessed with the Edinburgh Handedness Inventory, where laterality quotient (LQ) scores range from -100 (strong left handedness) to +100 (strong right handedness). Exclusion criteria included diagnosis of Type I Diabetes, diagnosis of Type II Diabetes, history of uncontrolled hypertension, history of limb amputation, chemotherapy, or neurological diseases (Alzheimer' Disease, Dementia, Huntington's Disease, Traumatic Brain Injury, Multiple Sclerosis, Parkinson's Disease, Paraproteinemic Demyelinating Neuropathy (PDN), Muscular Dystrophy, Carpal Tunnel Syndrome, Charcot-Marie-Tooth Disorder or other hereditary neuropathies), and pain in the extremities that limits activities of daily living. Participants were recruited from the greater Houston community. All subjects provided informed consent. This study was reviewed by the Institutional Review Board (IRB) at the University of Houston.

3.1.2 Main T2D Study Cohorts

Two groups were included in this portion of the study. Motor Unit Number Estimation of the first dorsal interosseous (FDI) from Allen, Kimpinski, Doherty, and Rice (2014) was used in a power analysis ($\mu_C = 107$, $\mu_{T2D} = 73$, $\sigma = 25$, $\alpha = 0.05$). This

analysis indicated that enrolling 13 participants in the T2D group would provide a power of 0.90. Thirteen (13) older adults with established T2D (T2D) and 12 age- and sex-matched control participants (Control) were recruited for this study. Inclusion criteria for the T2D groups consisted of: age 60 years and above, diagnosis of T2D, history of moderate range blood pressure (90/60 – 160/100 mmHg), and right-handed (assessed with the Edinburgh Handedness Inventory). For the Control group, inclusion criteria consisted of: 60 years and above, HbA_{1c} values in the range of 4.5 – 5.6%, history of normal blood pressure (90/60 – 120/80 mmHg), and right-handed. Exclusion criteria for both groups included diagnosis of T1D, diagnosis of T2D prior to age 18, history of uncontrolled hypertension, history of limb amputation, chemotherapy, or neurological diseases (Alzheimer' Disease, Dementia, Huntington's Disease, Traumatic Brain Injury, Multiple Sclerosis, Parkinson's Disease, Paraproteinemic Demyelinating Neuropathy (PDN), Muscular Dystrophy, Carpal Tunnel Syndrome, Charcot-Marie-Tooth Disorder or other hereditary neuropathies), and pain in the extremities that limits activities of daily living. Participants will be recruited from the greater Houston community. Participants for the T2D group were recruited through the American Diabetes Association (ADA) support groups in the area as well as a pool of participants currently involved in research with the CNBR. All subjects provided informed consent. This study was reviewed by the Institutional Review Board (IRB) at the University of Houston.

3.2 Protocol Overview

3.2.1 Young Healthy Controls

A methods verification study on young healthy controls was performed prior to the main study of this dissertation (see Table 3.1). Tests were performed during one two-hour session and took place at the Center for Neuromotor and Biomechanics Research (CNBR), Department of Health and Human Performance at the University of Houston (UH). The test session consisted of basic health questionnaire examinations, including a general medical history and the Edinburgh Handedness Inventory. Sensory function evaluation was performed using the Semmes-Weinstein Monofilament test and a biothesiometer. Motor function was evaluated through the performance of unilateral isometric muscle contractions of five upper extremity muscles (the Abductor Pollicis Brevis (APB), the Flexor Digitorum Superficialis (FDS), the Extensor Digitorum (ED), the Triceps Brachii (TB), and the Biceps Brachii (BB)) in addition to supramaximal nerve stimulation on each innervating nerve of the muscles of interest. All testing was performed on the dominant arm, as determined by the Edinburgh Handedness Inventory.

3.2.2 Main T2D Study

Testing was performed on two (2) different days, at the Center for Neuromotor and Biomechanics Research (CNBR), Department of Health and Human Performance at the University of Houston (UH). PwT2D and age-matched controls underwent the following two session protocol (see Table 3.1). The first testing session consisted of basic health questionnaire examinations, including general medical history, blood

pressure measurement, HbA_{1c} and cholesterol testing, and the Edinburgh Handedness Inventory. Following basic health examinations, sensory function was assessed using the Semmes-Weinstein Monofilament test and a biothesiometer. Motor function was evaluated through performance of unilateral isometric muscle contractions of five upper extremity muscles (APB, FDS, ED, TB, and BB) at varying levels of force (5%, 15%, 25%, 50%, 75%, and maximal) in addition to supramaximal nerve stimulation on each innervating nerve of the muscles of interest. Testing session one lasted approximately two hours. The second testing session consisted of a proprioceptive evaluation using a reach to pinch task in a motion analysis testing setup. All testing was performed on the dominant (right) arm, as determined by the Edinburgh Handedness Inventory.

Table 3.1 *List of testing conditions protocol by study cohort.*

	Young Healthy Control Study (Young, healthy controls)	**Main T2D Study (T2D and age- & sex-matched controls)**
Session 1	General medical history Edinburgh Handedness Inventory **Sensory Evaluation:** Tactile sensation, Vibrotactile sensation **Neuromuscular Evaluation:** Nerve Stimulation, Isometric contractions	General medical history Blood pressure, HbA1c, Cholesterol Edinburgh Handedness Inventory **Sensory Evaluation:** Tactile sensation, Vibrotactile sensation **Neuromuscular Evaluation:** Nerve Stimulation, Isometric contractions
Session 2	No Session 2 Testing	**Proprioceptive Evaluation:** Reach-to-Pinch Task

3.3 Sensory Evaluation

3.3.1 Tactile sensation

3.3.1.1 Young Healthy Controls

Tactile sensation of the dominant (right) hand was evaluated using the Semmes-Weinstein Monofilament test. The Semmes-Weinstein Monofilament test is an assessment of tactile function that uses monofilaments of various thickness and diameter to detect sensory loss of the hands and feet. Tactile sensation was tested at the tip of digit 1, digit 2, and digit 5, the hypothenar eminence, and the dorsum thenar of the right hand, as shown in Figure 3.1. Digit 1 and 2 have sensory innervations from the median nerve. Digit 5 and the hypothenar eminence have sensory innervations from the ulnar nerve and the dorsum has sensory innervation from the radial nerve.

Outcome measure: Tactile threshold (g)

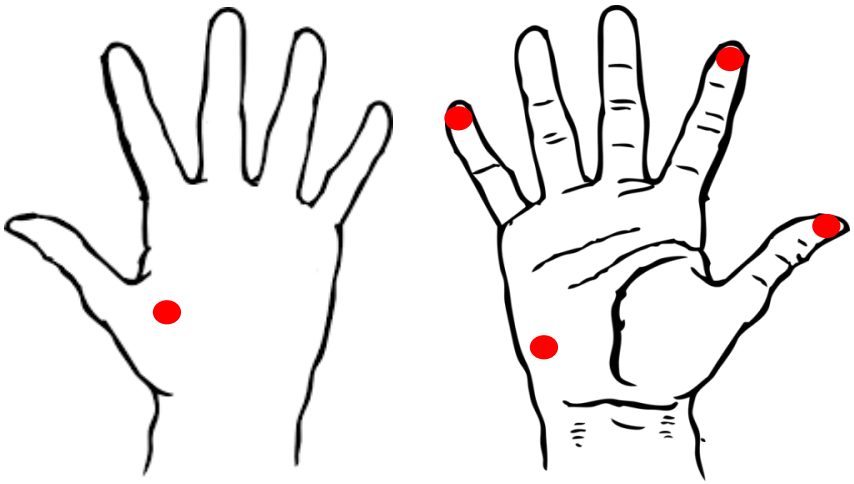

Figure 3.1 *Monofilament and Biothesiometer testing locations. On the top side of the hand: dorsum thenar. On the palm side of the hand: tip of digit 1, tip of digit 2, tip of digit 5, and the hypothenar eminence.*

3.3.1.2 Main T2D Study

The data collection process was the same as the EMG validation study, see previous section of text for full description. Additionally, tactile function of the dominant (right) foot was assessed using the Semmes-Weinstein Monofilament test. Testing sites, shown in Figure 3.2, include: 1st metatarsal head and distal hallux, 3rd metatarsal head and distal toe, 5th metatarsal head and distal toe, and distal heel pad. The 1st distal hallux, 1st metatarsal head, distal 3rd toe, and 3rd metatarsal head all have sensory innervations from the medial plantar nerve. The lateral plantar nerve innervates the distal 5th toe and 5th metatarsal head. The distal heel pad has sensory innervation from the tibial nerve.

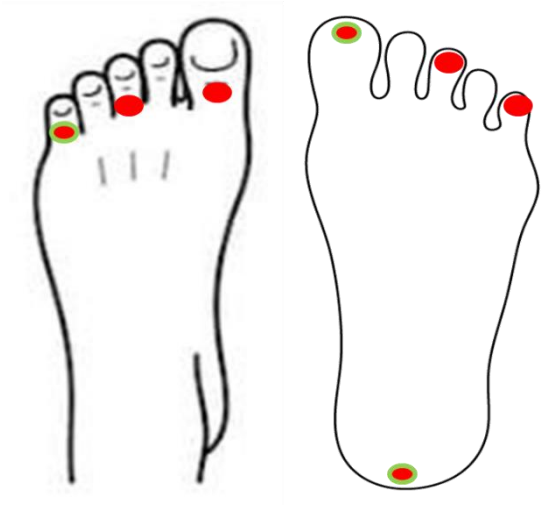

Figure 3.2 *Monofilament (in red) and Biothesiometer (in green) testing locations. On the top side of the foot:1st metatarsal head, 3rd metatarsal head, and 5th metatarsal head. On the bottom side of the foot: 1st distal hallux, 3rd distal toe, 5th distal toe, and distal heel pad.*

3.3.2 Vibrotactile Evaluation

3.3.2.1 Young Healthy Controls

Vibrotactile threshold of the dominant hand was evaluated using a biothesiometer device (BioMedical, Newbury, Ohio, USA). Vibrotactile threshold was evaluated at the same five sites used for Monofilament testing, see Figure 3.1. The vibration amplitude of the biothesiometer is gradually increased until the threshold of vibration perception is reached. If vibration was felt at the testing site, the participant indicated so with a verbal response. The voltage reading on the biothesiometer at this time was then recorded and converted to an amplitude. Next, the vibration amplitude was gradually reduced until the participant was no longer able to perceive it. This was performed three times per test site (one in increasing stimulation order, one in decreasing stimulation order, and one additional in increasing stimulation order). An average of the three values as indicated by study participants was used as the vibrotactile threshold.

Outcome measure: Vibrotactile threshold (μm)

3.3.2.2 Main T2D Study

The data collection process was the same as the EMG validation study, see previous section of text for full description. Additionally, vibrotactile threshold of the dominant foot was assessed using the same protocol as the hand. Testing sites include the 1st distal hallux, 5th metatarsal head, and distal heel pad, shown in Figure 3.2 in green.

3.3.3 Proprioceptive Evaluation

3.3.3.1 Young Healthy Controls

The participants in the equipment validation study did not perform the reach-to-pinch task.

3.3.3.2 Main T2D Study

Proprioceptive evaluation was performed during the second test session. Reflective markers were placed on the surface of the right hand and arm to acquire thumb (digit 1), index finger (digit 2) and wrist, and elbow kinematic data. Marker placement was inspired by Nataraj et al. (2014; 2015). One marker each was placed on the very distal tip of the distal phalanx of digit 1 and the very distal tip of the distal phalanx of digit 2. Two markers were placed on each side of the 1st carpometacarpal (CMC), 1st metacarpophalangeal (MCP1), 1st interphalangeal (IP1), 2nd metacarpophalangeal (MCP2), 2nd proximal interphalangeal (PIP2), and 2nd distal interphalangeal (DIP2) joints. A marker cluster was placed on the second metacarpal as a local hand reference coordinate system (G. Wu et al., 2005). Markers were also placed on the right acromioclavicular joint, the lateral epicondyle, and the radial and ulnar styloid processes. See Figure 3.3 for marker placement.

A VICON motion capture system was used to collect the three-dimensional positions of the reflective markers at 100 Hz (VICON, Oxford Metrics, Oxford, UK). Joint angles trajectories were collected for the CMC, MCP1, IP1, MCP2, PIP2, DIP2, flexion/extension and abduction/adduction of the wrist, and flexion/extension of the elbow.

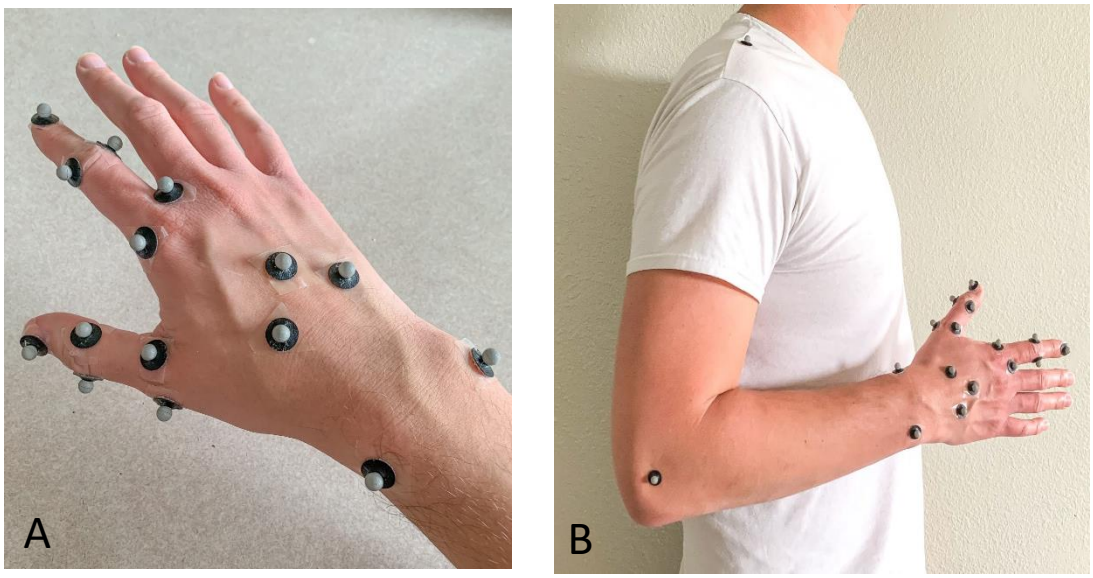

Figure 3.3 *A) Close up view of hand marker placement. 6.4 mm spherical reflective marker placement. One marker at the very tip of the distal phalanx of digit 1 and the very tip of the distal phalanx of digit 2. Two markers were placed on each side of the CMC, MCP1, IP1, MCP2. PIP2, and DIP2 joints. A "T" shaped marker cluster on the second metacarpal to be used as a local hand reference coordinate system. B) Marker placement of the right arm and hand. In addition to hand markers, markers are located at right acromioclavicular joint, the lateral epicondyle, and the radial and ulnar styloid processes.*

Subjects were asked to perform a reach-to pinch task with the thumb and index finger of their dominant (right) hand without visual feedback of the reaching hand but allowing for visualization of a spherical virtual target (Nataraj et al., 2014). While seated at a table, subjects sat to the left side of a high-resolution vertical mirror inserted into a custom made set up (Figure 3.4). The mirror blocked their view of the right side of the table. Also on the left side of the center of the mirror was a target placed on a stand (2 cm diameter, 10 cm from the surface of the table, 20 cm horizontally from the mirror, 20 cm from the edge of the table). From their seated position, the subject was able to view the target in the mirror, known as a "virtual

target". Subjects were asked to treat the virtual target in the mirror as if it were a real target that could be grasped with the right hand. The subject's reaching hand was located behind the mirror (the right side of the table set up), which prevented visual feedback. Additionally, the subject wore a right-side blinder to further prevent visual movement feedback of the upper arm during reaching.

An auditory metronome was used to control for speed across subjects. Subjects heard beeps at a frequency of 1 Hz throughout the experiment protocol. For each trial, they were asked to begin movement on a beep and finish the maneuver by the following beep. Each trial lasted for approximately 2 seconds, with a few seconds rest between trials. Subjects performed a total of 30 consecutive reach-pinch trials.

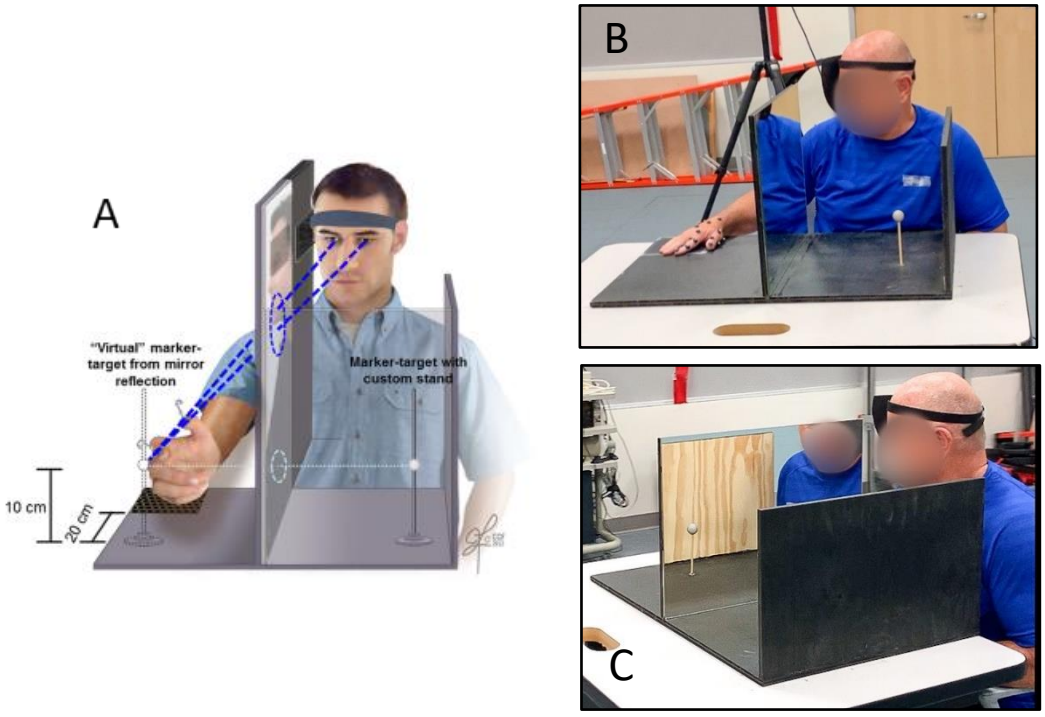

Figure 3.4 *A) Set up of Reach-to-Pinch task. Subjects viewed a virtual target in a mirror while performing a reach-to-pinch movement with their hand. The maneuver is performed without visual feedback of the moving arm and hand. Image from* Nataraj, R., Evans, P. J., Seitz, W. H., & Li, Z. M. (2014). Effects of carpal tunnel syndrome on reach-to-pinch performance. *PLoS ONE*, 9(3), 1–9. *B)Our study set up, front view. C) Our study set up, view of virtual target.*

Digit thickness was measured using calipers for both the thumb and index finger. This thickness was then used as the radius for a spherical model of the digit-pad and aperture. Pinch contact between the thumb and index finger was assumed to occur when inter-pad distance was below the threshold of 1 mm (Nataraj et al., 2014; Nataraj & Li, 2015).

Outcome measures: pinch contact location (aperture), path length (distance traveled by digit 1 and digit 2 distal tip of phalanges), trajectory variability, and joint angles (digit 1 interphalangeal joint, digit 1 metacarpophalangeal joint, digit 2 distal interphalangeal joint, digit 2 proximal interphalangeal joint, digit 2 metacarpophalangeal joint, radioulnar deviation, flexion/extension and abduction/adduction of the wrist, and flexion/extension of the elbow), accuracy error and accuracy precision.

3.4 EMG Recording

3.4.1 Young Healthy Controls

Multichannel surface electromyography (sEMG) was recorded from each muscle using a bioamplifier (FE234 Quad BioAmp, ADInstruments, Colorado Springs, CO, USA) and PowerLab data acquisition system (PowerLab 8/35, ADinstruments, Colorado Springs, CO, USA). Prior to attaching electrodes, the skin was cleaned with alcohol. The longitudinal axis of each of the muscles (APB, ED, FDS, TB and BB) was identified via palpation. Two monopolar recording electrodes were placed on the muscle belly, along the longitudinal axis of the muscle that is

underwent testing. A reference electrode was placed on a bony process located proximally to the muscle being tested while a ground electrode was placed distally. sEMG data was acquired using LabChart software (ADInstruments, Colorado Springs, CO, USA).

3.4.2 Main T2D Study

The sEMG set up was the same as the YHC study, see previous section of text for full description.

3.5 Neuromuscular Evaluation

3.5.1 Young Healthy Controls

3.5.1.1 MUNIX

3.5.1.1.a Nerve Stimulation and CMAP

Maximum CMAP for each muscle was obtained by supra-maximal stimulation of the innervating nerve (APB: median; FDS: median; ED: radial; TB: radial; BB: musculocutaneous), with a DS7A muscle current stimulator (Digitimer, United Kingdom). Stimulation intensity generally started around 5-30 mA and was increased in increments of approximately 20% until a maximal response was reached. The duration for a single pulse stimulation was 200 μs. The nerve was then be stimulated with 120% of the final intensity to confirm a maximum CMAP is reached and confirmed visually.

3.5.1.2b Isometric Contractions and surface EMG interference pattern (SIP)

While seated, YHC subjects performed three Maximum Voluntary Contraction (MVC) trials, with one minute of rest between trials. The highest MVC force was used to determine the target forces for the submaximal contraction trials. After MVC trials, subjects were asked to perform 30 second submaximal isometric contractions each at 5, 15, 25, 50, and 75% MVC. Force produced by the subject was used as visual feedback to maintain the contraction level. This testing procedure was performed for all five muscles (APB, ED, FDS, TB, and BB). The surface EMG interference pattern was recorded throughout each contraction at the varying levels of force. Motor unit number index (MUNIX) is a MUNE technique used to estimate a numerical index value relating to the number of motor units contained in a muscle and is performed using electromyography and supramaximal nerve stimulation. Though there are numerous techniques to estimate MUNE, MUNIX method uses the maximum CMAP and SIP recordings during voluntary isometric muscle contractions, see equations 2.1, 2.2, and 2.3. CMAP is representative of the size of the entire motor unit pool of the muscle while SIP is representative of whole muscle motor unit activity.

Additionally, motor unit size was estimated by calculating the motor unit size index (MUSIX), which is derived using MUNIX and CMAP values, shown in equation 3.1.

$$MUSIX = \frac{CMAP\ Amplitude}{MUNIX} \qquad (3.1)$$

Outcome measure: maximum CMAP amplitude, MUNIX, and MUSIX for each muscle

3.5.2 Main T2D Study

3.5.2.1 MUNIX

3.5.2.1.a Nerve stimulation

The data collection process was the same as the YHC study, see previous section of text for full description.

3.5.2.2.a Isometric Contractions and SIP

The data collection process was the same as the YHC study, see previous section of text for full description.

3.6 Statistical Analysis

SPSS version 26.0 (SPSS IBM, New York, NY, U.S.A) was used to perform statistical analysis.

3.6.1 Young Healthy Control Study

Sensory and neuromuscular data was analyzed using two-way ANOVAs with the level of significance set at $p \leq 0.05$. *Nerve* (median, ulnar, radial) was established as a within-subject factor for sensory data (tactile threshold and vibration perception threshold). Due to non-linearity, monofilament data were log transformed. Within-subject factor for neuromuscular evaluation was *Muscle* (APB, ED, FDS, TB, and BB) for the measures of CMAP, MUNIX, and MUSIX.

3.6.2 Main T2D Study

For all Main T2D study out measures, Automatic Linear Modeling (ALM) was used to select significant covariates from Health Status data using forward stepwise selection. Potential covariates included HbA_{1c}, blood pressure (systolic and diastolic, cholesterol (Total, HDL, LDL), duration of diagnosis (in months), PN status (via indicator variable), prediabetes status (via indicator variable), BMI, age, laterality quotient, and thumb and index finger size (for kinematic analysis only).

For sensory evaluation, monofilament data was log transformed due to non-linearity. Two- way analyses of covariance (ANCOVAs) were used to compare tactile threshold and vibration perception threshold of the hand and foot between *Groups* (T2D and Control). *Nerve* (for hand: median, radial, ulnar nerves; for foot; medial plantar, lateral planta, tibia nerves) was a within-subjects factor.

Neuromuscular data (CMAP, MUNIX, MUSIX) was analyzed using two-way ANCOVAs to compare between *Groups* (T2D and Control). *Muscle* (APB, ED, FDS, TB, and BB) was a within-subjects factor.

Similarly, kinematic data was analyzed using two-way ANCOVAs to compare between *Groups.* Data included mean trajectory values for each joint of interest in extension/flexion and abduction/adduction, trajectory pathlength of the hand, index finger, and thumb, mean aperture, and pinch contact location and precision.

4 RESULTS

4.1 Participants

A total of thirty-eight (38) adults took part in this study. Of those 38 participants, a cohort of thirteen young and healthy controls (24.5 ± 4.0 years old; 5 male, 8 female) participated in the equipment and methods validation leg of this dissertation, see Table 3.1. All YHC participants were right-handed determined via self-report and handedness laterality quotient was determined by the Edinburgh Handedness Inventory. YHC participants were excluded if they reported a history or diagnosis of neurological or musculoskeletal diseases including: T1D, T2D, hypertension, limb amputation, chemotherapy, Alzheimer' Disease, Dementia, Huntington's Disease, Traumatic Brain Injury, Multiple Sclerosis, Parkinson's Disease, Paraproteinemic Demyelinating Neuropathy (PDN), Muscular Dystrophy, Carpal Tunnel Syndrome, Charcot-Marie-Tooth Disorder or other hereditary neuropathies. YHC cohort characteristics can be found in Table 4.1.

Thirteen pwT2D (69.6 ± 6.7 years old; 7 male, 6 female) and twelve age- and sex-matched controls (68.1 ± 4.4 years old; 6 male, 6 female) participated in the Main T2D study, see Table 3.1. Inclusion for the T2D experimental group required diagnosis of T2D. Age- and sex-matched Control participants were excluded if they reported diagnosis of T2D. Participants were excluded from both groups if they reported diagnosis of T1D, diagnosis of T2D prior to age 18, history of uncontrolled hypertension, limb amputation, chemotherapy, or neurological diseases such as Alzheimer' Disease, Dementia, Huntington's Disease, Traumatic Brain Injury,

Multiple Sclerosis, Parkinson's Disease, Paraproteinemic Demyelinating Neuropathy (PDN), Muscular Dystrophy, Carpal Tunnel Syndrome, Charcot-Marie-Tooth Disorder or other hereditary neuropathies. All participants in the Main T2D study cohort were right-handed determined via self-report and handedness laterality quotient was determined by the Edinburgh Handedness Inventory. Main T2D cohort characteristics can be found in Table 4.1.

Table 4.1: *Study participant characteristics and health data*

	YHC	**pwT2D**	**Controls**
N	13	13	12
Age (y)	24.5 ± 4.0	69.6 ± 6.8	68.1 ± 4.5
BMI (kg/m^2)	23.8 ± 3.0	33.9 ± 7.2	30.6 ± 11.3
HbA_{1c} (mmol/L)	--	6.4 ± 0.7	5.6 ± 0.5
# females	8	6	6
LQ	80 ± 18	88 ± 12	92 ± 12
SYS (mmHg)	--	133 ± 22	139 ± 15
DIA (mmHg)	--	70 ± 11	78 ± 11
PN	--	4	0
Prediabetic	--	7	4
Disease duration (months)	--	104.3 ± 85.1	-
TC (mg/dL)	--	164 ± 27	194 ± 50
HDL (mg/dL)	--	55 ± 14	59 ± 9
LDL (mg/dL)	--	83 ± 18	105 ± 39

Values are mean ± SD or count. The entire study sample is 38 participants. HbA_{1c} = glycated hemoglobin, BMI = body mass index, DIA = diastole, HDL = high-density lipoprotein, LDL = low-density lipoprotein, LQ = laterality quotient, PN = peripheral neuropathy, SYS = systole, TC = total cholesterol

4.2 Health Data

4.2.1 Young Healthy Controls

Health status data was not collected for the YHC group, aside from BMI, see Table 4.1 above.

4.2.2 Main T2D Study

Using a commercially available wrist-worn device (Omron Intellisense 10 series Blood Pressure Monitor, Model BP785, Bannockburn, IL, USA), blood pressure was measured for the 25 participants in the Main T2D Study. Glycated hemoglobin (HbA_{1c}) values were measured using an A_{1c} Now+ kit (PTS Diagnostics, Indianapolis, IN, USA), which is also commercially available. Prediabetes status was determined via self-report, if the participant had ever been diagnosed as prediabetic by a physician. Total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were assessed using an at home analyzer device (CardioChek Cholesterol Analyzer, PTS Diagnostics, Indianapolis, IN, USA). Peripheral neuropathy status (PN) was determined by clinical examination or EMG/NCV testing (per physician). See Table 4.1 for health status average characteristics

4.3 Sensory Evaluations

4.3.1 Semmes-Weinstein Monofilament Test

Tactile sensation of the hand was assessed using the Semmes-Weinstein Monofilament test, with which tactile thresholds were detected. Nerves of interest included the median, radial, and ulnar nerves. Sites innervated by these nerves

included digit 1, digit 2, digit 5, hypothenar eminence, and dorsum of the right hand. Monofilament data was averaged across each nerve; digit 1 and digit 2 for median nerve, digit 5 and hypothenar for ulnar nerve, and dorsum for radial nerve. Tactile sensation of the foot was assessed in the T2D and age- and sex-matched control cohorts as well. Nerves of interest included the medial plantar, lateral planter and tibial nerves. Sites tested were the 1st distal hallux and metatarsal head, distal 3rd toe and metatarsal head, distal 5th toe and metatarsal head, and distal heal pad or the right foot. Similar to the hand sensitivity data, foot tactile threshold perception data was averaged across each nerve of interest; 1st distal hallux and metatarsal head, distal 3rd toe and metatarsal head for medial plantar nerve, distal 5th toe and metatarsal head for lateral plantar nerve, and distal heal pad for tibial nerve. Due to non-linearity of monofilament data, all monofilament data were log transformed (Log_{10}(Force(g)).

4.3.1.1 Young Healthy Controls

A one-way ANOVA was performed on Log_{10}Force data with *Nerve* (median, ulnar, radial) as a between-subjects factor. There was significant main effect of *Nerve* ($F_{2,38} = 3.60$, $p < 0.05$). Fischer's Least Significant Difference (LSD) post-hoc analysis revealed a significant difference in tactile threshold sensitivity of the median nerve compared to the radial nerve ($p < 0.05$) and in the ulnar nerve compared to the radial nerve ($p < 0.05$). There was not a significant difference in tactile threshold sensitivity between the median and ulnar nerves ($p = 1.00$). YHC Log_{10}Force values can be found in Table 4.2, below. Non-transformed monofilament force data are shown in Figure 4.1a.

Table 4.2: *YHC Hand Sensitivity Evaluation Data*

	Log_{10}(Force(g)	**Avg VPT Amp (µm)**
Nerve		
Median	-1.155 ± 0.053^{+}	0.101 ± 0.015
Ulnar	-1.155 ± 0.053*	0.106 ± 0.015
Radial	-0.98 ± 0.053*$^{+}$	0.084 ± 0.015
Mean	-1.097 ± 0.031	0.097 ± 0.009

Mean ± SE reported.
* denotes significant post hoc pairwise comparison, $p < 0.05$
$^{+}$ denotes significant post hoc pairwise comparison, $p < 0.05$

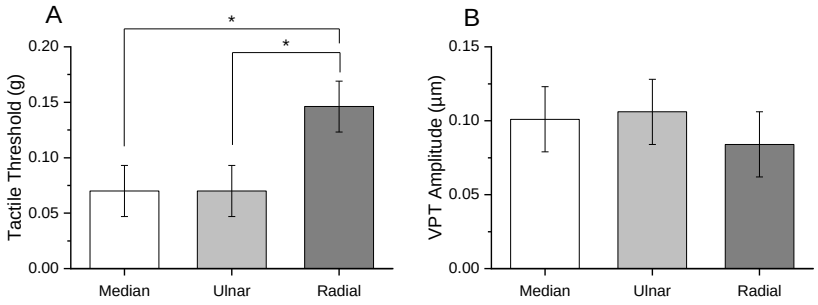

Figure 4.1 *A) Non-transformed YHC Semmes-Weinstein monofilament tactile sensory function data B) YHC Biothesiometer Vibration perception threshold data. Means ± SE shown for the nerves of the hand. * denotes p < 0.05 for log force transformed data.*

4.3.1.2 Main T2D Study

4.3.1.2.a Hand Tactile Threshold

Monofilament data was log transformed prior to statistical analysis. Automatic Linear Modeling (ALM) was used to determine covariates to include in the model. ALM did not indicate any covariates for the transformed Log_{10}Force data. A two-way ANOVA was performed on Log_{10}Force data with *Group* (T2D and Control) and *Nerve* (median, ulnar, radial) as between factors. Neither *Group* ($F_{1,69} = 1.063$, $p = 0.306$) nor *Nerve* ($F_{2,69} = 0.417$, $p = 0.660$) indicated significant differences in tactile perception

threshold between pwT2D and healthy, age-matched controls. There was not a significant *Group x Nerve* ($F_{2,69} = 1.683$, $p = 0.193$) interaction, either. Non-transformed monofilament group data averaged across nerves is reported in Figure 4.2a for ease of understanding. Additionally, non-transformed group mean tactile threshold values of the median, ulnar and radial nerves are shown below in Figure 4.3a.

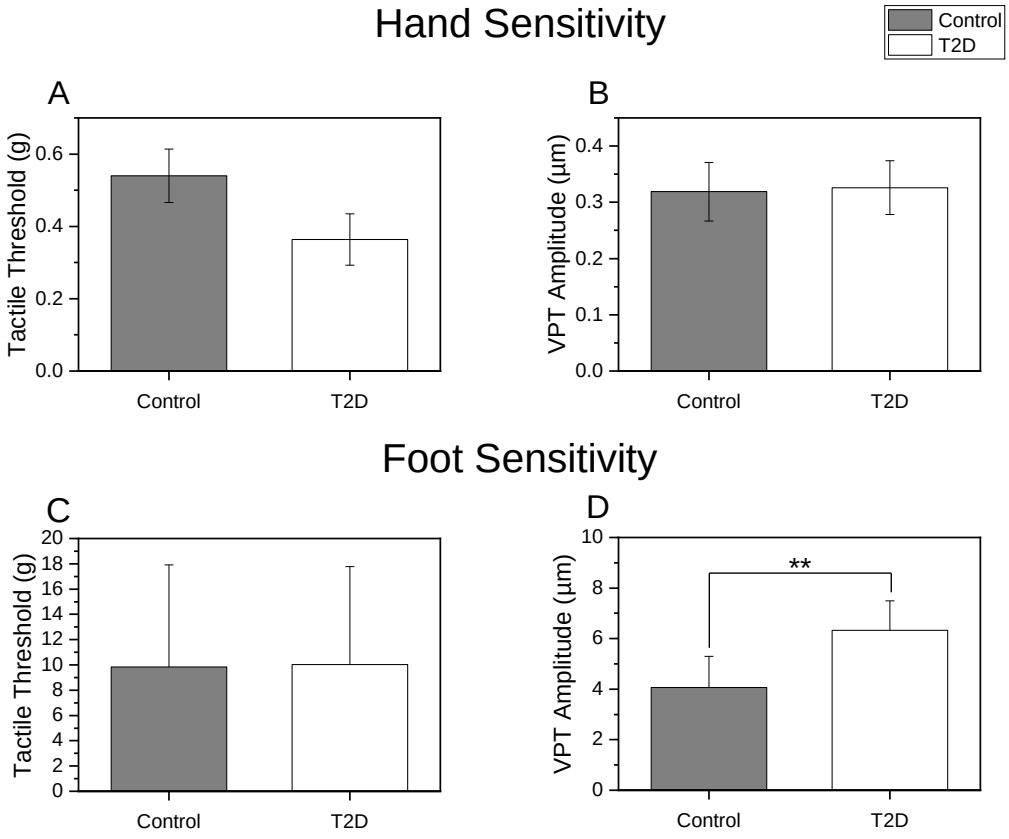

Figure 4.2 *A) Non-transformed Semmes-Weinstein monofilament tactile sensory function data for the right hand B) Biothesiometer Vibration perception threshold data for the right hand C) Non-transformed Semmes-Weinstein monofilament tactile sensory function data for the right foot D)) Biothesiometer Vibration perception threshold data for the right foot. Group Means ± SE reported. Control data in grey, T2D data in white. ** denotes p < 0.05 without covariates in the statistical model; with covariates the Group difference is no longer significant)*

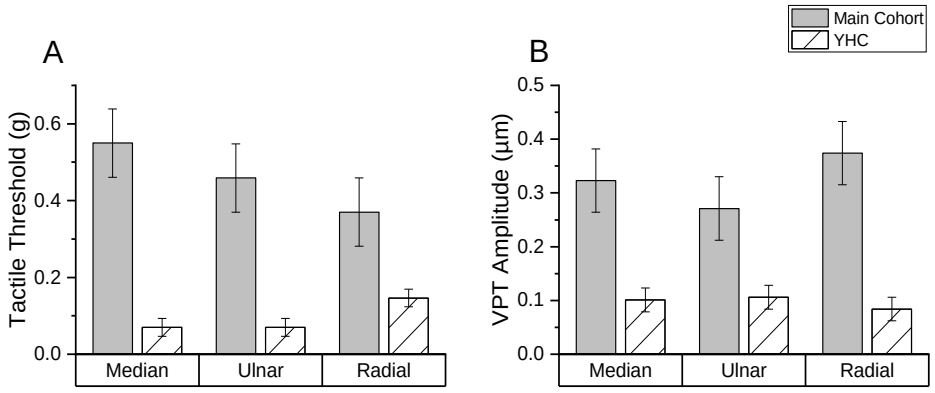

Figure 4.3 *A) Non-transformed Semmes-Weinstein monofilament tactile sensory function data B) Biothesiometer Vibration perception threshold data. Group Mean ± SE shown for nerves of the hand. Main cohort data is in grey and YHC data is striped (for comparison).*

4.3.1.2.b Foot Tactile Threshold

Foot monofilament data was log transformed and a two-way ANOVA was performed with between factors *Group* (T2D and Control) and *Nerve* (medial plantar, lateral plantar, tibial). ALM modeling did not identify significant covariates. Statistical analysis indicated a significant effect of *Nerve* ($F_{2,59} = 13.805$, $p < 0.001$) on foot tactile perception threshold, shown in Figure 4.4a such that the tibial nerve had a significantly higher tactile perception threshold compared to the medial plantar ($p < 0.001$) and lateral plantar ($p < 0.001$) nerves. *Group* ($F_{1,59} = 0.407$, $p = 0.526$) and *Group x Nerve* ($F_{2,59} = 1.02$, $p = 0.367$) were not significant, Figure 4.2c.

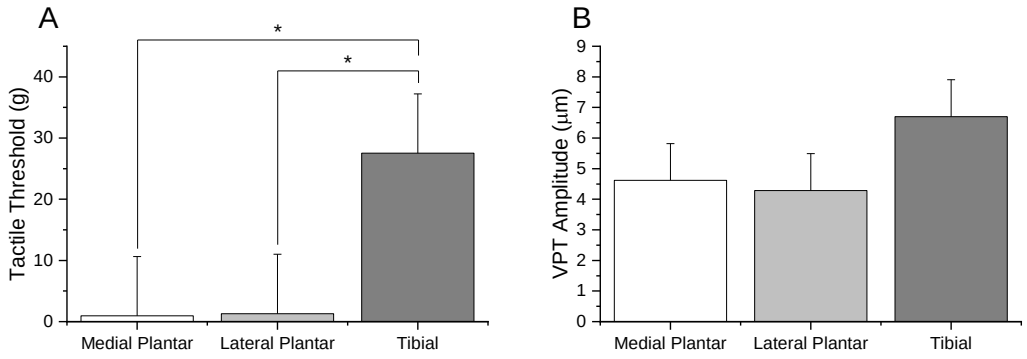

Figure 4.4 *A) Non-transformed Semmes-Weinstein monofilament tactile sensory function data across both T2D and Control groups B) Biothesiometer Vibration perception threshold data across both T2D and Control groups. Means ± SE shown for the nerves of the foot. * denotes $p < 0.001$ for log force transformed data.*

4.3.2 Vibrotactile sensation

Vibration perception of the hand was assessed using a Biothesiometer device (BioMedical, Newbury, Ohio, USA). Similar to tactile perception testing, nerves of interest included the median, radial, and ulnar nerves. Sites innervated by these nerves included digit 1, digit 2, digit 5, hypothenar eminence, and dorsum of the right hand. Vibration perception data was averaged across each nerve; digit 1 and digit 2 for median nerve, digit 5 and hypothenar for ulnar nerve, and dorsum for radial nerve. Vibration perception of the foot was assessed in the T2D and age and sex-matched control cohort as well. Nerves of interest included the medial plantar, lateral planter and tibial nerves. Sites tested were the 1st distal hallux (medial plantar nerve), 5th metatarsal head (lateral plantar nerve), and distal heal pad of the right foot (tibial nerve).

4.3.2.1 Young Healthy Controls

Ten of the thirteen YHC participants underwent hand vibration perception testing. A one-way ANOVA was performed on Vibration Perception Threshold average amplitude (AvgVPTAmp) data with *Nerve* (median, ulnar, radial) as a between-subjects factor. No main effects were found. Mean vibration perception threshold amplitudes were 0.101 ± 0.042, 0.106 ± 0.063, and 0.084 ± 0.037 μm for the median, ulnar and radial nerves, respectively (see Figure 4.1b, above).

4.3.2.2 Main T2D Study

4.3.2.2.a Hand Vibration Perception Threshold

ALM results did not identify significant covariates for hand VPT amplitude thus, a two-way ANOVA was performed on Vibration Perception Threshold with between factors of *Group* (T2D and Control) and *Nerve* (median, ulnar, radial). No main effects of *Group* ($F_{1,69}$ = 0.101, p = 0.751), *Nerve* ($F_{2,69}$ = .828, p = 0.441), nor interactions were found in hand vibration perception threshold. The mean value for vibration perception threshold amplitude of the right hand was 0.326 ± 0.247 and 0.319 ± 0.340 μm for the T2D and Control groups, respectively (figure 4.2b). Mean vibration perception threshold amplitudes were 0.323 ± 0.314, 0.271 ± 0.33, and 0.374 ± 0.222 μm for the Median, Ulnar and Radial nerves, as shown in figure 4.3b.

4.3.2.2.b Foot Vibration Perception Threshold

ALM results determined *Age* (t = 2.536, $p < 0.05$) and *PN* (t = 2.127, $p < 0.05$) to be significant covariates in the model. A two-way ANCOVA was performed on Vibration Perception Threshold for the right foot with between factors of *Group* (T2D and Control) and *Nerve* (medial plantar, lateral plantar, tibial) with *Age* and *PN* as covariates. There were no significant main effects or interactions in vibration perception threshold in the foot (*Group:* $F_{1,67} = 1.655$, $p = 0.203$; *Nerve:* $F_{2,67} = 1.289$, $p = 0.282$; *Group x Nerve*: $F_{2,67} = 1.857$, $p = 0.164$). Group mean values for vibration perception threshold amplitude of the right foot are shown in Figure 4.2d. However, when covariates are not included in the model, there is a significant *Group* ($F_{1,69} = 6.671$, $p < 0.05$) difference in foot vibration perception threshold such that pwT2D have a higher threshold for vibration perception. Mean vibration perception threshold amplitudes for the medial plantar, lateral plantar, tibial nerves are shown in Figure 4.4b. Pairwise comparisons did not reveal significant differences between lower limb nerves across both groups. Pairwise comparisons of each lower limb nerve between experimental groups, shown in Figure 4.5b, revealed a significant difference in VPT in the Tibial nerve ($p < 0.05$), though this was only significant without covariates.

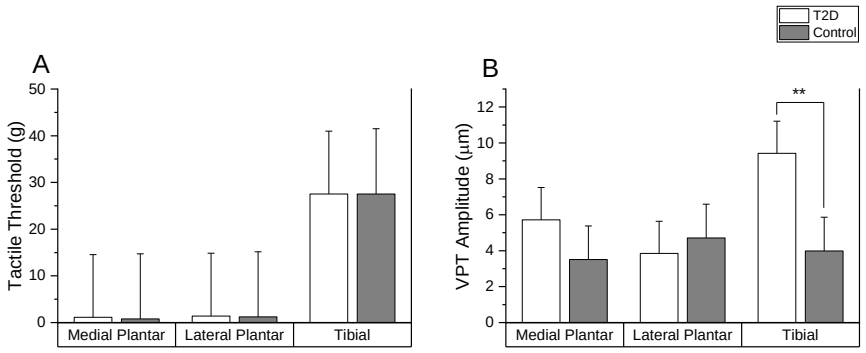

Figure 4.5 *A) Non-transformed Semmes-Weinstein monofilament tactile sensory function data B) Biothesiometer Vibration perception threshold data. Group Mean ± SE shown for nerves of the foot. T2D data is in white and Control data is dark grey. ** denotes $p < 0.05$ without covariates in the statistical model; with covariates the Group difference is no longer significant)*

4.4 Neuromuscular Data

Neuromuscular properties of five muscles in the right upper extremity (BB, TB, ED, FDS, and APB) were evaluated using the MUNIX method. Maximum CMAP amplitude for each muscle was obtained by supra-maximal stimulation of its innervating nerve. Surface EMG was then recorded while performing various levels of contractions. Motor unit size and number were estimated for each muscle using MUSIX and MUNIX calculations.

4.4.1 Young Healthy Controls

4.4.1.1 Motor Unit Number Estimation

4.4.1.1.a CMAP

A one-way ANOVA was performed for maximum CMAP amplitude with *Muscle* (BB, TB, ED, FDS, and APB) as the between factor. *Muscle* ($F_{4,34} = 2.484$, $p =$

0.065) was not found to be significant. However, pairwise comparisons revealed a significant difference in max CMAP amplitude between the FDS and BB muscles ($p < 0.05$) as well as the TB and FDS muscles ($p < 0.05$), see Figure 4.6. Maximum CMAP amplitude approached significant between the APB and TB muscles ($p = 0.059$).

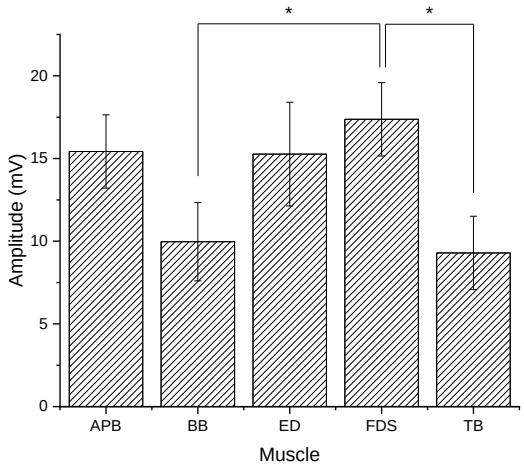

Figure 4.6 *YHC maximal compound muscle action potential (CMAP) amplitude for the abductor pollicis brevis (APB), biceps brachii (BB), extensor digitorum (ED), flexor digitorum superficialis (FDS), triceps brachii (TB) muscles. M ± SE reported. * denotes p < 0.05.*

4.4.1.1.b MUNIX

A one-way ANOVA was performed for MUNIX in the YHC group, with *Muscle* (BB, TB, ED, FDS, and APB) as the between factor. There was not a significant main effect of *Muscle* ($F_{4,34} = 2.484$, $p = 0.236$). Pairwise analysis did not reveal any significant differences between pairs of muscles, see Figure 4.7.

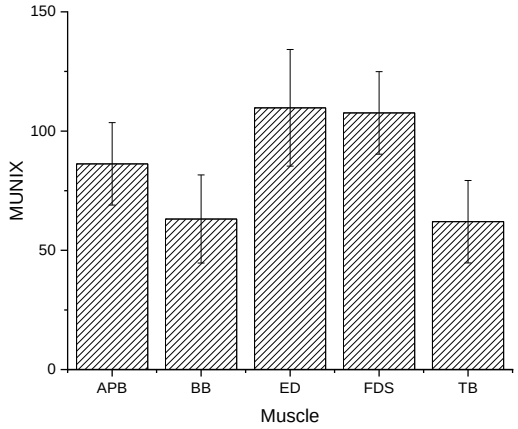

Figure 4.7 *YHC Motor Unit Number Index (MUNIX) for the APB, BB, ED, FDS, and TB muscles. M ± SE reported.*

4.4.1.1.c MUSIX

A one-way ANOVA was performed for MUSIX in the YHC group, with *Muscle* (BB, TB, ED, FDS, and APB) as the between factor. There was not a significant main effect of *Muscle* ($F_{4,34} = 2.484$, $p = 0.690$). Pairwise analysis did not reveal any significant differences between pairs of muscles (Figure 4.8).

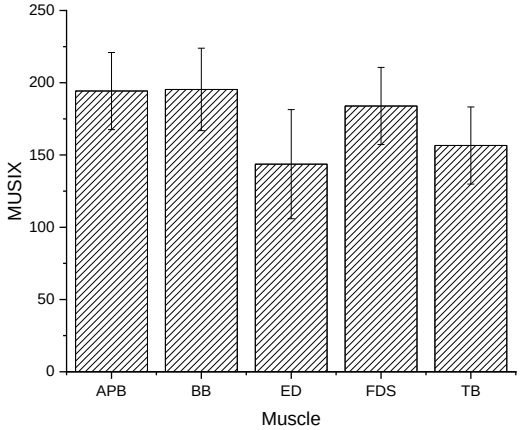

Figure 4.8 *YHC Motor Unit Size Index (MUSIX) for the APB, BB, ED, FDS, and TB muscles. M ± SE reported.*

4.4.2 Main T2D Study

4.4.2.1 Motor Unit Number Estimation

4.4.2.1.a CMAP

A two-way ANOVA was performed for maximum CMAP amplitude with *Group* (T2D and Control) and *Muscle* (BB, TB, ED, FDS, and APB) as between factors. *Group* ($F_{1,78} = 6.23$, $p < 0.05$) was found to be significantly different such that T2D (11.56 ± 0.698 mV) had significantly larger overall max CMAP amplitude compared to Controls (9.831 ± 0.712 mV). However, there was not a significant main effect of *Muscle* ($F_{1,78} = 1.37$, $p = 0.251$). ALM modeling indicated *Diastolic blood pressure* as a covariate in the CMAP amplitude model. A two-way ANCOVA was performed with *Group* and *Muscle* as between factors and *diastolic blood pressure* (t = -2.50, $p < 0.05$) as a covariate. Again, *Group* ($F_{1,77} = 9.21$, $p < 0.005$) was found to be significant while *Muscle* ($F_{1,77} = 1.37$, $p = 0.251$) was not. Maximum CMAP amplitude data is shown in Figure 4.9a and Figure 4.9b.

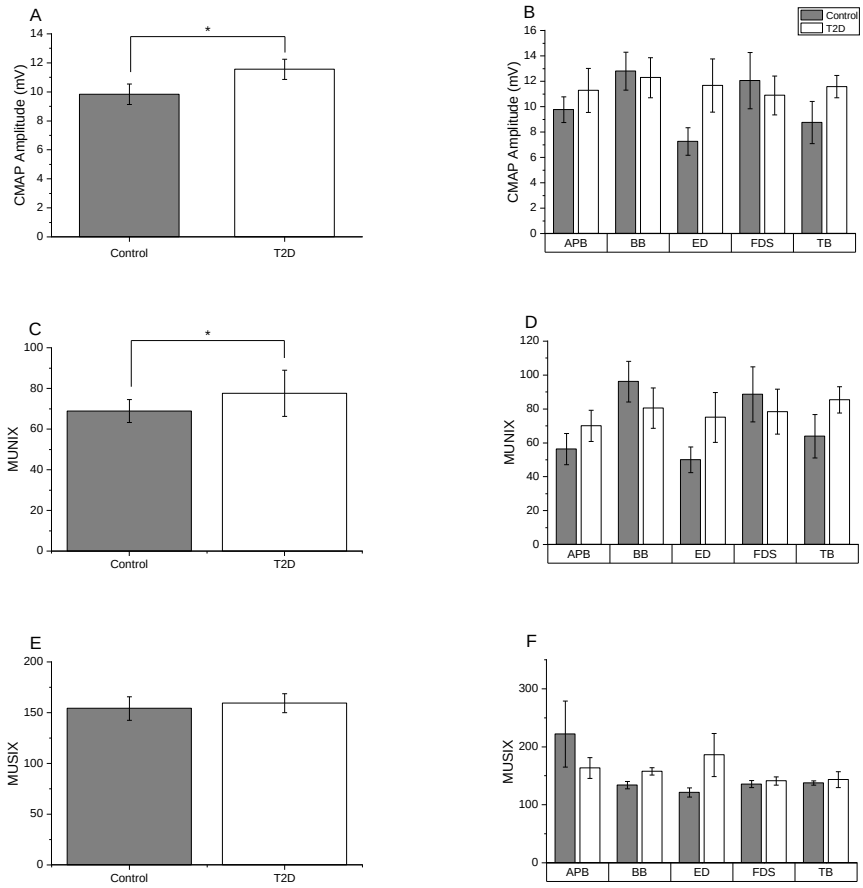

Figure 4.9 *A) maximal CMAP amplitude group data B) maximal CMAP amplitude for the APB, BB, ED, FDS, and TB muscles. C)MUNIX group values D) MUNIX values for the APB, BB, ED, FDS, and TB muscles E) MUSIX group values F) MUSIX for the APB, BB, ED, FDS, and TB muscles. M ± SE reported. * denotes p <0.05.*

4.4.2.1.b MUNIX

A two-way ANCOVA was performed for MUNIX with *Group* (T2D and Control) and *Muscle* (BB, TB, ED, FDS, and APB) as between factors. ALM identified *Diastolic blood pressure* ($t = 2.53$, $p < 0.05$) as a covariate for the MUNIX model. There was a significant main effect of *Group* ($F_{1,76} = 6.67$, $p < 0.05$), such that T2D had significantly higher MUNIX compared to Controls (Figure 4.8c) across all

muscles, indicating the T2D had more motor units overall. There was not a significant main effect of *Muscle* ($F_{1,76} = 2.01$, $p = 0.102$; figure 4.9d).

4.4.2.1.c MUSIX

A two-way ANOVA was performed for MUSIX with *Group* (T2D and Control) and *Muscle* (BB, TB, ED, FDS, and APB) as between factors. ALM did not identify any covariates for the MUSIX model. There was no significant main effect of *Group* ($F_{1,77} = 0.16$, $p = 0.690$) or *Muscle* ($F_{1,77} = 1.59$, $p = 0.186$) identified, indicating motor unit size was not significantly different between both groups or muscles, data is shown in figure 4.8e and figure 4.9f.

4.5 Kinematic Data

Proprioception status of the upper limb was evaluated using a motion capture system to analyze three dimensional kinematic measures while reaching for a virtual target. Kinematic measures included joint angles, aperture, trajectories for the thumb, index finger and hand, and accuracy of pinch location. Speed was controlled across all trials and all subjects via auditory metronome at a frequency of 1 Hz. For each trial, participants were instructed to begin on a beep and finish the pinch maneuver by the following beep. Kinematic data was processed in an original MATLAB program.

4.5.1 Young Healthy Controls

The YHC cohort did not participate in this portion of the study.

4.5.2 Main T2D Study

4.5.2.1 Joint Angles

Joint angles of interest included digit 1 carpometacarpal joint (CMC), digit 1 interphalangeal joint (IP1), digit 1 metacarpophalangeal joint (MCP1), digit 2 distal interphalangeal joint (DIP2), digit 2 proximal interphalangeal joint (PIP2), digit 2 metacarpophalangeal joint (MCP2), flexion/extension (W ex/fl) and abduction/adduction (W ab/ad) of the wrist, and flexion/extension of the elbow (Elb).

ANCOVAs were performed for each joint of interest, comparing mean values of the joint angle throughout the trajectory between groups. ALM was used to identify any significant covariates to include in the analysis. Covariates are listed in Table 4.3. Differences in mean joint angles are shown in Figure 4.10. The difference in mean joint angle for W ex/fl ($F_{1,16} = 25.73$, $p < 0.001$), W ab/ad ($F_{1,17} = 64.33$, $p < 0.001$), CMC ab/ad ($F_{1,15} = 17.42$, $p < 0.001$), and MCP2 ex/fl ($F_{1,16} = 23.01$, $p < 0.001$) were significantly different between groups. Mean IP1 and MCP2 ab/ad joint angles was significantly different between T2D and Control when covariates were not included in the statistical analysis, however this difference was no longer present with the addition of covariates, see Table 4.3 and Figure 4.10.

The variability (one standard deviation) about each mean joint angles throughout the reach and pinch movement was compared between T2D and Control groups using ANCOVAs. ALM was used to identify significant covariates, see Table 4.4. Variability about the mean joint angle trajectory was significantly different between the two groups for CMC ex/fl ($F_{1,14} = 14.26$, $p < 0.001$), MCP1 ab/ad ($F_{1,17} = 21.34$, $p < 0.001$), MCP2 ab/ad ($F_{1,18} = 9.50$, $p < 0.01$), and MCP2 ex/fl ($F_{1,16} = 21.46$,

$p < 0.001$), shown in Figure 4.11. Variability about mean W ex/fl was significantly different between T2D and Controls when covariates were not included in the statistical model. This significant result did not persist with the addition of covariates.

4.5.2.2 Trajectories

The pathlength (total distance traveled) and transport trajectories for the hand, thumb, and index finger were calculated, as was variability for each measure. Two participants from the Control group were excluded from this analysis as they did not complete the pinching task of the virtual object correctly despite practice beforehand and instructions throughout the task. These two participants reached for the mirror instead of reaching for a virtual target located on the right side of the experimental set up.

Table 4.3: *Joint angle ALM results, t- values listed*

	Age	*BMI*	*HbA_{1c}*	*LQ*	*SYS*	*DIA*	*PN*	*PD*	*DD*	*TC*	*HDL*	*LDL*	*Thumb*	*Index*	*Gender*
Elb		12.58 +			-5.62 +				3.46 ^						
W ex/fl					-2.74 *				-4.85 +						
W ab/ad							4.93 +		2.34 *						
CMC ex/fl		2.36 *													
CMC ab/ad		4.80 +											3.82 ^	-2.60 *	
MCP1 ex/fl												-3.20 ^			
MCP1 ab/ad															6.17 *
IP1			8.49 ^											8.55 ^	5.45 *
MCP2 ex/fl					-3.90 +	2.94 ^		-3.23 ^							
MCP2 ab/ad	-52.21 +				-36.39 +		2.82 ^								
PIP2				-2.53 *	-5.48 +	7.08 +								-3.22 ^	
DIP2							6.42 *								

HbA_{1c} = glycated hemoglobin, BMI = body mass index, DD = Disease Duration, DIA = diastole, HDL = high-density lipoprotein, Index = Index finger thickness, LDL = low-density lipoprotein, LQ = laterality quotient, PD = Prediabetes status, PN = peripheral neuropathy, SYS = systole, TC = total cholesterol, Thumb = Thumb thickness.

* denotes $p < 0.05$; ^ denotes $p < 0.01$; + denotes $p < 0.001$

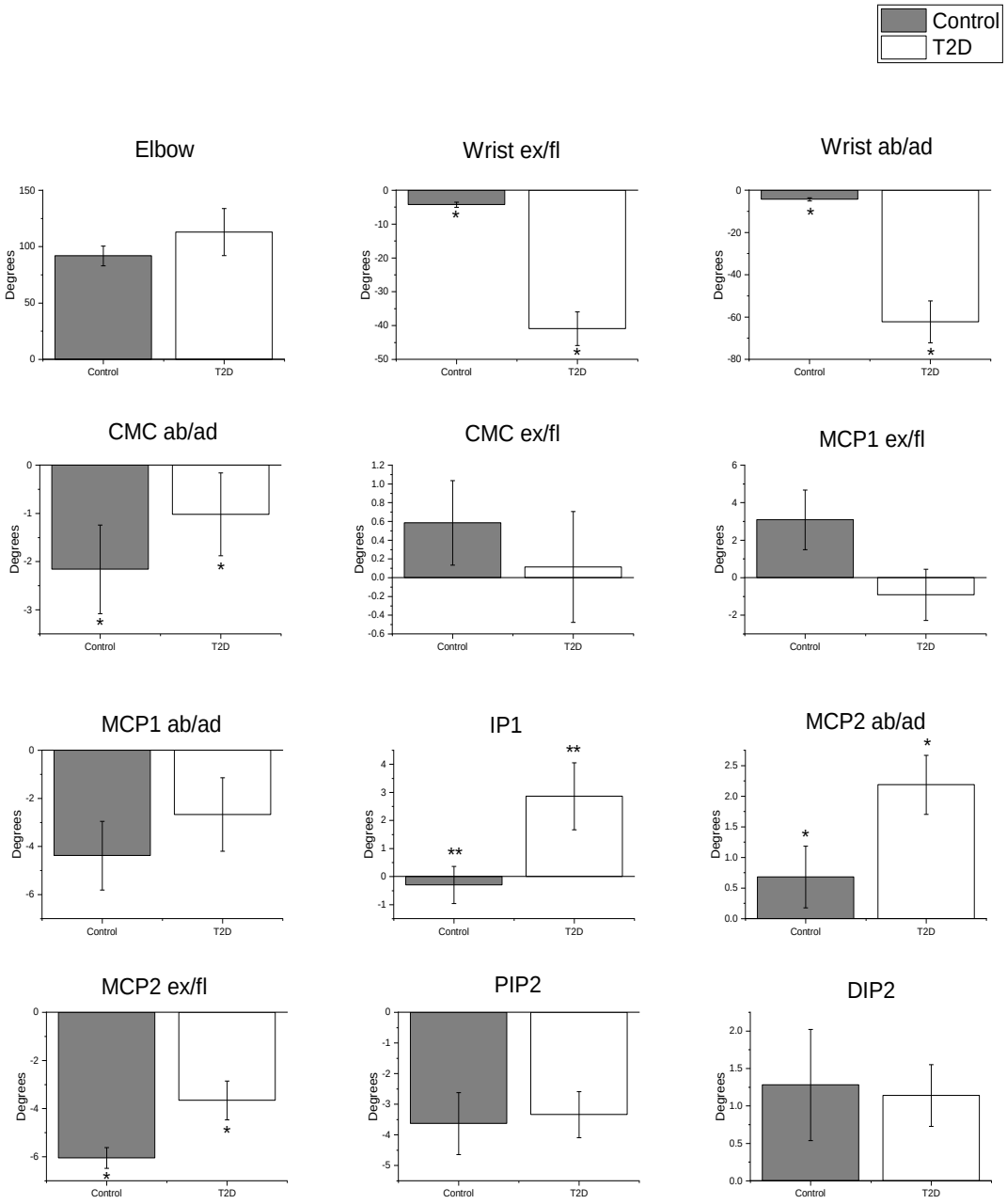

Figure 4.10 *Comparison of mean joint angles between T2D and Control groups.*

** denotes $p < 0.001$*

*** denotes $p < 0.05$ without covariates in the statistical model; with covariates the Group difference is no longer significant)*

Table 4.4: *Joint angle variability ALM results, t- values listed*

	Age	*BMI*	*HbA_{1c}*	*LQ*	*SYS*	*DIA*	*PN*	*PD*	*DD*	*TC*	*HDL*	*LDL*	*Thumb*	*Index*	*Gender*
Elb										2.77 *					
W ex/fl									4.09 +						
W ab/ad													-3.18 ^		
CMC ex/fl		8.19 +				-7.11 +	-4.09 +						3.59 ^	-5.16 +	
CMC ab/ad		4.39 +					13.44 ^								
MCP1 ex/fl						-3.92 +			-2.40 *						
MCP1 ab/ad		6.98 *						3.46 ^							
IP1		5.65 *											5.02 *		
MCP2 ex/fl		3.55 ^						3.17 ^			4.54 +				
MCP2 ab/ad													12.79 ^		
PIP2			2.58 *			-3.44 ^					3.21 ^	-2.14 *			3.03 ^
DIP2														10.45 ^	12.24 ^

HbA_{1c} = glycated hemoglobin, BMI = body mass index, DD = Disease Duration, DIA = diastole, HDL = high-density lipoprotein, Index = Index finger thickness, LDL = low-density lipoprotein, LQ = laterality quotient, PD = Prediabetes status, PN = peripheral neuropathy, SYS = systole, TC = total cholesterol, Thumb = Thumb thickness.

* denotes $p < 0.05$; ^ denotes $p < 0.01$; + denotes $p < 0.001$

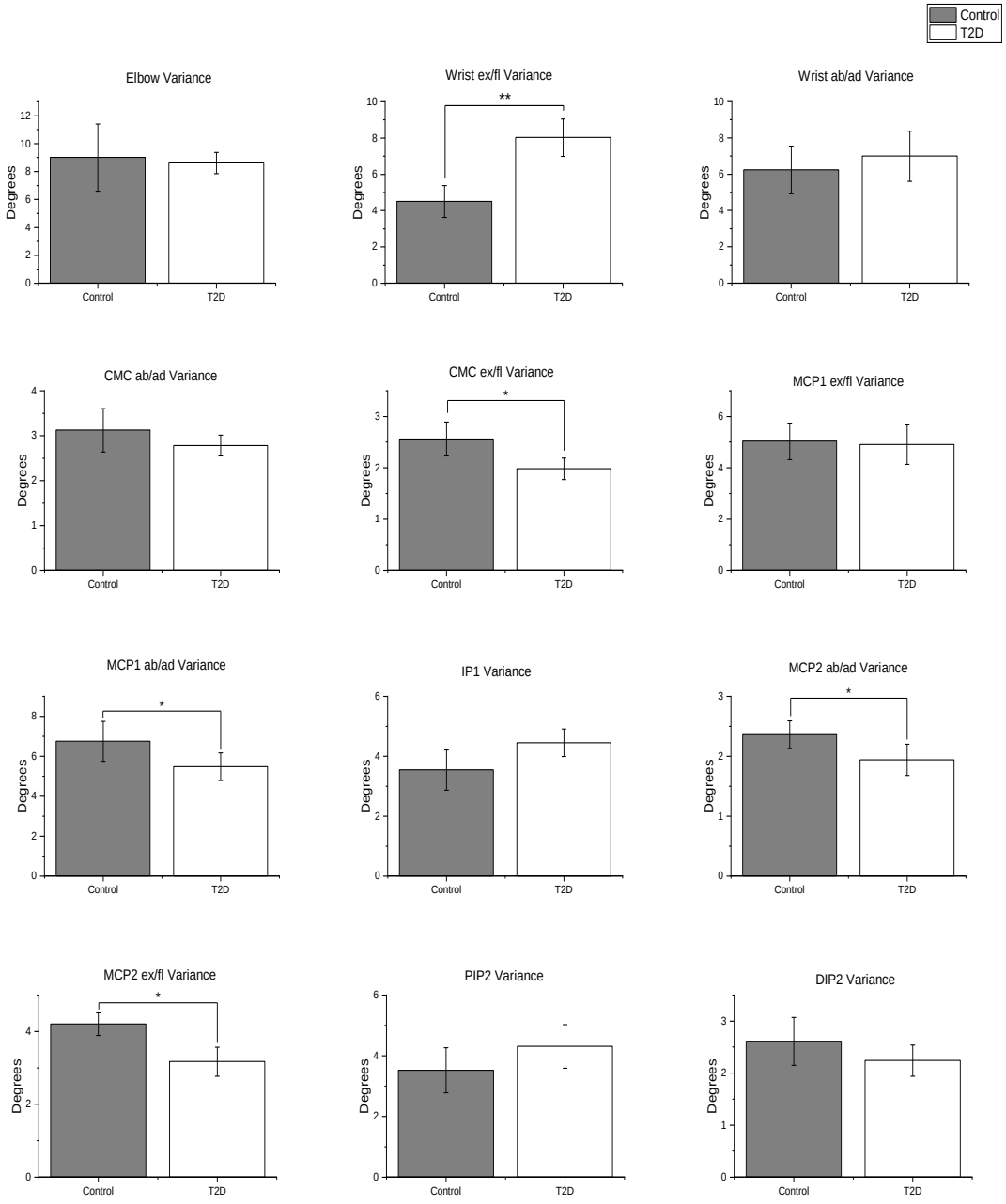

Figure 4.11 *Comparison of variability of mean joint angles throughout the reach and pinch trajectory between T2D and Control groups. Group mean variability ± SE reported.*

** denotes p < 0.01*

*** denotes p < 0.05 without covariates in the statistical model; with covariates the Group difference is no longer significant)*

Table 4.5: *Trajectory ALM results, t- values listed*

	Hand Trajectory	Variability Hand	Thumb Trajectory	Variability Thumb	Index Trajectory	Variability Index
BMI	3.53^	7.31^{+}	4.01^{+}		3.28^	
HbA_{1c}		-2.84*				
LQ		-5.98^{+}	-2.44^		-2.17*	
SYS						
DIA	-3.21^					
PN				3.65*		
Prediabetic						
Disease duration		9.74^{+}				
TC		7.13^{+}				
HDL		-4.04^				
LDL		-8.19^{+}				
Thumb thickness	4.30^{+}	4.47^{+}				
Index thickness	-3.67^					
Gender						2.93*

HbA_{1c} = glycated hemoglobin, BMI = body mass index, DIA = diastole, HDL = high-density lipoprotein, LDL = low-density lipoprotein, LQ = laterality quotient, PN = peripheral neuropathy, SYS = systole, TC = total cholesterol.
* denotes $p < 0.05$; ^ denotes $p < 0.01$; $^{+}$ denotes $p < 0.001$

An ANCOVA was performed for the kinematic variable Index Path Trajectory with the between subjects factor of *Group* (T2D, Control). From Table 4.5, ALM identified *BMI* and *LQ* to be significant covariates included in the analysis. Results indicated a significant difference in index finger trajectory between the two groups ($F_{1,16} = 72.27$, $p < 0.001$), such that index finger path length was shorter in the T2D group as compared to Controls. Index finger trajectory data is illustrated in Figure 4.12a.

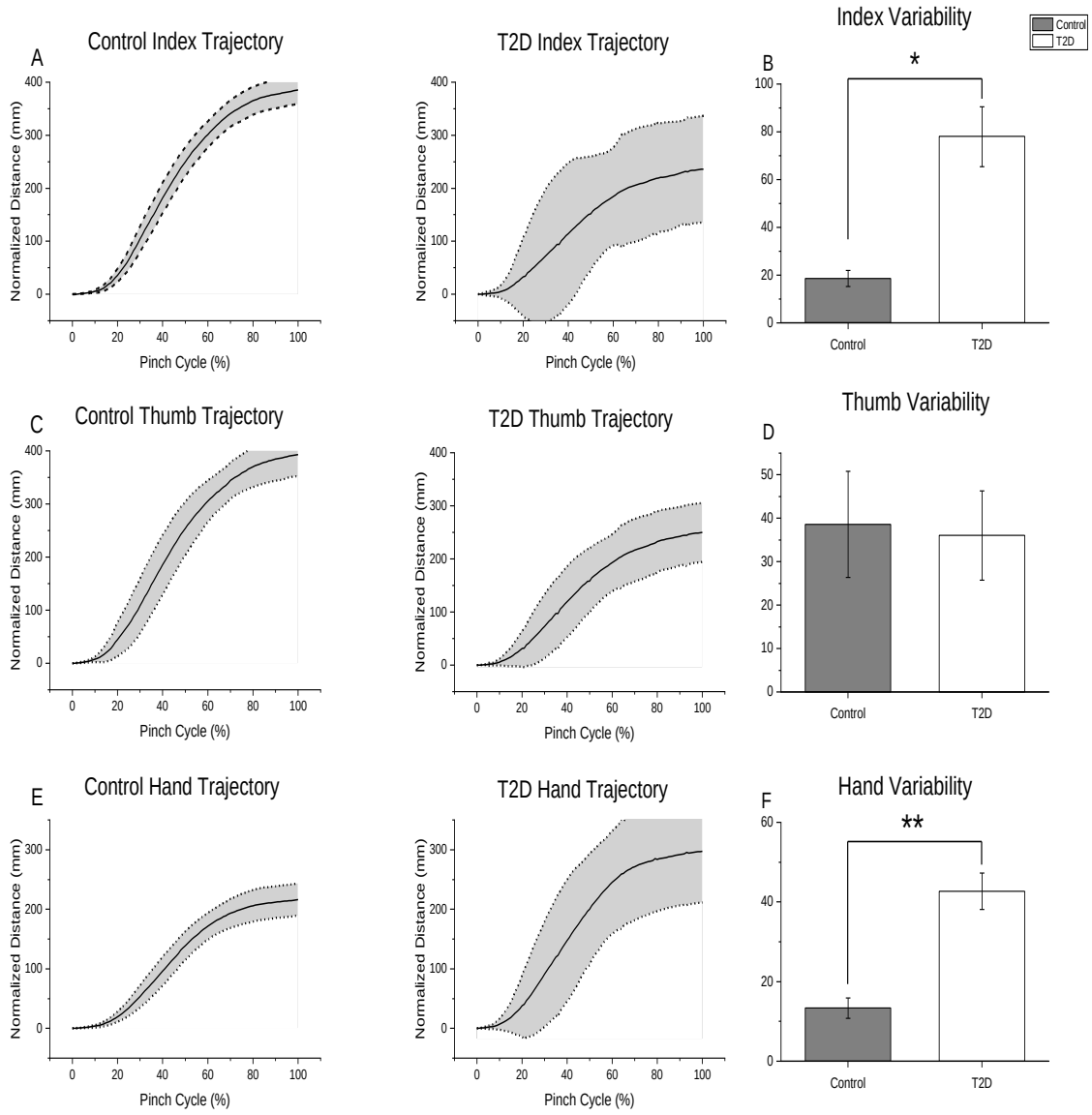

Figure 4.12 *Digit and hand path length accumulated over the pinch cycle A) Control Index finger and T2D Index finger B) variability of Index trajectory C) Control Thumb and T2D Thumb D) variability of Thumb trajectory E) Control Hand and T2D Hand F) variability of hand trajectory. Solid line denotes mean trajectory, dotted line denotes SD. Control group in dark grey, T2D group in white.*

** denotes p < 0.00,*

*** denotes p < 0.001 without covariates in the statistical model; with covariates the Group difference is no longer significant*

For the measure Index Path Trajectory variability, an ANCOVA was performed with *Group* (T2D, Control) as the between factor and the covariate, *Gender* (identified via ALM, Table 4.5). *Gender* was positively correlated with Index Path Trajectory variability indicating in general, women had greater variability. Shown in Figure 4.12b, index finger trajectory variability was significantly different ($F_{1,16}$ = 24.58, $p < 0.001$). T2D participants had significantly greater index finger trajectory variability compared to Control participants.

An ANCOVA was performed on the measure Thumb Path Trajectory with *Group* (T2D, Control) as a between factor. *BMI* and *LQ* were identified as covariates using ALM and included in the model. T2D participants had significantly shorter distance traveled by their thumb compared to Control participants ($F_{1,16}$ = 115.19, $p <$ 0.001), which can be seen in Figure 4.12c. However, an ANCOVA for Thumb path trajectory variability, shown in Figure 4.12d, was not significantly different between the two groups. *PN* status was included as a covariate for thumb path variability.

ALM modeling identified several covariates to include in the statistical models for hand path trajectory and hand path variability, identified in Table 4.5. ANCOVAs were performed for both Hand path trajectory and Hand Path variability with *Group* as a between factor. *BMI, DIA, Index thickness,* and *Thumb thickness* were included as significant covariates for Hand trajectory. A significant difference in Hand Trajectory ($F_{1,16}$ = 9.28, $p < 0.01$) between the two experimental groups was found and shown in Figure 4.12e. Overall, T2D participants hand traveled a significantly greater distance during the reach-to-pinch trial compared to healthy controls. *BMI, HbA_{1c}, LQ, Disease duration, TC, HDL, LDL, and Thumb thickness* included as covariates in the

hand path variability model. There was not a significant difference in variability of hand transport between the T2D and Control groups. However, without covariates added to the model, hand trajectory variability is significantly different between Controls and T2D ($F_{1,21} = 26.95$, $p < 0.001$). Hand trajectory path variability can be viewed in Figure 4.12f.

4.5.2.3 Aperture and Accuracy

Pinch location accuracy results are shown below in Table 4.6. As with the trajectory analysis, two participants from the Control group were excluded from the accuracy analysis as they did not complete the pinching task of the virtual object correctly. The same two participants from the Control group were excluded from this analysis as well for the same reason. These two participants reached for the mirror instead of reaching for a virtual target located on the right side of the experimental set up and thus, did not understand the objective of the task. ANCOVAS were performed for accuracy in each direction (x, y, z) with between factor of *Group* (T2D, Control). Covariates were as follows: Accuracy in *x*-direction – *Index thickness, BMI,* and *TC*; Accuracy in *y*-direction - *HDL*; there were no covariates found for Accuracy in *z*-direction. Mean pinch location for both experimental groups was distal (positive x-coordinate) and to the left (positive y-coordinate) of the target. However, pwT2D were below (negative z-coordinate) while Controls were slightly above (positive z-coordinate) the target. This difference in accuracy in the *z* direction was not significant.

Table 4.6: *Pinch Location Accuracy and Aperture Data*

	T2D	**Controls**
Mean pinch location from Target (x, mm)	12.75 ± 10.36	2.32 ± 12.43
Mean pinch location from Target (y, mm)	49.65 ± 11.42	21.69 ± 10.68
Mean pinch location from Target (z, mm)	-13.64 ± 28.49	1.37 ± 3.39
Mean pinch error (mm)	118.78 ± 10.39*	48.16 ± 8.70*
Mean pinch precision (mm)	6.33 ± 4.56	5.59 ± 2.31
Aperture at Pinch (mm)	15.60 ± 3.76	32.94 ± 5.60
Average Aperture (mm)	60.83 ± 3.22	47.05 ± 3.10

Group means ± SE reported. * denotes $p < 0.001$. + x forward, + y leftward, +z upward, relative to virtual target

ALM identified *BMI* as covariate to include in the model for mean pinch location, an ANCOVA was performed with between factor *Group.* Controls were more accurate with their overall pinch location error compared to target location compared to T2D, values can be found in Table 4.6 and visualized in Figure 4.13. This difference in accuracy was significant ($F_{1,16} = 20.90$, $p < 0.001$). No covariates were identified for pinch precision (variation about the target), thus an ANOVA was performed for this measure setting *Group* as a between factor. Pinch precision (one standard deviation about mean pinch error) did not differ significantly between groups.

Pinch Contact

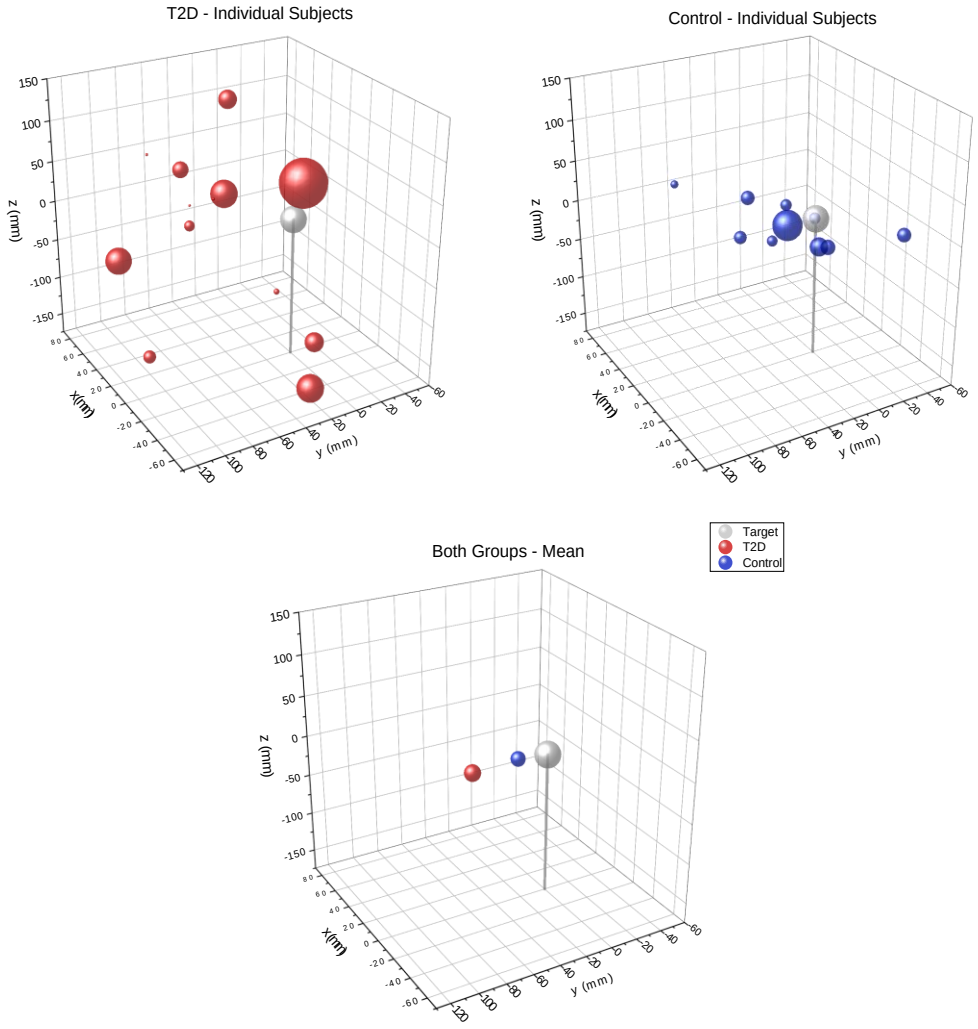

Figure 4.13 *Mean pinch location for individual and mean T2D and Control subjects with respect to the virtual target location (grey). Center of each sphere indicates pinch contact location while the radius indicates pinch precision (1 s.d.) about pinch contact location. + x forward, + y leftward, +z upward, relative to virtual target.*

An ANCOVA was performed for average aperture size throughout reach-to-pinch trial with *Group* as between subjects factor and *Disease Duration* and *PN* status included as covariates (identified by ALM). There was no significant difference in

average aperture between the two groups. An ANCOVA was performed for aperture size at pinch location with *Group* as between subjects factor and HbA_{1c} and *BMI* included as covariates in the model. There was no significant different in aperture at pinch location between the two experimental groups. Aperture data is shown in Table 4.6.

4.5.2.4 Relationship between motor unit characteristics and sensorimotor measures.

The relationships between neuromuscular measures (MUNIX, MUSIX, and CMAP amplitude) and sensory (tactile and vibration perception thresholds) and kinematic (accuracy) outcome measures were examined using Pearson's Correlation coefficient. MUNIX correlation coefficients in each muscle are shown in Table 4.7. In the BB muscle, Log_{10}Force and MUNIX moderately positively correlated ($r(76) = 0.277$, $p < 0.05$). In the ED muscle, mean pinch error and MUNIX were strongly negatively correlated ($r(61) = -0.329$, $p < 0.01$). MUNIX was negatively correlated with Log_{10}Force ($r(67) = -0.312$, $p < 0.01$) and positively correlated with mean pinch error ($r(43) = .0339$, $p < 0.05$) in the FDS muscle. Vibration perception threshold (VPT) was not significantly correlated with MUNIX.

Table 4.8 contains MUSIX correlation coefficients for each muscle. In the APB ($r(79) = -0.229$, $p < 0.05$), BB ($r(76) = -0.251$, $p < 0.05$) and FDS ($r(67) = -0.289$, $p < 0.05$) muscles, Log_{10}Force was moderately negatively correlated with MUSIX. Vibration perception threshold and mean pinch error were not significantly correlated with MUSIX in any of the five muscles.

Table 4.7: *Pearson's Correlation coefficients between sensorimotor measures and MUNIX*

	APB	BB	ED	FDS	TB
Log_{10}Force	-.073 (79)	.277 (76) *	-.157 (73)	-.312 (67) ^	.116 (85)
Avg VPT Amp	-.092 (79)	.186 (76)	-.015 (73)	-.112 (67)	.068 (85)
Mean pinch error	-.096 (55)	-.047 (55)	-.329 (61) ^	.339 (43) *	.153(61)

Pearson's Correlation reported with (DOF). * denotes $p < 0.05$, ^ denotes $p < 0.01$.

Table 4.8: *Pearson's Correlation coefficients between sensorimotor measures and MUSIX*

	APB	BB	ED	FDS	TB
Log_{10}Force	-.229 (79) *	-.251 (76) *	.038 (73)	-.289 (67) *	-.061 (85)
Avg VPT Amp	-.184 (79)	-.121 (76)	.067 (73)	-.158 (67)	-.071 (85)
Mean pinch error	.073 (55)	-.093 (55)	.062 (61)	.144 (43)	-.117 (61)

Pearson's Correlation (r) reported with DOF. * denotes $p < 0.05$.

CMAP amplitude correlation coefficients are shown in Table 4.9. There was a strong negative correlation between Log_{10}Force and CMAP in the APB muscle ($r(79) = -0.291$, $p < 0.01$) while the BB muscle ($r(76) = 0.230$, $p < 0.05$) exhibited a moderate positive correlation. In the ED muscle, mean pinch error was moderately negatively correlated with CMAP ($r(61) = -0.302$, $p < 0.05$). In the FDS muscle,

Log_{10}Force and CMAP were found to be very strongly negatively correlated ($r(67) = -0.443$, $p < 0.001$). Also in the FDS, mean pinch error and CMAP were found to be strongly correlated ($r(43) = 0.388$, $p < 0.01$). VPT was not found to significantly correlate with CMAP in any of the upper limb muscles examined.

Table 4.9: *Pearson's Correlation coefficients between sensorimotor measures and CMAP*

	APB	**BB**	**ED**	**FDS**	**TB**
Log_{10}Force	-.291 (79) ^	.230 (76) *	-.133 (73)	-.443 (67) $^{+}$	.113 (88)
Avg VPT Amp	-.187 (79)	.165 (76)	.016 (73)	-.177 (67)	.051 (88)
Mean pinch error	.019 (55)	-.055 (55)	-.302 (61) *	.388 (43) ^	.067 (64)

Pearson's Correlation (r) reported with DOF. * denotes $p < 0.05$, ^ denotes $p < 0.01$, $^{+}$ denotes $p < 0.001$.

5 DISCUSSION

5.1 General Discussion

The purpose of this study was to examine upper limb muscle motor unit characteristics and proprioception in patients with Type II Diabetes (T2D) compared to healthy age- and sex-matched controls. In Hypothesis 1a, we expected T2D patients to exhibit increased movement variability of the digits, hand, and arm when compared to the control group. This hypothesis was partially supported. Those in the T2D group had significantly increased variability in transport trajectory of the index finger and whole hand when compared to controls. However, when examining joint angle trajectories, there were conflicting findings. Group differences in joint angle variability were only present in three joints and degrees of freedom, however these differences were not clear cut. T2D patients exhibited more variability in wrist extension/flexion while healthy controls exhibited more variability in CMC extension/flexion and MCP2 abduction/adduction. In Hypothesis 1b, we expected T2D patients to be less accurate and less precise relative to the virtual target location when compared to the control group. Hypothesis 1b was partially supported such that T2D patients had significantly more pinch location error, however mean pinch precision was not different between the two groups.

In our Hypothesis 2.1a we predicted T2D patients to have fewer motor units and increased motor unit size when compared to the control group. This hypothesis was not supported. In our study, T2D patients had significantly larger mean CMAP amplitude and mean MUNIX (indicating greater number of motor units) when

compared to healthy controls. However, motor unit size was not different between the two groups. Additionally, Hypothesis 2.2 was not supported. In Hypothesis 2c, we expected motor unit characteristics to differ in the muscle examined in patients with T2D such that impairment would be more pronounced in distal muscles compared to those more proximal. These differences were not found. Maximum CMAP amplitude, number of motor units, and size of motor units did not significantly differ between the APB, BB, ED, FDS, and TB muscles. Lastly, in Hypothesis 2.1b, we expected motor unit impairment to correlate with impaired sensorimotor measures. This hypothesis could not be confirmed nor denied. There were some significant correlations between these measures, however they were not consistent across muscles or motor unit properties (CMAP amp, MUNIX, MUSIX).

5.1.1 Tactile sensitivity and proprioceptive function

Tactile and proprioceptive functions were assessed using several tests, including the Semmes-Weinstein Monofilament Test, Biothesiometer vibration device, and a reach-to-pinch task. Contrary to most prior findings in the T2D population, neither hand nor foot tactile sensitivity as measured by the Semmes-Weinstein Monofilament test differed between groups. Previous findings in our lab have reported differences in tactile sensitivity between pwT2D and healthy controls, with a pronounced difference for the median nerve (Gorniak et al., 2014; Ochoa et al., 2016; Ochoa & Gorniak, 2014). In a large case control study, Gorniak et al. (2020) recently reported hand tactile differences not only in Group, but also between sexes, and with incidence of PN, with influences of handedness/laterality and HbA_{1c}. However, our

recent study in postmenopausal women with T2D did not find differences in hand tactile function compared to age- and sex-matched healthy controls (Pollonini, Cox, & Gorniak, 2020). The data within this dissertation also did not identify differences in upper limb nerve sensitivity. Sensitivity did differ by nerve however in the young healthy control group, with the Median and Ulnar nerves being more sensitive than the Radial nerve. Additionally, Automatic Linear Modeling did not identify any significant covariates for hand tactile sensitivity in our study. These findings challenge our understanding of sensory dysfunction and how it relates to the development and progression of T2D. More work is warranted in this area.

Vibratory thresholds of the hand and foot were measured using a Biothesiometer vibration device. Again, differences in group and site were not detected in our study. Together, lack of tactile and vibration sensitivity differences between pwT2D and healthy age- and sex-matched controls suggests that in our study cohort, mechanoreceptors responsible for light touch (Merkel's disks, located in the base of the epidermis, in the skin on the palms, fingers, and soles of the feet) and vibration (Meissner's corpuscles located in the upper dermis primarily in the fingertips and deeper Ruffini endings) are similarly intact, though slightly impaired when compared to young healthy controls.

Proprioception of the upper limb was assessed using a reach-to-pinch task. Specifically, effects of T2D on kinematics of reaching and grasping a virtual target object were examined. A similar study performed in patients with CTS reported greater variability about the mean angular trajectory in all joint degrees of freedom in addition to significant differences in mean trajectory values in a majority of joint

degrees of freedom (Nataraj et al., 2014). Within the current data set there were differences in some joint angle trajectories between experimental groups. Throughout the reach and pinch movement, pwT2D exhibited greater wrist extension and adduction, CMC abduction, IP1 flexion, and MCP2 extension and abduction compared to health age-and sex-matched controls. Additionally, pwT2D also exhibited greater variability in wrist extension/flexion and less variability in CMC extension/flexion and MCP2 extension/flexion and abduction/adduction. This could point towards a change in aiming strategy. Healthy older adults utilized a thumb and index finger strategy while pwT2D utilized a wrist or whole hand strategy. Potentially, the attenuated group difference in our study is a result of an intact median nerve while CTS is associated with median nerve compression.

Total distance traveled throughout the entire movement was reduced for the Index finger and Thumb in pwT2D as compared to healthy controls while whole hand distance traveled was increased. This suggests that throughout the reach and pinch movement, T2D patients were moving their index and thumb less, while using their whole hand to adjust and aim for the target, further supporting a change in aiming strategy associated with T2D. Despite moving their index finger and thumb less during the task, the T2D group exhibited greater variability in index finger and hand transport when compared to controls. Nataraj et al. (2014) reported similar increased variability in pwCTS. Our results indicate there is increased variability in the hand during reaching as well as a distinct aperture position associated with T2D.

As a functional performance outcome, pinch location accuracy and precision relative to the virtual target were measured. The reaching portion of the movement

brings the hand and fingers near to the target while the pinching portion of the movement relies on thumb and index finger movement. PwT2D exhibited worse mean accuracy than age- and sex-matched controls. Both pwT2D and Control participants were generally in front of and to the left of the target location. However, pwT2D pinched below the target while Controls pinched slightly above. There were no differences in precision associated with T2D. Our results support findings in a recent study examining knee proprioception. In this study, pwT2D demonstrated 46% greater knee joint position sense inaccuracy but no differences in precision (Ettinger, Boucher, & Simonovich, 2018). Other lower extremity T2D proprioception studies report impaired ankle joint movement perception and response to tendon vibration (Simoneau et al., 1996; van Deursen & Simoneau, 1999; van Deursen et al., 1998). Our results indicate that despite an absence of sensory impairment typically associated with T2D, proprioception of the upper extremity is altered in pwT2D.

5.1.2 Motor unit characteristics

Motor unit (MU) characteristics of the abductor pollicis brevis (APB), biceps brachii (BB), extensor digitorum (ED), flexor digitorum superficialis (FDS), and triceps brachii (TB) muscles of the upper limb were assessed utilizing MUNIX and MUSIX methods to quantify their number and average size. Supramaximal neuromuscular nerve stimulation was performed on each muscle's respective innervating nerve to elicit a maximum CMAP.

Smaller CMAP amplitudes and reduced MUNE has been reported in lower extremity muscles and FDI muscle of the hand in pwT2D (Allen et al., 2013, 2014;

Watanabe et al., 2013). To our knowledge, this is the first study to use the MUNIX method to characterize MUs in pwT2D. Contradictory to prior MUNE findings in pwT2D, our Control group had reduced mean MUNIX and mean maximum CMAP amplitude when compared to pwT2D overall, with this difference most prominent in the ED and TB muscles. In both statistical analyses, ALM identified Diastolic blood pressure to be a significant covariate in the model, a similar finding reported by Pollonini et al. (2020). Thus, this may be the driving factor of MU impairment in the older population and not necessarily diagnoses of T2D. In our study, the Control group had a mean blood pressure of 139/78 mm Hg, while the T2D group had a mean of 133/70 mm Hg, both categorized as Stage 1 Hypertension according to the American Heart Association (AHA, 2020). It is also important to note that in general, the pwT2D in this study were more commonly on blood pressure medication than their age- and -sex matched controls. This could be due to more regular doctor visits required for the maintenance of T2D, resulting in the identification of high blood pressure and prescription of medication. The presence of Hypertension within the control group being directly related to increased CMAP values reinforces CVD as the most likely source of neuromuscular complications. Additionally, we expected to see length-dependent neuromuscular impairment in the upper extremity, with the APB being more affected compared to the BB and TB. While Allen et al. (2014) reported this relationship between the Tibialis Anterior and FDI muscles, we did not see this effect within the upper limbs of our study participants.

MUSIX however, was not different between the two groups. Typically, in diseased populations where MUs are reduced, MU size must increase as a regulatory

mechanism to compensate for those lost. In an older population, especially with sarcopenia, reinnervation may be impaired. Thus, MU size may not increase to compensate in pwT2D despite MU loss (Cao, Gu, Zhang, & Hou, 2020; de Carvalho, Barkhaus, Nandedkar, & Swash, 2018; Hepple, 2003). This potentially pathological neuromuscular finding is novel and warrants additional investigation,

Though not statistically compared, in general, participants in the Main Study (older adults) had lower maximal CMAP amplitudes and reduced MUNIX values in the APB, ED, and FDS muscles when compared to the young healthy controls in our study. Cao et al. (2020) aimed to establish reference MUNIX values in various muscles of healthy controls, two of which were the APB and BB muscles. Similar to comparison of our YHCs, our older adults exhibited reduced MUNIX values and larger MUSIX values, indicating fewer number of motor units that are larger in size compared to reference values of healthy adults established by Cao et al, and confirming an inverse relationship between MUNIX and age (Cao et al., 2020).

5.1.3 Relationship between motor unit characteristics and sensorimotor measures.

We examined correlations between neuromuscular properties in the upper extremity and sensory and proprioceptive measures to determine if impairment in these areas were related. While vibration perception threshold of the hand did not correlate with CMAP, MUNIX or MUSIX, hand tactile threshold and mean pinch error did. Additionally, relationships between the two variables (average hand tactile threshold and mean pinch error) and MU properties were not clear cut and varied by

muscle and characteristic. Such conflicting findings in our data indicate impairment in these areas may not be related or may be more complicated than previously assumed.

5.1.4 Conclusion

Overall, the lack of sensory and neuromuscular dysfunction despite impaired proprioception in pwT2D is somewhat conflicting within the current data set. These data indicate that there may be more complex neuromuscular changes in pwT2D than has been previously assumed. Similar to other recently reported data sets, changes to MU properties may be driven by other health related conditions such as high blood pressure and sarcopenia, rather than a diagnosis of T2D. Our data suggest that impairment of mechanoreceptors involved in proprioception occurs prior to loss of MUs in muscles as well as more commonly assessed sensory modalities such as touch and vibration. However, it is possible that negative impacts to MUs develop concurrently with the development of deficits in tactile and vibration sensations. Additionally, altered upper extremity kinematics may point to changes in motor control in the central nervous system. Further research should examine changes to both the motor and sensory cortices in pwT2D. These findings help to understand proprioceptive and neuromuscular function of the upper limb in pwT2D, with implications to early identify patients who may need medical and lifestyle intervention.

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