ALTERED MYOFIBER FUNCTION AND PHYSIOLOGY IN TYPE 1 DIABETES

ALTERED MUSCLE FUNCTION AND MYOFIBER PHYSIOLOGY IN ADULTS WITH TYPE 1 DIABETES

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LAY ABSTRACT

Type 1 diabetes (T1D) is a disease that affects millions of adults worldwide by harming their ability to manage blood sugar levels. Insulin therapy has allowed for longer and healthier lives but requires constant attention manage blood sugar levels. Over time, the quality of life declines because of complications from T1D. Muscle is able to control blood sugar levels through exercise, but little is known about muscle in those with T1D. Therefore, the purpose of this work was to examine skeletal muscle health in people with T1D who do not have other complications. We found that adults with T1D exhibit signs of aging in their muscles earlier than non-diabetic people. Also, we observed that muscle from young adults with T1D recovered slower from exercise. Finally, we learned that people with T1D have more of a muscle-shrinking protein. This is the first evidence of dysfunctional muscle fibers at rest, after exercise, and with age in adults with T1D. This work aims to improve future guidelines for millions of adults with T1D.

ABSTRACT

The objective of this thesis was to examine muscle function and myofiber physiology in skeletal muscles in those with type 1 diabetes (T1D) by investigating the effects of diabetic myopathy on these metrics of muscle health under various conditions: at rest, after exercise and with increasing age. These works recruited adults from surrounding communities with T1D and non-diabetic counterparts (i.e. controls) matched for age, sex, body mass index, and self-reported physical activity levels. We hypothesized that adults with T1D would exhibit decreased muscle function (i.e. lower maximal strength) and altered myofiber physiology in each of these conditions. At rest, we observed that those with T1D exhibited more fast-twitch fibers and fewer satellite cells. After exercise, T1D muscles recovered less strength, showed higher amounts of myofiber damage, and delayed satellite cell proliferation. With increasing age, adults with T1D exhibited exaggerated signs of muscular aging compared to age-matched controls in the form of more abundant hybrid fibers and type 1 fiber grouping. Finally, individuals with T1D exhibited higher baseline expression of myostatin, a negative muscle growth regulator, compared to controls. Overall, our work provides the first evidence in muscle dysfunction from humans with T1D at various ages and after damaging exercise. Our findings provide novel insights on muscle health and its contribution to overall health during this lifelong, debilitating disease. Our work aims to guide future clinical & exercise guidelines with the ultimate purpose of improving the lives of millions of individuals living with T1D.

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To my child who is due to join us soon, please take this document as proof that your father once had intelligent things to say…

PREFACE

This thesis is a "sandwich" style thesis prepared in the format outlined in the School of Graduate Studies' Guide for the Preparation of Theses. Chapter 1 is original work that serves as the general introduction. Chapters 2 and 4 include original research papers published and presented as the electronic version of the published reprint. Chapter 3 is an electronic version of a manuscript accepted for publication. The candidate is the first author on all the papers. The declaration of academic achievement is found in the preface of each chapter including author contributions and the citation. Copyright permission is indicated at the beginning of each chapter and found in Appendix A. All chapters have been reproduced with the permission of each co-author.

TABLE OF CONTENTS

LAY ABSTRACT....iii
ABSTRACT....iv
ACKNOWLEDGEMENTS....v
PREFACE....vi
TABLE OF CONTENTS....vii
List of tables....ix
List of figures....ix
List of all abbreviations and symbols....ix
CHAPTER 1 – GENERAL INTRODUCTION....1
1 Type 1 diabetes....2
1.1 Epidemiology....2
1.2 Etiology....3
1.3 Pathophysiology....5
2 Skeletal muscle....6
2.1 Muscle structure....6
2.2 Myofilaments & muscle contraction....7
2.3 Muscle fiber-type composition....8
2.4 Neuromuscular junctions....11
2.5 Satellite cells....12
2.6 Capillaries....12
3 Skeletal muscle with type 1 diabetes....13
3.1 Muscle weakness & diabetic peripheral neuropathy....14
3.2 Decreased muscle mass....14
3.3 Fiber-type plasticity during type 1 diabetes....15
3.4 Reduced regenerative and satellite cell capacity....16
3.5 Vascular dysfunction....17
3.6 Dysregulation of myostatin & negative growth regulators....18
4 Addressing limitations of T1D skeletal muscle literature....19
4.1 Bridging clinical observations with preclinical models of T1D....19

4.2 Narrow scope of human T1D studies. 23
Objectives & hypotheses 25
Study #1 (Chapter 2) 25
Study #2 (Chapter 3) 25
Study #3 (Chapter 4) 26
References 27
CHAPTER 2 – IMPAIRED FUNCTION AND ALTERED MORPHOLOGY IN THE SKELETAL MUSCLES OF ADULT MEN AND WOMEN WITH TYPE 1 DIABETES 45
Preface 46
CHAPTER 3 – ALTERATIONS IN SKELETAL MUSCLE REPAIR IN YOUNG ADULTS WITH TYPE 1 DIABETES MELLITUS 65
Preface 66
CHAPTER 4 – MUSCLE AND SERUM MYOSTATIN EXPRESSION IN TYPE 1 DIABETES 95
Preface 96
CHAPTER 5 – OVERALL CONCLUSIONS 106
5.1 Significance of studies and conclusions 107
5.2 Future research 115
References 121
APPENDIX A: COPYRIGHT PERMISSIONS 129

LIST OF TABLES

CHAPTER 2
Table 2. 1 Participant characteristics, overall 49
Table 2. 2 Participant characteristics, split by age group 50

CHAPTER 3
Table 3.1. Participant characteristics. 71

CHAPTER 4
Table 4. 1 Participant characteristics 98
Table 4.2. Clinically relevant correlates of myostatin mRNA expression 103

LIST OF FIGURES

CHAPTER 2
Figure 2.1. Accelerated loss of maximal muscle strength in type 1 diabetics 53
Figure 2.2. Vascularity, satellite cell, and glycogen content of skeletal muscle in males and females across the adult lifespan 54
Figure 2.3. Altered fiber-types and myofiber size in T1D 56
Figure 2.4. Prevalence of hybrid fibers in T1D skeletal muscle 58
Figure 2.5. Exaggerated type 1 myofiber grouping in those with T1D. 59

CHAPTER 3
Figure 3.1 Systemic & skeletal muscle characteristics following eccentric exercise. 79
Figure 3.2 Satellite cell content and proliferation after eccentric contractions. 81
Figure 3.3. Skeletal muscle transcriptomics pre- and post-exercise. 83

CHAPTER 4
Figure 4.1. Elevated expression of serum myostatin and its correlates as measured by ELISA 100
Figure 4.2. Correlates of muscle myostatin protein expression with and without T1D. .101
Figure 4.3. Summary of correlative analyses in groupings of the present study 102

LIST OF ALL ABBREVIATIONS AND SYMBOLS

ATP	adenosine triphosphate
CEL	Affymetrix probe results file
CGM	continuous glucose monitor

CK	creatine kinase
CON	control
CTLA-4	encoding cytotoxic T lymphocyte-associate protein 4
DAPI	4′,6-diamidino-2-phenylindole
DPN	diabetic peripheral neuropathy
DYS	dystrophin
ECM	extracellular matrix
eGDR	estimated glucose disposal rate
ELISA	enzyme-linked immunosorbent assay
GEO	Gene Expression Omnibus
GLUT4	glucose transporter type 4
GSE	GEO Series
GSEA	gene set enrichment analysis
HLA	human leukocyte antigen
HYB12	myosin heavy chain, type 1/2 hybrid
HYB2AX	myosin heavy chain, type 2A/2X hybrid
IDDM2	insulin dependent diabetes mellitus 2
IL2Rα	interleukin-2 receptor-alpha
ISS	insulin sensitivity score
Ki67	marker of proliferation Ki67

LAM	laminin
MET	metabolic equivalent of task
MHC	myosin heavy chain
MHC1	myosin heavy chain, type 1
MHC2	myosin heavy chain, type 2
MHC2A	myosin heavy chain, type 2A
MHC2X	myosin heavy chain, type 2X
MVC	maximal voluntary contraction
NMJ	neuromuscular junction
NSAID	nonsteroidal anti-inflammatory drug
NSERC	Natural Sciences and Engineering Research Council of Canada
OCT	optimal cutting temperature
PAS	periodic acid-Schiff
Pax7	paired box 7
PTPN22	protein tyrosine phosphatase non-receptor type 22
RNA	ribonucleic acid
ROI	region of interest
SC	satellite cell
SD	standard deviation
SEM	standard error of the mean

T1D	type 1 diabetes mellitus
T2D	type 2 diabetes
TEM	transmission electron microscopy
TRIS	tris(hydroxymethyl)aminomethane
TSA	tyramide signal amplification

CHAPTER 1 – GENERAL INTRODUCTION

1 TYPE 1 DIABETES

Type 1 diabetes (T1D) is a disease precipitated by the autoimmune destruction of the insulin-producing β-cells of the pancreas. Loss of normal insulin function leads to a host of disease-associated complications, chief among which is the inability to regulate blood glucose (i.e. dysglycemia). The resultant dysregulation of glucose metabolism leads to various complications including nephropathy, neuropathy, retinopathy, and myopathy. While newer, more efficient therapies continue to lengthen the lives of those with T1D, there remains no cure for the disease. Thus, increased understanding of its effects on insulin-sensitive tissues remains of paramount importance to help control and/or prevent comorbidities and increase the healthy lifespan.

1.1 EPIDEMIOLOGY

Globally, the incidence of T1D has increased since the 1950s with an average annual increase of 3–4% over the most recent decades (Gale, 2002; Karvonen et al., 2000; Patterson et al., 2019). It is estimated that 300,000 Canadians live with the disease (Karvonen et al., 2000). T1D is the major type of diabetes experienced by youths, accounting for greater than 85% of cases under the age of 20 years (Thunander et al., 2008; Vandewalle et al., 1997). While it is possible for disease onset to occur as late as the ninth decade of life (Thunander et al., 2008), peak incidence of T1D onset appears to occur at 10-14 years of age (Weng et al., 2018). Race/ethnicity is often not the focus of epidemiological studies, in part because many studies lack the statistical power based on their sample size to examine rates by race/ethnicity. However, one study has provided specific data on the role of race/ethnicity on T1D in the United States. Mayer-Davis *et al.*

concluded that incidence of T1D in youths was highest in non-Hispanic white individuals, and that these rates were among the highest in the world (Mayer-Davis et al., 2009). With regards to sex, girls and boys are equally affected with T1D (Soltesz et al., 2007). However, many reports indicate that males are disproportionately affected post-puberty (Gale, 2002; Kyvik et al., 2004; Pundziute-Lyckå et al., 2002; Weets et al., 2002). However, the underlying physiological mechanisms driving sex-differences in T1D are not well known.

1.2 ETIOLOGY

The etiology of T1D is currently described by a pathogenetic model in which individuals with genetic predisposition are exposed to an environmental stimulus which triggers β-cell autoimmunity, ultimately progressing towards clinical presentation of T1D symptoms.

1.2.1 Genetic

Establishing which genes confer genetic susceptibility to T1D remains an active area of study. Since its discovery several decades ago, the human leukocyte antigen (HLA) region continues to be the strongest genetic contributor to T1D susceptibility, with the highest risk genotype having an odds ratio of 16 (Noble & Valdes, 2011). Additionally, a variant of the insulin gene leads to a lower number of repeats in the promoter region of the insulin-dependent diabetes mellitus 2 (IDDM2) locus, and is associated with increased susceptibility to T1D (Van Belle et al., 2011). Novel genes continue to be hypothesized as T1D susceptibility genes, such as protein tyrosine phosphatase non-receptor type 22 (PTPN22), interleukin-2 receptor-alpha (IL2Rα), and encoding cytotoxic T lymphocyte-associate protein 4 (CTLA-4). These genes have been found to mediate risk in other

autoimmune disease susceptibility in addition to T1D. However, the mechanisms by which these genes mediate T1D susceptibility are not currently well understood and/or have contradictory explanations (for review, see (Van Belle et al., 2011)).

1.2.2 Environmental

Despite elevated susceptibility, less than 10% of individuals with HLA-conferred genetic susceptibility to T1D present with overt disease (Knip et al., 2005; Van Belle et al., 2011). In the context of the pathogenetic model, this suggests that the exposure to T1D-inducing exogenous factors must be appropriately timed in order to enhance risk of T1D even in the presence of the other predispositions. Vitamin D deficiency has been posited as one of the triggers for T1D, supported by the observation that autoimmunity is triggered more commonly during colder seasons (Knip et al., 2005). Interestingly, northern Europe, a region with one of the lowest daily exposures to sunlight, has the highest incidence of T1D in the world (Karvonen et al., 2000). Also, HbA_{1c} levels appear to display seasonal variation with the highest values in the fall and winter (Käär et al., 1984; Nordfeldt & Ludvigsson, 2000). A second hypothesized trigger to T1D is a viral exposure in childhood. In particular, enterovirus exposure appears to be the most probable trigger of β-cell autoimmunity, supported by the strong temporal relationship between enteroviral infection and first appearance of autoantibodies in children (Hyöty et al., 1995; Lönnrot, Korpela, et al., 2000; Lönnrot, Salminen, et al., 2000; Salminen et al., 2003). Additionally, enterovirus infections are also more common in individuals who were newly diagnosed with T1D (33% of T1D patients vs. < 5% in control subjects) (Hyöty et al., 1995). Despite efforts in the area, there is still no direct evidence for any virus being causative to development of T1D.

1.3 PATHOPHYSIOLOGY

1.3.1 Diagnosis & early progression

Autoimmune destruction of β-cells was initially described by Eisenbarth to occur in a linear fashion over time (Eisenbarth, 1986). However, more recent and complex hypotheses involve a "relapse and remission" model where fluctuations in beta cell mass occur as waves of β-cell destruction over time (Atkinson et al., 2015; Bonifacio et al., 1999; Van Belle et al., 2011). In either case, it is believed that people with T1D do not present clinical with T1D until over 80% of the β-cells have been destroyed (Atkinson, 2012). Loss of normal insulin function progressively and severely dysregulates the glycemic environment. Clinically, patients usually present initially with a classic trio of symptoms (i.e., polydypsia, polyphagia, polyuria) alongside overt hyperglycemia (Atkinson, 2012). Historically, acute glycemic complications have been a significant cause of mortality in patients with T1D (Goodkin, 1975). In older patients, T1D more typically presents as mild fasting hyperglycemia with diminished glucose tolerance which can rapidly intensify to severe hyperglycemia/ketoacidosis during times of physiological stress (American Diabetes Association, 2014a).

1.3.2 Insulin therapy & current therapeutic challenges

The advent of life-saving recombinant insulin therapies in the 1980s (Quianzon & Cheikh, 2012) transformed T1D from a fatal disease into a manageable, life-long condition for the majority of patients. However, despite being able to live longer and healthier lives, people with T1D experience repetitive fluctuations outside of normal glycemic ranges (i.e. higher glycemic variability) (Šoupal et al., 2014). Dysglycemic episodes over time can lead

to the systemic perturbation of metabolism from T1D in the form of dyslipidemia and insulin resistance (Kaul et al., 2015), resulting in well-understood dysfunctions at insulin-sensitive tissues such as retinopathy, neuropathy and nephropathy (DiMeglio et al., 2018). However, our understanding of systemic complications resulting from T1D remains incomplete. Now, less understood complications of T1D such as myopathy are beginning to garner attention of researchers (D. D'Souza et al., 2013). Indeed, as a tissue that is a major contributor to glucose disposal in the post-prandial state (Meyer et al., 2002), it is extremely important that skeletal muscle is understood both in the context of normal health and during of T1D.

2 SKELETAL MUSCLE

Skeletal muscle is one of the most dynamic and plastic tissues in the body. It makes up to 40% of human body weight (Janssen et al., 2000) and serves as a major storage pool for up to 60% of total body protein in humans (Poortmans et al., 2012). The primary purpose of skeletal muscle is the production of mechanical force from chemical energy via the breakdown of ATP (Kuo & Ehrlich, 2015). The resultant force is used to maintain body posture, produce movement (i.e. locomotion) that is required for physical activity, and ultimately makes skeletal muscle a prime contributor to functional independence (Amigues et al., 2013; Foldvari et al., 2000; Santos et al., 2017).

2.1 MUSCLE STRUCTURE

Skeletal muscle is made of multinucleated myofibers that originated from the fusion of a large number of precursor cells called myoblasts (Bentzinger et al., 2012). Unique components of myofibers include an interlaced network of contractile elements known as

myofibrils and a large number of mitochondria between myofibrils required to support the high energy demands of muscle contraction (Dahl et al., 2015). Peripheral to these elements, there is another population of mitochondria located close to the sarcolemma (i.e. muscle membrane) that are particularly useful for energy production during exercise due to their proximity to the lattice-like network of capillaries and therefore the oxygen/nutrient supply (Gan et al., 2018). Also adjacent to the sarcolemma are many myonuclei and a unique population of resident stem cells known as satellite cells which are important for supporting muscle repair and regeneration (Frontera & Ochala, 2015). Finally, skeletal muscles are interfaced with the nervous system through neuromuscular junctions (NMJ) which allow for the transmission of synaptic information into contractile activity via the action potential (Tintignac et al., 2015).

2.2 MYOFILAMENTS & MUSCLE CONTRACTION

Myofibrils are comprised of two types of molecular contractile elements, known as myofilaments, which are arranged along the longitudinal axis of the myofiber. The thicker of the two filaments, myosin, was first described structurally in 1993 (Rayment et al., 1993) as a hexameric protein which is able to bind the thinner actin filament to form the actomyosin complex/crossbridge in the relaxed state (Frontera & Ochala, 2015). Briefly, contraction results when the myosin heavy chain (MHC) ATPase hydrolyzes adenosine triphosphate (ATP), into its constituent by-products adenosine diphosphate (ADP) and a high-energy phosphate group. Energy from this reaction detaches the actomyosin crossbridge, repriming the myofilaments for subsequent repeated crossbridge cycles needed for tetanic contraction. Notably, there exist multiple MHC ATPase enzyme

subtypes, which are categorized primarily by the speed at which they are able to catalyze the hydrolysis of ATP and re-initiate the cross-bridge cycle. The muscle fiber-type composition describes the distribution and abundance of different MHCs across a given muscle and is often used to broadly categorize the characteristics of the muscle fiber, and by extension categorize entire muscles.

2.3 MUSCLE FIBER-TYPE COMPOSITION

By determining the rate of ATP hydrolysis, the fiber-type of a muscle cell determines its inherent contractile speed and results in muscle fibers that exhibit slower or faster contraction, relatively. Together, these factors have given rise to the most common categorization of muscle fibers based on their contractile speed (i.e. fast vs. slow). However, the notion of muscle fiber-type diversity has rapidly progressed since the model of two muscle types. Since an initial observation by Edstrom and Kugelberg (Edström & Kugelberg, 1968) in 1968 of fibers with enhanced glycolytic or oxidative metabolism, it has become increasingly well understood that muscle fibers also differ on a metabolic continuum, and are often categorized in reference to their propensity for a certain type of metabolism. As our understanding of skeletal muscles increases, muscle fiber-types have been subcategorized using an expanding repertoire of methodologies (i.e. histochemical, biochemical, morphological, or physiologic characteristics) (Herbison et al., 1982; van Wessel et al., 2010). Importantly, these methods do not always agree. Thus, the need for more advanced and specific quantifications of fiber-types is still an important need in the field (for review, see (Schiaffino & Reggiani, 2011)).

2.3.1 Type 1 vs type 2 fibers

Type 1 fibers are those that are dominated by type 1 MHC (MHC1) filaments. These are often postural or tonically active muscles, including the soleus in the lower leg or the diaphragm (Johnson & Polgar, n.d.). These slow-twitch muscles are more fatigue-resistant and thus excel in repetitive or prolonged activities such as endurance exercise or the maintenance of proper posture (Wilson et al., 2012). In contrast, type 2 fibers, composed mostly of type 2 MHC (MHC2) filaments possess the ability to produce a higher maximal force at a quicker rate, but are less fatigue-resistant (Linssen et al., 1991) than their MHC1 counterparts. Generally, type 2 fibers are dominant in muscles that produce more powerful or explosive movements, including the gastrocnemius in the lower leg or the triceps in the upper arm (Johnson & Polgar, n.d.). These muscles excel at activities such as resistance training or plyometric activities (Wilson et al., 2012). Metabolically, type 1 fibers express a higher abundance of the insulin- and contraction-sensitive glucose transporter GLUT4 (Richter & Hargreaves, 2013) and have been found to have an enhanced glucose handling capability (Albers et al., 2015). The impact of fiber-type proportion on human health is emphasized in the case of certain metabolic diseases such as obesity and type 2 diabetes (T2D). In those with T2D, type 1 fiber proportions are smaller than in their control counterparts (Oberbach et al., 2006; Tanner et al., 2002), which has been associated with a reduction in oxidative enzyme activity in the muscle (Oberbach et al., 2006). Accordingly, the proportion of the two major fiber types in skeletal muscle often serves as an indicator of muscle function (i.e. oxidative vs. glycolytic capacity) and muscle health in different physiologic conditions of health and disease.

2.3.2 Hybrid fibers

As the quantification of muscle fiber-types has advanced, it has often been recognized that myofibers may not exist strictly as one type or another, but instead as hybrid fibers (i.e. expressing two or more MHC types). Hybrid fibers have been observed in many situations in human muscle and have shown the potential to help infer whether a muscle is in a transitionary state (Medler, 2019). For example, hybrid fibers tend to become more prevalent during detrimental changes such as disuse and aging (J. L. Andersen et al., 1999; Talmadge, 2000). Therefore, measuring hybrid fibers as a characteristic of myofiber morphology may help indicate the health of skeletal muscle. To our knowledge, no study to date has employed the measurement of hybrid fibers to elucidate the impact of metabolic disease, such as type 1 diabetes, on skeletal muscle health.

2.3.3 Type 1 fiber grouping

In addition to the proportion of major MHC types expressed across a muscle, the spatial relationship of different muscle fibers is an important yet less studied aspect of muscle literature. The clustering of several contiguous type 1 fibers (i.e. fiber grouping) is a characteristic that has been associated with neuromuscular aging. Specifically, denervation/reinnervation cycles lead to the progressive loss of motor units over the lifespan (Lexell & Downham, 1991). The surviving motor units that collaterally reinnervate new fibers tend to have slower twitches (Campbell et al., 1973), observed histologically as clustering or grouping of slow-twitch, type 1 myofibers in older adults (Kelly et al., 2018). Others have postulated that slower type 1 motor units are more efficient at reinnervation (Desypris & Parry, 1990) and thus show a greater propensity to reinnervate during these cycles. Indeed, over time, aging muscles are composed of progressively larger

groups of type 1 fibers (Campbell et al., 1973; Lexell et al., 1988; Lexell & Downham, 1991) and fewer type 2 fibers overall (Brunner et al., 2007). Functionally, age-related type 1 fiber grouping is associated with inefficient motor unit activation and greater physiologic difficulty to complete motor tasks (Kelly et al., 2017). Thus, if we consider the aforementioned negative aspects of advanced aging in skeletal muscle, the quantification of type 1 fiber grouping would be pertinent to apply to novel populations, particularly those that exhibit aspects of an aging phenotype (Monaco, Gingrich, et al., 2019).

2.4 NEUROMUSCULAR JUNCTIONS

As the primary effectors of movement and locomotion, skeletal muscles possess a unique interface with the nervous system, known as the neuromuscular junction (NMJ), which permits electrochemical signals from the motor neuron to be transformed to muscle contraction via the synapse. In addition to its integral role in muscle contraction, the NMJ signals a number of other important characteristics to the muscle fiber from motor neurons (called anterograde signaling) that indicate its 'role' in the muscular system. For example, a motor neuron that exists as part of a fast-twitch or slow-twitch motor unit will in turn encourage the propensity of the muscle fiber to exhibit a fast- or slow-twitch myofiber program (for review, see (Tintignac et al., 2015)). In addition to anterograde signaling from neuron to muscle, it is now increasingly understood that the health and stability of the post-synaptic NMJ (i.e. sarcolemmal portion) can translate effects from the muscle to the motor neuron as well. Evidence in this area comes from rodent muscles with transgenic overexpression of the peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), often called the master regulator of mitochondrial biogenesis, and known to be a key

factor in the formation and maintenance of slow muscle fibers (Schuler et al., 2006). Arnold *et al.* (Arnold et al., 2014) exhibited beneficial NMJ remodelling at both pre- and post-synaptic surfaces, as well as improved electrophysiological function in these mice. Meanwhile, others have found that PGC-1α overexpression in muscle was able to affect the phenotype of the motor neuron (Chakkalakal et al., 2010). Furthermore, the stability of the post-synaptic NMJ has been linked to the rate of age-related denervation in aging skeletal muscle (Bütikofer et al., 2011) and metabolic disease states can also disturb NMJ physiology (Garcia et al., 2012; Marques & Santo Neto, 2002).

2.5 SATELLITE CELLS

Adherent to the exterior of the sarcolemma are a population of progenitor cells known as muscle satellite cells. These cells serve as major contributors the muscle repair, regeneration and growth (Hawke & Garry, 2001; Kadi et al., 2004; Le Grand & Rudnicki, 2007), exemplified by muscles that have had satellite cells ablated and exhibit severely compromised muscle regeneration (Relaix & Zammit, 2012). Satellite cell populations are very plastic in response to external stimuli. For example, a single bout of high-intensity exercise has been shown to increase satellite cell activation (Crameri et al., 2004), while satellite cells have been found to decrease in type 2 fibers with age (Verdijk et al., 2007, 2014), and show less response to exercise in aged individuals (Dreyer et al., 2006). Interestingly, a suboptimal metabolic state of skeletal muscle can affect the functionality of these cells and their impact on muscle regeneration differentiation (Aguiari et al., 2008).

2.6 CAPILLARIES

Capillaries serve to match blood supply to the changing metabolic demands of the myofibers (Parise et al., 2020). Studies have long-suggested that a less dense network of capillaries (i.e. lower capillary density) in skeletal muscle is detrimental to skeletal muscle and whole-body metabolism, as seen in cases of T2D (Groen et al., 2014), obese (Gavin et al., 2005), and insulin resistant individuals (Lillioja et al., 1987). Conversely, capillaries per myofiber tend to increase during exercise training regimens, proportional to the growth of fiber size during training (Hermansen & Wachtlova, 1971; McCall et al., 1996). More recent work has also pointed to the close relationship between capillaries and satellite cell function, as active satellite cells are located anatomically closer to capillaries than quiescent satellite cells (Nederveen et al., 2016). Additionally, muscle fibers in young adults with a higher perfusion capacity exhibited enhanced activation of satellite cells following eccentric exercise (Nederveen et al., 2018).

3 SKELETAL MUSCLE WITH TYPE 1 DIABETES

Skeletal muscles represent the largest pool of insulin sensitive tissues in the human body. They are is responsible for up to 40% of systemic (i.e. non-liver) glucose uptake in the post-prandial state, representing 25% of total glucose uptake (Meyer et al., 2002). Under hyperinsulinemic conditions, this has been shown to rise to 80% (Thiebaud et al., 1982). Skeletal muscle is also able to uptake blood glucose in an insulin-independent manner, such as during contraction/exercise (for review, see (Richter & Hargreaves, 2013)), emphasizing the importance of skeletal muscle in whole-body glucose metabolism. Therefore, the optimization of skeletal muscle health is a necessary consideration in promoting healthy glucose metabolism in people with type 1 diabetes. Despite its

physiological importance for glucose handling in this disease, our understanding of changes to skeletal muscle health during acute and long-term T1D remains in its infancy.

3.1 MUSCLE WEAKNESS & DIABETIC PERIPHERAL NEUROPATHY

While a number of studies have shown reductions in skeletal muscle strength, most have not considered the effects of T1D independent from diabetic peripheral neuropathy (DPN) (H. Andersen et al., 1996, 1997; H. Andersen, 1998; H. Andersen et al., 2005; Andreassen et al., 2009; Orlando et al., 2017), a complication of T1D which can prevent physical activity due to insensitivity and/or pain resulting from neuronal damage (Tesfaye & Selvarajah, 2012). In long-standing T1D, impairments in force production have been correlated to the severity of neuropathic symptoms (H. Andersen, 1998; H. Andersen et al., 1996). These findings have led some to point to DPN as the root of T1D-induced muscle weakness. Understandably, the selection of study participants with longstanding T1D often necessitates recruitment of individuals with comorbidities such as DPN. However, it remains possible that people with T1D and DPN possess a conflating factor that can mask the effects of the disease on altered skeletal muscle in those with T1D. Therefore, there is present need to study skeletal muscle health in T1D participants in the absence of and/or preceding the observation of any neuropathic symptoms to truly uncover if muscle weakness is independent of DPN.

3.2 DECREASED MUSCLE MASS

Muscle loss during T1D has been demonstrated several times in rodent models of the disease. Rodents with experimental T1D exhibit significant decreases in myofiber area, particularly in fast-glycolytic fibers (Armstrong et al., 1975; Cotter et al., 1993; Klueber &

Feczko, 1994; Medina-Sanchez et al., 1991; Snow et al., 2005). Other studies reveal that these fibers also show inhibited growth in response to a hypertrophic stimulus (Armstrong & Ianuzzo, 1977), suggesting that the capacity for muscle growth is hindered in muscles with T1D. However, humans with T1D in recent decades have benefitted from widespread use of insulin therapies that are able to rescue T1D patients from the excessive atrophy that was historically observed in unmanaged diabetes (Jakobsen & Reske-Nielsen, 1986b). Despite this, studies in the era of insulin therapy continue to report a reduction in the myofiber size of both those recently diagnosed with T1D (Jakobsen & Reske-Nielsen, 1986b) and in middle-aged persons with T1D compared with non-diabetic participants (H. Andersen et al., 1997, 2004). Further, recently diagnosed people with T1D have also been shown to exhibit atrophy in myofibers preceding any detectable signs of neuropathy (Reske-Nielsen et al., 1977), suggesting that the muscle atrophy accompanies or perhaps precedes neuropathy in T1D people. However, the exact temporal relationship between muscle atrophy and other complications remains unclear. Therefore, particular attention in future studies should be dedicated to examining muscle mass in people with T1D before the presence of diabetic complications.

3.3 FIBER-TYPE PLASTICITY DURING TYPE 1 DIABETES

3.3.1 Elevated proportion of type 2 fibers

To date, few studies have morphologically examined myofibers in persons with T1D. Studies quantifying fiber-type proportions observed a greater proportion of type 2 fibers in distal leg muscles of people with T1D when compared to people with T2D and non-diabetic individuals (Andreassen et al., 2014). A similar shift towards type 2 fibers

was observed in younger adults with T1D (Fritzsche, Blüher, et al., 2008). A proposed mechanism states that insulin resistance may disturb the ratio of glycolytic-to-oxidative enzymes in skeletal muscle, as observed in other metabolic diseases like obesity and T2D (Crowther et al., 2003; He et al., 2001). Future studies need to confirm this putative mechanism in T1D, given that overt insulin resistance is not present in all patients.

3.3.2 Shift towards glycolytic profile

Adults with T1D also display higher levels of glycolytic enzymes observed histochemically (Fritzsche, Blüher, et al., 2008). Furthermore, the larger population of 'fast-glycolytic fibers' appeared to be expressed at the expense of primarily oxidative fibers (Fritzsche, Blüher, et al., 2008), suggesting a shift towards glycolytic metabolism that accompanies the shift in the predominant fiber-type. Interestingly, Cree-Green *et al.* (Cree-Green et al., 2015), also observed a lower rate of oxidative phosphorylation in adolescents with T1D, which was correlated to insulin resistance. Another investigation by Roch-Norlund *et al.* (Roch-Norlund et al., 1970) revealed that participants with diabetes (T1D and T2D) expressed less glycogen than control participants, further suggesting metabolic dysregulation of these muscles. However, the insulin-dependent (i.e. T1D) participants in this study were all recently diagnosed, with none exhibiting symptoms for longer than six months (Roch-Norlund et al., 1970). Accordingly, further studies are required to examine skeletal muscle glycogen content during longstanding T1D with insulin therapy as is now commonplace in management of the disease.

3.4 REDUCED REGENERATIVE AND SATELLITE CELL CAPACITY

The majority of evidence for altered regenerative capacity in T1D has been generated by rodent studies. Muscles from rodent models of T1D are more susceptible to damage (D. M. D'Souza et al., 2016a), exhibit delayed regeneration following damage (Krause et al., 2013), and are unable to properly facilitate muscle repair (Jeong et al., 2013a). Interestingly, D'Souza *et al.* observed that human (18-21 year old) T1D muscles also exhibit significant reductions in satellite cell content at rest (D. M. D'Souza et al., 2016a). The mechanisms affecting satellite cells in T1D muscles are unclear. It has been previously observed that hyperglycemia is able to induce changes to the muscle stem cell niche, namely oxidative stress and chronic inflammation, which have been shown to reduce satellite cell activity and subsequent muscle repair (Aragno et al., 2004; Gordin et al., 2008). To date, there have been no investigations of the acute muscle repair process in humans with T1D following exercise. Furthermore, there has been no literature that has measured the content or signalling of satellite cells in older adults with the disease. Therefore, there is a particular need to elucidate satellite cell physiology under known acute and chronic muscle stressors, such as the contexts of exercise and aging, to further our understanding of skeletal muscle alteration during T1D.

3.5 VASCULAR DYSFUNCTION

Perturbations to the skeletal muscle vasculature have been observed in pre-clinical rodent models which exhibit alterations to the capillary bed, disrupted diffusion to skeletal muscle (Kindig et al., 1998; Sexton et al., 1994), and have been correlated to the level of dysglycemia in T1D humans (Raskin et al., 1983; Rosenstock et al., 1988; Sosenko et al., 1984). Also, evidence from several T1D rodent models has demonstrated a decrease in

capillary-to-fiber ratio (Kivelä et al., 2006; Krause et al., 2009) as well as dysregulated angiogenesis that was unable to be rescued by exercise (Kivelä et al., 2006). Skeletal muscles from humans with T1D possess more permeable capillaries, exhibit diminished blood flow through the vessels, and a higher rate of diffusion into surrounding muscle tissue, while exhibiting no difference in the capillary density (Leinonen et al., 1982). With exercise, young patients with T1D were found to display blunted microvascular reactivity along with a lower mitochondrial respiratory capacity in skeletal muscles (Heyman et al., 2020). Fortunately, it appears that exercise can also elicit beneficial effects on vascular function in T1D individuals (Fuchsjäger-Mayrl et al., 2002). To date, however, observations of vascular dysfunction during T1D have been made in participants up to the fifth decade of life with age-matched control participants as comparators (Fuchsjäger-Mayrl et al., 2002). As the average lifespan for patients with T1D continues to increase, there is an evident need to examine the vascular content of T1D patients across a wide age range. Comparisons of younger and older T1D patients may give insight into the effects of long-term T1D on skeletal muscle vasculature.

3.6 DYSREGULATION OF MYOSTATIN & NEGATIVE GROWTH REGULATORS

Myostatin is a potent negative regulator of muscle growth which has emerged as a putative mediator of muscle mass during T1D. Primarily synthesized in skeletal muscle, myostatin binds to its receptor complex activin IIB on the cell surface resulting in a cascade of signaling from the cell surface to the nucleus (Elliott et al., 2012). Ultimately, myostatin targets a number of transcription factors that promote muscle atrophy (Elkina et al., 2011).

Elevations in myostatin expression have been observed repeatedly in humans with muscle wasting conditions (Elliott et al., 2012), in metabolic disorders like T2D (Brandt et al., 2012), and are associated with impaired insulin signaling/sensitivity (Brandt et al., 2012; Chen et al., 2009; Wang et al., 2012). Evidence in rodent models suggests that myostatin expression is elevated in T1D (Chen et al., 2009; Jeong et al., 2013a; Wieteska-Skrzeczynska et al., 2009). Satellite cells from STZ mice exhibit excessive levels of myostatin, while administration of a myostatin antagonist is able to rescue impaired skeletal muscle regeneration (Jeong et al., 2013a). Despite the accumulating evidence for a role of myostatin in the maintenance of skeletal muscle function and metabolic health, its expression in the blood and muscle of those with T1D has not previously been reported.

4 ADDRESSING LIMITATIONS OF T1D SKELETAL MUSCLE LITERATURE

4.1 BRIDGING CLINICAL OBSERVATIONS WITH PRECLINICAL MODELS OF T1D

To date, the majority of skeletal muscle research in those with T1D has delivered evidence in one of two categories.

4.1.1 Human observational studies

Firstly, non-invasive studies of T1D patients have provided insights into the muscle-specific effects of T1D, such as impaired glucose uptake (Nuutila et al., 1993; Peltoniemi et al., 2001), or dysregulated blood flow (Leinonen et al., 1982; Vervoort et al., 1999). However, these often lack any investigation of cellular characteristics which are vital to muscle health and function, such as morphology and fiber-type composition. There

are a few notable exceptions that make measurements at the myofiber-level, discussed in previous sections (Andreassen et al., 2014; Fritzsche, Blüher, et al., 2008).

Fritzsche et al. (Fritzsche, Blüher, et al., 2008) provide evidence that changes in fiber-type composition are associated with elevated levels of glycolytic enzymes in T1D patients, suggesting that changes occur in both the contractile and metabolic qualities of skeletal muscles in those with T1D. However, the classification of fiber-type in this study was done by combining metabolic and contractile enzyme activities. This technique relies on serial-sectioning (i.e. several contiguous sections) of skeletal muscle, which is much more difficult to perform and therefore to generalize the results of this study, as compared to MHC-antigen immunostaining which allows for discrete labelling of all muscle fiber-types including hybrid fibers in a single cross-section (Bloemberg & Quadrilatero, 2012). Furthermore, this study provides an imbalanced mixed-sex sample of young T1D patients (Controls, male/female = 4/6; T1D, male/female = 1/6) and presents myofiber data for the cohort overall, thereby potentially masking effects of that may be sex-specific in males with T1D. Given these factors, there remains the need to examine these characteristics in adults with T1D, giving particular attention to specific fiber-type composition, and utilizing a more balanced cohort of males and females to elucidate possible sex-differences.

Andreassen et al. (Andreassen et al., 2014) investigated myofiber size, composition, and capillary density in relation to neuropathy and muscle strength. Despite there being larger fibers and relatively more type 2 fibers in T1D patients, the authors concluded that each of these characteristics bears no relationship to neuropathy. However, the data for these measurements were provided in a mixed group of neuropathic and non-neuropathic

patients, thereby making it difficult to discern the contribution of neuropathy to the changes observed here. Interestingly, the authors state that the location of muscle biopsies (i.e. gastrocnemius) utilized in this study is more likely to observe damage due to macroangiopathy and more pronounced DPN. Therefore, it seems likely that the presence of neuropathy in some of the patients is even more likely to present a confounding factor in true differences to fiber size, composition, and capillary density. This presents the need for future investigations to study these characteristics in the absence of confounders (i.e. DPN), by observing more proximal muscles like the vastus lateralis which are less affected by neuropathy, or in patients that are uniformly non-neuropathic.

Beyond the clinical studies mentioned above, there are still a number of important myofiber characteristics that have not been adequately addressed in humans, such as satellite cells and myofiber ultrastructure. Currently, only one investigation has noted a decreased satellite cell content in T1D patients (D. M. D'Souza et al., 2016a), under resting conditions only. Therefore, there is still a paucity of knowledge of what happens to muscles during T1D after a regeneration/recovery stimulus (i.e. exercise). Additionally we do not know if muscle ultrastructure changes at rest or under any stimuli in those with T1D. Unfortunately, the ultrastructural investigations to date through transmission electron microscopy (TEM) have only been undertaken in one study focused on the muscle capillaries (Leinonen et al., 1982), providing little to no insight into the ultrastructure of T1D skeletal muscle per se.

4.1.2 Preclinical models of T1D

The second and larger category of skeletal muscle research in T1D involves a growing collection of literature in preclinical rodent models that has begun to unravel the perturbations to muscle physiology at a cellular/molecular level. These experimental models provide several pieces of information that contribute to our understanding of physiological changes to muscle during T1D, including altered regeneration/repair (D. M. D'Souza et al., 2016a; Jeong et al., 2013a; Krause et al., 2013) and angiogenic deficiency (Kivelä et al., 2006; Lehti et al., 2006). Unfortunately, the nature of experimental rodent models precludes data from being readily extrapolated to the clinical context of T1D. For example, one of the major rodent models of T1D historically has been in rats or mice administered experimental levels of streptozotocin (STZ), a compound which causes DNA damage followed by cell death of pancreatic β-cells, rendering the animal insulin deficient (Klueber & Feczko, 1994; Krause et al., 2009). Although the chemotoxic effects of STZ were initially thought to be specific to the β-cells, recent studies have shown this not to be the case, particularly in the case of skeletal muscle (Johnston et al., 2007; Krause et al., 2009). Therefore, off-target effects of STZ need to be considered when interpreting these data. An alternative model, the Akita mouse, offers improvements over STZ rodents as the loss of insulin is due to genetic modification of the insulin gene (Al-awar et al., 2016; Yoshioka et al., 1997), and the animals are not subject to off-target effects of STZ. Despite its improvements, these mice still do not exhibit any fluctuation in insulin levels but rather a constant hypoinsulinemia, and therefore do not accurately reflect the typical conditions of a patient with T1D who will treat with insulin therapy to offset dietary fluctuations in glucose (Carlsen et al., 2011; García-García et al., 2015; Griggs et al., 2020). Importantly,

skeletal muscle displays particular plasticity in terms of glucose tolerance and insulin sensitivity (Goodyear & Kahn, 1998; Holloszy, 2005). Therefore, use of animal models carries relevant weaknesses which necessitate veritable caution when generalizing to the human experience of T1D and its effects on skeletal muscle.

To overcome the constrained utility of preclinical T1D models, there is a particular need to expand upon the earliest cellular observations in myofibers using techniques to broaden our knowledge of disturbances in muscle function from humans with T1D. Studies of this nature will involve more invasive techniques, but the information gained will propel forward our understanding of human skeletal muscle directly, without being constrained by the limitations of rodent models. Further, more invasive studies will allow for the investigation of biochemical markers that could uncover potential biochemical mediators of diabetic myopathy in T1D.

4.2 NARROW SCOPE OF HUMAN T1D STUDIES.

While human studies of T1D circumvent the limitations of preclinical models, the majority of studies involving skeletal muscle during T1D have been under primarily baseline/resting conditions. Given the dramatic physiological changes that can occur under acute stressors like eccentric exercise (i.e. damage, regeneration, repair), or chronic stressors like age (i.e. muscle atrophy, strength loss, denervation/reinnervation), the dearth of literature in these areas leaves blind spots in our knowledge of human T1D muscle physiology.

4.2.1 Aging studies

Due to the scarcity of T1D research in aging adults, current treatment guidelines are based on T2D (Bispham et al., 2020). The striking lack of studies in the area has led to literature that is void of information regarding skeletal muscle health in older individuals with T1D. Recently it has been hypothesized that T1D skeletal muscle exhibits a form of accelerated aging (Monaco, Gingrich, et al., 2019) – exhibiting signs of aged muscle sooner than age-matched non-diabetic counterparts. Deleterious effects commonly observed in aged muscles are all observed to a higher degree in T1D skeletal muscle, such as decreased myofiber size (H. Andersen et al., 2004; Jakobsen & Reske-Nielsen, 1986b), increased fatigability (Kaya Mutlu et al., 2018; Lukács et al., 2012; Maratova et al., 2018; Mosher et al., 1998), and dysregulated metabolism (Cree-Green et al., 2015; Crowther et al., 2003; Monaco et al., 2018a; Saltin et al., 1979; Wallberg-Henriksson et al., 1984). These cross-sectional studies offer a snapshot comparison with age-matched control participants. However, to date, no studies have examined skeletal muscle in participants from multiple age groups with age-matched controls. In the absence of longitudinal studies which take considerable resources to undertake, there is a need for studies which use age as a comparator to understand T1D over the lifespan. It should also be noted that advancements in insulin therapies and their implications in skeletal muscle need to be appreciated or addressed. Currently, this is an area understudied and ripe for future investigative research.

4.2.2 Exercise studies and the impact on skeletal muscle morphology

The focus of exercise studies in T1D patients in the recent decades have been with regards to the effects of exercise on glycemia (Guelfi et al., 2005; Riddell & Perkins, 2006; Yardley et al., 2012, 2013). While some studies have examined resistance exercise in

individuals with T1D (Ramalho et al., 2006; Toghi-Eshghi & Yardley, 2019; Turner et al., 2015; Yardley et al., 2012, 2013), these were investigations of overall health and glycemic modulation, rather than physiological changes of myofibers during T1D. Given the reduced regenerative capacity of skeletal muscle in T1D rodents (D. M. D'Souza et al., 2016a; Krause et al., 2013), there is still the need to understand human muscle physiology in response to a damaging or eccentric exercise stimulus.

OBJECTIVES & HYPOTHESES

The purpose of this body of work is to address the aforementioned gaps in the literature. It is the aim of this work to expand understanding of the cellular skeletal muscle environment during T1D under stressors of age and eccentric damage, and to attempt to observe potential molecular mediators of diabetic myopathy in humans.

STUDY #1 (CHAPTER 2)

The objective of this study was to examine skeletal muscle function in those with T1D across a wide age range while using supporting histological/immunofluorescent measures to characterize myofiber morphological traits over a significant portion of the adult lifespan.

It was hypothesized that compared to control participants: 1) strength would be decreased in all age groups in individuals with T1D. 2) muscles would shift towards a faster, more glycolytic fiber-type composition in people with T1D.

STUDY #2 (CHAPTER 3)

The objective of this study was to investigate the early timepoints of muscle regeneration following an eccentric exercise stimulus in young adults with T1D for the first

time in order to gain an understanding of satellite cell content/proliferation responses, as well as the degree of ultrastructural and sarcolemmal damage in these participants.

It was hypothesized that compared to control participants: 1) satellite cell content/proliferation would be lower across all timepoints of regeneration, and 2) ultrastructural and membrane damage would be increased in T1D participants.

STUDY #3 (CHAPTER 4)

This study aimed to observe circulating and skeletal muscle myostatin levels in a large, diverse cohort of T1D participants in order to expand findings in rodent models that position myostatin as a potential mediator of muscle loss during T1D.

It was hypothesized that T1D participants would exhibit higher circulating and skeletal muscle myostatin compared to control participants.

REFERENCES

Aguiari, P., Leo, S., Zavan, B., Vindigni, V., Rimessi, A., Bianchi, K., Franzin, C., Cortivo, R., Rossato, M., Vettor, R., Abatangelo, G., Pozzan, T., Pinton, P., & Rizzuto, R. (2008). High glucose induces adipogenic differentiation of muscle-derived stem cells. *Proceedings of the National Academy of Sciences*, *105*(4), 1226–1231. https://doi.org/10.1073/pnas.0711402105

Al-awar, A., Kupai, K., Veszelka, M., Szűcs, G., Attieh, Z., Murlasits, Z., Török, S., Pósa, A., & Varga, C. (2016). Experimental diabetes mellitus in different animal models. *Journal of Diabetes Research*, *2016*, e9051426. https://doi.org/10.1155/2016/9051426

Albers, P. H., Pedersen, A. J. T., Birk, J. B., Kristensen, D. E., Vind, B. F., Baba, O., Nøhr, J., Højlund, K., & Wojtaszewski, J. F. P. (2015). Human muscle fiber type-specific insulin signaling: impact of obesity and type 2 diabetes. *Diabetes*, *64*(2), 485–497. https://doi.org/10.2337/db14-0590

American Diabetes Association. (2014). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, *37 Suppl 1*, S81-90. https://doi.org/10.2337/dc14-S081

Amigues, I., Schott, A.-M., Amine, M., Gelas-Dore, B., Veerabudun, K., Paillaud, E., Beauchet, O., Rolland, Y., Canouï Poitrine, F., & Bonnefoy, M. (2013). Low skeletal muscle mass and risk of functional decline in elderly community-dwelling women: the prospective EPIDOS study. *Journal of the American Medical Directors Association*, *14*(5), 352–357. https://doi.org/10.1016/j.jamda.2012.12.002

Andersen, H. (1998). Muscular endurance in long-term IDDM patients. *Diabetes Care*, *21*(4), 604–609. https://doi.org/10.2337/diacare.21.4.604

Andersen, H., Gadeberg, P. C., Brock, B., & Jakobsen, J. (1997). Muscular atrophy in diabetic neuropathy: A stereological magnetic resonance imaging study. *Diabetologia*, *40*(9), 1062–1069. https://doi.org/10.1007/s001250050788

Andersen, H., Gjerstad, M. D., & Jakobsen, J. (2004). Atrophy of foot muscles: A measure of diabetic neuropathy. *Diabetes Care*, *27*(10), 2382–2385. https://doi.org/10.2337/diacare.27.10.2382

Andersen, H., Poulsen, P. L., Mogensen, C. E., & Jakobsen, J. (1996). Isokinetic muscle strength in long-term IDDM patients in relation to diabetic complications. *Diabetes*, *45*(4), 440–445. https://doi.org/10.2337/diab.45.4.440

Andersen, H., Schmitz, O., & Nielsen, S. (2005). Decreased isometric muscle strength after acute hyperglycaemia in type 1 diabetic patients. *Diabetic Medicine: A Journal of*

the British Diabetic Association, *22*(10), 1401–1407. https://doi.org/10.1111/j.1464-5491.2005.01649.x

Andersen, J. L., Terzis, G., & Kryger, A. (1999). Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. *Muscle & Nerve*, *22*(4), 449–454. https://doi.org/10.1002/(SICI)1097-4598(199904)22:4<449::AID-MUS4>3.0.CO;2-2

Andreassen, C. S., Jakobsen, J., Flyvbjerg, A., & Andersen, H. (2009). Expression of neurotrophic factors in diabetic muscle—Relation to neuropathy and muscle strength. *Brain: A Journal of Neurology*, *132*(Pt 10), 2724–2733. https://doi.org/10.1093/brain/awp208

Andreassen C. S., Jensen J. M., Jakobsen J., Ulhøj B. P., & Andersen H. (2014). Striated muscle fiber size, composition, and capillary density in diabetes in relation to neuropathy and muscle strength. *Journal of Diabetes*, *6*(5), 462–471. https://doi.org/10.1111/1753-0407.12124

Aragno, M., Mastrocola, R., Catalano, M. G., Brignardello, E., Danni, O., & Boccuzzi, G. (2004). Oxidative stress impairs skeletal muscle repair in diabetic rats. *Diabetes*, *53*(4), 1082–1088.

Armstrong, R. B., Gollnick, P. D., & Ianuzzo, C. D. (1975). Histochemical properties of skeletal muscle fibers in streptozotocin-diabetic rats. *Cell and Tissue Research*, *162*(3), 387–393. https://doi.org/10.1007/BF00220185

Armstrong, R. B., & Ianuzzo, C. D. (1977). Compensatory hypertrophy of skeletal muscle fibers in streptozotocin-diabetic rats. *Cell and Tissue Research*, *181*(2), 255–266. https://doi.org/10.1007/BF00219985

Arnold, A.-S., Gill, J., Christe, M., Ruiz, R., McGuirk, S., St-Pierre, J., Tabares, L., & Handschin, C. (2014). Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1α. *Nature Communications*, *5*(1), 3569. https://doi.org/10.1038/ncomms4569

Atkinson, M. A. (2012). The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harbor Perspectives in Medicine*, *2*(11), a007641. https://doi.org/10.1101/cshperspect.a007641

Atkinson, M. A., von Herrath, M., Powers, A. C., & Clare-Salzler, M. (2015). Current concepts on the pathogenesis of type 1 diabetes—Considerations for attempts to prevent and reverse the disease. *Diabetes Care*, *38*(6), 979–988. https://doi.org/10.2337/dc15-0144

Bentzinger, C. F., Wang, Y. X., & Rudnicki, M. A. (2012). Building muscle: molecular regulation of myogenesis. *Cold Spring Harbor Perspectives in Biology*, *4*(2), a008342. https://doi.org/10.1101/cshperspect.a008342

Bispham, J. A., Hughes, A. S., Driscoll, K. A., & McAuliffe-Fogarty, A. H. (2020). Novel challenges in aging with type 1 diabetes. *Current Diabetes Reports*, *20*(5), 15. https://doi.org/10.1007/s11892-020-01298-9

Bloemberg, D., & Quadrilatero, J. (2012). Rapid determination of myosin heavy chain expression in rat, mouse, and human skeletal muscle using multicolor immunofluorescence analysis. *PLoS ONE*, *7*(4), e35273. https://doi.org/10.1371/journal.pone.0035273

Bonifacio, E., Scirpoli, M., Kredel, K., Füchtenbusch, M., & Ziegler, A. G. (1999). Early autoantibody responses in prediabetes are IgG1 dominated and suggest antigen-specific regulation. *Journal of Immunology (Baltimore, Md.: 1950)*, *163*(1), 525–532.

Brandt, C., Nielsen, A. R., Fischer, C. P., Hansen, J., Pedersen, B. K., & Plomgaard, P. (2012). Plasma and muscle myostatin in relation to type 2 diabetes. *PloS One*, *7*(5), e37236. https://doi.org/10.1371/journal.pone.0037236

Brunner, F., Schmid, A., Sheikhzadeh, A., Nordin, M., Yoon, J., & Frankel, V. (2007). Effects of aging on type ii muscle fibers: a systematic review of the literature. *Journal of Aging and Physical Activity*, *15*(3), 336–348. https://doi.org/10.1123/japa.15.3.336

Bütikofer, L., Zurlinden, A., Bolliger, M. F., Kunz, B., & Sonderegger, P. (2011). Destabilization of the neuromuscular junction by proteolytic cleavage of agrin results in precocious sarcopenia. *The FASEB Journal*, *25*(12), 4378–4393. https://doi.org/10.1096/fj.11-191262

Campbell, M. J., McComas, A. J., & Petito, F. (1973). Physiological changes in ageing muscles. *Journal of Neurology, Neurosurgery & Psychiatry*, *36*(2), 174–182. https://doi.org/10.1136/jnnp.36.2.174

Carlsen, S., Petersen, P. H., Skeie, S., Skadberg, Ø., & Sandberg, S. (2011). Within-subject biological variation of glucose and HbA1c in healthy persons and in type 1 diabetes patients. *Clinical Chemistry and Laboratory Medicine*, *49*(9), 1501–1507. https://doi.org/10.1515/CCLM.2011.233

Chakkalakal, J. V., Nishimune, H., Ruas, J. L., Spiegelman, B. M., & Sanes, J. R. (2010). Retrograde influence of muscle fibers on their innervation revealed by a novel marker

for slow motoneurons. *Development (Cambridge, England)*, *137*(20), 3489–3499. https://doi.org/10.1242/dev.053348

Chen, Y., Cao, L., Ye, J., & Zhu, D. (2009). Upregulation of myostatin gene expression in streptozotocin-induced type 1 diabetes mice is attenuated by insulin. *Biochemical and Biophysical Research Communications*, *388*(1), 112–116. https://doi.org/10.1016/j.bbrc.2009.07.129

Cotter, M. A., Cameron, N. E., Robertson, S., & Ewing, I. (1993). Polyol pathway-related skeletal muscle contractile and morphological abnormalities in diabetic rats. *Experimental Physiology*, *78*(2), 139–155. https://doi.org/10.1113/expphysiol.1993.sp003675

Crameri, R. M., Langberg, H., Magnusson, P., Jensen, C. H., Schrøder, H. D., Olesen, J. L., Suetta, C., Teisner, B., & Kjaer, M. (2004). Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *The Journal of Physiology*, *558*(1), 333–340. https://doi.org/10.1113/jphysiol.2004.061846

Cree-Green, M., Newcomer, B. R., Brown, M. S., Baumgartner, A. D., Bergman, B., Drew, B., Regensteiner, J. G., Pyle, L., Reusch, J. E. B., & Nadeau, K. J. (2015). Delayed skeletal muscle mitochondrial ADP recovery in youth with type 1 diabetes relates to muscle insulin resistance. *Diabetes*, *64*(2), 383–392. https://doi.org/10.2337/db14-0765

Crowther, G. J., Milstein, J. M., Jubrias, S. A., Kushmerick, M. J., Gronka, R. K., & Conley, K. E. (2003). Altered energetic properties in skeletal muscle of men with well-controlled insulin-dependent (type 1) diabetes. *American Journal of Physiology. Endocrinology and Metabolism*, *284*(4), E655-662. https://doi.org/10.1152/ajpendo.00343.2002

Dahl, R., Larsen, S., Dohlmann, T. L., Qvortrup, K., Helge, J. W., Dela, F., & Prats, C. (2015). Three-dimensional reconstruction of the human skeletal muscle mitochondrial network as a tool to assess mitochondrial content and structural organization. *Acta Physiologica*, *213*(1), 145–155. https://doi.org/10.1111/apha.12289

Desypris, G., & Parry, D. J. (1990). Relative efficacy of slow and fast alpha-motoneurons to reinnervate mouse soleus muscle. *American Journal of Physiology-Cell Physiology*, *258*(1), C62–C70. https://doi.org/10.1152/ajpcell.1990.258.1.C62

DiMeglio, L. A., Evans-Molina, C., & Oram, R. A. (2018). Type 1 diabetes. *Lancet (London, England)*, *391*(10138), 2449–2462. https://doi.org/10.1016/S0140-6736(18)31320-5

Dreyer, H. C., Blanco, C. E., Sattler, F. R., Schroeder, E. T., & Wiswell, R. A. (2006). Satellite cell numbers in young and older men 24 hours after eccentric exercise. *Muscle & Nerve*, *33*(2), 242–253. https://doi.org/10.1002/mus.20461

D'Souza, D., Al-Sajee, D., & Hawke, T. (2013). Diabetic myopathy: Impact of diabetes mellitus on skeletal muscle progenitor cells. *Frontiers in Physiology*, *4*, 379. https://doi.org/10.3389/fphys.2013.00379

D'Souza, D. M., Zhou, S., Rebalka, I. A., MacDonald, B., Moradi, J., Krause, M. P., Al-Sajee, D., Punthakee, Z., Tarnopolsky, M. A., & Hawke, T. J. (2016). Decreased satellite cell number and function in humans and mice with type 1 diabetes is the result of altered Notch signaling. *Diabetes*, *65*(10), 3053–3061. https://doi.org/10.2337/db15-1577

Edström, L., & Kugelberg, E. (1968). Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. *Journal of Neurology, Neurosurgery, and Psychiatry*, *31*(5), 424–433. https://doi.org/10.1136/jnnp.31.5.424

Eisenbarth, G. S. (1986). Type I diabetes mellitus. A chronic autoimmune disease. *The New England Journal of Medicine*, *314*(21), 1360–1368. https://doi.org/10.1056/NEJM198605223142106

Elkina, Y., von Haehling, S., Anker, S. D., & Springer, J. (2011). The role of myostatin in muscle wasting: An overview. *Journal of Cachexia, Sarcopenia and Muscle*, *2*(3), 143–151. https://doi.org/10.1007/s13539-011-0035-5

Elliott, B., Renshaw, D., Getting, S., & Mackenzie, R. (2012). The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiologica*, *205*(3), 324–340. https://doi.org/10.1111/j.1748-1716.2012.02423.x

Foldvari, M., Clark, M., Laviolette, L. C., Bernstein, M. A., Kaliton, D., Castaneda, C., Pu, C. T., Hausdorff, J. M., Fielding, R. A., & Singh, M. A. F. (2000). Association of muscle power with functional status in community-dwelling elderly women. *The Journals of Gerontology: Series A*, *55*(4), M192–M199. https://doi.org/10.1093/gerona/55.4.M192

Fritzsche, K., Blüher, M., Schering, S., Buchwalow, I. B., Kern, M., Linke, A., Oberbach, A., Adams, V., & Punkt, K. (2008). Metabolic profile and nitric oxide synthase expression of skeletal muscle fibers are altered in patients with type 1 diabetes. *Experimental and Clinical Endocrinology & Diabetes*, *116*(10), 606–613. https://doi.org/10.1055/s-2008-1073126

Frontera, W. R., & Ochala, J. (2015). Skeletal muscle: A brief review of structure and function. *Calcified Tissue International*, *96*(3), 183–195. https://doi.org/10.1007/s00223-014-9915-y

Fuchsjäger-Mayrl, G., Pleiner, J., Wiesinger, G. F., Sieder, A. E., Quittan, M., Nuhr, M. J., Francesconi, C., Seit, H.-P., Francesconi, M., Schmetterer, L., & Wolzt, M. (2002). Exercise training improves vascular endothelial function in patients with type 1 diabetes. *Diabetes Care*, *25*(10), 1795–1801. https://doi.org/10.2337/diacare.25.10.1795

Gale, E. A. M. (2002). The rise of childhood type 1 diabetes in the 20th century. *Diabetes*, *51*(12), 3353–3361. https://doi.org/10.2337/diabetes.51.12.3353

Gan, Z., Fu, T., Kelly, D. P., & Vega, R. B. (2018). Skeletal muscle mitochondrial remodeling in exercise and diseases. *Cell Research*, *28*(10), 969–980. https://doi.org/10.1038/s41422-018-0078-7

Garcia, C. C., Potian, J. G., Hognason, K., Thyagarajan, B., Sultatos, L. G., Souayah, N., Routh, V. H., & McArdle, J. J. (2012). Acetylcholinesterase deficiency contributes to neuromuscular junction dysfunction in type 1 diabetic neuropathy. *American Journal of Physiology - Endocrinology and Metabolism*, *303*(4), E551–E561. https://doi.org/10.1152/ajpendo.00622.2011

García-García, F., Kumareswaran, K., Hovorka, R., & Hernando, M. E. (2015). Quantifying the acute changes in glucose with exercise in type 1 diabetes: A systematic review and meta-analysis. *Sports Medicine*, *45*(4), 587–599. https://doi.org/10.1007/s40279-015-0302-2

Gavin, T. P., Stallings, H. W., Zwetsloot, K. A., Westerkamp, L. M., Ryan, N. A., Moore, R. A., Pofahl, W. E., & Hickner, R. C. (2005). Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans. *Journal of Applied Physiology*, *98*(1), 315–321. https://doi.org/10.1152/japplphysiol.00353.2004

Goodkin, G. (1975). Mortality factors in diabetes. A 20 year mortality study. *Journal of Occupational Medicine.*, *17*(11), 716–721.

Goodyear, L. J., & Kahn, B. B. (1998). Exercise, glucose transport, and insulin sensitivity. *Annual Review of Medicine*, *49*(1), 235–261. https://doi.org/10.1146/annurev.med.49.1.235

Gordin, D., Forsblom, C., Ronnback, M., Parkkonen, M., Waden, J., Hietala, K., & Groop, P.-H. (2008). Acute hyperglycaemia induces an inflammatory response in young

patients with type 1 diabetes. *Annals of Medicine*, *40*(8), 627–633. https://doi.org/10.1080/07853890802126547

Griggs, S., Redeker, N. S., Jeon, S., & Grey, M. (2020). Daily variations in sleep and glucose in adolescents with type 1 diabetes. *Pediatric Diabetes*, *21*(8), 1493–1501. https://doi.org/10.1111/pedi.13117

Groen, B. B. L., Hamer, H. M., Snijders, T., van Kranenburg, J., Frijns, D., Vink, H., & van Loon, L. J. C. (2014). Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *116*(8), 998–1005. https://doi.org/10.1152/japplphysiol.00919.2013

Guelfi, K. J., Jones, T. W., & Fournier, P. A. (2005). The decline in blood glucose levels is less with intermittent high-intensity compared with moderate exercise in individuals with type 1 diabetes. *Diabetes Care*, *28*(6), 1289–1294. https://doi.org/10.2337/diacare.28.6.1289

Hawke, T. J., & Garry, D. J. (2001). Myogenic satellite cells: Physiology to molecular biology. *Journal of Applied Physiology*, *91*(2), 534–551. https://doi.org/10.1152/jappl.2001.91.2.534

He, J., Watkins, S., & Kelley, D. E. (2001). Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes*, *50*(4), 817–823. https://doi.org/10.2337/diabetes.50.4.817

Herbison, G. J., Jaweed, M. M., & Ditunno, J. F. (1982). Muscle fiber types. *Archives of Physical Medicine and Rehabilitation*, *63*(5), 227–230.

Hermansen, L., & Wachtlova, M. (1971). Capillary density of skeletal muscle in well-trained and untrained men. *Journal of Applied Physiology*, *30*(6), 860–863. https://doi.org/10.1152/jappl.1971.30.6.860

Heyman, E., Daussin, F., Wieczorek, V., Caiazzo, R., Matran, R., Berthon, P., Aucouturier, J., Berthoin, S., Descatoire, A., Leclair, E., Marais, G., Combes, A., Fontaine, P., & Tagougui, S. (2020). Muscle oxygen supply and use in type 1 diabetes, from ambient air to the mitochondrial respiratory chain: is there a limiting step? *Diabetes Care*, *43*(1), 209–218. https://doi.org/10.2337/dc19-1125

Holloszy, J. O. (2005). Exercise-induced increase in muscle insulin sensitivity. *Journal of Applied Physiology*, *99*(1), 338–343. https://doi.org/10.1152/japplphysiol.00123.2005

Hyöty, H., Hiltunen, M., Knip, M., Laakkonen, M., Vähäsalo, P., Karjalainen, J., Koskela, P., Roivainen, M., Leinikki, P., Hovi, T., & Åkerblom, H. K. (1995). A prospective study of the role of Coxsackie B and other enterovirus infections in the pathogenesis of IDDM. *Diabetes*, *44*(6), 652–657. https://doi.org/10.2337/diab.44.6.652

Jakobsen, J., & Reske-Nielsen, E. (1986). Diffuse muscle fiber atrophy in newly diagnosed diabetes. *Clinical Neuropathology*, *5*(2), 73–77.

Janssen, I., Heymsfield, S. B., Wang, Z., & Ross, R. (2000). Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology*, *89*(1), 81–88. https://doi.org/10.1152/jappl.2000.89.1.81

Jeong, J., Conboy, M. J., & Conboy, I. M. (2013). Pharmacological inhibition of myostatin/TGF-β receptor/pSmad3 signaling rescues muscle regenerative responses in mouse model of type 1 diabetes. *Acta Pharmacologica Sinica*, *34*(8), 1052–1060. https://doi.org/10.1038/aps.2013.67

Johnson, M. A., & Polgar, J. (n.d.). *Data on the Distribution of Fibre Types in Thirty-six Human Muscles An Autopsy Study*. 19.

Johnston, A. P. W., Campbell, J. E., Found, J. G., Riddell, M. C., & Hawke, T. J. (2007). Streptozotocin induces G2 arrest in skeletal muscle myoblasts and impairs muscle growth in vivo. *American Journal of Physiology-Cell Physiology*, *292*(3), C1033–C1040. https://doi.org/10.1152/ajpcell.00338.2006

Käär, M. L., Akerblom, H. K., Huttunen, N. P., Knip, M., & Säkkinen, K. (1984). Metabolic control in children and adolescents with insulin-dependent diabetes mellitus. *Acta Paediatrica Scandinavica*, *73*(1), 102–108. https://doi.org/10.1111/j.1651-2227.1984.tb09906.x

Kadi, F., Schjerling, P., Andersen, L. L., Charifi, N., Madsen, J. L., Christensen, L. R., & Andersen, J. L. (2004). The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. *The Journal of Physiology*, *558*(3), 1005–1012. https://doi.org/10.1113/jphysiol.2004.065904

Karvonen, M., Viik-Kajander, M., Moltchanova, E., Libman, I., LaPorte, R., & Tuomilehto, J. (2000). Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care*, *23*(10), 1516–1526. https://doi.org/10.2337/diacare.23.10.1516

Kaul, K., Apostolopoulou, M., & Roden, M. (2015). Insulin resistance in type 1 diabetes mellitus. *Metabolism*, *64*(12), 1629–1639. https://doi.org/10.1016/j.metabol.2015.09.002

Kaya Mutlu, E., Unver, B., Taskiran, H., & Ozgen, I. T. (2018). An investigation of the foot ankle joint mobility, muscle strength, and foot structure in adolescent with type 1 diabetes. *International Journal of Diabetes in Developing Countries*, *38*(1), 108–114. https://doi.org/10.1007/s13410-017-0556-3

Kelly, N. A., Hammond, K. G., Bickel, C. S., Windham, S. T., Tuggle, S. C., & Bamman, M. M. (2017). Effects of aging and Parkinson's disease on motor unit remodeling: Influence of resistance exercise training. *Journal of Applied Physiology*, *124*(4), 888–898. https://doi.org/10.1152/japplphysiol.00563.2017

Kelly, N. A., Hammond, K. G., Stec, M. J., Bickel, C. S., Windham, S. T., Tuggle, S. C., & Bamman, M. M. (2018). Quantification and characterization of grouped type 1 myofibers in human aging. *Muscle & Nerve*, *57*(1), E52–E59. https://doi.org/10.1002/mus.25711

Kindig, C. A., Sexton, W. L., Fedde, M. R., & Poole, D. C. (1998). Skeletal muscle microcirculatory structure and hemodynamics in diabetes. *Respiration Physiology*, *111*(2), 163–175. https://doi.org/10.1016/S0034-5687(97)00122-9

Kivelä, R., Silvennoinen, M., Touvra, A.-M., Lehti, T. M., Kainulainen, H., & Vihko, V. (2006). Effects of experimental type 1 diabetes and exercise training on angiogenic gene expression and capillarization in skeletal muscle. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *20*(9), 1570–1572. https://doi.org/10.1096/fj.05-4780fje

Klueber, K. M., & Feczko, J. D. (1994). Ultrastructural, histochemical, and morphometric analysis of skeletal muscle in a murine model of type I diabetes. *The Anatomical Record*, *239*(1), 18–34. https://doi.org/10.1002/ar.1092390104

Knip, M., Veijola, R., Virtanen, S. M., Hyöty, H., Vaarala, O., & Åkerblom, H. K. (2005). Environmental triggers and determinants of type 1 diabetes. *Diabetes*, *54*(suppl 2), S125–S136. https://doi.org/10.2337/diabetes.54.suppl_2.S125

Krause, M. P., Al-Sajee, D., D'Souza, D. M., Rebalka, I. A., Moradi, J., Riddell, M. C., & Hawke, T. J. (2013). Impaired macrophage and satellite cell infiltration occurs in a muscle-specific fashion following injury in diabetic skeletal muscle. *PloS One*, *8*(8), e70971. https://doi.org/10.1371/journal.pone.0070971

Krause, M. P., Riddell, M. C., Gordon, C. S., Imam, S. A., Cafarelli, E., & Hawke, T. J. (2009). Diabetic myopathy differs between Ins2Akita+/- and streptozotocin-induced type 1 diabetic models. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *106*(5), 1650–1659. https://doi.org/10.1152/japplphysiol.91565.2008

Kuo, I. Y., & Ehrlich, B. E. (2015). Signaling in muscle contraction. *Cold Spring Harbor Perspectives in Biology*, *7*(2). https://doi.org/10.1101/cshperspect.a006023

Kyvik, K. O., Nystrom, L., Gorus, F., Songini, M., Oestman, J., Castell, C., Green, A., Guyrus, E., Ionescu-Tirgoviste, C., McKinney, P. A., Michalkova, D., Ostrauskas, R., & Raymond, N. T. (2004). The epidemiology of type 1 diabetes mellitus is not the same in young adults as in children. *Diabetologia*, *47*(3), 377–384. https://doi.org/10.1007/s00125-004-1331-9

Le Grand, F., & Rudnicki, M. A. (2007). Skeletal muscle satellite cells and adult myogenesis. *Current Opinion in Cell Biology*, *19*(6), 628–633. https://doi.org/10.1016/j.ceb.2007.09.012

Lehti, T. M., Silvennoinen, M., Kivelä, R., Kainulainen, H., & Komulainen, J. (2006). Effects of streptozotocin-induced diabetes and physical training on gene expression of extracellular matrix proteins in mouse skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, *290*(5), E900–E907. https://doi.org/10.1152/ajpendo.00444.2005

Leinonen, H., Matikainen, E., & Juntunen, J. (1982). Permeability and morphology of skeletal muscle capillaries in type 1 (insulin-dependent) diabetes mellitus. - Abstract—Europe PMC. *Diabetologia*, *22*(3), 158–162. https://doi.org/10.1007/BF00283744

Lexell, J., & Downham, D. Y. (1991). The occurrence of fibre-type grouping in healthy human muscle: A quantitative study of cross-sections of whole vastus lateralis from men between 15 and 83 years. *Acta Neuropathologica*, *81*(4), 377–381. https://doi.org/10.1007/BF00293457

Lexell, J., Taylor, C. C., & Sjöström, M. (1988). What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. *Journal of the Neurological Sciences*, *84*(2–3), 275–294. https://doi.org/10.1016/0022-510x(88)90132-3

Lillioja, S., Young, A. A., Culter, C. L., Ivy, J. L., Abbott, W. G., Zawadzki, J. K., Yki-Järvinen, H., Christin, L., Secomb, T. W., & Bogardus, C. (1987). Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *Journal of Clinical Investigation*, *80*(2), 415–424.

Linssen, W. H. J. P., Stegeman, D. F., Joosten, E. M. G., Binkhorst, R. A., Merks, M. J. H., Laak, H. J. T., & Notermans, S. L. H. (1991). Fatigue in type I fiber predominance: A muscle force and surface EMG study on the relative role of type I

and type II muscle fibers. *Muscle & Nerve*, *14*(9), 829–837. https://doi.org/10.1002/mus.880140906

Lönnrot, M., Korpela, K., Knip, M., Ilonen, J., Simell, O., Korhonen, S., Savola, K., Muona, P., Simell, T., Koskela, P., & Hyöty, H. (2000). Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: The Finnish Diabetes Prediction and Prevention Study. *Diabetes*, *49*(8), 1314–1318. https://doi.org/10.2337/diabetes.49.8.1314

Lönnrot, M., Salminen, K., Knip, M., Savola, K., Kulmala, P., Leinikki, P., Hyypiä, T., Akerblom, H. K., & Hyöty, H. (2000). Enterovirus RNA in serum is a risk factor for beta-cell autoimmunity and clinical type 1 diabetes: A prospective study. Childhood Diabetes in Finland (DiMe) Study Group. *Journal of Medical Virology*, *61*(2), 214–220.

Lukács, A., Mayer, K., Juhász, E., Varga, B., Fodor, B., & Barkai, L. (2012). Reduced physical fitness in children and adolescents with type 1 diabetes. *Pediatric Diabetes*, *13*(5), 432–437. https://doi.org/10.1111/j.1399-5448.2012.00848.x

Maratova, K., Soucek, O., Matyskova, J., Hlavka, Z., Petruzelkova, L., Obermannova, B., Pruhova, S., Kolouskova, S., & Sumnik, Z. (2018). Muscle functions and bone strength are impaired in adolescents with type 1 diabetes. *Bone*, *106*, 22–27. https://doi.org/10.1016/j.bone.2017.10.005

Marques, M. J., & Santo Neto, H. (2002). Acetylcholine receptors and nerve terminal distribution at the neuromuscular junction of non-obese diabetic mice. *The Anatomical Record*, *267*(2), 112–119. https://doi.org/10.1002/ar.10091

Mayer-Davis, E. J., Bell, R. A., Dabelea, D., D'Agostino, R., Imperatore, G., Lawrence, J. M., Liu, L., & Marcovina, S. (2009). The many faces of diabetes in American youth: type 1 and type 2 diabetes in five race and ethnic populations: The search for diabetes in youth study. *Diabetes Care*, *32*(Supplement 2), S99–S101. https://doi.org/10.2337/dc09-S201

McCall, G. E., Byrnes, W. C., Dickinson, A., Pattany, P. M., & Fleck, S. J. (1996). Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. *Journal of Applied Physiology*, *81*(5), 2004–2012. https://doi.org/10.1152/jappl.1996.81.5.2004

Medina-Sanchez, M., Rodriguez-Sanchez, C., Vega-Alvarez, J. A., Menendez-Pelaez, A., & Perez-Casas, A. (1991). Proximal skeletal muscle alterations in streptozotocin-diabetic rats: A histochemical and morphometric analysis. *American Journal of Anatomy*, *191*(1), 48–56. https://doi.org/10.1002/aja.1001910105

Medler, S. (2019). Mixing it up: The biological significance of hybrid skeletal muscle fibers. *Journal of Experimental Biology*, *222*(23). https://doi.org/10.1242/jeb.200832

Meyer, C., Dostou, J. M., Welle, S. L., & Gerich, J. E. (2002). Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *American Journal of Physiology-Endocrinology and Metabolism*, *282*(2), E419–E427. https://doi.org/10.1152/ajpendo.00032.2001

Monaco, C. M. F., Gingrich, M. A., & Hawke, T. J. (2019). Considering type 1 diabetes as a form of accelerated muscle aging. *Exercise and Sport Sciences Reviews*, *47*(2), 98–107. https://doi.org/10.1249/JES.0000000000000184

Monaco, C. M. F., Hughes, M. C., Ramos, S. V., Varah, N. E., Lamberz, C., Rahman, F. A., McGlory, C., Tarnopolsky, M. A., Krause, M. P., Laham, R., Hawke, T. J., & Perry, C. G. R. (2018). Altered mitochondrial bioenergetics and ultrastructure in the skeletal muscle of young adults with type 1 diabetes. *Diabetologia*, *61*(6), 1411–1423. https://doi.org/10.1007/s00125-018-4602-6

Mosher, P. E., Nash, M. S., Perry, A. C., LaPerriere, A. R., & Goldberg, R. B. (1998). Aerobic circuit exercise training: Effect on adolescents with well-controlled insulin-dependent diabetes mellitus. *Archives of Physical Medicine and Rehabilitation*, *79*(6), 652–657. https://doi.org/10.1016/s0003-9993(98)90039-9

Nederveen, J. P., Joanisse, S., Snijders, T., Ivankovic, V., Baker, S. K., Phillips, S. M., & Parise, G. (2016). Skeletal muscle satellite cells are located at a closer proximity to capillaries in healthy young compared with older men. *Journal of Cachexia, Sarcopenia and Muscle*, *7*(5), 547–554. https://doi.org/10.1002/jcsm.12105

Nederveen, J. P., Joanisse, S., Snijders, T., Thomas, A. C. Q., Kumbhare, D., & Parise, G. (2018). The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men. *The Journal of Physiology*, *596*(6), 1063–1078. https://doi.org/10.1113/JP275155

Noble, J. A., & Valdes, A. M. (2011). Genetics of the HLA region in the prediction of type 1 diabetes. *Current Diabetes Reports*, *11*(6), 533–542. https://doi.org/10.1007/s11892-011-0223-x

Nordfeldt, S., & Ludvigsson, J. (2000). Seasonal variation of HbA1c in intensive treatment of children with type 1 diabetes. *Journal of Pediatric Endocrinology & Metabolism: JPEM*, *13*(5), 529–535. https://doi.org/10.1515/jpem.2000.13.5.529

Nuutila, P., Knuuti, J., Ruotsalainen, U., Koivisto, V. A., Eronen, E., Teras, M., Bergman, J., Haaparanta, M., Voipio-Pulkki, L. M., Viikari, J., & et, al. (1993). Insulin resistance is localized to skeletal but not heart muscle in type 1 diabetes. *American*

Journal of Physiology-Endocrinology and Metabolism, *264*(5), E756–E762. https://doi.org/10.1152/ajpendo.1993.264.5.E756

Oberbach, A., Bossenz, Y., Lehmann, S., Niebauer, J., Adams, V., Paschke, R., Schön, M. R., Blüher, M., & Punkt, K. (2006). Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. *Diabetes Care*, *29*(4), 895–900. https://doi.org/10.2337/diacare.29.04.06.dc05-1854

Orlando, G., Balducci, S., Bazzucchi, I., Pugliese, G., & Sacchetti, M. (2017). The impact of type 1 diabetes and diabetic polyneuropathy on muscle strength and fatigability. *Acta Diabetologica*, *54*(6), 543–550. https://doi.org/10.1007/s00592-017-0979-9

Parise, G., Murrant, C. L., Cocks, M., Snijders, T., Baum, O., & Plyley, M. J. (2020). Capillary facilitation of skeletal muscle function in health and disease. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *45*(5), 453–462. https://doi.org/10.1139/apnm-2019-0416

Patterson, C. C., Harjutsalo, V., Rosenbauer, J., Neu, A., Cinek, O., Skrivarhaug, T., Rami-Merhar, B., Soltesz, G., Svensson, J., Parslow, R. C., Castell, C., Schoenle, E. J., Bingley, P. J., Dahlquist, G., Jarosz-Chobot, P. K., Marčiulionytė, D., Roche, E. F., Rothe, U., Bratina, N., … Green, A. (2019). Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989–2013: A multicentre prospective registration study. *Diabetologia*, *62*(3), 408–417. https://doi.org/10.1007/s00125-018-4763-3

Peltoniemi, P., Yki-Järvinen, H., Oikonen, V., Oksanen, A., Takala, T. O., Rönnemaa, T., Erkinjuntti, M., Knuuti, M. J., & Nuutila, P. (2001). Resistance to exercise-induced increase in glucose uptake during hyperinsulinemia in insulin-resistant skeletal muscle of patients with type 1 diabetes. *Diabetes*, *50*(6), 1371–1377. https://doi.org/10.2337/diabetes.50.6.1371

Poortmans, J. R., Carpentier, A., Pereira-Lancha, L. O., & Lancha, A. (2012). Protein turnover, amino acid requirements and recommendations for athletes and active populations. *Brazilian Journal of Medical and Biological Research*, *45*(10), 875–890. https://doi.org/10.1590/S0100-879X2012007500096

Pundziute-Lyckå, A., Dahlquist, G., Nyström, L., Arnqvist, H., Björk, E., Blohmé, G., Bolinder, J., Eriksson, J. W., Sundkvist, G., Ostman, J., & Swedish Childhood Diabetes Study Group. (2002). The incidence of type I diabetes has not increased but shifted to a younger age at diagnosis in the 0-34 years group in Sweden 1983-1998. *Diabetologia*, *45*(6), 783–791. https://doi.org/10.1007/s00125-002-0845-2

Quianzon, C. C., & Cheikh, I. (2012). History of insulin. *Journal of Community Hospital Internal Medicine Perspectives*, *2*(2). https://doi.org/10.3402/jchimp.v2i2.18701

Ramalho, A. C., de Lourdes Lima, M., Nunes, F., Cambuí, Z., Barbosa, C., Andrade, A., Viana, A., Martins, M., Abrantes, V., Aragão, C., & Temístocles, M. (2006). The effect of resistance versus aerobic training on metabolic control in patients with type-1 diabetes mellitus. *Diabetes Research and Clinical Practice*, *72*(3), 271–276. https://doi.org/10.1016/j.diabres.2005.11.011

Raskin, P., Pietri, A. O., Unger, R., & Shannon, W. A. (1983). The effect of diabetic control on the width of skeletal-muscle capillary basement membrane in patients with type I diabetes mellitus. *New England Journal of Medicine*, *309*(25), 1546–1550. https://doi.org/10.1056/NEJM198312223092504

Rayment, I., Rypniewski, W. R., Schmidt-Base, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G., & Holden, H. M. (1993). Three-dimensional structure of myosin subfragment-1: A molecular motor. *Science*, *261*(5117), 50–58. https://doi.org/10.1126/science.8316857

Relaix, F., & Zammit, P. S. (2012). Satellite cells are essential for skeletal muscle regeneration: The cell on the edge returns centre stage. *Development*, *139*(16), 2845–2856. https://doi.org/10.1242/dev.069088

Reske-Nielsen, E., Harmsen, A., & Vorre, P. (1977). Ultrastructure of muscle biopsies in recent, short-term and long-term juvenile diabetes. *Acta Neurologica Scandinavica*, *55*(5), 345–362. https://doi.org/10.1111/j.1600-0404.1977.tb05654.x

Richter, E. A., & Hargreaves, M. (2013). Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological Reviews*, *93*(3), 993–1017. https://doi.org/10.1152/physrev.00038.2012

Riddell, M. C., & Perkins, B. A. (2006). Type 1 diabetes and vigorous exercise: applications of exercise physiology to patient management. *Canadian Journal of Diabetes*, *30*(1), 63–71. https://doi.org/10.1016/S1499-2671(06)01010-0

Roch-Norlund, A. E., Bergström, J., Castenfors, H., & Hultman, E. (1970). Muscle glycogen in patients with diabetes mellitus. *Acta Medica Scandinavica*, *187*(1–6), 445–453. https://doi.org/10.1111/j.0954-6820.1970.tb02969.x

Rosenstock, J., Challis, P., Strowig, S., & Raskin, P. (1988). Improved diabetes control reduces skeletal muscle capillary basement membrane width in insulin-dependent diabetes mellitus. *Diabetes Research and Clinical Practice*, *4*(3), 167–175. https://doi.org/10.1016/s0168-8227(88)80014-7

Salminen, K., Sadeharju, K., Lönnrot, M., Vähäsalo, P., Kupila, A., Korhonen, S., Ilonen, J., Simell, O., Knip, M., & Hyöty, H. (2003). Enterovirus infections are associated with the induction of beta-cell autoimmunity in a prospective birth cohort study. *Journal of Medical Virology*, *69*(1), 91–98. https://doi.org/10.1002/jmv.10260

Saltin, B., Houston, M., Nygaard, E., Graham, T., & Wahren, J. (1979). Muscle fiber characteristics in healthy men and patients with juvenile diabetes. *Diabetes*, *28 Suppl 1*, 93–99. https://doi.org/10.2337/diab.28.1.s93

Santos, L. dos, Cyrino, E. S., Antunes, M., Santos, D. A., & Sardinha, L. B. (2017). Sarcopenia and physical independence in older adults: The independent and synergic role of muscle mass and muscle function. *Journal of Cachexia, Sarcopenia and Muscle*, *8*(2), 245–250. https://doi.org/10.1002/jcsm.12160

Schiaffino, S., & Reggiani, C. (2011). Fiber types in mammalian skeletal muscles. *Physiological Reviews*, *91*(4), 1447–1531. https://doi.org/10.1152/physrev.00031.2010

Schuler, M., Ali, F., Chambon, C., Duteil, D., Bornert, J.-M., Tardivel, A., Desvergne, B., Wahli, W., Chambon, P., & Metzger, D. (2006). PGC1alpha expression is controlled in skeletal muscles by PPARbeta, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. *Cell Metabolism*, *4*(5), 407–414. https://doi.org/10.1016/j.cmet.2006.10.003

Sexton, W. L., Poole, D. C., & Mathieu-Costello, O. (1994). Microcirculatory structure-function relationships in skeletal muscle of diabetic rats. *American Journal of Physiology-Heart and Circulatory Physiology*, *266*(4), H1502–H1511. https://doi.org/10.1152/ajpheart.1994.266.4.H1502

Snow, L. M., Sanchez, O. A., McLoon, L. K., Serfass, R. C., & Thompson, L. V. (2005). Myosin heavy chain isoform immunolabelling in diabetic rats with peripheral neuropathy. *Acta Histochemica*, *107*(3), 221–229. https://doi.org/10.1016/j.acthis.2005.06.003

Soltesz, G., Patterson, C. C., Dahlquist, G., & EURODIAB Study Group. (2007). Worldwide childhood type 1 diabetes incidence—What can we learn from epidemiology? *Pediatric Diabetes*, *8 Suppl 6*, 6–14. https://doi.org/10.1111/j.1399-5448.2007.00280.x

Sosenko, J. M., Miettinen, O. S., Williamson, J. R., & Gabbay, K. H. (1984). Muscle capillary basement-membrane thickness and long-term glycemia in type I diabetes mellitus. *The New England Journal of Medicine*, *311*(11), 694–698. https://doi.org/10.1056/NEJM198409133111102

Šoupal, J., Škrha, J., Fajmon, M., Horová, E., Mráz, M., Škrha, J., & Prázný, M. (2014). Glycemic variability is higher in type 1 diabetes patients with microvascular complications irrespective of glycemic control. *Diabetes Technology & Therapeutics*, *16*(4), 198–203. https://doi.org/10.1089/dia.2013.0205

Talmadge, R. J. (2000). Myosin heavy chain isoform expression following reduced neuromuscular activity: Potential regulatory mechanisms. *Muscle & Nerve*, *23*(5), 661–679. https://doi.org/10.1002/(SICI)1097-4598(200005)23:5<661::AID-MUS3>3.0.CO;2-J

Tanner, C. J., Barakat, H. A., Dohm, G. L., Pories, W. J., MacDonald, K. G., Cunningham, P. R. G., Swanson, M. S., & Houmard, J. A. (2002). Muscle fiber type is associated with obesity and weight loss. *American Journal of Physiology-Endocrinology and Metabolism*, *282*(6), E1191–E1196. https://doi.org/10.1152/ajpendo.00416.2001

Tesfaye, S., & Selvarajah, D. (2012). Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. *Diabetes/Metabolism Research and Reviews*, *28*(S1), 8–14. https://doi.org/10.1002/dmrr.2239

Thiebaud, D., Jacot, E., Defronzo, R. A., Maeder, E., Jequier, E., & Felber, J.-P. (1982). The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes*, *31*(11), 957–963. https://doi.org/10.2337/diacare.31.11.957

Thunander, M., Petersson, C., Jonzon, K., Fornander, J., Ossiansson, B., Torn, C., Edvardsson, S., & Landin-Olsson, M. (2008). Incidence of type 1 and type 2 diabetes in adults and children in Kronoberg, Sweden. *Diabetes Research and Clinical Practice*, *82*(2), 247–255. https://doi.org/10.1016/j.diabres.2008.07.022

Tintignac, L. A., Brenner, H.-R., & Rüegg, M. A. (2015). Mechanisms regulating neuromuscular junction development and function and causes of muscle wasting. *Physiological Reviews*, *95*(3), 809–852. https://doi.org/10.1152/physrev.00033.2014

Van Belle, T. L., Coppieters, K. T., & Von Herrath, M. G. (2011). Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiological Reviews*, *91*(1), 79–118. https://doi.org/10.1152/physrev.00003.2010

van Wessel, T., de Haan, A., van der Laarse, W. J., & Jaspers, R. T. (2010). The muscle fiber type–fiber size paradox: Hypertrophy or oxidative metabolism? *European Journal of Applied Physiology*, *110*(4), 665–694. https://doi.org/10.1007/s00421-010-1545-0

Vandewalle, C. L., Coeckelberghs, M. I., De Leeuw, I. H., Du Caju, M. V., Schuit, F. C., Pipeleers, D. G., & Gorus, F. K. (1997). Epidemiology, clinical aspects, and biology

of IDDM patients under age 40 years. Comparison of data from Antwerp with complete ascertainment with data from Belgium with 40% ascertainment. The Belgian Diabetes Registry. *Diabetes Care*, *20*(10), 1556–1561. https://doi.org/10.2337/diacare.20.10.1556

Verdijk, L. B., Koopman, R., Schaart, G., Meijer, K., Savelberg, H. H. C. M., & van Loon, L. J. C. (2007). Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *American Journal of Physiology. Endocrinology and Metabolism*, *292*(1), E151-157. https://doi.org/10.1152/ajpendo.00278.2006

Verdijk, L. B., Snijders, T., Drost, M., Delhaas, T., Kadi, F., & van Loon, L. J. C. (2014). Satellite cells in human skeletal muscle; from birth to old age. *AGE*, *36*(2), 545–557. https://doi.org/10.1007/s11357-013-9583-2

Vervoort, G., Wetzels, J. F., Lutterman, J. A., van Doorn, L. G., Berden, J. H., & Smits, P. (1999). Elevated skeletal muscle blood flow in noncomplicated type 1 diabetes mellitus. *Hypertension*, *34*(5), 1080–1085. https://doi.org/10.1161/01.HYP.34.5.1080

Wallberg-Henriksson, H., Gunnarsson, R., Henriksson, J., Östman, J., & Wahren, J. (1984). Influence of physical training on formation of muscle capillaries in type i diabetes. *Diabetes*, *33*(9), 851–857. https://doi.org/10.2337/diab.33.9.851

Wang, F., Liao, Y., Li, X., Ren, C., Cheng, C., & Ren, Y. (2012). Increased circulating myostatin in patients with type 2 diabetes mellitus. *Journal of Huazhong University of Science and Technology*, *32*(4), 534–539. https://doi.org/10.1007/s11596-012-0092-9

Weets, I., De Leeuw, I. H., Du Caju, M. V. L., Rooman, R., Keymeulen, B., Mathieu, C., Rottiers, R., Daubresse, J.-C., Rocour-Brumioul, D., Pipeleers, D. G., Gorus, F. K., & Belgian Diabetes Registry. (2002). The incidence of type 1 diabetes in the age group 0-39 years has not increased in Antwerp (Belgium) between 1989 and 2000: Evidence for earlier disease manifestation. *Diabetes Care*, *25*(5), 840–846. https://doi.org/10.2337/diacare.25.5.840

Weng, J., Zhou, Z., Guo, L., Zhu, D., Ji, L., Luo, X., Mu, Y., & Jia, W. (2018). Incidence of type 1 diabetes in China, 2010-13: Population based study. *BMJ*, *360*, j5295. https://doi.org/10.1136/bmj.j5295

Wieteska-Skrzeczynska, W., Grzelkowska-Kowalczyk, K., Jank, M., & Maciejewski, H. (2009). Transcriptional dysregulation of skeletal muscle protein metabolism in streptozotocin-diabetic mice. *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society*, *60 Suppl 1*, 29–36.

Wilson, J. M., Loenneke, J. P., Jo, E., Wilson, G. J., Zourdos, M. C., & Kim, J.-S. (2012). The effects of endurance, strength, and power training on muscle fiber type shifting. *The Journal of Strength & Conditioning Research*, *26*(6), 1724–1729. https://doi.org/10.1519/JSC.0b013e318234eb6f

Yardley, J. E., Kenny, G. P., Perkins, B. A., Riddell, M. C., Balaa, N., Malcolm, J., Boulay, P., Khandwala, F., & Sigal, R. J. (2013). Resistance versus aerobic exercise: acute effects on glycemia in type 1 diabetes. *Diabetes Care*, *36*(3), 537–542. https://doi.org/10.2337/dc12-0963

Yardley, J. E., Kenny, G. P., Perkins, B. A., Riddell, M. C., Malcolm, J., Boulay, P., Khandwala, F., & Sigal, R. J. (2012). Effects of performing resistance exercise before versus after aerobic exercise on glycemia in type 1 diabetes. *Diabetes Care*, *35*(4), 669–675. https://doi.org/10.2337/dc11-1844

Yoshioka, M., Kayo, T., Ikeda, T., & Koizuni, A. (1997). A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. *Diabetes*, *46*(5), 887–894. https://doi.org/10.2337/diab.46.5.887

CHAPTER 2 – IMPAIRED FUNCTION AND ALTERED MORPHOLOGY IN THE SKELETAL MUSCLES OF ADULT MEN AND WOMEN WITH TYPE 1 DIABETES

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PREFACE

Author contributions

AGD, CMFM, and TJH designed the experiments. **AGD** and TJH wrote the manuscript. **AGD** performed the experiments. **AGD** and TPP analysed the data. AGD and TJH interpreted the data. MAT performed muscle biopsies, neurological evaluations, and blood sampling. **AGD**, CMFM and GKG collected and processed muscle samples. All authors edited the manuscript. All authors provided final approval of the version to be published. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. TJH is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of data and the accuracy of the data analysis.

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Clinical Research Article

Impaired Function and Altered Morphology in the Skeletal Muscles of Adult Men and Women With Type 1 Diabetes

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Abstract

Context. Previous investigations on skeletal muscle health in type 1 diabetes (T1D) have generally focused on later stages of disease progression where comorbidities are present and are posited as a primary mechanism of muscle dysfunction.
Objective. To investigate skeletal muscle function and morphology across the adult lifespan in those with and without T1D.
Design. Participants underwent maximal contraction (MVC) testing, resting muscle biopsy, and venous blood sampling.
Setting. Procedures in this study were undertaken at the McMaster University Medical Centre.
Participants. Sixty-five healthy adult (18-78 years old) men/males and women/females (T1D = 34; control = 31) matched for age/biological sex/body mass index; self-reported physical activity levels were included.
Main Outcome Measures. Our primary measure in this study was MVC, with supporting histological/immunofluorescent measures.
Results. After 35 years of age ("older adults"), MVC declined quicker in T1D subjects compared to controls. Loss of strength in T1D was accompanied by morphological changes associated with accelerated aging. Type 1 myofiber grouping was higher in T1D, and the groups were larger and more numerous than in controls. Older T1D females exhibited more myofibers expressing multiple myosin heavy chain isoforms (hybrid fibers) than controls, another feature of accelerated aging. Conversely, T1D males exhibited a shift toward type 2 fibers, with less evidence of myofiber grouping or hybrid fibers.

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Conclusions. These data suggest impairments to skeletal muscle function and morphology exist in T1D. The decline in strength with T1D is accelerated after 35 years of age and may be responsible for the earlier onset of frailty, which characterizes those with diabetes.

Key Words: T1DM, T1D, muscle morphometrics, function, diabetic myopathy, skeletal muscle function

Type 1 diabetes (T1D) is a metabolic disease caused by the autoimmune destruction of the insulin-producing beta cells of the pancreas (1). Therapeutic regimens are being continually improved, including more closely monitored blood glucose and diet. Further, there have been recent advancements in automated insulin delivery, such as the hybrid closed loop system coupled with continuous interstitial glucose monitoring (2-4). The advent of these and other adjuvant therapies, while not a cure, have allowed those with T1D to live longer than ever before, in large part due to improvements in the modulation of modifiable risk factors for mortality such as renal and cardiovascular impairments (5). What this means clinically is that the risk of geriatric conditions, particularly frailty, are becoming more evident and need to be considered in addition to the traditional complications that characterize T1D (neuropathy, cardiovascular disease, nephropathy) (6). In fact, evidence is accumulating that those with diabetes (T1D and T2D) are much more likely to be frail in later life and are more likely to suffer from fatigue and weakness, as well as loss of muscle mass and quality than age-matched nondiabetic controls (7).

Despite the primary role for skeletal muscle in both whole body physical and metabolic capacity, the effects of T1D on skeletal muscle are not well understood. There is, however, accumulating evidence of metabolic abnormalities in the skeletal muscles of those with T1D, such as delayed adenosine 5′-triphosphate synthesis (8), altered glycolysis (9), mitochondrial dysfunction (10), and abnormal mitochondrial ultrastructure (10,11). Based upon the aforementioned evidence, it has been hypothesized that the skeletal muscle of those with T1D may be exhibiting a phenotype of accelerated aging (12). This hypothesis has yet to be tested and much of the current literature investigating morphological changes to skeletal muscles in those with T1D has investigated persons with known neuropathy (13,14), combined male and female data together (13,14), or was investigated before the current standard of care (eg, aggressive insulin therapy). Of note, though, an electron micrograph examination of muscle biopsies from newly diagnosed juvenile patients (among others) prior to insulin therapy found evidence for myofiber atrophy and morphological disruption of their sarcomeres and mitochondria (15). Taken together, these findings highlight the important, but limited data currently available pointing to potential deficits in skeletal muscle health even in the presence of current treatment standards.

In addition to changes in muscle mass, studies evaluating skeletal muscle at the myofiber level have noted alterations to the fiber-type composition of muscle with T1D in the form of an increased proportion of fast-glycolytic (16) or type 2 fibers (17) in middle-aged adults with T1D. However, the existing data have yet to examine how muscle fiber composition and morphology may be part of the aforementioned advanced neuromuscular aging of those with T1D. Interestingly, recent findings have highlighted the importance of morphological indicators of muscle fibers that may display the more understated changes that occur with increasing age. For example, type 1 myofiber grouping (ie, the clustering of several contiguous type 1 fibers) is a characteristic associated with neuromuscular aging. Specifically, age-related denervation/reinnervation events (18) gradually see denervated type 2 fibers reinnervated with type 1 motor units, ultimately changing the muscle composition toward one with larger "groups" of type 1 fibers and fewer type 2 fibers overall. Type 1 fiber grouping becomes significantly elevated with age (19) and is associated with inefficient motor unit activation and greater physiologic difficulty to complete motor tasks (20). Additionally, as methods to identify fiber types have improved, it has often been recognized that myofibers may not exist as one type or another but instead as hybrid fibers [ie, those expressing 2 or more myosin heavy chain (MHC) types]. Hybrid fibers have been observed in many situations in human muscle and have shown the potential to help infer whether a muscle is in a transitionary state (21). Often, hybrid fibers become more prevalent during detrimental changes such as disuse and aging (22,23). Therefore, measuring this characteristic of muscle morphology may aid in the determination of aging status in the skeletal muscle of different populations. To our knowledge, no study to date has employed these techniques to elucidate the impact of T1D on adult skeletal muscle.

Therefore, the purpose of this study was to investigate skeletal muscle function and morphology across the adult lifespan in those with and without T1D. Herein, we investigated maximal voluntary contractions (MVCs) and many aspects of skeletal muscle morphology in male and female T1D subjects across a wide age span (18-78 years old). Importantly, these

subjects were free of significant diabetic complications (particularly neuropathy) to understand the changes to muscle health in the absence of confounding comorbidities.

Methods

Participants

Adult men/women (hereafter referred to as males/females) with type 1 diabetes (female n = 21, male n = 13) and matched control (CON) participants without diabetes (female n = 18, male n = 13) between 18 and 78 years of age were recruited for this study. Participants were matched for age, sex, body mass index and self-reported physical activity levels and were told to refrain from exercise 24 h preceding the study visit. In some instances, multiple T1D participants were matched to 1 CON subject. During neurological examination, 20% of older adult subjects exhibited evidence of a mild sensory neuropathy (ie, decreased vibration sensitivity and/or absent ankle reflexes) but there were no cases of moderate/severe neuropathy, nor was there any evidence of proximal limb motor deficit. Detailed subject characteristics and exclusion criteria for the overall cohort and per age group are available in Tables 1 and 2, respectively.

Ethics

Prior to giving written informed consent, all participants were given oral and written information about the experimental procedures. All procedures herein were approved by the Hamilton Integrated Research Ethics Board (REB #5344 and #5355) and conformed to all declarations on the use of human participants as research subjects.

Study Protocol

Participants visited the laboratory for assessment of height, weight, and a baseline maximal strength measurement procedure (MVC) using a Biodex dynamometer (Biodex System 3; Biodex Medical Systems, Shirley, NY, USA). All participants older than 35 years of age (controls: 11 female, 7 male; T1D: 11 female, 7 male) underwent neurological examination of their motor, reflex, and sensory function by an experienced neuromuscular disorders clinician (MAT), where scores were averaged between left and right legs. Participants were instructed to refrain from caffeine and alcohol 24 h prior to visiting the laboratory and were instructed to consume a standardized meal 1.5 to 2 h prior to their visit. Participants with T1D were also instructed to continue their habitual use of insulin.

Muscle and Blood Measurements

Participants underwent a resting biopsy of the vastus lateralis muscle and a venous blood sample. Biopsies were obtained from the mid-portion of the vastus lateralis under local anesthetic (2% lidocaine) using manual suction, as previously described (24). Immediately following the muscle biopsy procedure, a venous blood sample was obtained from the antecubital vein. Hemoglobin A1c levels were determined by the Hamilton Health Sciences clinical core lab facility at McMaster University.

Frozen Muscle Sections

Fresh muscle samples were mounted in optimal cutting temperature (OCT) compound and frozen in liquid nitrogen cooled 2-methylbutane. The OCT embedded

Table 1. Participant characteristics, overall

	CON		T1D	
	Female	Male	Female	Male
n	18	13	21	13
Age (year)	42 ± 4	42 ± 5	37 ± 3	43 ± 5
Body mass index (kg/m^2)	26 ± 0.85	25 ± 0.69	27 ± 1.37	27 ± 0.94
MVC (n)	179 ± 11.2	273 ± 14.68[a]	175 ± 9.29	266 ± 21.27[b]
Hemoglobin A1c (%)	5.3 ± 0.06	5.3 ± 0.14	7.6 ± 0.28[a]	7.3 ± 0.25[a]
T1D duration (years)	—	—	19 ± 3	29 ± 4

Adult men/women with type 1 diabetes and matched control participants without diabetes were recruited for this study. Participants were not eligible to participate in the study if they matched any one of the following criteria: smoke or use any tobacco products, prediabetes, type 2 diabetes, statin-induced myopathy or experience myalgia, use assistive walking devices, chronically medicate with any analgesic or anti-inflammatory drug(s), corticosteroids or non-steroidal anti-inflammatories or prescription strength acne medications, use medications known to affect muscle metabolism (with the exception of insulin), heart disease, rheumatoid arthritis, poor lung function, uncontrolled hypertension, stage 3 neuropathy, severe retinopathy, or any health conditions that put the subject at risk during this study. Data are expressed as mean ± SEM.

[a]Significantly different *vs* same-sex control, $P < 0.05$.

[b]Significantly different *vs* female of same group, $P < 0.05$.

Table 2. Participant characteristics, split by age group

	CON				T1D			
	Female		Male		Female		Male	
	Younger	Older	Younger	Older	Younger	Older	Younger	Older
n	7	11	6	7	10	11	6	7
Age (years)	25 ± 2	53 ± 3[c]	26 ± 2	56 ± 4[c]	26 ± 2	49 ± 2[c]	27 ± 2	57 ± 3[c]
Body mass index (kg/m^2)	25 ± 1.017	26 ± 1.21	24 ± 1.15	25 ± 0.88	28 ± 2.14	27 ± 1.84	26 ± 1.37	27 ± 1.31
MVC (n)	187 ± 21.60	175 ± 21.60	288 ± 25.94[b]	262 ± 17.53[b]	192 ± 16.83	161 ± 8.33	290 ± 18.49[b]	249 ± 33.79[b]
Hemoglobin A1c (%)	5.2 ± 0.08	5.4 ± 0.08	5.4 ± 0.17	5.1 ± 0.22	7.9 ± 0.53[a]	7.3 ± 0.22[a]	7.1 ± 0.38[a]	7.6 ± 0.32[a]
T1D duration (years)	—	—	—	—	10 ± 2	27 ± 4	16 ± 3	39 ± 3
7-day average blood glucose (mM)	—	—	—	—	—	8.4 ± 0.34	—	9.1 ± 0.74
7-day time in target range (%)	—	—	—	—	—	58 ± 5	—	54 ± 6
7-day time in hyperglycemia (%)	—	—	—	—	—	38 ± 5	—	39 ± 7
7-day time in hypoglycemia (%)	—	—	—	—	—	4 ± 1	—	7 ± 2

Study participants, as described in Table 1, split into younger (<35 years old) and older (>35 years old) groups. Data are expressed as mean ± SEM.
[a]Significantly different *vs* same-sex control, $P < 0.05$.
[b]Significantly different *vs* female of same group, $P < 0.05$.
[c]Significantly different *vs* younger group of same sex, $P < 0.05$.

samples were stored at −80°C prior to sectioning. All muscles were cut in cross-section at a thickness of 6 µm and allowed to air dry before histology/immunofluorescence was performed.

Double Antibody Immunofluorescence

Details pertaining to the primary and secondary antibodies used for immunofluorescent staining described in the following discussion are detailed in the supplementary material in (25). Sections were fixed in 4% paraformaldehyde for 10 min. Sections were then rinsed and incubated in blocking solution (5% normal goat serum, 5% fetal bovine serum, 2% bovine serum albumin, 0.1% sodium azide, and 0.2% Triton-X-100 in phosphate buffered saline) for 90 min at room temperature (RT). Tissue sections were then incubated in primary antibodies against Pax7 and CD31 at 4°C overnight. Following primary antibody incubation, sections were incubated in secondary antibody cocktail. Tissue sections were then refixed for 5 min, incubated for 2 h in the primary antibody against myosin heavy chain 1 (MHC1) and laminin, and then incubated in the secondary antibodies 2 h at RT. All washes between incubations were performed using Tris-buffered saline. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole for 5 min at RT. Sections were mounted with Fluoromount (Sigma-Aldrich, F4680) and coverslips added.

Fiber-type Immunofluorescence

Details pertaining to primary and secondary antibodies used for fiber-type immunofluorescent staining are detailed in the supplementary material in (25). Sections were incubated in blocking solution (5% normal goat serum) for 1 h at RT. Tissue sections were then incubated the primary antibody cocktail against MHC1, MHC2A, and MHC2X at 4°C overnight. Following primary antibody incubation, sections were incubated in the secondary antibody cocktail for 1 h at RT. Washes between incubations were performed using Tris-buffered saline. Sections were mounted with Fluoromount and coverslips added.

Periodic Acid-Schiff Stain

Sections were initially rinsed with running tap water and then incubated with 0.5% periodic acid for 5 min, followed immediately by 2 min in Schiff's solution (Sigma-Aldrich, 3952016). Sections were then washed via warm running tap water for 5 min. After which, sections were mounted with Permount (Fisher Scientific, SP15500) and coverslips added.

Microscopy and Image Analysis

All images were captured with a Nikon 90i Eclipse microscope (Nikon Inc, Melville, NY, USA) and analyzed

using Nikon Elements AR software v4.6. At least 100 muscle fibers per subject were analyzed for each histological/immunofluorescence analysis. Laminin was used as a surrogate plasma membrane marker for quantification of minimum feret diameter. Fiber type analysis was performed by quantifying the total number of cells that predominantly expressed MHC1/2A/2X. A subset of fibers expressing high levels of MHC1 and MHC2A/X were identified has type 1/2 hybrids (HYB12). Likewise, fibers co-expressing MHC2A/X were identified has type 2AX hybrids (HYB2AX). Detailed definitions of hybrid fibers are found in the supplementary material in (25). Capillaries were identified as CD31-positive cells adjacent to myofibers. Thirty myofibers from each sample were chosen to quantify capillary-to-fiber ratio (ie, the number of adjacent capillaries surrounding a myofiber) and capillary density (ie, number of capillaries per 30 fibers divided by total fiber area), calculated based on previous work by Hepple et al (26). Satellite cells were identified via co-staining of Pax7 and 4′,6-diamidino-2-phenylindole, and localization beneath the basal lamina (laminin). Fiber-type and satellite cells (SCs) were expressed as a percentage of total muscle fibers analyzed. Analyses of grouped type 1 myofibers were performed as described previously by Kelly et al (19). Briefly, the expected mean number of like myofibers touching a given type 1 myofiber was calculated as the product of the total number of myofibers touching a given myofiber and type 1 myofiber proportion. To qualify as a myofiber group, the number of like myofibers touching a given type 1 myofiber must exceed the mean plus 1 SD around the mean for at least 2 contiguous myofibers in the core of a group. Subsequently, the number of type 1 myofibers touching any other type 1 myofiber was counted until the edge of the group or a border of the muscle section was reached. Although this method partially controls for type 1 myofiber distribution, the influence is not completely removed (19,27). Glycogen content was quantified by measuring mean pixel saturation values from NIS Elements software from periodic acid-Schiff (PAS)-stained myofibers that were individually circled. All immunofluorescence and histological analyses were completed in a blinded fashion.

Continuous Glucose Monitoring

Seven-day glycemic status was measured in a subset of older participants (>35 years old) with T1D via continuous glucose monitoring. These participants were offered use of the Dexcom G5 Mobile CGM System (DexCom Inc., San Diego, CA, USA) between their 2 study visits. Participants who already utilized continuous glucose monitoring were permitted to continue use of their continuous glucose monitoring for the study period and provided their data for our analysis following the study period.

Activity Tracking by Accelerometry

Older adults were provided with, and instructed to wear, a Polar M430 watch for activity tracking via accelerometry. Polar Flow® software was used to extract summary values indicating total time spent in each of 5 activity intensity ranges (eg, resting, sitting, low, medium, high). Total activity time in minutes was divided by the total amount of time each participant wore the activity tracker to represent relative activity time in each zone.

Statistical Analysis

Bar graphs are presented as mean ± standard error of the mean (SEM) with datapoints overlaid. Participant characteristics are expressed as mean ± SEM. Younger and older age groups were defined as those younger and older than 35 years of age, respectively, based on the time point when the MVC of T1D participants began to decline quicker than CON participants. Effect sizes between groups were assessed by Hedge's *g* (see following discussion). To compare age groups (ie, younger *vs* older), CON and T1D were collapsed into their respective age group and then compared with Hedge's *g*. Distributions of minimum feret diameter between groups were compared by a 2-sample Kolmogorov-Smirnov test. Outliers were defined as datapoints outside of the mean ± 1.5 times the interquartile range. All effect sizes (*g*) and *P*-values (for 2-sample Kolmogorov-Smirnov tests) are displayed within graphical summaries. In the interest of research transparency, we have noted *g*- and *P*-values for all of the relevant comparisons and have commented in the text on those that were deemed of particular interest or importance. Correlations were assessed by Spearman's rho (herein denoted as *r*). All analyses were carried out in the R environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

Assessment of Effect Sizes

Despite this being one of the most comprehensive muscle biopsy studies in participants with T1D, our sample size is small in the scope relative to other less invasive clinical studies. Therefore, we have utilized Hedge's *g* (ie, corrected effect sizes) to delineate the magnitude of differences in our comparisons. Effect sizes of ≥0.2, ≥0.5, and ≥0.8 are denoted as small, moderate, and large effect sizes, respectively, whereas those effect sizes <0.2 were deemed negligible based on thresholds by Cohen (28). These represent the most

commonly used thresholds in the literature and are used commonly in clinical trials (29,30), preclinical scientific studies (31-34), and meta-analyses (35,36) as a means of interpreting magnitude of changes, particularly in investigations where the results are extremely novel and cannot be readily compared with findings from other literature, such as in the current study (for a review, see (37)). We aimed to make our results more generalizable and interpretable for a wide audience. However, these interpretations should not be viewed rigidly, and more studies in the area are required to determine the true clinical significance of the physiological changes observed in each case.

Results

Accelerated Loss of Muscular Strength in Older Adults With T1D

Analysis of MVC over the lifespan revealed that people with T1D began to exhibit a loss of strength after 35 years of age compared to CON participants (Fig. 1A). This separation in muscle function provided the basis to divide cohorts into 2 age groups (ie, younger, defined as less than 35 years, and older, defined as greater than 35 years of age) to determine differences in skeletal muscle health between groups with and without a decline in function. Indeed, while CON participants exhibited a small decline in maximal strength between age groups (–10%, $g = -0.4$), older adults with T1D exhibited a moderate decline (–17%, $g = -0.6$) in MVC force (Fig. 1B). Notably, the loss of MVC force appears to be moderate in females with T1D (–17%, $g = -0.6$), whereas the decline in their CON counterparts ($g = -0.2$) was small (Fig. 1B). Conversely, there was a moderate age-related decline in MVC that was similar in males with/without T1D (CON: –9%, $g = -0.7$; T1D: –18%, $g = -0.7$).

No Evidence for Changes in Capillary Density and Satellite Cell Content in Those With T1D

We utilized immunofluorescence to identify capillary density and SC content (Fig. 2A). Older females in this study exhibited moderately elevated capillary density *vs* the younger cohort (27%, $g = 0.7$) (Fig. 2B), while males did not exhibit a change ($g = -0.2$) (Fig. 2B). Capillary-to-fiber ratio was comparable between CON and T1D, although older females again moderately increased capillary content relative to younger females (14%, $g = 0.6$) (Fig. 2C). Older males overall exhibited a small elevation in (SC) content (+5%, $g = 0.2$) (Fig. 2D).

Lower Glycogen Content Is Correlated With Increased Seated Time in Older T1D Adults

In older adults, we assessed glycogen content at rest, quantified as pixel saturation by PAS stain (Fig. 2E). Skeletal muscle glycogen content was found to be lower in the skeletal muscle of older adults with T1D: moderately in females ($g = -0.7$) (Fig. 2F) and with a large effect size in males ($g = -1.2$) (Fig. 2F). Next, we utilized accelerometry to assess physical activity in the older adults in our cohort. Older adults with T1D exhibited a large increase in the amount of time spent in the seated activity range, suggesting more sedentary behavior from these individuals (female $g = 1.10$, male $g = 1.04$) (Fig. 2G). Also, older females and males with T1D exhibited small and large reductions in time spent resting, respectively (female $g = -0.31$, male $g = -1.03$) (Fig. 2G), a finding that suggests a decreased sleep duration as the majority of "resting" data was captured between the hours of 12 AM and 8 AM (for representative tracing, see supplementary material in (25)). There was a particularly strong negative correlation between muscle glycogen and time spent seated in this cohort ($r = -0.63$, $P < 0.01$) (Fig. 2H), suggesting that more sedentary time may play a role in the reduction of muscle glycogen levels in these adults. We next attempted to discern if any other important covariates discussed in this study (ie, age, MVC, hemoglobin A1c, 7-day average blood glucose) were associated with glycogen status in these individuals. However, we found no notable correlations between these variables and glycogen content, as displayed in the supplementary material in (25).

Fiber-type Differences Between Males and Females with Type 1 Diabetes

Muscle biopsies were analyzed for morphology via immunofluorescence, revealing altered distributions of fiber sizes in skeletal muscle with T1D, as measured by minimum feret diameter (Fig. 3A). On average, there was a moderate minimum feret diameter reduction in older females relative to their younger counterparts (–8%, $g = -0.5$) (Fig. 3B), and older males exhibited a larger decline (–13%, $g = -0.9$) (Fig. 3B). Interestingly, older females with T1D showed a small decline in minimum feret diameter compared to CON (–5%, $g = -0.2$) (Fig. 3B), whereas males showed a moderate elevation of minimum feret diameter relative to their same-sex controls (8%, $g = 0.5$) (Fig. 3B). In the younger cohort, there were no noteworthy differences in minimum feret diameter between CON and T1D participants ($g < 0.2$) (Fig. 3B). Quantification of type 1 and 2 fiber proportions revealed disparate effects of T1D

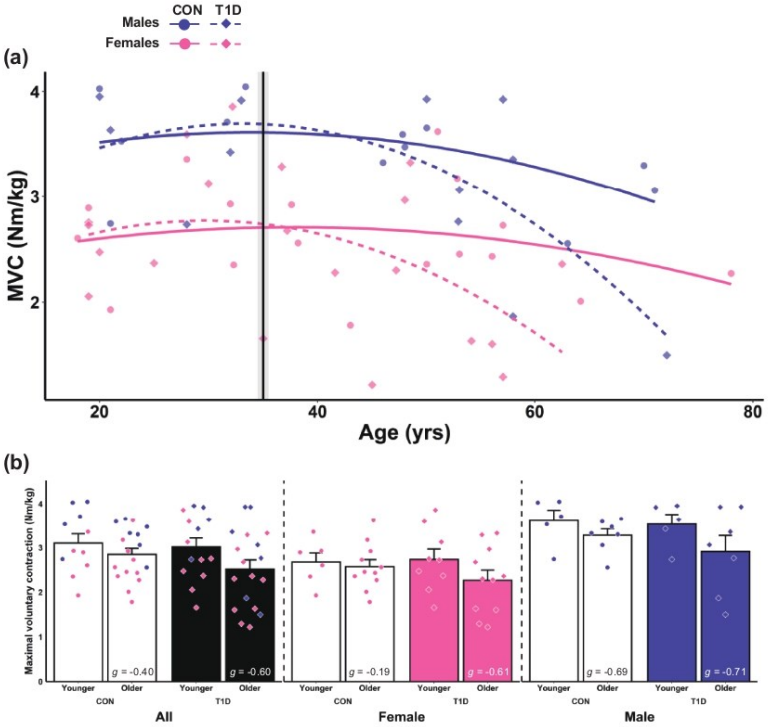

Figure 1. Accelerated loss of maximal muscle strength in type 1 diabetics. (A) Maximal voluntary contraction (MVC) strength in males and females with type 1 diabetes (T1D) and their nondiabetic counterparts (CON), from 18 to 78 years of age. (B) MVC strength, compared between younger (<35 years old) and older (>35 years old) groups. For individual datapoints, circles indicate CON participants and diamonds indicate T1D. Blue and pink coloring indicate males and females, respectively. For trend lines (A), solid lines indicate CON and dashed lines indicate T1D, vertical black line indicates division between Younger and Older groups (ie, 35 years old). In bar plot (B), data are expressed as mean ± SEM.

on male and female muscle composition. Type 2 fiber proportions showed a small decrease with age in both females (−5%, $g = -0.4$) (Fig. 3C) and males (−6%, $g = -0.3$) (Fig. 3C). Young females with T1D exhibited similar fiber proportions relative to CON ($g < 0.2$), while older females with T1D displayed a large reduction in type 2 fibers compared to their CON counterparts (−14%, $g = -0.8$) (Fig. 3C). Conversely, males with T1D exhibited a large relative increase in type 2 fibers in younger participants (25%, $g = 1.5$) (Fig. 3C), and older males with T1D also exhibited a largely elevated proportion of type 2 fibers compared to CON males (16%, $g = 0.8$) (Fig. 3C).

Increased Prevalence of Hybrid Muscle Fibers in Older Adults With Type 1 Diabetes

Recently, the importance of hybrid myofibers has gained attention in the literature as an important aspect of muscle phenotype (21). Thus, given the observed changes in muscle fiber-type composition (ie, type 1 *vs* type 2), we attempted to delineate the fraction of each specific MHC type (Fig. 4A) and subsequently the prevalence of hybrid fibers in skeletal muscle in older T1D and CON participants.

When considering MHC isoforms (ie, 1/2A/2X), examination of minimum feret diameter revealed the distribution of minimum feret diameter was different in T1D participants for each fiber type, regardless of sex ($P < 0.05$) (Fig. 4B). Most notably, 2X myofibers were substantially larger in males with T1D (36%) (Fig. 4B). In females, there was a moderate gain in the proportion of type 1 ($g = 0.5$) (Fig. 4C) and type 2X ($g = 0.5$) (Fig. 4C), with a large decrease in type 2A fibers ($g = -0.8$) (Fig. 4C). Similarly, in males, there was a moderate loss of type 2A fibers ($g = -0.6$; Fig. 4C) and concomitant gain in type 2X fibers ($g = 0.7$) (Fig. 4C), while there

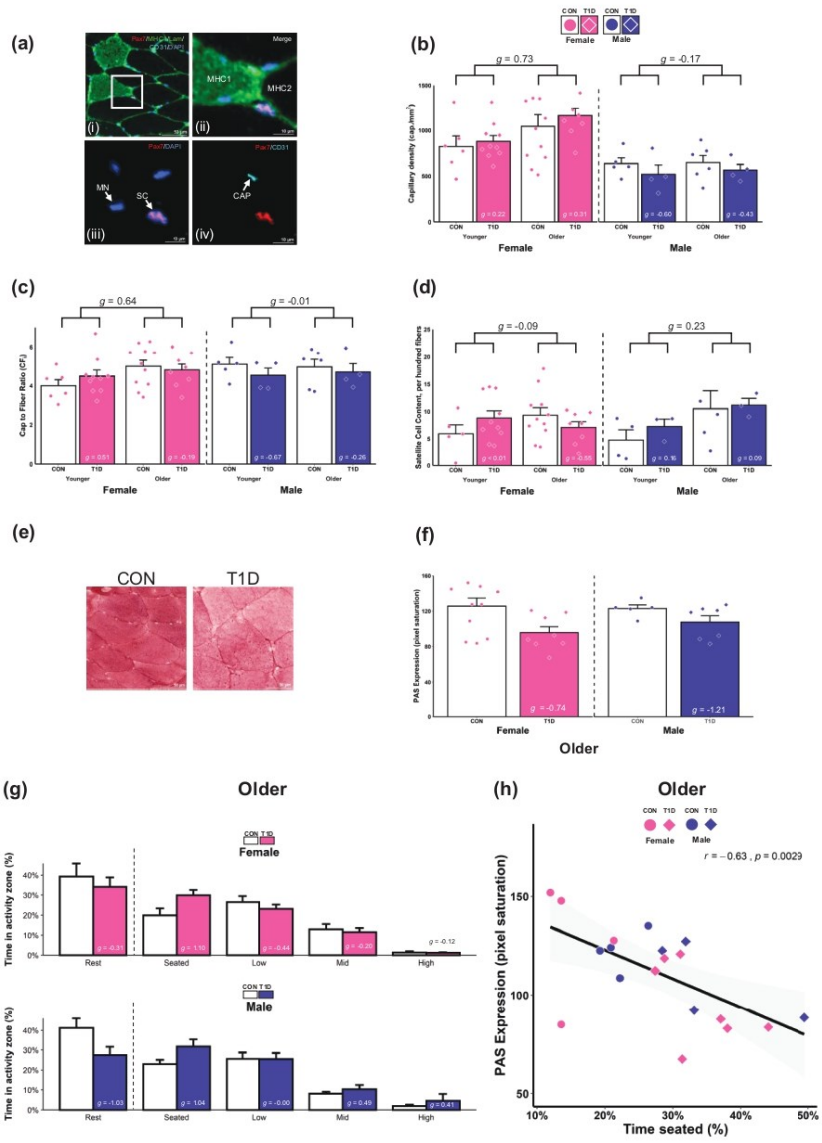

Figure 2. Vascularity, satellite cell, and glycogen content of skeletal muscle in males and females across the adult lifespan. (A) Representative immunofluorescence images including (i) low magnification merge (ii) higher magnification merge depicting type 1 (MHC1) and type 2 (MHC2) fibers, (iii) Pax7+ [ie, satellite cell (SC)] and Pax7– myonuclei (MN), and (iv) CD31 positive cells [ie, capillaries (CAP)]. (B) Representative images of periodic acid-Schiff (PAS) stain. (C) Capillary density (ie, number of capillaries per square millimeter) between disease (CON *vs* T1D) and age (younger *vs* older) groups. (D) Capillary-to-fiber ratio [ie, number of capillaries in contact with an individual myofiber (CF_i)] between disease and age groups. (E) Satellite cell content (ie, number of satellite cells expressed per 100 fibers) between disease and age groups. (F) Glycogen content, as measured by PAS stain. (G) Time spent in physical activity intensity zones, expressed as percentage of total time. (H) Scatter plot depicting the relationship

were negligible changes to the proportion of type 1 fibers ($g < 0.2$) (Fig. 4C). Further, the changes in fiber-type proportions were accompanied by an increased abundance of hybrid fibers in participants with T1D (Fig. 4D). This increase was driven by a large increase in females with T1D (26%, $g = 0.9$) (Fig. 4E). Analysis of specific hybrid fiber-types in females with T1D revealed ~2 times more HYB2AX ($g = 0.8$) (Fig. 4F) and ~3 times more HYB12 ($g = 0.9$) (Fig. 4F). On the other hand, males with T1D appeared to express a small decrease in HYB12 fibers (g = HYB2AX 0.4) (Fig. 4F) with no notable change in HYB2AX fibers ($g < 0.2$).

Increased Grouping of Type 1 Myofibers in T1D Muscle

Due to the observed perturbations in myofiber type proportions, we next investigated the prevalence of type 1 myofiber grouping in our participants' muscle. Similar to previous observations in comparing younger and older adults (19), the proportion of grouped fibers was elevated in older individuals by over 3 times that of younger participants in the study (females $g = 1.23$, males $g = 1.5$) (Fig. 5A). Older females, in particular, exhibited a largely elevated proportion of grouped type 1 myofibers relative to their CON counterparts (15%, $g = 1.1$) (Fig. 5A). Also, males with T1D exhibited an elevated proportion of grouped myofibers, although the effect size was small (25%, $g = 0.3$) (Fig. 5A).

Additionally, older male participants displayed myofiber groups that were much larger (ie, 17 times larger) on average (ie, more fibers per cluster) than younger males ($g = 1.1$) (Fig. 5B), but there was a negligible effect of T1D ($g < 0.2$) (Fig. 5B). Meanwhile, females did not exhibit larger fiber groups with age ($g < 0.2$) (Fig. 5B). However, there was a small elevation in myofiber group size in T1D women relative to their CON counterparts in younger ($g = 0.2$) and older ($g = 0.3$) cohorts.

Lastly, the quantity of type 1 myofiber groups was elevated by ~4 times in older participants (large effect size for females $g = 1.86$ and males $g = 1.3$) (Fig. 5C). The age-related increase in grouped myofiber quantity was exacerbated by T1D in older females (35%, $g = 0.9$) (Fig. 5C) but not older males ($g < 0.2$) (Fig. 5C).

Discussion

The current study presents skeletal muscle morphometrics from 60 years of the adult T1D lifespan and is, to date, the most expansive investigation of skeletal muscle morphology and function in those with T1D. Our novel findings highlight a greater decline in muscle strength, along with concomitant changes in hybrid fiber proportions and type 1 myofiber grouping in older adults with T1D—characteristics of accelerated aging, as has been previously speculated (12). Furthermore, we have noted sexual dimorphism between the morphology of skeletal muscle in males and females with T1D—a particularly understudied component of the T1D literature—where females with T1D exhibited emphasized signs of aging phenotype compared to male counterparts. Taken together, these findings highlight significant changes to the muscles of older adults with T1D even in the absence of overt diabetic complications, particularly neuropathy.

Age-related Loss of Muscle Strength in Adults With T1D

Our assessment of MVC strength revealed a sharper decline in maximal strength in the skeletal muscle of T1D participants after 35 years of age. We separated our sample into younger (ie, <35 years old) and older (ie, >35 years old) cohorts based on this delineation in an attempt to further determine the underlying factors that may be contributing to accelerated decline in muscle strength we observed in T1D adults. Older T1D participants exhibited a decline almost twice that (~170%) of CON participants that resulted in a greater effect size when compared to their CON counterparts. While the exact mechanism(s) underlying these changes are still being examined, hyperglycemia has previously been demonstrated to contribute to the diminished muscle strength in older adults (38). Furthermore, previous studies have made note of more substantial decrements in maximal strength in knee extensors (39), and the distal extensor/flexor muscles of the lower limb (39,40) correlated to the degree of neuropathy present in T1D patients. Interestingly, while distal polyneuropathy patients were the focus of previous investigations (39), it is possible that our participants did not exhibit knee extensor

Figure 2: continued

between relative time spent seated and PAS expression. All values are expressed as mean ± SEM. Younger cohort is defined as <35 years old. For individual datapoints, circles indicate CON participants and diamonds indicate T1D. Blue and pink coloring indicate males and females, respectively. For scatter plot, black line indicates regression line and grey shaded area indicates SEM. Effect sizes (g) *vs* CON are displayed within bars. Effect sizes *vs* younger cohort are displayed over square brackets.

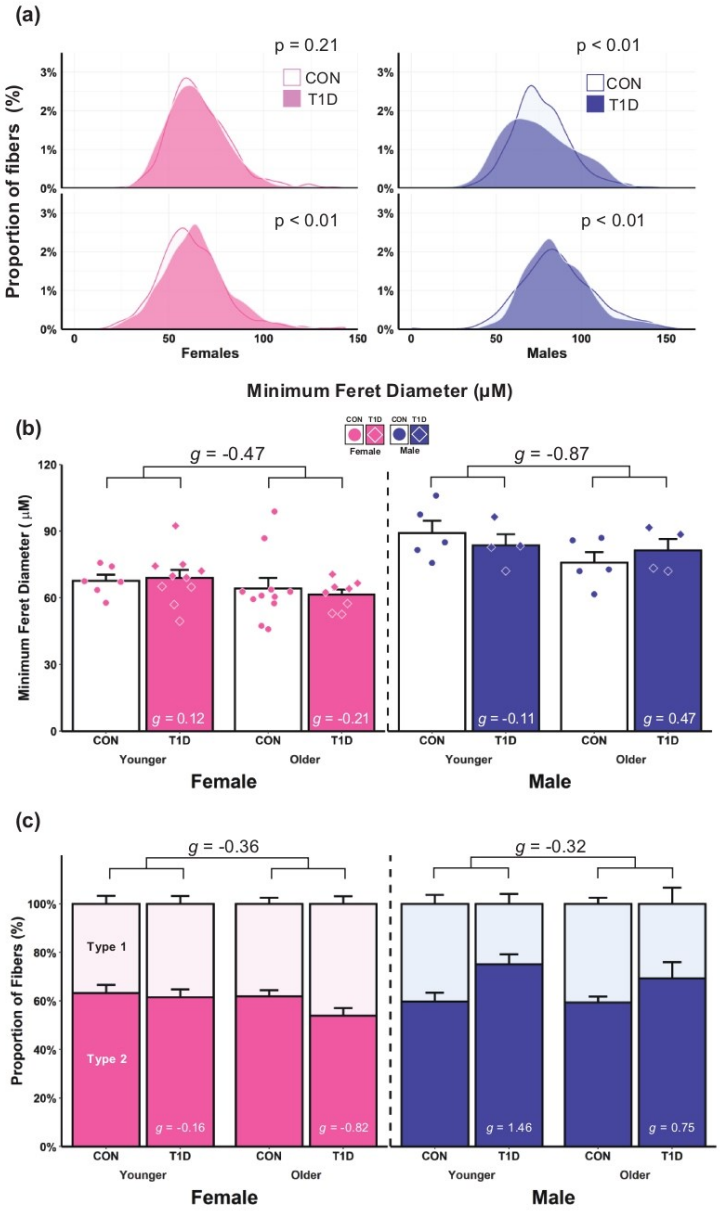

Figure 3. Altered fiber-types and myofiber size in T1D. (A) Distributions of type 1 and type 2 myofiber size, as measured by minimum feret diameter. (B) Average minimum feret diameter per participant between disease (CON *vs* T1D) and age (younger *vs* older) groups. (C) Muscle fiber composition, between disease and age groups. Light and dark shaded areas represent type 1 and type 2 fiber proportions, respectively, and are labeled within

deficits to the same degree due to the exclusion of neuropathic patients in the current study. Further, it should be noted that previous studies have utilized electrophysiological techniques to measure neurological dysfunction in the distal limb (17), whereas here we interrogated proximal limb muscles. Here, our clinical examination of neuropathy revealed no significant motor dysfunction between any of the groups. Therefore, it remains possible that neurological motor dysfunction of the proximal limb is still progressing in these adults with T1D, yet it is unlikely that the differences observed between adults with/without T1D are neurogenic in nature. Still, these findings highlight the need for future studies to interrogate proximal limb denervation with denervation-specific biomarkers, such as embryonic myosin heavy chain or the sodium channel $Nav_{1.5}$ (41) to more fully elucidate its presence in T1D skeletal muscle. Nevertheless, the exacerbation in age-related strength loss observed here seemingly precedes the development of neurogenic motor deficits, suggesting that T1D elicits negative effects on skeletal muscle function throughout adult life, perhaps earlier than other key comorbidities.

The Role of Satellite Cell and Capillary Content in Aging Muscle of Those With T1D

Some studies have suggested the age-related loss in type 2 fibers to occur concomitantly with a loss of SCs in skeletal muscle (42,43), and their importance in age-related strength and muscle loss (ie, sarcopenia) is a topic of intense study (for a review, see (44)). In the context of T1D, D'Souza et al showed that young males (18-22 years of age) with T1D had a lower SC content as a proportion of total myonuclei compared to age-matched nondiabetic males (45). Interestingly, in the current study, we observed only small or negligible changes in SC content between those with and without T1D. The discrepancy between these and previous data may be the different methodology for quantification used here (ie, SC per 100 myofibers) increased age of our younger male participants (27 ± 1.37 years) *vs* the previous study (45) or that our current cohort is significantly larger and more diverse than previously reported. To our knowledge, there are no other observations of SC in human muscle with T1D. Additionally, we interrogated muscle capillary content, as its importance has been noted for its contribution to muscle endurance capacity (46), which is reportedly lacking in muscle with T1D (47). We did not detect decrements in capillary content in muscle with T1D, similar to previous investigations (17). Furthermore, older females in the present study tended toward higher capillary content than their younger counterparts, which is contrary to recent observations of angiogenic potential in aged females (48). While the reason(s) underlying this difference are currently unknown, we did observe that capillary density was negatively correlated with muscle fiber size (minimum feret diameter) in older females ($R = -0.62$, $P < 0.01$), which may suggest that elevated capillary density may be due, in part, to the smaller minimum feret diameter of older females with T1D, who are therefore expressing more capillaries per unit area in the muscle. Further, due to the elevated proportion of type 1 fiber grouping in older women with T1D, there may have been an increased propensity to measure capillaries surrounded by more type 1 fibers, which tend to be more highly vascularized. Indeed, there was indications that capillary density was correlated with the proportion of grouped type 1 myofibers in this cohort ($r = 0.69$, $P = 0.07$).

Altered Glycogen Expression in T1D as a Potential Outcome of Elevated Glycolysis

In older participants, we observed lower glycogen content at rest in T1D myofibers *vs* those of their CON counterparts, regardless of sex. The current finding of diminished muscle glycogen in T1D muscle complements the literature, which suggests metabolism tends toward increased glycolysis during exercise and at rest in T1D skeletal muscle (8,9,16,49,50). Indeed, previous investigations have shown that T1D myofibers exhibit higher levels of the glycolytic enzyme glycerol-3-phosphate dehydrogenase at rest (16), while others have observed elevated anerobic glycolysis in muscle of T1D adolescents during exercise through noninvasive measures (8,49,50). Alternatively, it may be posited that T1D muscles are exhibiting defects in glycogen synthesis. However, earlier investigations have observed normal protein/mRNA content as well as total activity of enzymes involved in glycogen synthetic pathways (51). Diminished muscle glycogen may in fact represent another metabolic perturbation that resembles accelerated aging in T1D muscle, in addition to previous observations of mitochondrial dysfunction during T1D (10,52,53) (for reviews, see (54-56)), since

Figure 3: continued

graphical summary. Minimum feret diameter distributions in (A) are expressed as a distribution of all fibers of each respective fiber type. Bar plots in (B and C) are expressed as mean ± SEM. For individual datapoints, circles indicate CON participants and diamonds indicate T1D. Blue and pink coloring indicate males and females, respectively. For comparisons of distributions (A), *P*-values are displayed within graphical summary. Effect size (*g*) *vs* CON are displayed within bars. Effect size *vs* younger cohort are displayed over square brackets.

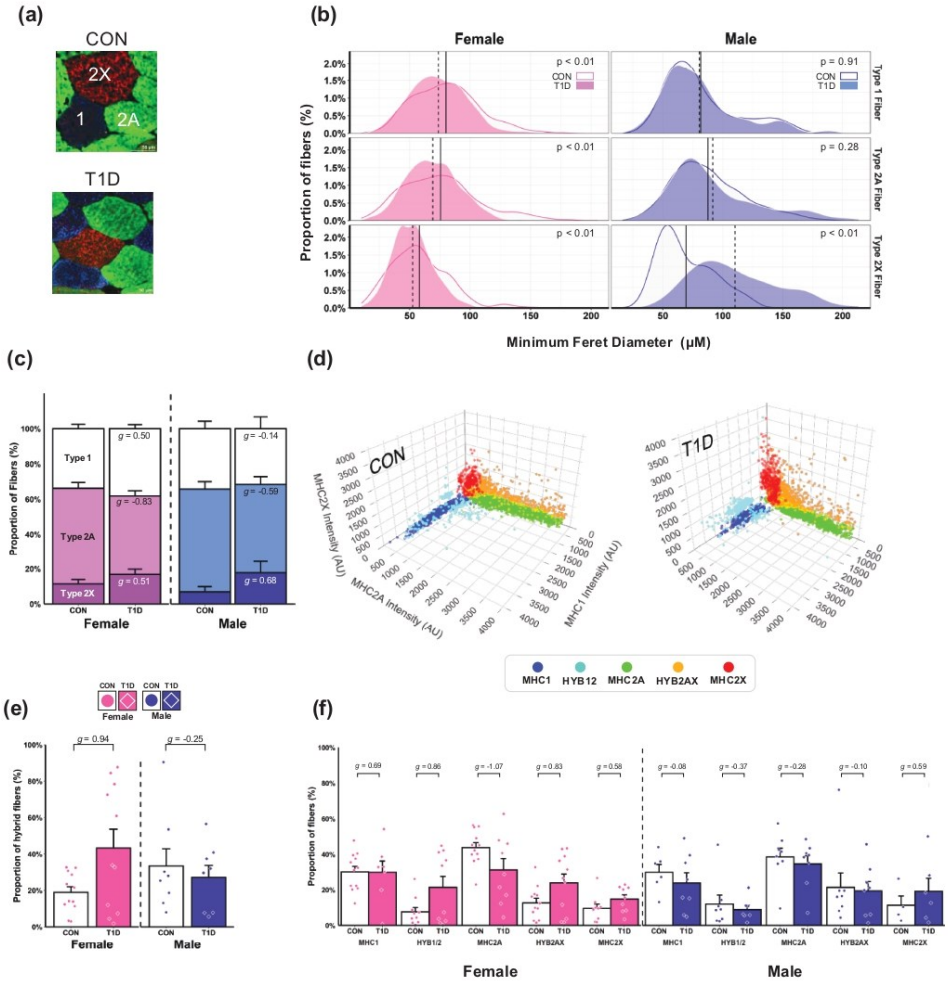

Figure 4. Prevalence of hybrid fibers in T1D skeletal muscle. (A) Representative immunofluorescent images of fiber type stain. Examples of type 1, 2A and 2X fibers labeled within image. (B) Muscle fiber size distributions, as measured by minimum feret diameter. (C) Muscle fiber composition, as measured by immunofluorescence. Light, medium, and dark shaded bars represent type 1, 2A, and 2X fiber proportions, respectively, and are labeled within graphical summary. (D) Three-dimensional scatter plot displaying MHC co-expression, as measured by average pixel intensity [arbitrary units (AU)]. Each point represents a myofiber, and each color represents a myofiber type (legend under graphical summaries). (E) Proportion of hybrid fibers (ie, co-expressing 2 or more MHC types; for hybrid fiber criteria, see supplementary material in (25)). (F) Proportion of specific hybrid fiber types in males and females with and without T1D. Minimum feret diameter distributions in (B) are expressed as a distribution of all counted myofibers. Solid and dashed vertical lines indicate mean values for CON and T1D, respectively. Bar plots (C, E, and F) are expressed as mean ± SEM. For individual datapoints within dot/bar plots (E and F), circles indicate CON participants and diamonds indicate T1D. Blue and pink coloring indicate males and females, respectively. For comparisons of distributions (B), *P*-values are displayed within graphical summary. Effect sizes (*g*) *vs* CON are displayed within (A) or over bars (E and F).

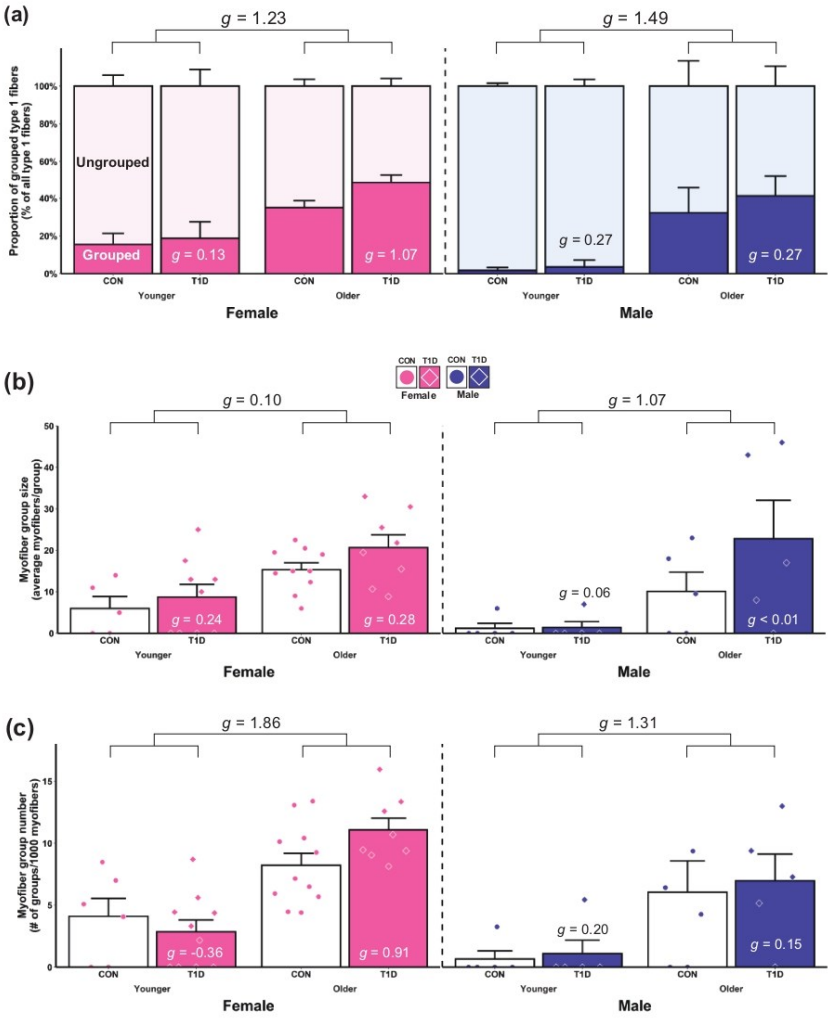

Figure 5. Exaggerated type 1 myofiber grouping in those with T1D. (A) Proportions of grouped/ungrouped type 1 myofibers, between disease (CON *vs* T1D) and age (younger *vs* older) groups. Light and dark shaded areas represent ungrouped and grouped type 1 fiber proportions, respectively, and are labeled within graphical summary. (B) Myofiber group number between disease and age groups. (C) Myofiber group size between disease and age groups. All data are expressed as mean ± SEM. For individual datapoints within dot/bar plots (B and C), circles indicate CON participants and diamonds indicate T1D. Blue and pink coloring indicate males and females, respectively. Effect sizes (*g*) *vs* CON are displayed within bars.

diminished muscle glycogen stores are found to be lower in skeletal muscle in older adults (57-59). Although, contrasting findings have shown intramyocellular glycogen, as measured by magnetic resonance spectroscopy, to be similar in T1D patients and control individuals, in a cohort that was of similar age to our younger adults (60). Our observations indicate there is a lower level of muscle glycogen in muscle with T1D compared to CON, albeit this observation was in older adults than previous studies (current = 52 ± 2 years *vs* Bally et al (60) = 26 ± 2 years) and were measured by different methods. Thus, the current data suggest that greater glycolytic metabolism

during T1D may contribute to a diminished level of glycogen storage. Also, the relationship between glycogen content and time spent seated in our cohort was evident and suggests a potential link with sedentary status of individuals that warrants future study. Whether or not insulin resistance experienced by some T1D adults plays a role in diminished muscle glycogen remains an open question. However, early investigations of those with T1D and T2D suggested that the lack of insulin sensitivity was an important contributor to low muscle glycogen concentration (61). Others have found that diminished glycogen synthase activity was secondary to a reduction in glucose uptake in T1D muscle (62). Further, observations of individuals with T2D, which exhibit insulin resistance as a primary pathology, have been observed to have diminished muscle glycogen—in a pattern that varies between muscle fiber types (63). We aimed to discern if the altered fiber-type characteristics in T1D participants (ie, grouped and/or hybrid fibers) may be associated with glycogen content on PAS stain intensity but were unable to find any apparent relationship between PAS intensity and type 1 muscle fiber grouping proportion, number of grouped type 1 fibers, hybrid 1/2 proportion, or hybrid 2A/X proportion (data not shown). Whether or not the diminished glycogen levels observed here warrant a health risk for T1D patients remains to be determined.

Putative Effect of Sleep Loss in Older Adults With T1D

Our accelerometry data suggests that older adults with T1D spend less time sleeping (as noted by reduced time spent in a "resting zone" between 12 AM and 8 AM). For an example of a 24-h physical activity tracing, see supplementary material in (25). This phenomenon has been previously observed in persons with T1D who report that nonsevere hypoglycemic episodes disrupt their sleep and impair their ability to fall back asleep following treatment of these events (64,65). Hyperglycemia can also exert negative effects on hormones that regulate circadian rhythm (66). Previous investigations have demonstrated the detrimental effects of sleep deprivation on muscle protein synthesis rates (67) and glycogen content (68), which may contribute to the loss of muscle function seen in older T1D participants in this study. It should be noted as well that the link between sleep loss and neurogenesis deficits have been established in humans (69). While currently speculative, it may be that altered sleep patterns in those with T1D elicit degenerative effects on motor neuron function (ie, muscle strength) as well.

Accelerated Loss of Type 2 Fibers in Adults With T1D

Our histological examination of muscle fibers in adults with T1D aimed to determine whether there were changes to muscle fiber composition that would affect muscle size and strength in these muscles. The proportion and distribution of slow-twitch (type 1) and fast-twitch (type 2) fibers is dynamic during the aging process. With advancing age, muscle size gradually decreases due to a reduction in both number and size of type 2 fibers (70-73), suggesting that the type 2 fiber proportion earlier in life may be important to determining age-related loss of muscle mass. Interestingly, in our participants, only those with T1D exhibited the expected decrease in type 2 fiber proportion with age, suggesting that the rate of type 2 myofiber loss is greater than that of nondiabetic individuals, who appeared to maintain fiber composition over the same time period. In this study, we found that these alterations to the proportions of type 1 and 2 fibers occur in a sexually dimorphic manner. On one hand, younger males with T1D exhibited an elevated proportion of type 2 fibers relative to younger CON males, similar to previous observations (17). The exact reason for the shift toward type 2 fibers in T1D remains unclear, although others have suggested it is coordinated with the shift toward glycolytic metabolism in these muscles (16,17). Importantly, our data suggest that the aggregation of male and female fiber-type proportions by Andreassen et al (17) may have resulted in a generalized finding that is sexually dimorphic. Indeed, here we did not observe an elevation in type 2 fibers in females with T1D. Instead, older females with T1D exhibited relatively less type 2 fibers than their nondiabetic counterparts. Given the importance of type 2 fibers in producing force/power, a diminished fraction of type 2 fibers may have detrimental effects on the ability of older women with T1D to perform activities of daily living (72).

Increased Prevalence of Hybrid Fibers in Older Females With T1D

Observation of the altered fast/slow fiber-type proportions (ie, type 1/2) warranted deeper examination of the specific MHC isoforms (ie, 1/2A/2X) in these participants. Generally, trends in the data indicated that T1D participants gained type 2X fibers, seemingly at the expense of type 2A fibers in males and females with T1D. Additionally, it was observed that there was a larger proportion of hybrid fibers (ie, those co-expressing 2 or more MHC types) only in females with T1D. An increased prevalence of hybrid fibers is often associated with states in skeletal muscle such as disuse or aging (22,23). During aging, in particular, the

expression of hybrid fibers becomes increasingly prevalent (22). Andersen et al (22) proposed a mechanism by which age-related denervation/reinnervation events cause affected fibers to receive neural inputs that contradict their original myogenic lineage, thus providing conflicting signals and eventually giving rise to a mixed expression of MHC isoforms. In conjunction with our data, it again insinuates that females with T1D display a phenotype that resembles a transition toward increasingly "aged" muscle.

Elevated Myofiber Grouping in Aging Females With T1D

In addition to higher relative amounts of type 1 fibers (ie, diminished type 2 fiber proportion) and elevated hybrid fibers, we observed an elevated amount of type 1 myofiber grouping in older women with T1D *vs* their CON counterparts. The grouping of type 1 myofibers is a phenotype that has been associated with age-related denervation/reinnervation events (18,19) as well as inefficient motor unit activation and greater physiologic difficulty to complete motor tasks (20). Interestingly, type 1 myofiber grouping may be initiated by neuromuscular remodeling in the form of denervation of type 2 myofibers either centrally (ie, death of type 2 α-motor neuron) or peripherally (ie, loss of neuromuscular junction integrity) (74-76). A small handful of preclinical studies have shown the postsynaptic environment (ie, skeletal muscle) heavily affects the neuromuscular junction and is even able to modulate the motor unit type (77-79). Thus, the possibility remains that neuromuscular dysfunction in T1D may be driven by muscular alterations that elicit retrograde effects on the neuromuscular junction and, ultimately, motor neurons. Indeed, this may be the case here, as we did not observe any obvious cases of angulated fibers or grouped myofiber atrophy, which would have been more indicative of neurogenic denervation (74,80) as opposed to dysfunction originating in skeletal muscles. Our observations of elevated type 1 myofiber grouping in T1D women support the ongoing hypothesis that T1D muscle, apparently more so in females, may be susceptible to a form of accelerated neuromuscular aging (12). Interestingly, it appears that similar mechanisms of age-related denervation/reinnervation may impact the prevalence of hybrid and grouped type 1 myofibers, at least in our study cohort. To date, this study represents the first effort to interrogate myofiber grouping as a component of neuromuscular health in adults with T1D. The exact mechanism by which myofibers in females respond differentially to T1D is unknown and warrants future study.

Limitations

The current study has a smaller sample size of males relative to females. Therefore, our ability to make conclusions based on statistical hypothesis tests may be limited in the comparisons involving only males. Further, some measurements such as continuous glucose and activity monitoring were only performed on the older adult cohort of the study. Given these limitations, we are not able to fully ascertain whether differences in activity/blood glucose control were underlying mechanisms to the differences between young and older cohorts herein. For the sake of transparency, we have attempted to display every comparison between males, females, or all participants combined. Second, although the method for quantifying myofiber grouping partially controls for type 1 myofiber distribution, the influence is not completely removed ($R = 0.46$; data not shown). Therefore, type 1 fiber proportion may partially account for measurements of myofiber grouping, as previously noted (19,27). Nevertheless, to our knowledge, the methodology used here represents the most comprehensive and statistically stringent method for histologically quantifying type 1 fiber grouping. Finally, the storage of our muscle biopsies in OCT medium was chosen as it allowed us the most breadth in analyses between histological and immunofluorescent techniques but limited other biochemical analyses. To maximize validity of our analyses, we analyzed the maximum available surface of each muscle biopsy. Nevertheless, the possibility for differences in muscle architecture remains, which may, in theory, affect some of the measurements of staining intensity (ie, PAS staining) Thus, there remains opportunity for future studies to investigate muscle glycogen by other biochemical means that are less reliant on internal cellular structure between comparator groups and/or in other experimental conditions (ie, before and after muscle function tests). Muscle insulin sensitivity/resistance was beyond the scope of the measurements planned for this study. Nevertheless, there are several open questions with regards to insulin sensitivity and the modulation of muscle metabolism during T1D. Thus, the study of insulin sensitivity in T1D and its link to altered fuel metabolism in skeletal muscle remains a promising area for future study.

Conclusions

The current work has demonstrated that, during the progression of T1D, decrements in skeletal muscle strength appear before that of other comorbidities (such as clinically diagnosed neuropathy), and it diminishes at a quicker rate than nondiabetic counterparts. Interestingly,

changes in skeletal muscle fiber composition appear to be sexually dimorphic. On one hand, males exhibit a shift towards fast-twitch type 2 muscle fibers, similar to previous findings (16,17). Meanwhile, compared to their CON counterparts, females with T1D exhibit more type 1 fibers, with higher proportions of them being grouped and co-expressing multiple MHC isoforms (ie, hybrid fibers). In all, older T1D women in this study exhibited multiple signs associated with accelerated neuromuscular aging (18-20,22,27). Our data suggest future investigations are warranted to determine the underlying cause(s) of these effects that are exacerbated in the muscle of females with T1D. Finally, the diminished glycogen content observed here appears to complement other observations of glycolysis abnormalities in T1D muscle (8,16,49,50). Further, diminished glycogen content may signal an age-associated metabolic perturbation in skeletal muscle that is not present in persons without T1D. Future studies investigating the convergence of pathways regulating muscle fiber composition and metabolite networks are warranted.

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Author Contributions: AGD, CMFM, and TJH designed the experiments. AGD and TJH wrote the manuscript. AGD performed the experiments. AGD and TPP analyzed the data. AGD and TJH interpreted the data. MAT performed muscle biopsies, neurological evaluations, and blood sampling. AGD, CMFM, and GKG collected and processed muscle samples. All authors edited the manuscript. All authors provided final approval of the version to be published. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. TJH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of data and the accuracy of the data analysis.

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Disclosures: M.A.T. is the founder and CEO of Exerkine Corporation/Stay Above Nutrition and they sell nutritional products for aging and obesity but have neither products nor plans to develop products for type 1 diabetes. C.M.F.M is an employee of Medtronic. No other conflicts are reported.

Data Availability: Restrictions apply to some or all the availability of data generated or analyzed during this study to preserve patient confidentiality. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

References

1. Mobasseri M, Shirmohammadi M, Amiri T, Vahed N, Hosseini Fard H, Ghojazadeh M. Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis. *Health Promot Perspect.* 2020;**10**(2):98-115.
2. Thabit H, Hovorka R. Closed-loop insulin delivery in type 1 diabetes. *Endocrinol Metab Clin North Am.* 2012;**41**(1):105-117.
3. Zaharieva DP, Messer LH, Paldus B, O'Neal DN, Maahs DM, Riddell MC. Glucose control during physical activity and exercise using closed loop technology in adults and adolescents with type 1 diabetes. *Can J Diabetes.* 2020;**44**(8):740-749.
4. Lal RA, Ekhlaspour L, Hood K, Buckingham B. Realizing a closed-loop (artificial pancreas) system for the treatment of type 1 diabetes. *Endocr Rev.* 2019;**40**(6):1521-1546.
5. Distiller LA. Why do some patients with type 1 diabetes live so long? *World J Diabetes.* 2014;**5**(3):282-287.
6. Katsarou A, Gudbjörnsdottir S, Rawshani A, et al. Type 1 diabetes mellitus. *Nat Rev Dis Primers.* 2017;**3**:17016.
7. Morley JE, Malmstrom TK, Rodriguez-Mañas L, Sinclair AJ. Frailty, sarcopenia and diabetes. *J Am Med Dir Assoc.* 2014;**15**(12):853-859.
8. Cree-Green M, Newcomer BR, Brown MS, et al. Delayed skeletal muscle mitochondrial ADP recovery in youth with type 1 diabetes relates to muscle insulin resistance. *Diabetes.* 2015;**64**(2):383-392.
9. Dutta T, Kudva YC, Persson XM, et al. Impact of long-term poor and good glycemic control on metabolomics alterations in type 1 diabetic people. *J Clin Endocrinol Metab.* 2016;**101**(3):1023-1033.
10. Monaco CMF, Hughes MC, Ramos SV, et al. Altered mitochondrial bioenergetics and ultrastructure in the skeletal muscle of young adults with type 1 diabetes. *Diabetologia.* 2018;**61**(6):1411-1423.
11. Karakelides H, Asmann YW, Bigelow ML, et al. Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. *Diabetes.* 2007;**56**(11):2683-2689.
12. Monaco CMF, Gingrich MA, Hawke TJ. Considering type 1 diabetes as a form of accelerated muscle aging. *Exerc Sport Sci Rev.* 2019;**47**(2):98-107.
13. Andersen H, Gadeberg PC, Brock B, Jakobsen J. Muscular atrophy in diabetic neuropathy: a stereological magnetic resonance imaging study. *Diabetologia.* 1997;**40**(9):1062-1069.
14. Andersen H, Gjerstad MD, Jakobsen J. Atrophy of foot muscles: a measure of diabetic neuropathy. *Diabetes Care.* 2004;**27**(10):2382-2385.
15. Reske-Nielsen E, Harmsen A, Vorre P. Ultrastructure of muscle biopsies in recent, short-term and long-term juvenile diabetes. *Acta Neurol Scand.* 1977;**55**(5):345-362.
16. Fritzsche K, Blüher M, Schering S, et al. Metabolic profile and nitric oxide synthase expression of skeletal muscle fibers are altered in patients with type 1 diabetes. *Exp Clin Endocrinol Diabetes.* 2008;**116**(10):606-613.
17. Andreassen CS, Jensen JM, Jakobsen J, Ulhøj BP, Andersen H. Striated muscle fiber size, composition, and capillary density

in diabetes in relation to neuropathy and muscle strength. *J Diabetes*. 2014;**6**(5):462-471.
18. Lexell J, Downham DY. The occurrence of fibre-type grouping in healthy human muscle: a quantitative study of cross-sections of whole vastus lateralis from men between 15 and 83 years. *Acta Neuropathol*. 1991;**81**(4):377-381.
19. Kelly NA, Hammond KG, Stec MJ, et al. Quantification and characterization of grouped type I myofibers in human aging. *Muscle Nerve*. 2018;**57**(1):E52-E59.
20. Kelly NA, Hammond KG, Bickel CS, Windham ST, Tuggle SC, Bamman MM. Effects of aging and Parkinson's disease on motor unit remodeling: influence of resistance exercise training. *J Appl Physiol (1985)*. 2018;**124**(4):888-898.
21. Medler S. Mixing it up: the biological significance of hybrid skeletal muscle fibers. *J Exp Biol*. 2019;**222**(23):jeb200832.
22. Andersen JL, Terzis G, Kryger A. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. *Muscle Nerve*. 1999;**22**(4):449-454.
23. Talmadge RJ. Myosin heavy chain isoform expression following reduced neuromuscular activity: potential regulatory mechanisms. *Muscle Nerve*. 2000;**23**(5):661-679.
24. Tarnopolsky MA, Pearce E, Smith K, Lach B. Suction-modified Bergström muscle biopsy technique: experience with 13,500 procedures. *Muscle Nerve*. 2011;**43**(5):717-725.
25. Dial AG, Monaco CMF, Grafham GK, Patel TP, Tarnopolsky MA, Hawke TJ. Electronic supplementary material from impaired function and altered morphology in the skeletal muscles of adult men and women with type 1 diabetes. *Figshare*. 2021. doi:10.6084/m9.figshare.13293239.v1.
26. Hepple RT, Mackinnon SL, Goodman JM, Thomas SG, Plyley MJ. Resistance and aerobic training in older men: effects on VO2peak and the capillary supply to skeletal muscle. *J Appl Physiol (1985)*. 1997;**82**(4):1305-1310.
27. Lavin KM, Sealfon SC, McDonald MN, et al. Skeletal muscle transcriptional networks linked to type I myofiber grouping in Parkinson's disease. *J Appl Physiol (1985)*. 2020;**128**(2):229-240.
28. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Erlbaum; 1988.
29. van Baar ME, Assendelft WJ, Dekker J, Oostendorp RA, Bijlsma JW. Effectiveness of exercise therapy in patients with osteoarthritis of the hip or knee: a systematic review of randomized clinical trials. *Arthritis Rheum*. 1999;**42**(7):1361-1369.
30. Davies GM, Watson DJ, Bellamy N. Comparison of the responsiveness and relative effect size of the western Ontario and McMaster Universities Osteoarthritis Index and the short-form Medical Outcomes Study Survey in a randomized, clinical trial of osteoarthritis patients. *Arthritis Care Res*. 1999;**12**(3):172-179.
31. Vu Q, Xie K, Eckert M, Zhao W, Cramer SC. Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. *Neurology*. 2014;**82**(14):1277-1286.
32. Papp KV, Rentz DM, Orlovsky I, Sperling RA, Mormino EC. Optimizing the preclinical Alzheimer's cognitive composite with semantic processing: the PACC5. *Alzheimers Dement (N Y)*. 2017;**3**(4):668-677.
33. Guilcher SJT, Voth J, Ho C, et al. Characteristics of non-traumatic spinal cord dysfunction in Canada using administrative health data. *Top Spinal Cord Inj Rehabil*. 2017;**23**(4):343-352.
34. Wilson JR, Voth J, Singh A, et al. Defining the pathway to definitive care and surgical decompression after traumatic spinal cord injury: results of a Canadian population-based cohort study. *J Neurotrauma*. 2016;**33**(10):963-971.
35. Field-Fote EC, Roach KE. Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: a randomized clinical trial. *Phys Ther*. 2011;**91**(1):48-60.
36. Demetriou EA, Lampit A, Quintana DS, et al. Autism spectrum disorders: a meta-analysis of executive function. *Mol Psychiatry*. 2018;**23**(5):1198-1204.
37. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol*. 2013;**4**:863.
38. Kalyani RR, Metter EJ, Egan J, Golden SH, Ferrucci L. Hyperglycemia predicts persistently lower muscle strength with aging. *Diabetes Care*. 2015;**38**(1):82-90.
39. Andersen H, Poulsen PL, Mogensen CE, Jakobsen J. Isokinetic muscle strength in long-term IDDM patients in relation to diabetic complications. *Diabetes*. 1996;**45**(4):440-445.
40. Andersen H. Muscular endurance in long-term IDDM patients. *Diabetes Care*. 1998;**21**(4):604-609.
41. Rowan SL, Rygiel K, Purves-Smith FM, Solbak NM, Turnbull DM, Hepple RT. Denervation causes fiber atrophy and myosin heavy chain co-expression in senescent skeletal muscle. *PloS One*. 2012;**7**(1):e29082.
42. Verdijk LB, Koopman R, Schaart G, Meijer K, Savelberg HH, van Loon LJ. Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab*. 2007;**292**(1):E151-E157.
43. Sajko S, Kubínová L, Cvetko E, Kreft M, Wernig A, Erzen I. Frequency of M-cadherin-stained satellite cells declines in human muscles during aging. *J Histochem Cytochem*. 2004;**52**(2):179-185.
44. Snijders T, Verdijk LB, van Loon LJ. The impact of sarcopenia and exercise training on skeletal muscle satellite cells. *Ageing Res Rev*. 2009;**8**(4):328-338.
45. D'Souza DM, Zhou S, Rebalka IA, et al. Decreased satellite cell number and function in humans and mice with type 1 diabetes is the result of altered notch signaling. *Diabetes*. 2016;**65**(10):3053-3061.
46. Terzis G, Spengos K, Manta P, Sarris N, Georgiadis G. Fiber type composition and capillary density in relation to submaximal number of repetitions in resistance exercise. *J Strength Cond Res*. 2008;**22**(3):845-850.
47. Orlando G, Balducci S, Bazzucchi I, Pugliese G, Sacchetti M. The impact of type 1 diabetes and diabetic polyneuropathy on muscle strength and fatigability. *Acta Diabetol*. 2017;**54**(6):543-550.
48. Olsen LN, Hoier B, Hansen CV, et al. Angiogenic potential is reduced in skeletal muscle of aged women. *J Physiol*. 2020;**598**(22):5149-5164.
49. Jenni S, Oetliker C, Allemann S, et al. Fuel metabolism during exercise in euglycaemia and hyperglycaemia in patients with type 1 diabetes mellitus: a prospective single-blinded randomised crossover trial. *Diabetologia*. 2008;**51**(8):1457-1465.
50. Crowther GJ, Milstein JM, Jubrias SA, Kushmerick MJ, Gronka RK, Conley KE. Altered energetic properties in skeletal muscle of men with well-controlled insulin-dependent

(type 1) diabetes. *Am J Physiol Endocrinol Metab*. 2003;**284**(4):E655-E662.
51. Vestergaard H, Andersen PH, Lund S, Vedel P, Pedersen O. Expression of glycogen synthase and phosphofructokinase in muscle from type 1 (insulin-dependent) diabetic patients before and after intensive insulin treatment. *Diabetologia*. 1994;**37**(1):82-90.
52. Lanza IR, Zhang S, Ward LE, Karakelides H, Raftery D, Nair KS. Quantitative metabolomics by H-NMR and LC-MS/MS confirms altered metabolic pathways in diabetes. *PloS One*. 2010;**5**(5):e10538.
53. Robinson MM, Dasari S, Karakelides H, Bergen HR 3rd, Nair KS. Release of skeletal muscle peptide fragments identifies individual proteins degraded during insulin deprivation in type 1 diabetic humans and mice. *Am J Physiol Endocrinol Metab*. 2016;**311**(3):E628-E637.
54. Sivitz WI, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxid Redox Signal*. 2010;**12**(4):537-577.
55. Alway SE. Mitochondrial dysfunction: linking type 1 diabetes and sarcopenia. *Exerc Sport Sci Rev*. 2019;**47**(2):63.
56. Ruegsegger GN, Creo AL, Cortes TM, Dasari S, Nair KS. Altered mitochondrial function in insulin-deficient and insulin-resistant states. *J Clin Invest*. 2018;**128**(9):3671-3681.
57. Meredith CN, Frontera WR, Fisher EC, et al. Peripheral effects of endurance training in young and old subjects. *J Appl Physiol (1985)*. 1989;**66**(6):2844-2849.
58. Cartee GD. Aging skeletal muscle: response to exercise. *Exerc Sport Sci Rev*. 1994;**22**:91-120.
59. Cartee GD. Influence of age on skeletal muscle glucose transport and glycogen metabolism. *Med Sci Sports Exerc*. 1994;**26**(5):577-585.
60. Bally L, Buehler T, Dokumaci AS, Boesch C, Stettler C. Hepatic and intramyocellular glycogen stores in adults with type 1 diabetes and healthy controls. *Diabetes Res Clin Pract*. 2015;**109**(1):e1-e3.
61. Roch-Norlund AE, Bergström J, Castenfors H, Hultman E. Muscle glycogen in patients with diabetes mellitus. *Acta Med Scand*. 1970;**187**(1-6):445-453.
62. Koivisto VA, Yki-Järvinen H. Changes in muscle glucose metabolism in type 1 diabetes. *Ann Med*. 1990;**22**(3):201-205.
63. He J, Kelley DE. Muscle glycogen content in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab*. 2004;**287**(5):E1002-E1007.
64. Brod M, Pohlman B, Wolden M, Christensen T. Non-severe nocturnal hypoglycemic events: experience and impacts on patient functioning and well-being. *Qual Life Res*. 2013;**22**(5):997-1004.
65. Brod M, Christensen T, Bushnell DM. Impact of nocturnal hypoglycemic events on diabetes management, sleep quality, and next-day function: results from a four-country survey. *J Med Econ*. 2012;**15**(1):77-86.
66. Amaral FG, Turati AO, Barone M, et al. Melatonin synthesis impairment as a new deleterious outcome of diabetes-derived hyperglycemia. *J Pineal Res*. 2014;**57**(1):67-79.
67. Lamon S, Morabito A, Arentson-Lantz E, et al. The effect of acute sleep deprivation on skeletal muscle protein synthesis and the hormonal environment. *Physiol Rep*. 2021;**9**(1):e14660.
68. Skein M, Duffield R, Edge J, Short MJ. Intermittent-sprint performance and muscle glycogen after 30 h of sleep deprivation. *Med Sci Sports Exerc*. 2011;**43**(7):1301-1311.
69. Meerlo P, Mistlberger RE, Jacobs BL, Heller HC, McGinty D. New neurons in the adult brain: the role of sleep and consequences of sleep loss. *Sleep Med Rev*. 2009;**13**(3):187-194.
70. Evans WJ, Lexell J. Human aging, muscle mass, and fiber type composition. *J Gerontol Ser A* 1995;**50A**(Spec no. 11-6). doi:10.1093/gerona/50a.special_issue.11
71. Lexell J, Henriksson-Larsén K, Winblad B, Sjöström M. Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. *Muscle Nerve*. 1983;**6**(8):588-595.
72. Brunner F, Schmid A, Sheikhzadeh A, Nordin M, Yoon J, Frankel V. Effects of aging on Type II muscle fibers: a systematic review of the literature. *J Aging Phys Act*. 2007;**15**(3):336-348.
73. Bougea A, Papadimas G, Papadopoulos C, Paraskevas GP, Kalfakis N, Manta P, Kararizou E. An age-related morphometric profile of skeletal muscle in healthy untrained women. *J Clin Med*. 2016;**5**(11):97.
74. Aare S, Spendiff S, Vuda M, et al. Failed reinnervation in aging skeletal muscle. *Skelet Muscle*. 2016;**6**(1):29.
75. Bishop DL, Milton RL. The effects of denervation location on fiber type mix in self-reinnervated mouse soleus muscles. *Exp Neurol*. 1997;**147**(1):151-158.
76. Haddix SG, Lee YI, Kornegay JN, Thompson WJ. Cycles of myofiber degeneration and regeneration lead to remodeling of the neuromuscular junction in two mammalian models of Duchenne muscular dystrophy. *PloS One*. 2018;**13**(10):e0205926.
77. Arnold AS, Gill J, Christe M, et al. Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1α. *Nat Commun*. 2014;**5**:3569.
78. Handschin C, Kobayashi YM, Chin S, Seale P, Campbell KP, Spiegelman BM. PGC-1alpha regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy. *Genes Dev*. 2007;**21**(7):770-783.
79. Chakkalakal JV, Nishimune H, Ruas JL, Spiegelman BM, Sanes JR. Retrograde influence of muscle fibers on their innervation revealed by a novel marker for slow motoneurons. *Development*. 2010;**137**(20):3489-3499.
80. Spendiff S, Vuda M, Gouspillou G, et al. Denervation drives mitochondrial dysfunction in skeletal muscle of octogenarians. *J Physiol*. 2016;**594**(24):7361-7379.

CHAPTER 3 – ALTERATIONS IN SKELETAL MUSCLE REPAIR IN YOUNG ADULTS WITH TYPE 1 DIABETES MELLITUS

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PREFACE

Author contributions:

GKG, CMFM, and TJH designed the experiments. **AGD**, GKG, and TJH wrote the manuscript. GKG, CMFM, LAB and MAT performed sample collection. GKG and **AGD** performed sample analysis. **AGD**, GKG, JV and TJH analyzed and interpreted the data. All authors edited the manuscript. All authors provided final approval of the version to be published. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. TJH is the guarantor of this work and, as such, had full access to the data in this study and takes responsibility for the integrity of such data and the accuracy of data analysis.

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ABSTRACT

Though preclinical models of type 1 diabetes (T1D) exhibit impaired muscle regeneration, this has yet to be investigated in humans with T1D. Here we investigated the impact of damaging exercise (eccentric quadriceps contractions) in eighteen physically-active young adults with and without T1D. Pre- and post-exercise (48h and 96h), participants provided blood samples, *vastus lateralis* biopsies and performed maximal voluntary quadriceps contractions (MVC). Skeletal muscle sarcolemmal integrity, extracellular matrix content (ECM), and satellite cell (SC) content/proliferation were assessed by immunofluorescence. Transmission electron microscopy was used to quantify ultrastructural damage.

MVC was comparable between T1D and controls before exercise. Post-exercise, MVC was decreased in both groups, but T1D subjects exhibited moderately lower strength recovery at both 48h and 96h. Serum creatine kinase, an indicator of muscle damage, was moderately higher in T1D participants at rest, and exhibited a small elevation 96h post-exercise. T1D participants showed lower SC content at all timepoints and demonstrated a moderate delay in SC proliferation after exercise. A greater number of myofibers exhibited sarcolemmal damage (disrupted dystrophin) and increased ECM (laminin) content in participants with T1D despite no differences between groups in ultrastructural damage as assessed by electron microscopy. Finally, transcriptomic analyses revealed dysregulated gene networks involving RNA translation and mitochondrial respiration, providing potential explanations for previous observations of mitochondrial dysfunction in similar T1D cohorts. Our findings indicate that skeletal muscle in young adults with moderately-

controlled T1D is altered after damaging exercise; suggesting that longer recovery times following intense exercise may be necessary.

TWEET

Evidence for delays in #muscle repair in those with #T1D #Myotwitter #Exercise

KEYWORDS

Skeletal muscle, Type 1 Diabetes, T1D, Myopathy, Regeneration, Functional capacity, Satellite cells, ultrastructure

ABBREVIATIONS

- CON, Control
- CK, creatine kinase
- ECM, extracellular matrix
- GSEA, gene set enrichment analysis
- MET, metabolic equivalent of task
- NSAID, nonsteroidal anti-inflammatory drug
- SC, satellite cell
- T1D, type 1 diabetes

INTRODUCTION

Dysregulation of glucose metabolism in Type 1 Diabetes (T1D) leads to dysfunction in various body tissues (D. D'Souza et al., 2013; Harding et al., 2019) and, thus, regular physical activity is recommended for those with T1D to mitigate complications progression (Chimen et al., 2012). While increased physical activity is beneficial, muscle damage following intense exercise is common and repair from this damage is critical for effective muscle growth and adaptation (Chargé & Rudnicki, 2004). Unfortunately, murine models report that the T1D environment creates an unfavourable situation for muscle repair, with prolonged fibrosis (Krause et al., 2011), altered satellite cell behaviour (Jeong et al., 2013b), and delayed myofiber maturation (Krause et al., 2013, p. 201). However, due to continually evolving insulin therapies (Lal et al., 2019; Thabit & Hovorka, 2012, p.; Zaharieva et al., 2020) leading to longer, healthier lives of those with T1D, the phenotype of animal T1D models are increasingly divergent from the human condition. Still, in humans, T1D also confers alterations to skeletal muscle morphology, metabolism and function (Dial et al., 2021; Monaco et al., 2018b). However, the capacity of muscle to repair following damaging exercise has never been studied. Thus, the purpose of this study was to evaluate the initial stages of skeletal muscle repair in otherwise healthy young adults with T1D after eccentric exercise. We hypothesized that T1D participants would exhibit more muscle damage, delayed strength recovery and attenuated muscle repair.

METHODS

Participants

Participants were young, physically-active adults with (*N*, male/female=4/5) and without T1D (*N*, male/female=4/5) were matched for sex, age and BMI (±30%) and self-reported physical activity levels (Table 1). Exclusion criteria included other chronic health conditions, smoking, use of anti-inflammatory medication(s), and/or orthopedic disability preventing performance of the exercise intervention. All participants gave informed written consent. Experimental procedures were approved by the Hamilton Health Sciences Integrated Research Ethics Board (ID no. 5505) and conformed to the guidelines outlined in the *Declaration of Helsinki.*

	Control		Type 1 diabetes	
Characteristic	Male	Female	Male	Female
n	4	5	4	5
Age, years	22.0 (1.08)	21.6 (1.75)	24.0 (2.31)	21.0 (1.76)
BMI, kg/m^2	22.90 (0.40)	23.07 (0.42)	25.17 (1.69)	25.01 (1.54)
HbA1c, mmol/mol	36.50 (1.65)	33.40 (1.21)	53.75 (4.87)*	61.40 (10.28)*
HbA1c, %	5.50 (0.58)	5.24 (0.12)	7.05 (0.46)*	7.74 (0.95)*
Diabetes duration, years	–	–	12.75 (1.79)	6.60 (2.98)
Physical Activity, MET minutes/week	3669 (550)	2995 (725)	3928 (680)	2786 (612)
Maximal Isometric Strength, Nm/kg	3.50 (0.27)†	2.66 (0.23)	3.87 (0.24)†	2.72 (0.25)

Table 3.1. Participant characteristics.
Data are presented as means (SEM). Physical activity was computed using the International Physical Activity Questionnaire. Significant difference from same-sex control group, *p<0.05. Significant difference from females, †p<0.05. MET, metabolic equivalent of task.

Study design

Participants were instructed to consume a standardized meal 1.5-2h prior to each visit, and those with T1D continued their habitual blood glucose management. Participants were instructed to refrain from exercising 48h prior to their initial visit and throughout the duration of the study.

One leg per participant was randomly selected to complete the protocols described below. Participants performed a knee extensor MVC and a pre-exercise muscle biopsy and venous blood sample were collected. Approximately ten days later, participants completed the eccentric exercise protocol followed immediately by another MVC. At 48h and 96h post-eccentric exercise, participants completed a MVC, and a muscle biopsy and venous blood sample were collected. No adverse events were reported.

Muscle biopsy

Vastus lateralis biopsies were collected using a 5mm Bergstrom needle as previously described (Tarnopolsky et al., 2011). Muscle samples were divided and stored as described below.

Blood collection

Blood samples were collected from the antecubital vein for HbA_{1c} and serum creatine kinase analyses (performed by the McMaster Medical Centre Core Laboratory).

MVC and eccentric exercise protocol

MVCs of the knee extensors were performed on the Biodex Dynamometer (Biodex-System 3, Biodex Medical Systems, Shirley, USA) at a knee joint angle of 90°. Movements at the shoulders, hips, and thigh were restrained. Each MVC test consisted of three 5-

second stationary maximal knee extensions, with 30s of rest between attempts. Peak torque for the set was taken as the participant's MVC. The MVC was performed before biopsy and blood draw procedures to minimize the influence of systemic factors on strength.

Eccentric quadriceps contractions were performed on a Biodex Dynamometer with contractions performed at a rate of 90°/s over a range of 60° of movement. Thirty sets of 10 contractions (1-minute of rest between sets) were performed for a total of 300 contractions, as previously described (MacIntyre et al., 1996).

Immunohistochemistry

Pax7/Laminin/Ki67. Flash-frozen muscle samples were fixed in 4% paraformaldehyde overnight, embedded in paraffin wax, and cut into 6μm thick cross-sections. Primary antibodies for Pax7 (1:1, Pax7-s, RRID:AB_2299243, Developmental Studies Hybridoma Bank, Iowa City, USA), laminin (1:500, ab11575, Abcam, Cambridge, UK), and Ki67 (1:300, CRM 325, RRID:AB_298179, Biocare Medical, Pacheco, USA) were diluted in 5% bovine serum albumin in TRIS-buffered saline (TBS). Secondary antibodies included biotinylated goat anti-mouse IgG1 antibody (1:300, 115-065-205, RRID:AB_2338571, Jackson ImmunoResearch Laboratories, West Grove, USA), horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:300, ab7171, RRID:AB_955396, Abcam, Cambridge, UK) and TSA Plus Cyanine 3 system (NEL744001KT, Akoya Biosciences). Sections were counterstained with DAPI and then incubated in Sudan Black B to remove autofluorescence.

Dystrophin. Muscle samples were mounted in optimal cutting temperature (OCT) compound and frozen in liquid nitrogen-cooled 2-methylbutane. The OCT-embedded samples were stored at -80°C prior to being cut into 6μm thick cross-sections and allowed to air dry. Dystrophin antibody (1:250, ab15277, RRID:AB_301813, Abcam, Cambridge, UK) was diluted in 5% (wt/vol) normal goat serum in TBS. A secondary antibody was applied (1:500, A-11008, RRID:AB_143165, Thermo Fisher Scientific, Waltham, USA) before being counterstained with DAPI.

Microscopy & Image Analysis

Fluorescent images were captured with a Nikon 90 Eclipse microscope (Nikon Instruments, Inc., Melville, USA) and analysed using Nikon Elements AR software v4.6. Greater than 100 myofibers/subject/timepoint were included in the analyses. Laminin area, sarcolemmal disruption, and ultrastructural damage were examined before and 48h post-exercise, while satellite cell content/proliferation were examined pre-exercise, at 48h and 96h post exercise.

Laminin content. Regions of interest (ROI) were drawn around the areas of each muscle biopsy cross-section that were free of experimental artefact. Each image was similarly thresholded to highlight areas stained with laminin antibody. Data are represented as the area of laminin staining as a percentage of total ROI area.

Sarcolemmal disruption. Data are shown as the quantity of myofibers exhibiting portion(s), of disrupted dystrophin staining, expressed as a percentage of total fibers (Lovering & De Deyne, 2004).

Total/proliferating satellite cells. Pax7+ nuclei and Ki67+/Pax7+ nuclei were identified as total and proliferating satellite cells, respectively. Data are presented as a percentage of total myofibers. Missing observations were replaced with the group median with analyses completed in a blinded fashion.

Transmission electron microscopy (TEM). Biopsies were processed as previously described (Nilsson et al., 2015). Fibers were imaged and categorized based on the greatest level of disruption observed (focal, moderate, extreme). 10-30 fibers per participant were analyzed in a blinded fashion, excluding hypercontracted fibers. Data are presented as the percentage of myofibers in each category of damage (Gibala et al., 1995).

RNA Preparation & Microarray

For a subset of participants (*N,* CON/T1D = 4F/4F) pre- and 48h post-exercise, RNA was extracted from ~10-25 mg frozen muscle using the AllPrep DNA/RNA/miRNA Universal Kit including homogenization with the TissueRuptor II according to manufacturer's protocol (Qiagen GmbH, Hilden, Germany). RNA samples were quantified using the Qubit RNA HS Kit (Thermo Fisher Scientific, Waltham, USA) and quality was assessed using the Bioanalyzer Total RNA Pico Kit (Agilent Technologies, Santa Clara, USA). RNA samples were normalized and processed according to the Clariom™ S Pico Assay protocol which includes poly-A reverse-transcription, cRNA amplification and conversion to biotinylated cDNA and hybridization to the Clariom™ S human GeneChip and detection using the GeneChip Scanner 3000 system (Thermo Fisher Scientific, Waltham, USA). CEL files were generated using the GeneChip Instrument

Control Software and analyzed using Transcriptome Analysis Control software (Thermo Fisher Scientific, Waltham, USA). Expression data can be found in GEO (GSE182494).

Transcriptomic Analyses

Gene set enrichment analysis (GSEA) (Subramanian et al., 2005) was performed on a pre-ranked gene list from the differential expression results between participants with and without T1D at each timepoint, with duplicated gene sets removed (http://baderlab.org/genesets). The group-by-timepoint interaction term was used to examine if there were differential effects of eccentric exercise on the muscle transcriptome across groups, as provided by Transcriptome Analysis Control software. The EnrichmentMap plug-in of Cytoscape (Shannon et al., 2003) was used to create a visualization of the GSEA results. Enriched gene sets (q<0.1) were used as input, and a conservative overlap coefficient (0.5) was used to build the EnrichmentMap. AutoAnnotate identified and annotated clusters with ≥3 connected gene sets.

Statistical analysis

Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria). Quantitative data was compared via Hedge's g to compute group differences using the *effsize* package (see below), and 95% confidence intervals are noted in-text. Qualitative/count data were evaluated using chi-square tests, where p<0.05 was accepted as statistically significant. Data are presented as means ± standard error of the mean (SEM), unless otherwise stated. Individual data points are depicted for continuous data, and chi-square tests are visualized by group means only.

Assessment of effect sizes

Given the invasive nature of the study protocol, our sample size was limited. Thus, we utilized a Hedge's g (i.e. corrected effect sizes) statistical approach to delineate the magnitude of differences in our comparisons. Effect sizes of ≥ 0.2, ≥ 0.5, and ≥ 0.8 are denoted as small, moderate, and large effect sizes, whereas those effect sizes <0.2 were deemed negligible based on thresholds by Cohen (Cohen, 1988). These represent the most commonly used thresholds in the literature and are typically used in clinical trials, pre-clinical studies, and meta-analyses to interpret magnitude of changes when results are extremely novel and cannot be readily compared with other literature, such as in the current study (Lakens, 2013). We aimed to make our results generalizable and interpretable for a wide audience. However, these interpretations should not be viewed rigidly, and more studies in the area are required to determine the true clinical significance of the physiological changes observed in each case. Our sample size was statistically powered to detect differences amongst the whole cohort. Unfortunately, we were not powered to detect sex differences or bivariate relationships.

RESULTS

Maximal strength and serum CK changes

Pre-exercise, MVC was comparable between T1D and matched-controls (Table 1). MVC decreased substantially immediately post-exercise for both groups (~55% decrease). T1D subjects exhibited moderately attenuated force recovery at 48h (g=-0.52, CI=-1.49-0.45; Fig.1a) and 96h post-exercise (g=-0.55, CI=-1.51-0.42; Fig.1a).

Serum CK was moderately higher pre-exercise in T1D (g=0.72, CI = -0.25 to 1.71; Fig.1b). CK levels were ~75-times higher than resting levels when measured at 96h post-exercise. T1D subjects exhibited a small degree of increase at 96h (g=0.42, CI=-0.43-1.39; Fig.1b).

Sarcolemmal integrity, ECM and ultrastructure alterations

T1D participants displayed higher laminin content pre-exercise (g=2.11, CI=0.67-3.55; Fig.1c,d) and 48h post-exercise (g=0.81, CI=-1.83-0.20; Fig.2b). T1D participants exhibited 71% higher incidence of dystrophin disruption pre-exercise ($\chi2$=3.61, p=0.06; Fig.1e,f) and 87% higher 48h post-exercise ($\chi2$=15.44, p<0.05; Fig.1e,f).

Both groups exhibited myofibrillar disruption after exercise (Fig.1g,h). Incidence of T1D myofibers that exhibited some degree of ultrastructural damage increased by 18-times ($\chi2$=39.99, p<0.05; Fig.2g,h) versus 9-times ($\chi2$=59.03, p<0.05; Fig.2g,h) in controls. However, there were no significant group differences within specific damage categories.

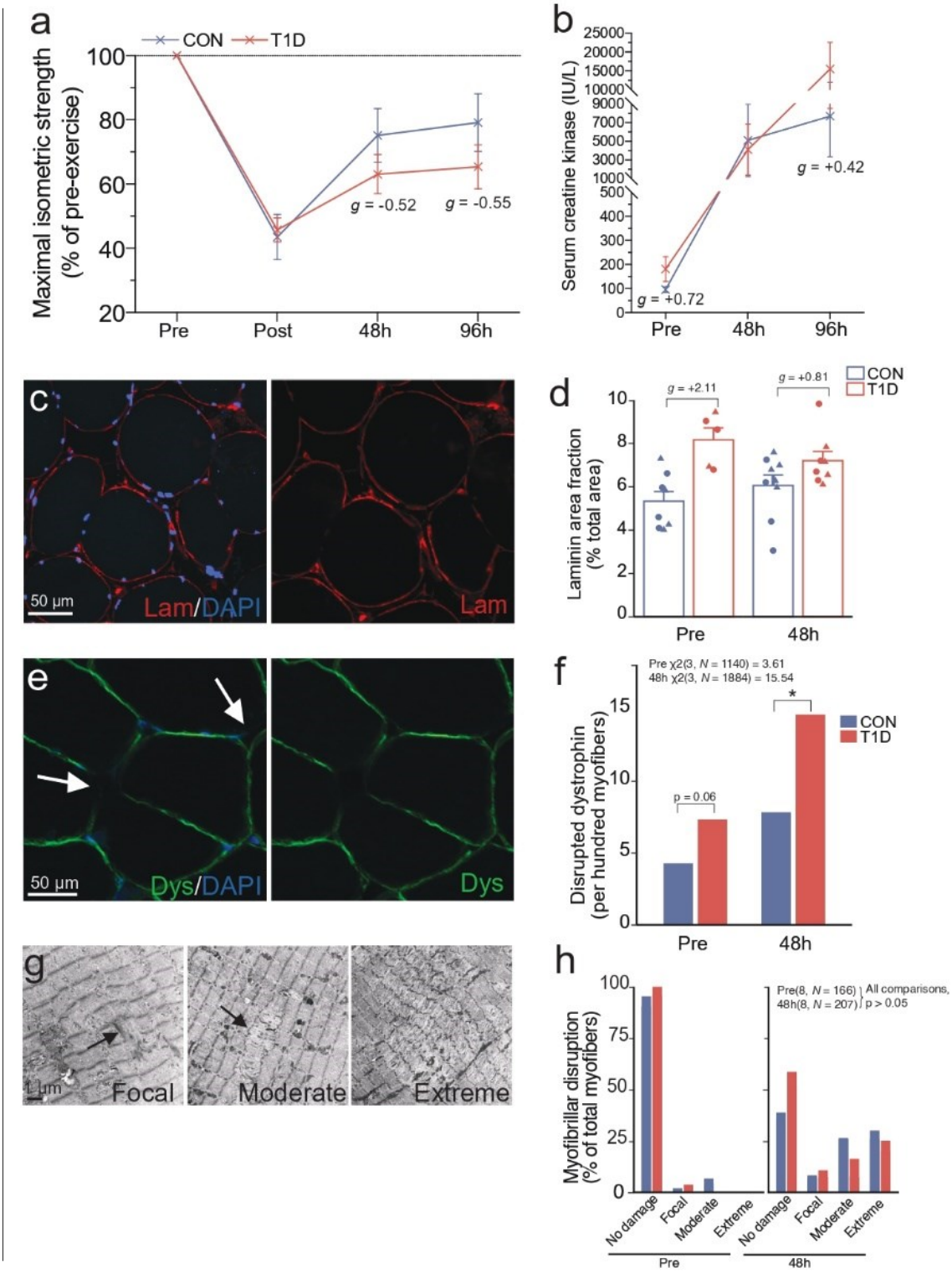
a
CON
T1D
Maximal isometric strength (% of pre-exercise)
100
80
60
40
20
Pre
Post
48h
96h
g = -0.52
g = -0.55
b
Serum creatine kinase (IU/L)
25000
20000
15000
10000
9000
7000
5000
3000
1000
500
400
300
200
100
0
g = +0.72
g = +0.42
Pre
48h
96h
c
50 µm
Lam/DAPI
Lam
d
Laminin area fraction (% total area)
g = +2.11
g = +0.81
CON
T1D
10
8
6
4
2
0
Pre
48h
e
50 µm
Dys/DAPI
Dys
f
Pre χ2(3, N = 1140) = 3.61
48h χ2(3, N = 1884) = 15.54
Disrupted dystrophin (per hundred myofibers)
15
10
5
0
p = 0.06
*
CON
T1D
Pre
48h
g
1 µm
Focal
Moderate
Extreme
h
Myofibrillar disruption (% of total myofibers)
100
75
50
25
0
Pre(8, N = 166)
48h(8, N = 207)
All comparisons, p > 0.05
No damage
Focal
Moderate
Extreme
Pre
48h

Figure 3.1 Systemic & skeletal muscle characteristics following eccentric exercise. (**a**) Characterization of strength recovery from pre-exercise (Pre, 100%), immediately following the intervention (Post), and at 48-hours and 96-hours after eccentric exercise in CON (n, male/female=4/5) and T1D(n, male/female=4/5). Effect sizes are displayed below each comparison. **(c,d)** Representative images(c) and graphical summary(d) of ECM content (assessed by laminin area/total area) at rest (CON, n=8[4M/4F]; T1D, n=5[3M/2F]), and 48h post-exercise (CON, n=9[4M/5F]; T1D, n=8[4M/4F]). Circles, males; triangles, females. Effect sizes are displayed above each comparison. **(e,f)** Representative images(e) and graphical summary(f) of damaged sarcolemma (assessed by incidence of myofibers with discontinuous dystrophin) at rest and 48h post-exercise (CON, n=3[2 male/1 female]; T1D, n=3[1 male/2 female]). **(g,h)** Representative images(g) and graphical summary(h) of post-exercise ultrastructural disruption based on the severity and number of disrupted myofibers in CON and T1D participants (CON, n=8; T1D, n=8). For chi-squared analyses (f,h), data are displayed as disrupted dystrophin or myofibrillar disruption per hundred myofibers. p-values are displayed within graphical summary. *, $p<0.05$. CON, blue; T1D, red.

Satellite cell content and proliferation

Pre-exercise SC quantity was -17% lower in T1D ($\chi2=4.07$, $p<0.05$; Fig.2a,b), 48h post-exercise (-12%, $\chi2=4.45$, $p<0.05$; Fig.2b), and 96h post-exercise (-17%, $\chi2=5.77$, $p<0.05$; Fig.2a,b) versus CON.

T1D displayed slower induction of SC proliferation, with highest levels 96h post-exercise versus 48h in CON (Fig.2a,c) with maximal proliferation 33% lower in T1D versus CON.

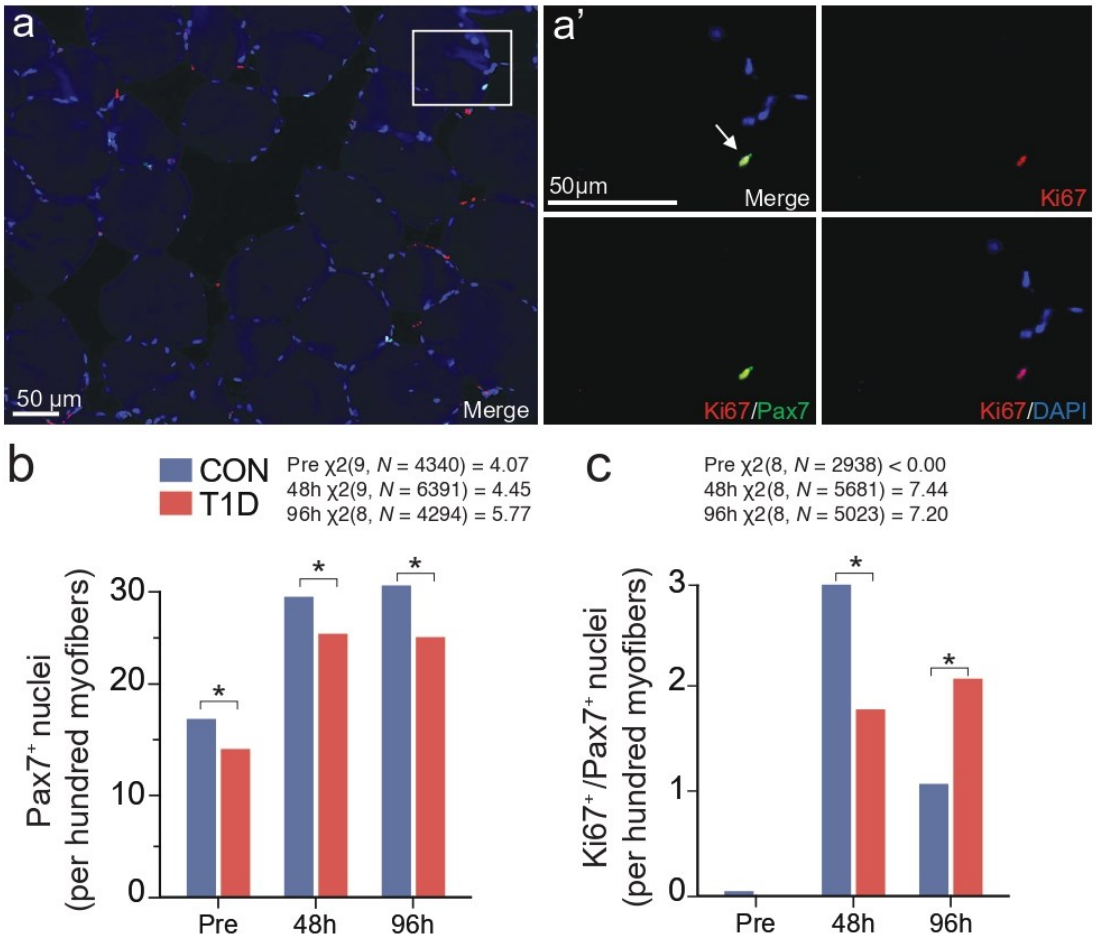

Figure 3.2 Satellite cell content and proliferation after eccentric contractions.

(**a**) Representative image of a Ki67/Pax7/DAPI stained muscle cross-section. (**a'**) Higher magnification image (from white box). Channel views of Ki67; Ki67/Pax7; Ki67/DAPI. Arrow indicates proliferating SC co-expressing Ki67/Pax7/DAPI. (**b,c**) Change in post-exercise SC content and proliferation in CON and T1D(CON, n=9[4M/5F]; T1D, n=9[4M/5F]). Data are expressed as the number of Pax7+ (b), or Pax7+/Ki67+ nuclei (c) per hundred myofibers. *, $p<0.05$. CON, blue bars; T1D, red bars.

Gene Set Enrichment Analysis

Pre-exercise, T1D participants exhibited 65 positively enriched gene sets ($q<0.1$) arranged into functionally themed subnetworks of translation, electron transport chain (ETC) and mitochondrial protein-targeting (Fig.3a). Post-exercise, many of these gene sets (53/65, 82%) became negatively enriched versus control, organized into similar subnetworks as the pre-exercise comparison (Fig.3a). In T1D, approximately 32% of gene sets became attenuated (vs. pre-exercise), while ~68% of the significantly upregulated gene sets pre-exercise became downregulated relative to controls (Fig.3b-d). Genes with the most divergent exercise-responses included mitochondrial ribosomal proteins (MRPL13/22), ribosomal proteins (RPL39L), ETC subunits (SDHD), and mitochondrial membrane transport proteins (TIMM8A; Fig.3e).

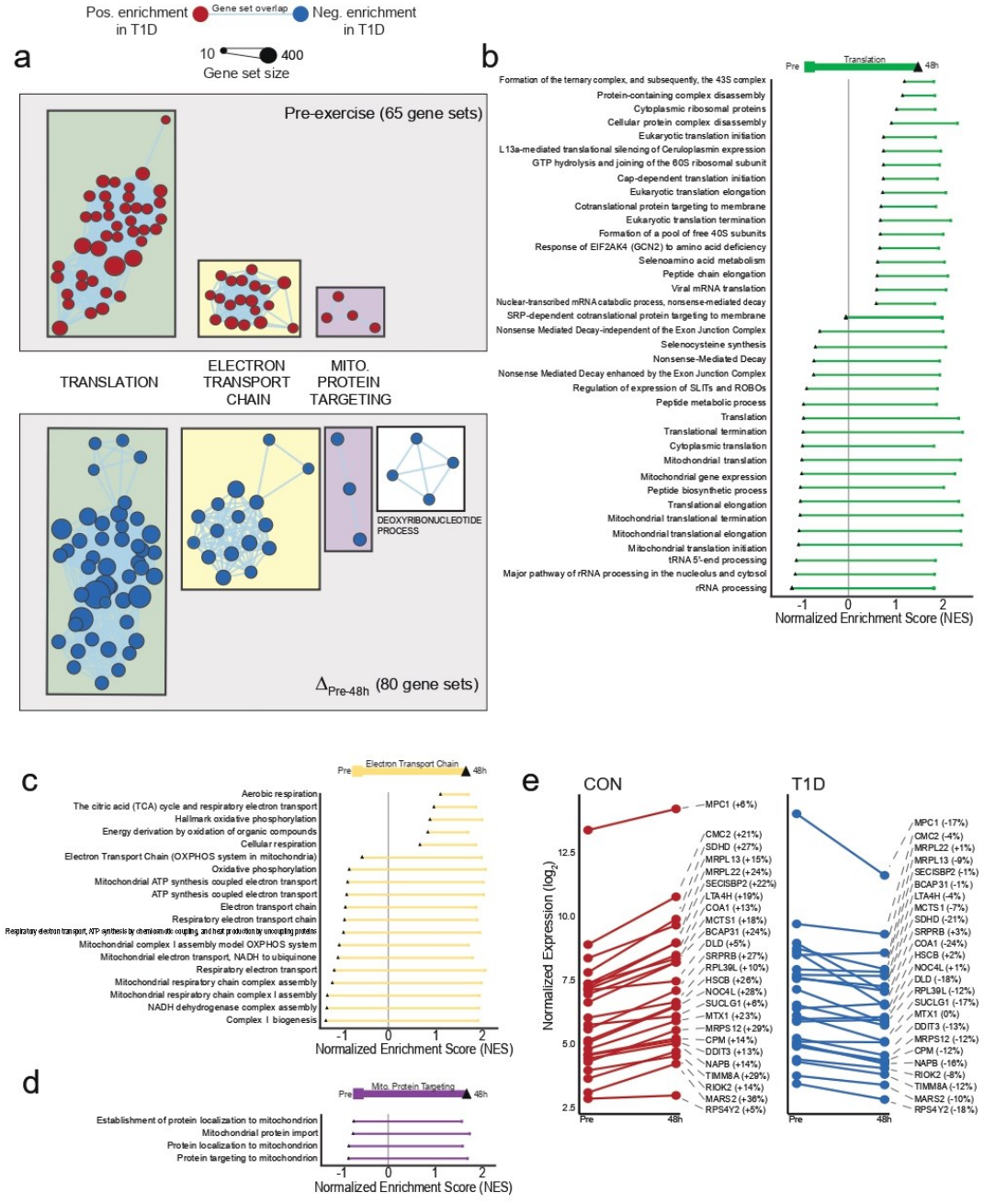

Figure 3.3. Skeletal muscle transcriptomics pre- and post-exercise.

Figure 3.4. Skeletal muscle transcriptomics pre- and post-exercise. **(a)** EnrichmentMap summarizing the result of GSEA at rest (top panel) and differential response to eccentric exercise between groups (bottom panel; CON, n=4F; T1D, n=4F). Red nodes indicate positive enrichment (i.e. upregulation) and blue nodes indicate negative enrichment (i.e. downregulation) in T1D group. The size of each node indicates the size (i.e. number of genes) of each gene set. Edges between nodes indicate gene sets with overlapping/common genes. Colored boxes indicate subnetworks of genes with similar functional themes. **(b-d)** Characterization of gene sets from GSEA pre- and post-exercise in T1D, divided into subnetworks observed in (a). Colors reflect the subnetworks denoted in (a). Colored squares and black triangles indicate pre- and post-exercise enrichment levels for T1D versus CON. Subnetworks with ≥3 nodes and $q<0.1$ are shown. **(e)** Expression of 25 transcripts with most divergent responses to exercise between groups.

DISCUSSION

Following damaging resistance exercise, persons with T1D exhibited decreased strength recovery, greater ECM content, a higher incidence of damaged sarcolemma, and altered SC content/proliferation. GSEA revealed a network of translational and mitochondrial genes that were dysregulated post-exercise in persons with T1D compared to matched controls. Taken together, these novel findings suggest that young adults with T1D may exhibit an altered response to damaging exercise and delayed reparative response.

Preserving muscle mass and quality is key to maintaining mobility and mitigating comorbidities in T1D, similar to other chronic diseases (Srikanthan et al., 2010). While decreased baseline strength has been observed in uncontrolled T1D (Jakobsen & Reske-Nielsen, 1986a), we found baseline strength comparable. However, post-exercise strength recovery was lower for T1D compared to controls. Previous studies suggest muscles of T1D subjects exhibit higher proportions of type 2X myofibers than non-diabetic muscles (Fritzsche, Bluher, et al., 2008), which are preferentially damaged by eccentric exercise

(Macaluso et al., 2012). Thus, adults with T1D may be more susceptible to muscle damage and prolonged reductions in strength during the recovery process. While we did not observe evidence of exaggerated ultrastructural damage, methodological limitations (e.g. no fiber typing) may have masked group differences. Clearly, more investigation into the association of fiber-type distribution and muscle damage in persons with T1D is needed. We did however see greater evidence of sarcolemmal disruption and laminin content in T1D participants. Similarly, its possible that higher laminin content could stiffen myofiber-ECM interactions making muscles of those with T1D more prone to damage, as seen in dystrophic muscles (Huebner et al., 2008).

Elevated resting serum CK in T1D, as observed here, has been previously observed and proposed to be an early sign of myopathy in adults with T1D (Jevric-Causevic et al., 2006). The substantial rise in post-exercise serum CK is both groups was strong evidence that the eccentric protocol elicited muscle damage. What is clinically concerning, and requires further study however, is the degree of muscle damage elicited and how the rise in muscle cytoplasmic contents in the serum (e.g. myoglobin) could affect kidney function of those with T1D, particularly given the prevalence of diabetic nephropathy (Dabla, 2010).

Laminin and dystrophin are both integral contributors to skeletal muscle integrity, at rest and during contractions (Holmberg & Durbeej, 2013; Petrof et al., 1993). T1D participants exhibited higher resting and post-exercise laminin content and more observations of damaged sarcolemma. While no studies have previously measured laminin content in human muscle, rodent models of T1D display elevated expression of ECM genes

(Kivelä et al., 2006) as well as worsened membrane repair after damage(Howard et al., 2011).

It has been shown that mice with untreated hyperglycemia not only have fewer satellite cells, but are unable to properly facilitate muscle repair (D. M. D'Souza et al., 2016b; Krause et al., 2011). Previously, we observed less SC content in young males with poorly controlled T1D (HbA_{1c}=8.4% or 68±2.2mmol/mol) (D. M. D'Souza et al., 2016b). Here, we observed less SC content/proliferation in T1D versus CON even with moderate-to-well controlled glycemia (HbA_{1c}=7.5% or 58±5.9mmol/mol). While the governing mechanism is unclear, earlier work from our lab found that T1D individuals expressed higher myostatin levels, a known inhibitor of satellite cell proliferation (Thomas et al., 2000). A similar mechanism may underpin *in vitro* findings where high-glucose environments impede SC proliferation. Nevertheless, delays in SC proliferation may ultimately lead to inability for muscles to repair from exercise, similar to injured mouse muscle (Pavlidou et al., 2019).

Our transcriptomic interrogation is the first, to our knowledge, in human muscle of persons with T1D. We observed a suite of dysregulated genes in T1D related to muscle mitochondrial translation, respiration, and protein-targeting that may contribute to our understanding of previously observed mitochondrial abnormalities in similar T1D cohorts (Monaco et al., 2018b). Mitochondrial translational dysregulation may suggest that muscle is adapting to a stressor, like oxidative stress (Monaco et al., 2018b), at this early stage of T1D. Accordingly, a rise in the production of mitochondrial proteins has been observed during periods of oxidative stress in human cells (Lee et al., 2000), suggesting that similar

mechanisms may play out *in vivo*. However, similar mitochondrial protein content in young adults with/without T1D (Monaco et al., 2018b) suggests that current observations reflect a compensatory adaptation in T1D muscle, rather than overt dysfunction. Previously, elevated protein turnover has been observed in those with poorly-controlled T1D (Nair et al., 1983). Here, the upregulation of mitochondrial translational pathways at rest may be affected by insulin therapy in our T1D cohort, a known stimulator of mitochondrial protein synthesis (Boirie et al., 2001). However, absence of mitochondrial protein content changes in similar cohorts suggest that worsened translational efficiency in T1D muscle may also be at play (McGlory et al., 2017) ultimately contributing to the diminished strength recovery post-exercise. Though we did not measure protein synthesis, the current transcriptomic data offers novel insight regarding muscle health in T1D, supporting the need for such measures in future investigations.

In conclusion, we provide the first evidence for differences in response to exercise-induced damage in persons with T1D. Notably, the alterations here were observed in individuals who self-report exceeding current national physical activity recommendations and were practicing glycemic control regimens. More work is clearly needed to fully appreciate the full spectrum of changes, particularly: (i) a more intensive assessment of specific reparative outcomes and (ii) a time-course study to full recovery – resulting in more practical recommendations of time to recovery after damaging/intense exercise. Nevertheless, these results provide foundational evidence to suggest that a longer recovery time may be warranted to ensure adequate recovery for persons with T1D when undertaking vigorous exercise programs.

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DATA AVAILABILITY

All relevant data are included in the article.

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CONTRIBUTION STATEMENT

GKG, CMFM, and TJH designed the experiments. AGD, GKG, and TJH wrote the manuscript. GKG, CMFM, LAB and MAT performed sample collection. GKG performed the experiments. AGD, GKG, JV and TJH analyzed and interpreted the data. All authors edited the manuscript. All authors provided final approval of the version to be published.

All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. TJH is the guarantor of this work and, as such, had full access to the data in this study and takes responsibility for the integrity of such data and the accuracy of data analysis.

REFERENCES

Boirie, Y., Short, K. R., Ahlman, B., Charlton, M., & Nair, K. S. (2001). Tissue-specific regulation of mitochondrial and cytoplasmic protein synthesis rates by insulin. *Diabetes*, *50*(12), 2652–2658. https://doi.org/10.2337/diabetes.50.12.2652

Chargé, S. B. P., & Rudnicki, M. A. (2004). Cellular and Molecular Regulation of Muscle Regeneration. *Physiological Reviews*, *84*(1), 209–238. https://doi.org/10.1152/physrev.00019.2003

Chimen, M., Kennedy, A., Nirantharakumar, K., Pang, T. T., Andrews, R., & Narendran, P. (2012). What are the health benefits of physical activity in type 1 diabetes mellitus? A literature review. *Diabetologia*, *55*(3), 542–551. https://doi.org/10.1007/s00125-011-2403-2

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed). L. Erlbaum Associates.

Dabla, P. K. (2010). Renal function in diabetic nephropathy. *World Journal of Diabetes*, *1*(2), 48–56. https://doi.org/10.4239/wjd.v1.i2.48

Dial, A. G., Monaco, C. M. F., Grafham, G. K., Patel, T. P., Tarnopolsky, M. A., & Hawke, T. J. (2021). Electronic supplementary material from Impaired Function and Altered Morphology in the Skeletal Muscles of Adult Men and Women with Type 1 Diabetes. *Figshare*. https://doi.org/10.6084/m9.figshare.13293239.v1

D'Souza, D., Al-Sajee, D., & Hawke, T. (2013). Diabetic myopathy: Impact of diabetes mellitus on skeletal muscle progenitor cells. *Frontiers in Physiology*, *4*, 379. https://doi.org/10.3389/fphys.2013.00379

D'Souza, D. M., Zhou, S., Rebalka, I. A., MacDonald, B., Moradi, J., Krause, M. P., Al-Sajee, D., Punthakee, Z., Tarnopolsky, M. A., & Hawke, T. J. (2016). Decreased Satellite Cell Number and Function in Humans and Mice With Type 1 Diabetes Is the Result of Altered Notch Signaling. *Diabetes*, *65*(10), 3053–3061. https://doi.org/10.2337/db15-1577

Fritzsche, K., Bluher, M., Schering, S., Buchwalow, I. B., Kern, M., Linke, A., Oberbach, A., Adams, V., & Punkt, K. (2008). Metabolic profile and nitric oxide synthase expression of skeletal muscle fibers are altered in patients with type 1 diabetes. *Experimental and Clinical Endocrinology & Diabetes : Official Journal, German Society of Endocrinology [and] German Diabetes Association*, *116*(10), 606–613. https://doi.org/10.1055/s-2008-1073126

Gibala, M. J., MacDougall, J. D., Tarnopolsky, M. A., Stauber, W. T., & Elorriaga, A. (1995). Changes in human skeletal muscle ultrastructure and force production after acute resistance exercise. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *78*(2), 702–708. https://doi.org/10.1152/jappl.1995.78.2.702

Harding, J. L., Pavkov, M. E., Magliano, D. J., Shaw, J. E., & Gregg, E. W. (2019). Global trends in diabetes complications: A review of current evidence. *Diabetologia*, *62*(1), 3–16. https://doi.org/10.1007/s00125-018-4711-2

Holmberg, J., & Durbeej, M. (2013). Laminin-211 in skeletal muscle function. *Cell Adhesion & Migration*, *7*(1), 111–121. https://doi.org/10.4161/cam.22618

Howard, A. C., McNeil, A. K., Xiong, F., Xiong, W.-C., & McNeil, P. L. (2011). A novel cellular defect in diabetes: Membrane repair failure. *Diabetes*, *60*(11), 3034–3043. https://doi.org/10.2337/db11-0851

Huebner, K. D., Jassal, D. S., Halevy, O., Pines, M., & Anderson, J. E. (2008). Functional resolution of fibrosis in mdx mouse dystrophic heart and skeletal muscle by halofuginone. *American Journal of Physiology. Heart and Circulatory Physiology*, *294*(4), H1550-1561. https://doi.org/10.1152/ajpheart.01253.2007

Jakobsen, J., & Reske-Nielsen, E. (1986). Diffuse muscle fiber atrophy in newly diagnosed diabetes. *Clinical Neuropathology*, *5*(2), 73–77.

Jeong, J., Conboy, M. J., & Conboy, I. M. (2013). Pharmacological inhibition of myostatin/TGF-β receptor/pSmad3 signaling rescues muscle regenerative responses in mouse model of type 1 diabetes. *Acta Pharmacologica Sinica*, *34*(8), 1052–1060. https://doi.org/10.1038/aps.2013.67

Jevric-Causevic, A., Malenica, M., & Dujic, T. (2006). Creatine kinase activity in patients with diabetes mellitus type I and type II. *Bosnian Journal of Basic Medical Sciences*, *6*(3), 5–9. https://doi.org/10.17305/bjbms.2006.3135

Kivelä, R., Silvennoinen, M., Touvra, A.-M., Lehti, T. M., Kainulainen, H., & Vihko, V. (2006). Effects of experimental type 1 diabetes and exercise training on angiogenic gene expression and capillarization in skeletal muscle. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *20*(9), 1570–1572. https://doi.org/10.1096/fj.05-4780fje

Krause, M. P., Al-Sajee, D., D'Souza, D. M., Rebalka, I. A., Moradi, J., Riddell, M. C., & Hawke, T. J. (2013). Impaired macrophage and satellite cell infiltration occurs in a muscle-specific fashion following injury in diabetic skeletal muscle. *PloS One*, *8*(8), e70971. https://doi.org/10.1371/journal.pone.0070971

Krause, M. P., Riddell, M. C., & Hawke, T. J. (2011). Effects of type 1 diabetes mellitus on skeletal muscle: Clinical observations and physiological mechanisms. *Pediatric Diabetes*, *12*(4 Pt 1), 345–364. https://doi.org/10.1111/j.1399-5448.2010.00699.x

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*. https://doi.org/10.3389/fpsyg.2013.00863

Lal, R. A., Ekhlaspour, L., Hood, K., & Buckingham, B. (2019). Realizing a Closed-Loop (Artificial Pancreas) System for the Treatment of Type 1 Diabetes. *Endocrine Reviews*, *40*(6), 1521–1546. https://doi.org/10.1210/er.2018-00174

LEE, H.-C., YIN, P.-H., LU, C.-Y., CHI, C.-W., & WEI, Y.-H. (2000). Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells. *Biochemical Journal*, *348*(2), 425–432. https://doi.org/10.1042/bj3480425

Lovering, R. M., & De Deyne, P. G. (2004). Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *American Journal of Physiology-Cell Physiology*, *286*(2), C230–C238. https://doi.org/10.1152/ajpcell.00199.2003

Macaluso, F., Isaacs, A. W., & Myburgh, K. H. (2012). Preferential type II muscle fiber damage from plyometric exercise. *Journal of Athletic Training*, *47*(4), 414–420. https://doi.org/10.4085/1062-6050-47.4.13

MacIntyre, D. L., Reid, W. D., Lyster, D. M., Szasz, I. J., & McKenzie, D. C. (1996). Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *80*(3), 1006–1013. https://doi.org/10.1152/jappl.1996.80.3.1006

McGlory, C., Devries, M. C., & Phillips, S. M. (2017). Skeletal muscle and resistance exercise training; the role of protein synthesis in recovery and remodeling. *Journal of Applied Physiology*, *122*(3), 541–548. https://doi.org/10.1152/japplphysiol.00613.2016

Monaco, C. M. F., Hughes, M. C., Ramos, S. V, Varah, N. E., Lamberz, C., Rahman, F. A., McGlory, C., Tarnopolsky, M. A., Krause, M. P., Laham, R., Hawke, T. J., & Perry, C. G. R. (2018). Altered mitochondrial bioenergetics and ultrastructure in the skeletal muscle of young adults with type 1 diabetes. *Diabetologia*, *61*(6), 1411–1423. https://doi.org/10.1007/s00125-018-4602-6

Nair, K. S., Garrow, J. S., Ford, C., Mahler, R. F., & Halliday, D. (1983). Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia*, *25*(5), 400–403. https://doi.org/10.1007/BF00282518

Nilsson, M. I., MacNeil, L. G., Kitaoka, Y., Suri, R., Young, S. P., Kaczor, J. J., Nates, N. J., Ansari, M. U., Wong, T., Ahktar, M., Brandt, L., Hettinga, B. P., & Tarnopolsky, M. A. (2015). Combined aerobic exercise and enzyme replacement therapy rejuvenates the mitochondrial-lysosomal axis and alleviates autophagic blockage in Pompe disease. *Free Radical Biology & Medicine*, *87*, 98–112. https://doi.org/10.1016/j.freeradbiomed.2015.05.019

Pavlidou, T., Marinkovic, M., Rosina, M., Fuoco, C., Vumbaca, S., Gargioli, C., Castagnoli, L., & Cesareni, G. (2019). Metformin Delays Satellite Cell Activation and Maintains Quiescence. *Stem Cells International*, *2019*, e5980465. https://doi.org/10.1155/2019/5980465

Petrof, B. J., Shrager, J. B., Stedman, H. H., Kelly, A. M., & Sweeney, H. L. (1993). Dystrophin protects the sarcolemma from stresses developed during muscle contraction. *Proceedings of the National Academy of Sciences*, *90*(8), 3710–3714. https://doi.org/10.1073/pnas.90.8.3710

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, *13*(11), 2498. https://doi.org/10.1101/gr.1239303

Srikanthan, P., Hevener, A. L., & Karlamangla, A. S. (2010). Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: Findings from the National Health and Nutrition Examination Survey III. *PloS One*, *5*(5), e10805. https://doi.org/10.1371/journal.pone.0010805

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., & Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, *102*(43), 15545–15550. https://doi.org/10.1073/pnas.0506580102

Tarnopolsky, M. A., Pearce, E., Smith, K., & Lach, B. (2011). Suction-modified Bergström muscle biopsy technique: Experience with 13,500 procedures. *Muscle & Nerve*, *43*(5), 717–725. https://doi.org/10.1002/mus.21945

Thabit, H., & Hovorka, R. (2012). Closed-loop Insulin Delivery in Type 1 Diabetes. *Endocrinology and Metabolism Clinics of North America*, *41*(1), 105–117. https://doi.org/10.1016/j.ecl.2011.12.003

Thomas, M., Langley, B., Berry, C., Sharma, M., Kirk, S., Bass, J., & Kambadur, R. (2000). Myostatin, a Negative Regulator of Muscle Growth, Functions by Inhibiting

Myoblast Proliferation. *Journal of Biological Chemistry*, *275*(51), 40235–40243. https://doi.org/10.1074/jbc.M004356200

Zaharieva, D. P., Messer, L. H., Paldus, B., O'Neal, D. N., Maahs, D. M., & Riddell, M. C. (2020). Glucose Control During Physical Activity and Exercise Using Closed Loop Technology in Adults and Adolescents with Type 1 Diabetes. *Canadian Journal of Diabetes*, *44*(8), 740–749. https://doi.org/10.1016/j.jcjd.2020.06.003

CHAPTER 4 – MUSCLE AND SERUM MYOSTATIN EXPRESSION IN TYPE 1 DIABETES

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PREFACE

Author Contributions:

AGD, CMFM, and TJH designed the experiments. **AGD** and TJH wrote the manuscript. **AGD** and CMFM performed the experiments. **AGD** analysed the data. **AGD**, CMFM, EK and TJH interpreted the data. JAS and NR developed and validated the antibody and assisted in Western blotting. MAT and CGRP performed muscle biopsies. **AGD**, CMFM and GKG collected and processed muscle samples. All authors edited the manuscript. All authors provided final approval of the version to be published. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. TJH is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of data and the accuracy of the data analysis.

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ORIGINAL RESEARCH

Physiological Reports

Muscle and serum myostatin expression in type 1 diabetes

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Abstract

Type 1 diabetes (T1D) has been reported to negatively affect the health of skeletal muscle, though the underlying mechanisms are unknown. Myostatin, a myokine whose increased expression is associated with muscle-wasting diseases, has not been reported in humans with T1D but has been demonstrated to be elevated in preclinical diabetes models. Thus, the purpose of this study was to determine if there is an elevated expression of myostatin in the serum and skeletal muscle of persons with T1D compared to controls. Secondarily, we aimed to explore relationships between myostatin expression and clinically important metrics (e.g., HbA_{1c}, strength, lean mass) in women and men with ($N = 31$)/without T1D ($N = 24$) between 18 and 72 years old. Body composition, baseline strength, blood sample and vastus lateralis muscle biopsy were evaluated. Serum, but not muscle, myostatin expression was significantly elevated in those with T1D versus controls, and to a greater degree in T1D women than T1D men. Serum myostatin levels were not significantly associated with HbA_{1c} nor disease duration. A significant correlation between serum myostatin expression and maximal voluntary contraction (MVC) and body fat mass was demonstrated in control subjects, but these correlations did not reach significance in those with T1D (MVC: $R = 0.64$ controls vs. $R = 0.37$ T1D; Body fat: $R = -0.52$ controls/$R = -0.02$ T1D). Collectively, serum myostatin was correlated with lean mass ($R = 0.45$), and while this trend was noted in both groups separately, neither reached statistical significance ($R = 0.47$ controls/$R = 0.33$ T1D). Overall, while those with T1D exhibited elevated serum myostatin levels (particularly females) myostatin expression was not correlated with clinically relevant metrics despite some of these relationships existing in controls (e.g., lean/fat mass). Future studies will be needed to fully understand the mechanisms underlying increased myostatin in T1D, with relationships to insulin dosing being particularly important to elucidate.

KEYWORDS

GDF-8, myostatin, skeletal muscle, T1D, TGF-family members

1 | INTRODUCTION

Skeletal muscle, by virtue of its mass, is the largest metabolic organ in the body. Nearly 80% of insulin-stimulated glucose uptake after a meal occurs in muscle (DeFronzo, 2009; Honka et al., 2018). As such, alterations to the health of skeletal muscle would have profound effects on glycemic control. Despite the major role of skeletal muscle in both our physical capacity for exercise and activities of daily living, our knowledge of changes to muscle health during type 1 diabetes (T1D) is currently limited. T1D has been associated with decreased muscle mass and strength (Maratova et al., 2018; Mori et al., 2017; Orlando, Balducci, Bazzucchi, Pugliese, & Sacchetti, 2017), as well as impaired muscle ultrastructure and energy production even in the absence of other co-morbidities (Cree-Green et al., 2015; Monaco et al., 2019). However, the underlying mechanism(s) behind these alterations to skeletal muscle health remain to be elucidated.

Myostatin is a potent negative regulator of muscle growth. Primarily synthesized in skeletal muscle, myostatin binds to its receptor complex activin IIB on the cell surface resulting in a cascade of signaling from the cell surface to the nucleus (Elliott et al., 2012). Ultimately, myostatin targets a number of transcription factors that promote muscle atrophy (Elkina, von Haehling, Anker, & Springer, 2011). Elevations in myostatin expression have been observed repeatedly in humans with muscle wasting conditions (Elliott et al., 2012) and in metabolic disorders like type 2 diabetes (T2D) (Brandt et al., 2012). Evidence in rodent models suggests that myostatin expression is elevated in T1D (Chen, Cao, Ye, & Zhu, 2009) and elevated myostatin expression is associated with impaired insulin signaling/sensitivity (Brandt et al., 2012; Chen et al., 2009; Wang et al., 2012).

Despite accumulating evidence for a role of myostatin in the maintenance of skeletal muscle function and metabolic health, its expression in the blood and muscle of those with T1D has not previously been reported. Thus, the purpose of the current study was to investigate the expression of myostatin in men and women with T1D and, secondarily, explore relationships between myostatin expression and clinically important health metrics.

2 | MATERIALS AND METHODS

2.1 | Participants

Adult men/women with T1D ($n = 31$; 21 female, 10 male) and matched control participants without diabetes ($n = 24$; 15 female, 9 male) 18–72 years of age were recruited for this study. Participants were matched for age, sex, and self-reported physical activity levels, and were told to refrain from exercise 24 hr preceding either study visit. Detailed subject characteristics and exclusion criteria are available in Table 1.

2.2 | Ethics

Prior to giving written informed consent, all participants were given oral and written information about the experimental procedures. All procedures herein were approved by the Research Ethics Board at York University (REB #e2013-032) and Hamilton Integrated Research Ethics Board (REB #5344 and #5355) and conformed to all declarations on the use of human participants as research participants.

	CON		T1D	
Characteristic	**Female**	**Male**	**Female**	**Male**
n	15	9	21	10
Age (years)	32 ± 3.2	27 ± 2.5	32 ± 2.4	32 ± 4.9
Height (m)	1.64 ± 0.02	1.77 ± 0.01†	1.64 ± 0.01	1.77 ± 0.02†
Weight (kg)	62.55 ± 2.95	77.45 ± 2.01†	70.45 ± 3.48	84.06 ± 3.94†
BMI (kg/m^2)	23.2 ± 0.79	24.64 ± 0.68	26.04 ± 1.17*	26.66 ± 1.04
HbA1c (%)	5.21 ± 0.08	5.42 ± 0.16	7.94 ± 0.31*	7.18 ± 0.28*
T1DM Duration (years)	—	—	16.13 ± 2.49	21.18 ± 4.71
Diabetes Onset (years of age)	—	—	16.52 ± 2.15	11.00 ± 1.94

Data are expressed as mean ± *SEM*.

*Significantly different versus same-sex control, $p < 0.05$.

†Significantly different versus female of same group, $p < 0.05$.

TABLE 1 Subject characteristics. Adult men/women with type 1 diabetes and matched control participants without diabetes were recruited for this study. Participants were not eligible to participate in the study if they matched any one of the following criteria: smoke or use any tobacco products, prediabetes, type 2 diabetes, statin-induced myopathy or experience myalgia, use assistive walking devices, chronically medicate with any analgesic or anti-inflammatory drug(s), corticosteroids or non-steroidal anti-inflammatories or prescription strength acne medications, use medications known to affect muscle metabolism, heart disease, rheumatoid arthritis, poor lung function, uncontrolled hypertension, stage 3 neuropathy, severe retinopathy, or any health conditions that put the subject at risk during this study.

2.3 | Study protocol

Participants visited the laboratory for baseline strength measurement procedure (maximal voluntary contractions, MVCs) using a Biodex dynamometer (Biodex System 3; Biodex Medical Systems) and a subset of participants (Controls: 11 female, 6 male; T1D: 12 female, 5 male) underwent body composition assessment via dual-energy X-ray absorptiometry (DEXA; GE Healthcare). Participants were instructed to refrain from caffeine and alcohol 24 hr prior to visiting the laboratory and were instructed to consume a standardized meal 1.5 to 2 hr prior to their visit. Participants with T1D were also instructed to continue their habitual use of insulin.

2.4 | Muscle and blood measurements

Participants underwent a biopsy of the vastus lateralis muscle and a venous blood sample. Biopsies were obtained from the mid-portion of the vastus lateralis under local anesthetic (1% lidocaine) using manual suction. A resting blood sample was obtained from the antecubital vein immediately following the muscle biopsy. HbA_{1c} levels were determined by the local core lab facility at McMaster University.

2.5 | Myostatin measurements

Serum myostatin was measured using a commercially available ELISA kit (R&D Systems, DGDF80). Muscle myostatin was measured by Western blot from skeletal muscle lysates using a custom-derived myostatin antibody (gifted by Dr. Jeremy Simpson), and a 15 kDa band representing myostatin in its monomeric form was quantified, as described previously (Dasarathy, Dodig, Muc, Kalhan, & McCullough, 2004). More detailed information including a representative blot with control is shown in ESM Figure 1.

2.6 | Microarray data mining and analysis

Microarray datasets were accessed through the Gene Expression Omnibus (GEO) online database according to the following accession codes and were given shorthand names based on the author of original publications: GSE22309 (Wu), GSE18732 (Gallagher), GSE121 (Yang), GSE19420 (vanTienen), GSE25462 (Patti). GSE121 was preprocessed as described in its source publication. All other datasets were preprocessed using Affy package in an R environment with RMA background correction, quantile normalization and $\log_2$ transformation (Gautier, Cope, Bolstad, & Irizarry, 2004). GSE25462 contains multiple experimental groups of which only two were used for contrasts—T2D participants versus control participants with no family history of T2D. For each dataset, gene symbols were obtained using the AnnotationDBI package in R. Differential expression analysis was performed using limma package in R (Ritchie et al., 2015).

2.7 | Statistical analysis

Bar graphs are presented as mean ± *SEM* with data points overlaid. Data points are presented as circles (controls) and triangles (T1D) with purple (female) and blue (male) colors to define sex. Participant characteristics are expressed as mean ± *SEM*. Not all samples were available for all analyses and the specific number of participants for each group are highlighted in the overlain dot plots and expressly written out in the figure legend. Due to multiple non-normally distributed variables, nonparametric statistics were utilized in this study. Wilcoxon–Mann–Whitney tests were performed to compare myostatin measurements between groups (i.e., CON and T1D). Kruskal–Wallis was used for comparisons of multiple groups (i.e., group and sex), followed by post hoc Wilcoxon–Mann–Whitney tests with Bonferroni–Holm correction for multiple comparisons. Correlational analyses were performed with Spearman's rho (herein denoted as *R*). Statistical significance was established at $p < 0.05$. All analyses were carried out in the R environment for statistical computing (R Foundation for Statistical Computing).

3 | RESULTS

3.1 | Circulating myostatin expression is elevated in adults with type 1 diabetes

Serum myostatin was significantly elevated in adults with T1D compared to matched persons without diabetes (Figure 1a). When separated by sex, women had 30%–40% less myostatin in circulation than men, independent of disease (Figure 1b), consistent with previous observations (Nishikawa et al., 2017). In women with T1D, serum myostatin was significantly higher relative to control women. There was also a notably elevated serum myostatin expression in men with T1D versus controls (~33%) though this difference did not meet statistical significance ($p = 0.08$). We then investigated the ratio of serum myostatin to lean mass to determine if the absolute muscle mass of participants influenced circulating myostatin concentrations and could be contributing to the sex differences observed. Interestingly, when normalized to muscle mass, expression remained 33% higher in those with T1D ($p = 0.09$; Figure 1c). When separated by sex, a ~20%–40% elevated expression was observed between T1D and the same-sex control counterparts, but

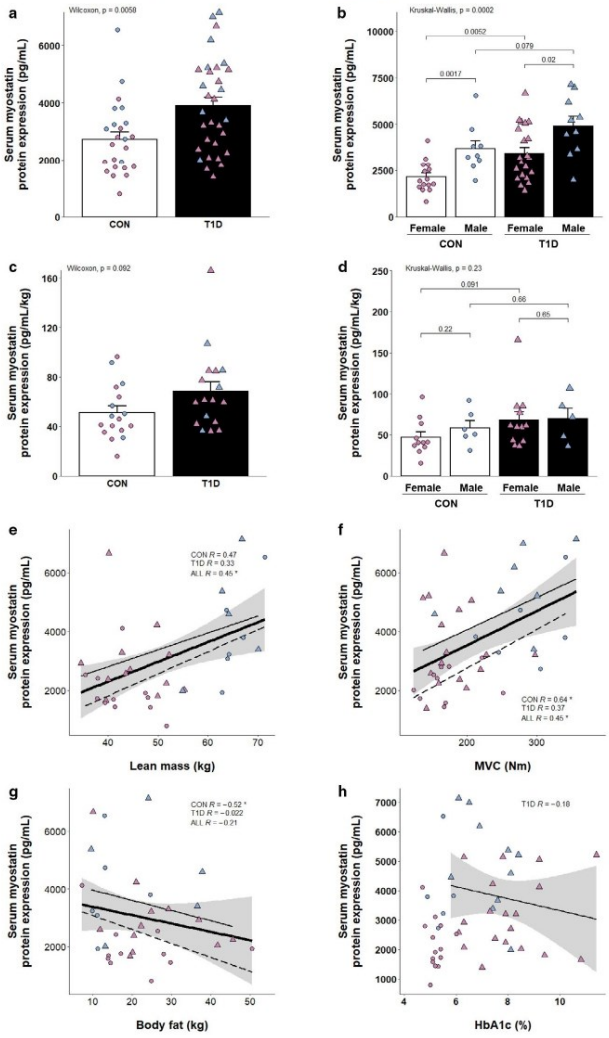

FIGURE 1 Elevated expression of serum myostatin and its correlates in T1D. (a) Serum myostatin expression as measured by ELISA (CON, $n = 24$; T1D, $n = 31$). (b) Serum myostatin expression as measured by ELISA, men and women analyzed separately (CON, $n = 24$; T1D, $n = 31$). (c) Serum myostatin expression as measured by ELISA normalized to lean mass (CON, $n = 17$; T1D, $n = 17$). (d) Serum myostatin expression as measured by ELISA normalized to lean mass, men and women analyzed separately (CON, $n = 17$; T1D, $n = 17$). Scatter plots summarizing the relationship between myostatin and various metrics are shown in (e–h). (e) Serum myostatin versus lean mass in kilograms (CON, $n = 17$; T1D, $n = 17$). (f) Serum myostatin versus maximal voluntary contraction strength (MVC; CON, $n = 16$; T1D, $n = 23$). (g) Serum myostatin versus whole-body fat in kilograms (CON, $n = 17$; T1D, $n = 17$). (h) Serum myostatin versus HbA_{1c} (T1D, $n = 29$). For individual data points, circles indicate control participants, triangles indicate T1D, blue fill indicates men, purple fill indicates women. For regression lines, dashed lines indicate control participants, solid thin lines indicate T1D, solid thick lines indicate overall regression line, gray shading indicates standard error. Spearman's rho (displayed as R) and p-values are displayed within graphical summary. *$p < 0.05$

these differences were not statistically significant ($p = 0.09$ females, p =0 .66 males; Figure 1d).

3.2 | Elevated serum myostatin levels are associated with increased lean mass, and maximal strength

When all participants were combined, serum myostatin showed a significant positive correlation with lean mass and MVC (Figure 1e,f). There were no observable differences in MVC between groups ($p = 0.54$; ESM Figure 2), yet, when separated by group, controls displayed a significant positive correlation with MVC while the weaker association in T1D participants fell short of the threshold for significance (Figure 1f). In line with this, serum myostatin was significantly, and negatively, associated with body fat mass, again only in control participants ($R = -0.52$; $p = 0.03$; Figure 1g). Interestingly, we observed that circulating myostatin expression was not associated with HbA_{1c}

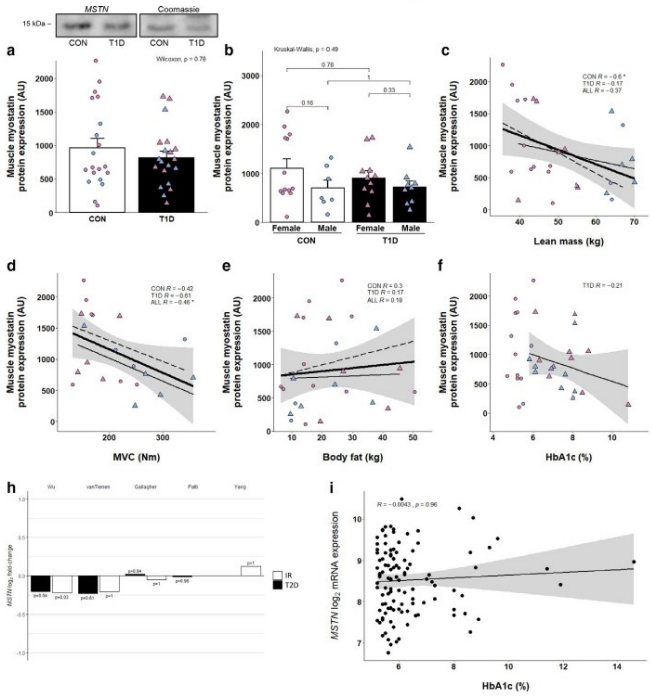

FIGURE 2 Correlates of muscle myostatin protein expression with and without T1D. (a) Skeletal muscle myostatin expression as measured by Western blot (CON, $n = 20$; T1D, $n = 20$), with inlaid representative blot displaying 15 kDa band representing monomeric myostatin, and Coomassie Blue loading control. (b) Skeletal muscle myostatin expression, men and women analyzed separately (CON, $n = 20$; T1D, $n = 20$). Scatter plots summarizing the relationship between myostatin and various metrics are shown in (c–f). (c) Skeletal muscle myostatin versus lean mass in kilograms (CON, $n = 14$; T1D, $n = 12$). (d) Skeletal muscle myostatin versus MVC (CON, $n = 10$; T1D, $n = 10$). (e) Skeletal muscle myostatin versus body fat in kilograms (CON, $n = 14$; T1D, $n = 12$). (f) Skeletal muscle myostatin versus HbA_{1c} in percent (T1D, $n = 19$). Analysis of publicly available microarray datasets are shown in (h, i). (h) Bar plot of muscle myostatin mRNA log_2 fold change values relative to healthy control group from each respective study. White bars indicate insulin-resistant (IR) groups, while black bars indicate T2D groups. p-values are displayed outside of each bar. (i) Scatter plot depicting the correlation between muscle myostatin log_2 mRNA expression and HbA_{1c} in percent. Data obtained from GSE18732. For individual data points, circles indicate control participants, triangles indicate T1D, blue fill indicates men, purple fill indicates women. For regression lines, dashed lines indicate control participants, solid thin lines indicate T1D, solid thick lines indicate overall regression line, gray shading indicates standard error. Spearman's rho (displayed as R) and p-values are displayed within graphical summary. $^*p < 0.05$

($R = -0.18$; Figure 1h) nor duration of disease in those with T1D ($R = 0.04$; ESM Figure 3).

3.3 | T1D and non-diabetic muscle express comparable myostatin protein

In contrast to circulating myostatin, skeletal muscle myostatin levels were not differentially affected by disease or sex (Figure 2a,b). Muscle myostatin was significantly and negatively associated with lean mass in control participants ($R = -0.6$; $p = 0.03$; Figure 2c) but not in T1D ($R = -0.17$; Figure 2c). In line with this, elevated myostatin expression was also associated with decreased MVC in all participants ($R = -0.46$; $p = 0.04$; Figure 2d). Contrary to serum myostatin expression, muscle myostatin levels were not significantly associated with body fat or HbA_{1c} (Figure 2e,f).

3.4 | Myostatin expression in diabetic muscle – context from microarray studies

The question arose of whether there was a relationship between insulin sensitivity and myostatin expression, in part

FIGURE 3 Summary of correlative analyses in groupings of the present study. Heat map summarizing strength of correlation between clinical metrics in this study and myostatin protein expression in the serum (S, top) and muscle (M, top). Variables are displayed along the left side including: maximal voluntary contraction (MVC), age of T1D diagnosis (T1D Onset), duration of T1D (T1D Duration), HbA_{1c}, absolute lean body mass (Abs. LM), absolute body fat (Abs. BF), relative lean body mass (% LM), relative body fat (% BF), body mass index (BMI), height, weight, and age. Participants have been grouped as healthy controls (CON), diabetic participants (T1D), males (MALE), females (FEMALE), and overall cohort (ALL). For each square, red fill represents positively correlated variables, blue fill represents negative correlation. Intensity of fill represents the strength of correlation. $*p < 0.05$; $\bullet p < 0.1$

because the current data differed from previous studies in T2D where muscle myostatin mRNA and/or protein is elevated in the diabetic state (Brandt et al., 2012; Hittel, Berggren, Shearer, Boyle, & Houmard, 2009). Unfortunately, we did not have direct results of insulin sensitivity in the present study. However, to further contextualize our results to the broader literature, we surveyed GEO for microarray experiments involving skeletal muscle biopsies from diabetic participants. While we were unable to find any such datasets with muscle biopsies from those with T1D, we were able to examine a cohort of microarray datasets involving muscle biopsies from participants with insulin-resistance (IR) and/or T2D. Interestingly, muscle myostatin mRNA was expressed to similar levels in insulin-resistant/T2D muscle in 4 out of 5 of the experiments analyzed (Figure 2h). Gallagher and colleagues (Gallagher et al., 2010) have included an excellent set of participant data as part of GSE18732 that allowed for correlational analyses between myostatin mRNA expression and variables related to IR/diabetes and overall metabolic health. The data from GSE18732, as well as the data from the current study suggest there is not a significant association between HbA_{1c} and muscle myostatin in human participants overall (Figure 2f,i), nor when divided into experimental groups (ESM Figure 4). However, myostatin expression from GSE18732 was positively correlated with metrics of insulin resistance such as the Homeostasis Model Assessment (HOMA) 1/2 (Table 2). An expanded table summarizing a selection of correlates of myostatin mRNA expression from this GSE18732 can be found in Table 2.

A heat map summarizing the correlative data analyzed in the current study can be found in Figure 3.

4 | DISCUSSION

Here, we report for the first time, the myostatin expression levels in the blood and muscle of persons with T1D. Our data reveal that people with T1D exhibited elevated circulating myostatin levels, with the relative increase in females greater than that in males. In contrast, protein expression of myostatin in the skeletal muscle was similar between cohorts and not different between sexes. Furthermore, we show that glycemic control (as measured by HbA_{1c}) was not associated with myostatin expression in the serum, nor the skeletal muscle.

In this study, we observed, collectively, that elevated serum myostatin was associated with leaner and stronger participants (Figure 1e-g). This is in line with a recent observation in which elevated myostatin levels were observed in elderly men who were more active and less frail (Arrieta et al., 2019), but is contrary to other findings (Chew et al., 2019). Furthermore, a positive correlation between myostatin expression and adiposity has previously been observed in obese participants (Hittel et al., 2009). The reason(s) underlying why conditions such as obesity exhibit

TABLE 2 Clinically relevant correlates of myostatin mRNA expression. A selection of subject characteristics recorded in GSE18732 is shown, summarized by Spearman's rho. Experimental groups analyzed separately (normal glucose tolerance, CON; impaired glucose tolerance IR; type 2 diabetic, T2D), as well as the entire cohort (ALL)

Variable	ALL	CON	IR	T2D
Age	−0.17	−0.31*	−0.16	−0.03
BMI	0.29*	0.30*	0.50*	0.12
Glucose, baseline	0.11	0.17	0.13	0.05
HbA1c	0.02	−0.05	0.00	−0.08
HOMA1 IR	0.35*	0.33*	0.71*	0.33*
HOMA2 IR	0.41*	0.33*	0.74*	0.39*
Insulin, baseline	0.39*	0.32*	0.72*	0.34*
Total Fat Mass	0.22*	0.30*	0.38	−0.02
Total Lean Mass	0.25*	0.22	0.49*	0.09
Weight	0.29*	0.32*	0.55*	0.06

*$p < 0.05$.

an increase in muscle myostatin expression versus the relationship observed here are unknown, but may pertain to the role of myostatin in insulin sensitivity previously reported (Hittel et al., 2009) and supported by some, but not all of our correlative analysis of myostatin expression (such as in GSE18732; Figure 2i; Table 2). Likely, insulin-resistance is a less severe trait in the current cohort of participants, who were otherwise healthy, active individuals without other co-morbidities. Interestingly, neither serum nor muscle myostatin expression was related to HbA_{1c} (Figures 1h and 2f,i) lending support for a potential role of myostatin in the development of insulin resistance, as previously suggested (Amor et al., 2018; Brandt et al., 2012; Hittel et al., 2009), that is distinct from its effects on systemic glycemic control as measured by HbA_{1c}. Thus, these current protein expression data in skeletal muscle adds an important piece of evidence to the T1D literature. The lack of association between myostatin protein expression and HbA_{1c} in T1D suggests that (a) the involvement of myostatin in the development insulin resistance does not necessarily extend to an association with HbA_{1c}, and/or (b) obesogenic factors that are often associated with T2D participants may be a key feature of the pathology that is driving a higher myostatin expression, as suggested by previous work in obese individuals (Amor et al., 2018; Hittel et al., 2009). Indeed, the average BMI of T1D patients in the current study was ~26 as opposed to ~30 in previous work where elevated muscle myostatin was observed in T2D (Brandt et al., 2012).

Taken together, the data suggest that myostatin expression covaries with muscle mass. In fact, in a stable disease condition, such as the current study cohort, it appears that serum myostatin expression reflects the amount of muscle mass producing myostatin in the body. Therefore, differences in muscle mass between cohorts may be important to consider whenever interpreting circulating myostatin expression levels in humans with or without a pathology. For example, situations where atrophy is a prevalent symptom may display higher (Barreiro & Jaitovich, 2018; Man et al., 2010; Plant et al., 2010), lower (Loumaye et al., 2015) or comparable expression of myostatin (Brandt et al., 2012) in circulation. Equivocal findings in this area suggest that serum expression of myostatin may have disease-specific responses that are secondary to each pathology. Caution is warranted when interpreting circulating expression levels as a singular diagnostic signal of poor muscle health.

Historically, it has often been posited that elevated myostatin expression elicits negative effects on metrics of muscle health and metabolism. However, the analyses here along with the growing number of studies observing myostatin as a biomarker in the context of chronic and metabolic diseases reveals the complex and somewhat unpredictable behavior of the myokine (Arrieta et al., 2019; Brandt et al., 2012; Elliott et al., 2012; Hittel et al., 2009). Our interrogation of available microarray data suggest a relationship between muscle myostatin mRNA and measures of insulin resistance are present in T2D, and though not investigated here, future studies defining the relationship between myostatin expression and the development of insulin resistance in persons with T1D would be of significant interest to help combat the comorbidities associated with insulin resistance in this population.

5 | CONCLUSIONS

In conclusion, this is the first observation of myostatin expression in persons with T1D and reveals that the myokine is expressed to a higher degree in the serum of persons with T1D, with leaner individuals displaying the highest levels of expression. Furthermore, we also observed a sexual dimorphic response with T1D women exhibiting a larger relative increase in serum myostatin (versus control females) compared to the same comparison in men. Overall, data here and elsewhere (Arrieta et al., 2019) suggest that, in our otherwise healthy cohort of persons with T1D, myostatin may be operating as a homeostatic, rather than pathological, regulator of muscle mass and may be associated with a leaner, more muscular phenotype in these men and women.

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CONFLICT OF INTEREST

The authors declare that there is no duality of interest associated with this manuscript.

AUTHOR CONTRIBUTION

AGD, CMFM, and TJH designed the experiments. AGD and TJH wrote the manuscript. AGD and CMFM performed the experiments. AGD analyzed the data. AGD, CMFM, EK, and TJH interpreted the data. JAS and NR developed and validated the antibody and assisted in western blotting. MAT and CGRP performed muscle biopsies. AGD, CMFM, and GKG collected and processed muscle samples. All authors edited the manuscript. All authors provided final approval of the version to be published. All people designated as authors qualify for authorship, and all those who qualify for authorship are listed. TJH is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of data and the accuracy of the data analysis.

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REFERENCES

Amor, M., Itariu, B. K., Moreno-Viedma, V., Keindl, M., Jürets, A., Prager, G., … Stulnig, T. M. (2018). Serum myostatin is upregulated in obesity and correlates with insulin resistance in humans. *Experimental and Clinical Endocrinology & Diabetes*, *127*(08), 550–556. https://doi.org/10.1055/a-0641-5546

Arrieta, H., Hervás, G., Rezola-Pardo, C., Ruiz-Litago, F., Iturburu, M., Yanguas, J. J., … Irazusta, J. (2019). Serum myostatin levels are higher in fitter, more active, and non-frail long-term nursing home residents and increase after a physical exercise intervention. *Gerontology*, *65*, 229–239. https://doi.org/10.1159/000494137

Barreiro, E., & Jaitovich, A. (2018). Muscle atrophy in chronic obstructive pulmonary disease: Molecular basis and potential therapeutic targets. *Journal of Thoracic Disease*, *10*, S1415–S1424. https://doi.org/10.21037/jtd.2018.04.168

Brandt, C., Nielsen, A. R., Fischer, C. P., Hansen, J., Pedersen, B. K., & Plomgaard, P. (2012). Plasma and muscle myostatin in relation to type 2 diabetes. *PLoS One*, *7*, e37236. https://doi.org/10.1371/journal.pone.0037236

Chen, Y., Cao, L., Ye, J., & Zhu, D. (2009). Upregulation of myostatin gene expression in streptozotocin-induced type 1 diabetes mice is attenuated by insulin. *Biochemical and Biophysical Research Communications*, *388*, 112–116. https://doi.org/10.1016/j.bbrc.2009.07.129

Chew, J., Tay, L., Lim, J. P., Leung, B. P., Yeo, A., Yew, S., … Lim, W. S. (2019). Serum myostatin and IGF-1 as gender-specific biomarkers of frailty and low muscle mass in community-dwelling older adults. *The Journal of Nutrition, Health & Aging*, *23*, 979–986. https://doi.org/10.1007/s12603-019-1255-1

Cree-Green, M., Newcomer, B. R., Brown, M. S., Baumgartner, A. D., Bergman, B., Drew, B., … Nadeau, K. J. (2015). Delayed skeletal muscle mitochondrial ADP recovery in youth with type 1 diabetes relates to muscle insulin resistance. *Diabetes*, *64*, 383–392. https://doi.org/10.2337/db14-0765

Dasarathy, S., Dodig, M., Muc, S. M., Kalhan, S. C., & McCullough, A. J. (2004). Skeletal muscle atrophy is associated with an increased expression of myostatin and impaired satellite cell function in the portacaval anastamosis rat. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *287*, G1124–1130. https://doi.org/10.1152/ajpgi.00202.2004

DeFronzo, R. A. (2009). From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*, *58*, 773–795. https://doi.org/10.2337/db09-9028

Elkina, Y., von Haehling, S., Anker, S. D., & Springer, J. (2011). The role of myostatin in muscle wasting: An overview. *Journal of Cachexia, Sarcopenia and Muscle*, *2*, 143–151. https://doi.org/10.1007/s13539-011-0035-5

Elliott, B., Renshaw, D., Getting, S., & Mackenzie, R. (2012). The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiologica*, *205*, 324–340. https://doi.org/10.1111/j.1748-1716.2012.02423.x

Gallagher, I. J., Scheele, C., Keller, P., Nielsen, A. R., Remenyi, J., Fischer, C. P., … Timmons, J. A. (2010). Integration of microRNA changes in vivo identifies novel molecular features of muscle insulin resistance in type 2 diabetes. *Genome Medicine*, *2*, 9. https://doi.org/10.1186/gm130

Gautier, L., Cope, L., Bolstad, B. M., & Irizarry, R. A. (2004). affy–analysis of Affymetrix GeneChip data at the probe level. *Bioinforma*, *20*, 307–315. https://doi.org/10.1093/bioinformatics/btg405

Hittel, D. S., Berggren, J. R., Shearer, J., Boyle, K., & Houmard, J. A. (2009). Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. *Diabetes*, *58*, 30–38. https://doi.org/10.2337/db08-0943

Honka, M.-J., Latva-Rasku, A., Bucci, M., Virtanen, K. A., Hannukainen, J. C., Kalliokoski, K. K., & Nuutila, P. (2018). Insulin-stimulated glucose uptake in skeletal muscle, adipose tissue and liver: A positron emission tomography study. *European Journal of Endocrinology*, *178*, 523–531. https://doi.org/10.1530/EJE-17-0882

Loumaye, A., de Barsy, M., Nachit, M., Lause, P., Frateur, L., van Maanen, A., … Thissen, J.-P. (2015). Role of activin A and myostatin in human cancer cachexia. *Journal of Clinical Endocrinology and Metabolism*, *100*, 2030–2038. https://doi.org/10.1210/jc.2014-4318

Man, W.- D.-C., Natanek, S. A., Riddoch-Contreras, J., Lewis, A., Marsh, G. S., Kemp, P. R., & Polkey, M. I. (2010). Quadriceps myostatin expression in COPD. *European Respiratory Journal*, *36*, 686–688. https://doi.org/10.1183/09031936.00032510

Maratova, K., Soucek, O., Matyskova, J., Hlavka, Z., Petruzelkova, L., Obermannova, B., … Sumnik, Z. (2018). Muscle functions and bone strength are impaired in adolescents with type 1 diabetes. *Bone*, *106*, 22–27. https://doi.org/10.1016/j.bone.2017.10.005

Monaco, C. M. F., Bellissimo, C. A., Hughes, M. C., Ramos, S. V., Laham, R., Perry, C. G. R., & Hawke, T. J. (2019). Sexual dimorphism in human skeletal muscle mitochondrial bioenergetics in response to type 1 diabetes. *American Journal of Physiology-Endocrinology and Metabolism*, https://doi.org/10.1152/ajpendo.00411.2019

Mori, H., Kuroda, A., Araki, M., Suzuki, R., Taniguchi, S., Tamaki, M., … Matsuhisa, M. (2017). Advanced glycation end-products are a risk for muscle weakness in Japanese patients with type 1 diabetes. *Journal of Diabetes Investigation*, *8*, 377–382. https://doi.org/10.1111/jdi.12582

Nishikawa, H., Enomoto, H., Ishii, A., Iwata, Y., Miyamoto, Y., Ishii, N., … Nishiguchi, S. (2017). Elevated serum myostatin level is

associated with worse survival in patients with liver cirrhosis. *Journal of Cachexia, Sarcopenia and Muscle*, *8*, 915–925. https://doi.org/10.1002/jcsm.12212

Orlando, G., Balducci, S., Bazzucchi, I., Pugliese, G., & Sacchetti, M. (2017). The impact of type 1 diabetes and diabetic polyneuropathy on muscle strength and fatigability. *Acta Diabetologica*, *54*, 543–550. https://doi.org/10.1007/s00592-017-0979-9

Plant, P. J., Brooks, D., Faughnan, M., Bayley, T., Bain, J., Singer, L., … Batt, J. (2010). Cellular markers of muscle atrophy in chronic obstructive pulmonary disease. *American Journal of Respiratory Cell and Molecular Biology*, *42*, 461–471. https://doi.org/10.1165/rcmb.2008-0382OC

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*, e47. https://doi.org/10.1093/nar/gkv007

Wang, F., Liao, Y., Li, X., Ren, C., Cheng, C., & Ren, Y. (2012). Increased circulating myostatin in patients with type 2 diabetes mellitus. *Journal of Huazhong University of Science and Technology*, *32*, 534–539. https://doi.org/10.1007/s11596-012-0092-9

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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CHAPTER 5 – OVERALL CONCLUSIONS

5.1 SIGNIFICANCE OF STUDIES AND CONCLUSIONS

Despite the advancing efficacy of glucose management, the lifelong management of glycemia during T1D remains challenging for patients and requires adjuvant modalities to assist in the mitigation of life-limiting comorbidities. Exercise has long been shown to systemically benefit the body in ways which mirror the negative impacts of T1D such as improved insulin sensitivity, glucose homeostasis, functional independence in older age, and longevity (Garber et al., 2011). Skeletal muscle is a primary effector for modulating glycemia at rest and during exercise, therefore making it a promising candidate to enhance the health of people with T1D. Despite our knowledge of its importance and the emerging role of diabetic myopathy in T1D, our understanding of cellular muscle physiology during T1D remains in its infancy.

Exercise studies have examined the different durations, intensities, and modalities of exercise primarily with respect to their associated effect on glycemia (Galassetti & Riddell, 2013; Kennedy et al., 2013; Lu & Zhao, 2020; Moser et al., 2020; Riddell & Perkins, 2006). Skeletal muscle has not been ignored in these studies. Skeletal muscle is responsible for a large portion of glucose uptake and disposal following a meal (Meyer et al., 2002), and this portion becomes even greater following an insulinemic or contractile stimulus such as exercise (Richter & Hargreaves, 2013), as previously noted. Unfortunately, skeletal muscle structure and physiology have seldom been the primary outcome measures of human T1D literature. Consequently, cellular muscle abnormalities in T1D patients – to the extent which they have been observed to date – are apparently considered a secondary attribute of other systemic dysfunctions like neuropathy (H.

Andersen, 1998; Andreassen et al., 2009, 2014; Fritzsche, Blüher, et al., 2008). Clearly the need still exists to interrogate tissue-specific dysfunctions involved in diabetic myopathy, in order to properly understand its contribution to whole-body physiology during T1D.

Therefore, the purpose of this dissertation was to examine myofiber physiology under acute and chronic muscle stressors such as eccentric exercise and aging. Further, we aimed to extend our preclinical knowledge of myostatin as a potential mediator of muscle dysfunction into the clinical context of humans with T1D.

Broadly, the work presented here displayed that maximal strength declines more quickly in adults with T1D and was associated with altered myofiber morphology, fiber-type composition, and glycogen expression over a wide age range in adults with T1D. Further, we observed that strength recovery is hindered, and muscle regenerative capacity is disturbed in young adults with T1D after an acute eccentric damage stimulus. Finally, we observed that adults with T1D exhibit higher circulating levels of myostatin than control participants in young adulthood.

Study 1

In study 1, we observed accelerated strength decline in adults with T1D during 6 decades of life (18-78 years old). In older participants with T1D (>35 years old), loss of strength was accompanied by more/larger clusters of type 1 myofibers and expressed less muscle glycogen in T1D participants than controls. Older T1D females exhibited more hybrid fibers (i.e. expressing multiple MHC isoforms) than age-matched controls. Conversely, T1D males exhibited a shift towards type 2 fibers, with less evidence of myofiber grouping or hybrid fibers.

Our results suggested that we may have observed a previously posited accelerated muscular aging phenotype (Monaco, Gingrich, et al., 2019) in the form of elevated proportions of hybrid myofibers and type 1 myofiber grouping in older T1D versus control participants; since both of these phenomena become increasingly prevalent during aging (J. L. Andersen et al., 1999; Kelly et al., 2017, 2018; Lexell & Downham, 1991). With regards to hybrid fibers, others have proposed that age-related denervation/reinnervation events cause affected fibers to receive contradictory neural inputs upon reinnervation, thus providing conflicting signals and eventually giving rise to a mixed expression of MHC isoforms (e.g. hybrid fibers) (J. L. Andersen et al., 1999). Similarly, the grouping of type 1 myofibers has also been associated with age-related denervation/reinnervation events (Kelly et al., 2018; Lexell & Downham, 1991) that result in inefficient motor unit activation and greater physiologic difficulty to complete motor tasks (Kelly et al., 2017). Additionally, diminished muscle glycogen may in fact represent another metabolic perturbation that resembles accelerated aging in T1D muscle since diminished muscle glycogen stores are found to be lower in skeletal muscle in older adults (Cartee, 1994a, 1994b; Meredith et al., 1989).

In addition to this study being the most expansive investigation of skeletal muscle morphology and function in adults with T1D of a wide age range, we also observed sex differences in which females with T1D exhibited emphasized signs of the aforementioned aging phenotype. To our knowledge, this is the first study observing myofiber-specific measurements from multiple age groups of T1D adults. Therefore, it is difficult to readily compare our results to other T1D human muscle studies. However, sexually dimorphic

changes to mitochondrial bioenergetics, another key component of muscle health, have been observed previously by our laboratory (Monaco, Bellissimo, et al., 2019a). Therefore, it stands to reason that sex may be an important consideration in forthcoming studies about T1D muscle physiology. Altogether, these findings highlight significant changes to the muscles of older adults with T1D even in the absence of overt diabetic complications and provides novel evidence of accelerated muscular aging in human muscles from adults with T1D.

Study 2

In study 2, we observed diminished strength recovery, lower SC content, delayed SC proliferation, greater ECM content, and a higher incidence of damaged sarcolemma in young adults with T1D (mean = 22, SEM = 0.9 years). Transcriptomic analyses revealed a network of translational and mitochondrial genes that were dysregulated post-exercise in persons with T1D compared to matched controls. Taken together, these novel findings suggest that young adults with T1D may exhibit an altered response to damaging exercise and delayed reparative response.

In T1D participants, we observed diminished strength recovery in the absence of a resting strength deficit, which suggests that the delayed strength recovery may be due to intrinsic alterations to the muscle. Previous studies have observed a greater proportion of type 2X muscle fibers (Fritzsche, Bluher, et al., 2008) which are preferentially damaged by eccentric exercise (Macaluso et al., 2012). Thus, persons with T1D may be more susceptible to muscle fiber damage leading to prolonged reductions in strength. Indeed, we did observe markers of damage at the sarcolemma (i.e. disrupted dystrophin) and laminin

content, indicating that T1D participants exhibited more myofiber damage and more abundant ECM. Accompanied by elevated serum creatine kinase activity, our observations here may be indicative of an early underlying metabolic myopathy, as previously suggested (Jevric-Causevic et al., 2006). Additionally, we observed less satellite cell content and delayed satellite cell proliferation in those with T1D. Decreased content of SCs has been observed previously in our laboratory in young males with poorly controlled T1D (HbA_{1c}=68 ± 2.2 mmol/mol) (D. M. D'Souza et al., 2016b). However, to our knowledge, this is the first observation of delayed SC proliferations in T1D individuals following an exercise stimulus. Dysglycemia has previously been shown to contribute to a disadvantageous environment for SC activity (i.e. perturbed satellite cell niche) (Aragno et al., 2004; Gordin et al., 2008). Given participants in the present study had more well-managed T1D (HbA_{1c} = 7.5 ± 2.7%) than previous observations (D. M. D'Souza et al., 2016b), it is noteworthy that we were still able to observe significant changes to SC activity in these adults.

Our gene set enrichment analysis (GSEA) of T1D skeletal muscle is, to our knowledge, the first in skeletal muscles from humans with T1D. We hypothesized we would observe dysregulated molecular pathways that underpinned the observations in this study (i.e. diminished SC content/proliferation, damaged sarcolemma, etc.). Interestingly, we instead observed networks of gene sets involving translation and mitochondrial oxidative phosphorylation in muscles from T1D participants. These findings were notable, given recent findings from our laboratory and others which noted alterations in mitochondrial bioenergetics in similar cohorts with T1D (Cree-Green et al., 2015, p.;

Monaco, Bellissimo, et al., 2019b; Monaco et al., 2018b), which suggests that the current observation of upregulated mitochondrial translational genes at rest may represent compensatory adaptation to some form of mitochondrial dysfunction or stress in the early decades of living with T1D. To date, no studies have revealed dysregulated expression of translational genes in humans with T1D. However, previous investigations have shown dysregulation of protein synthesis/breakdown in those with poorly managed type 1 diabetes (i.e. without insulin therapy) (Nair et al., 1983). Unfortunately, we did not measure *in vivo* protein synthesis in this or previous cohorts of young adults with diabetes (Dial et al., 2020; Monaco, Bellissimo, et al., 2019b; Monaco et al., 2018b), so any mechanistic connection between protein synthesis/breakdown and dysregulated gene expression in T1D muscle remains speculative at this point. However, the current transcriptomic data offers novel insight regarding potential pathways mediating changes to muscle health in T1D and suggests the need for such measures in future investigations.

Overall, this study was the first to provide evidence of dysfunctional skeletal muscle repair in young adults with T1D. The prolonged loss of strength in these individuals warrants caution for those engaging in intense exercise muscle-lengthening exercises and suggests that a particular focus on muscle recovery in exercise regimens may benefit the lifelong muscle health of these individuals.

Study 3

Study 3 was the first study to reveal that circulating myostatin is expressed to a higher degree in T1D participants than non-diabetic participants. Myostatin expression was correlated to body composition such that leaner individuals exhibited the highest levels of

expression. Also, we observed a sexually dimorphic effect of T1D, where women displayed a larger relative increase in serum myostatin versus control females than did T1D males compared their control counterparts. Other investigations have suggested that insulin resistance is associated with higher myostatin expression in populations enduring other metabolic diseases, such as T2D and obesity (Amor et al., 2018; Brandt et al., 2012; Hittel et al., 2009). However, this association does not extrapolate similarly to glycated hemoglobin (HbA_{1c}) levels, as evidenced by lack of correlative relationships between myostatin and HbA_{1c} in our T1D participants or in T2D participants from the work of Gallagher *et al.* (Gallagher et al., 2010). While these data are correlative only, they suggest that insulin sensitivity may be a better indicator of dysregulations in myokine expression than HbA_{1c}. Overall, data from this study provided evidence for irregular circulating myostatin expression in adults with T1D, and suggests the need to measure insulin sensitivity in participants with T1D to corroborate or dispute if it is a potential driver of enhanced myostatin expression in conditions of metabolic disturbance (Amor et al., 2018; Brandt et al., 2012; Hittel et al., 2009).

Conclusion. In conclusion, this is the first body of work to comprehensively profile muscle physiology and morphology at the level of the myofiber in those with T1D under different physiological stressors of age and eccentric damage. The observations of dysfunctional muscle repair from an acute eccentric damage stimulus and/or dysregulated myokine expression at rest in otherwise healthy young adults with T1D alerts patients, researchers and clinicians that muscle health may be compromised before the observations of any clinical signs of dysfunction. The compounding effect of these dysfunctions over

the lifespan may indeed lead to fundamental alterations in muscle health that resemble an accelerated form of muscular deterioration as suggested by the higher risk of disability and institutionalization in older adults with diabetes (Kalyani et al., 2010; Morley, 2008; Wong et al., 2013).

5.2 FUTURE RESEARCH

This research advanced our knowledge of cellular physiology of muscles with T1D. This was the first body of work to investigate myofiber physiology at rest, after exercise, and with increasing age. Naturally, the work here has prompted potential research directions, given the data presented herein.

5.2.1 Examining insulin sensitivity as a driver of muscle dysfunction in T1D

Being the first in-depth observations of myofiber physiology in T1D muscles, interpretations from our data were often viewed through the lens of other metabolic diseases, such as obesity and T2D, as they occupy a much larger footprint in the literature and thus have had more in-depth studies associating muscle physiology with factors such as insulin sensitivity. Studies from these areas seem to converge upon a theory that altered insulin sensitivity drives physiological dysfunction in muscles enduring either metabolic disease (Brandt et al., 2012; DeFronzo & Tripathy, 2009; He et al., 2001; Hittel et al., 2009; Petersen & Shulman, 2002; Sayer et al., 2005). Ultimately, the loss of insulin sensitivity has been attributed to (1) increased glycolytic enzyme activity (Goodpaster et al., 1997; J.-A. Simoneau & Kelley, 1997), (2) higher proportions of fast-twitch type 2 fibers (Hickey et al., 1995; Kelley et al., 1999; J. A. Simoneau et al., 1999), impaired muscle regeneration (Akhmedov & Berdeaux, 2013; Sinha et al., 2017), and (3) elevated myostatin expression (Brandt et al., 2012; Hittel et al., 2009) – all major findings observed in the present studies of adults with T1D. While insulin resistance is not the primary pathophysiological factor of T1D, it is becoming increasingly recognized as an important complication of T1D (Cree-

Green et al., 2018; Donga et al., 2015; Greenbaum, 2002) (e.g. "double diabetes"), and has since been recognized as a risk factor for progression of T1D (Fourlanos et al., 2004).

Unfortunately, the lengthy and/or invasive nature of the protocols involved in these human studies limited the quantity of protocols these participants underwent. Consequently, we did not prioritize measurement of insulin sensitivity in these cohorts. Instead we chose to rely on HbA_{1c} as the standard clinical measure of moderate-term glycemic regulation in T1D patients (American Diabetes Association, 2014b; Cheng, 2013; Rewers et al., 2014), in order to readily generalize our findings to clinical outcomes and/or recommendations. However, given the apparent parallels between our findings and that of T2D/obesity muscle physiology, it seems that the hypothesized link between insulin resistance and muscle dysfunction should be confirmed by: (1) euglycemic-hyperinsulinemic clamp (DeFronzo et al., 1979), and/or (2) insulin sensitivity surrogate indices – such as the Homeostatic Model Assessment (HOMA) (Singh & Saxena, 2010). Glucose clamp techniques, while considered the gold standard for pharmacokinetic/pharmacodynamic studies in diabetes, requires constant supervised attention by a trained physician, and entails requisite vigilance when being performed on T1D patients. On the other hand, surrogate measures of insulin sensitivity employ an approximation described by an equation, resulting in a surrogate index of insulin resistance. Popular indices for insulin resistance require measurement of fasting insulin and fasting glucose in circulation, which is problematic in a T1D population that would have no fasting insulin. In light of this, other formulas have been used, despite their limitations. One example is estimated glucose disposal rate (eGDR) which uses the inputs which are

inversely related to insulin resistance: waist-to-hip ratio, history of systemic arterial hypertension, and the Hb_{A1c} (Williams et al., 2000). Another formula evaluates insulin sensitivity score (ISS) based on: waist size, Hb_{A1c} and triglycerides (Dabelea et al., 2011). In cases where fasting is required of T1D participants, caution and supervision are warranted given that challenged glycemic regulation is a hallmark of the disease. Nevertheless, under expert supervision, it is possible to measure one or more of these outcomes in T1D patients. These outcomes, accompanied by in-depth profiling of the muscle alterations observed herein would provide valuable insight to further advance our knowledge of the impact of insulin metabolism on muscle in patients with T1D.

5.2.2 Larger, longer, and more specific muscle regeneration studies

Study 2 provided the first insight into cellular muscle physiology following an eccentric exercise stimulus. Given the invasive nature of this study protocol in particular, it was designed with a sample size that was statistically powered to detect differences amongst the whole cohort (Nederveen et al., 2018). Thus, our study was not powered to detect the presence of sexually dimorphic effects. Furthermore, this study looked at only the early time points of muscle degeneration/regeneration from zero to ninety-six hours following eccentric exercise, rather than the full reparative process, which has been shown to take upwards of two weeks in rodent models (Forcina et al., 2020), and even longer in rodents with experimental T1D (Vignaud et al., 2007). The muscle recovery process also encompasses several overlapping subprocesses, such as inflammation. However, Study 2 did not focus inflammation, nor other regenerative subprocesses, thereby making these fertile ground for future discovery of muscular perturbations in T1D patients. As an

example, relatively simple immunohistological measures can be employed to investigate macrophage content and polarity over the time course of regeneration, given that T1D rodent models exhibit slower recovery of the inflammatory response following exercise (Bortolon et al., 2020). In summary, in order to achieve more practical and accurate exercise recommendations for adults with T1D, we recommend that future studies employ larger cohorts to detect sex differences, a longer time course following an eccentric damage stimulus to encompass muscle recovery to a fuller extent, and/or investigation of specific muscle recovery subprocesses to deepen our understanding of T1D-specific changes to skeletal muscle.

5.2.3 Quantification of age-related denervation/reinnervation cycles

Study 1 revealed a host of characteristics (e.g. hybrid fibers, type 1 fiber grouping) resemblant of a muscular aging phenotype. Both of these features have been previously suggested to arise from age-related cycles of denervation and collateral reinnervation of myofibers (Hepple & Rice, 2016; Kanda & Hashizume, 1989; Lexell & Downham, 1991). In the case of hybrid fibers, Andersen *et al.* (J. L. Andersen et al., 1999) posited that myofiber reinnervation from a different motor unit type (i.e. type 1 vs type 2) results in signals to the muscle contradicting its original myogenic lineage, ultimately changing the predominant MHC-type of the myofiber, and ultimately the fiber-type composition of skeletal muscle over time.

Measurement of denervation in human muscles is challenging. Human skeletal muscle NMJs are difficult to sample via Bergstrom muscle biopsy due to their constrained location on the *vastus lateralis* – most often in the middle-third of the muscle (Nishimune &

Shigemoto, 2018). Some accounts state that modified Bergstrom biopsies observe NMJs in only 16.7% of samples at an average frequency of 2 NMJs per sample (Aubertin-Leheudre et al., 2020). However, a recently reported method by Aubertin-Leheudre *et al.* (Aubertin-Leheudre et al., 2020) entitled BeeNMJs (*B*iopsy using *E*lectrostimulation for *E*nhanced *NMJ S*ampling), uses surface electrodes to locate NMJs by observing twitch response via electrical stimulation – determining the location of highest NMJ density (i.e. biopsy location) as the one that produced the greatest twitch (Aubertin-Leheudre et al., 2020). Authors of this study report observations of NMJs in 53.3% of samples at an average frequency of 30 NMJs per sample. While this methodology still has room for improvement, this represents a vast improvement in NMJ sampling via biopsy and allows for direct morphological assessment of NMJs in human muscle from other populations such as those with T1D.

In the absence of direct observation of NMJs, researchers have primarily relied on denervated rodent models (Aare et al., 2016; Spendiff et al., 2016), or use of a histochemical markers which become elevated with denervation such as tetrodotoxin-resistant sodium channels ($Na_v1.5$) (Rowan et al., 2012), or neural cell adhesion molecule (NCAM) (Sonjak et al., 2019). Still, these measures are not without their limitations, and their elevated expression with denervation is observed equivocally (Hendrickse et al., 2018).

We believe the combined use of specific biopsy techniques and histochemical markers of NMJs to be potentially fruitful measures of denervation in humans, particularly accompanied by other surrogate measures of denervation such as the muscle

morphometrics measured in this body of work which would. This avenue of research would make necessary progress by reinforcing or disputing denervation as a central driver for myofiber morphological and physiological changes in T1D.

REFERENCES

Aare, S., Spendiff, S., Vuda, M., Elkrief, D., Perez, A., Wu, Q., Mayaki, D., Hussain, S. N. A., Hettwer, S., & Hepple, R. T. (2016). Failed reinnervation in aging skeletal muscle. *Skeletal Muscle*, *6*(1), 29. https://doi.org/10.1186/s13395-016-0101-y

Akhmedov, D., & Berdeaux, R. (2013). The effects of obesity on skeletal muscle regeneration. *Frontiers in Physiology*, *4*, 371. https://doi.org/10.3389/fphys.2013.00371

American Diabetes Association. (2014). Standards of medical care in diabetes—2014. *Diabetes Care*, *37 Suppl 1*, S14-80. https://doi.org/10.2337/dc14-S014

Amor, M., Itariu, B. K., Moreno-Viedma, V., Keindl, M., Jürets, A., Prager, G., Langer, F., Grablowitz, V., Zeyda, M., & Stulnig, T. M. (2018). Serum myostatin is upregulated in obesity and correlates with insulin resistance in humans. *Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology [and] German Diabetes Association*. https://doi.org/10.1055/a-0641-5546

Andersen, H. (1998). Muscular endurance in long-term IDDM patients. *Diabetes Care*, *21*(4), 604–609. https://doi.org/10.2337/diacare.21.4.604

Andersen, J. L., Terzis, G., & Kryger, A. (1999). Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. *Muscle & Nerve*, *22*(4), 449–454. https://doi.org/10.1002/(SICI)1097-4598(199904)22:4<449::AID-MUS4>3.0.CO;2-2

Andreassen, C. S., Jakobsen, J., Flyvbjerg, A., & Andersen, H. (2009). Expression of neurotrophic factors in diabetic muscle—Relation to neuropathy and muscle strength. *Brain: A Journal of Neurology*, *132*(Pt 10), 2724–2733. https://doi.org/10.1093/brain/awp208

Andreassen C. S., Jensen J. M., Jakobsen J., Ulhøj B. P., & Andersen H. (2014). Striated muscle fiber size, composition, and capillary density in diabetes in relation to neuropathy and muscle strength. *Journal of Diabetes*, *6*(5), 462–471. https://doi.org/10.1111/1753-0407.12124

Aragno, M., Mastrocola, R., Catalano, M. G., Brignardello, E., Danni, O., & Boccuzzi, G. (2004). Oxidative stress impairs skeletal muscle repair in diabetic rats. *Diabetes*, *53*(4), 1082–1088.

Aubertin-Leheudre, M., Pion, C. H., Vallée, J., Marchand, S., Morais, J. A., Bélanger, M., & Robitaille, R. (2020). Improved human muscle biopsy method to study neuromuscular junction structure and functions with aging. *The Journals of Gerontology: Series A*, *75*(11), 2098–2102. https://doi.org/10.1093/gerona/glz292

Bortolon, J. R., Murata, G. M., Borges, L., Weimann, E., Silva, M. B. B., Dermargos, A., & Hatanaka, E. (2020). Recovery of diabetic rats after physical exhaustion: kinetic alterations in muscle inflammation and muscle-signaling proteins to atrophy and hypertrophy. *Frontiers in Physiology*, *11*, 1411. https://doi.org/10.3389/fphys.2020.573416

Brandt, C., Nielsen, A. R., Fischer, C. P., Hansen, J., Pedersen, B. K., & Plomgaard, P. (2012). Plasma and muscle myostatin in relation to type 2 diabetes. *PloS One*, *7*(5), e37236. https://doi.org/10.1371/journal.pone.0037236

Cartee, G. D. (1994a). Aging skeletal muscle: Response to exercise. *Exercise and Sport Sciences Reviews*, *22*, 91–120.

Cartee, G. D. (1994b). Influence of age on skeletal muscle glucose transport and glycogen metabolism. *Medicine and Science in Sports and Exercise*, *26*(5), 577–585.

Cheng, A. Y. Y. (2013). Clinical practice guidelines for the prevention and management of diabetes in Canada. Introduction. *Canadian Journal of Diabetes*, *37*, S1–S3. https://doi.org/10.1016/j.jcjd.2013.01.009

Cree-Green, M., Newcomer, B. R., Brown, M. S., Baumgartner, A. D., Bergman, B., Drew, B., Regensteiner, J. G., Pyle, L., Reusch, J. E. B., & Nadeau, K. J. (2015). Delayed skeletal muscle mitochondrial ADP recovery in youth with type 1 diabetes relates to muscle insulin resistance. *Diabetes*, *64*(2), 383–392. https://doi.org/10.2337/db14-0765

Cree-Green, M., Stuppy, J. J., Thurston, J., Bergman, B. C., Coe, G. V., Baumgartner, A. D., Bacon, S., Scherzinger, A., Pyle, L., & Nadeau, K. J. (2018). Youth with type 1 diabetes have adipose, hepatic, and peripheral insulin resistance. *The Journal of Clinical Endocrinology & Metabolism*, *103*(10), 3647–3657. https://doi.org/10.1210/jc.2018-00433

Dabelea, D., D'Agostino, R. B., Mason, C. C., West, N., Hamman, R. F., Mayer-Davis, E. J., Maahs, D., Klingensmith, G., Knowler, W. C., & Nadeau, K. (2011). Development, validation and use of an insulin sensitivity score in youths with diabetes: The SEARCH for Diabetes in Youth study. *Diabetologia*, *54*(1), 78–86. https://doi.org/10.1007/s00125-010-1911-9

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. *American Journal of Physiology-Endocrinology and Metabolism*, *237*(3), E214. https://doi.org/10.1152/ajpendo.1979.237.3.E214

DeFronzo, R. A., & Tripathy, D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care*, *32*(suppl 2), S157–S163. https://doi.org/10.2337/dc09-S302

Dial, A. G., Monaco, C. M. F., Grafham, G. K., Romanova, N., Simpson, J. A., Tarnopolsky, M. A., Perry, C. G. R., Kalaitzoglou, E., & Hawke, T. J. (2020). Muscle and serum myostatin expression in type 1 diabetes. *Physiological Reports*, *8*(13), e14500. https://doi.org/10.14814/phy2.14500

Donga, E., Dekkers, O. M., Corssmit, E. P. M., & Romijn, J. A. (2015). Insulin resistance in patients with type 1 diabetes assessed by glucose clamp studies: Systematic review and meta-analysis. *European Journal of Endocrinology*, *173*(1), 101–109. https://doi.org/10.1530/EJE-14-0911

D'Souza, D. M., Zhou, S., Rebalka, I. A., MacDonald, B., Moradi, J., Krause, M. P., Al-Sajee, D., Punthakee, Z., Tarnopolsky, M. A., & Hawke, T. J. (2016). Decreased satellite cell number and function in humans and mice with type 1 diabetes is the result of altered Notch signaling. *Diabetes*, *65*(10), 3053–3061. https://doi.org/10.2337/db15-1577

Forcina, L., Cosentino, M., & Musarò, A. (2020). Mechanisms regulating muscle regeneration: insights into the interrelated and time-dependent phases of tissue healing. *Cells*, *9*(5), 1297. https://doi.org/10.3390/cells9051297

Fourlanos, S., Narendran, P., Byrnes, G. B., Colman, P. G., & Harrison, L. C. (2004). Insulin resistance is a risk factor for progression to type 1 diabetes. *Diabetologia*, *47*(10), 1661–1667. https://doi.org/10.1007/s00125-004-1507-3

Fritzsche, K., Blüher, M., Schering, S., Buchwalow, I. B., Kern, M., Linke, A., Oberbach, A., Adams, V., & Punkt, K. (2008). Metabolic profile and nitric oxide synthase expression of skeletal muscle fibers are altered in patients with type 1 diabetes. *Experimental and Clinical Endocrinology & Diabetes*, *116*(10), 606–613. https://doi.org/10.1055/s-2008-1073126

Fritzsche, K., Bluher, M., Schering, S., Buchwalow, I. B., Kern, M., Linke, A., Oberbach, A., Adams, V., & Punkt, K. (2008). Metabolic profile and nitric oxide synthase expression of skeletal muscle fibers are altered in patients with type 1 diabetes. *Experimental and Clinical Endocrinology & Diabetes : Official Journal, German*

Society of Endocrinology [and] German Diabetes Association, *116*(10), 606–613. https://doi.org/10.1055/s-2008-1073126

Galassetti, P., & Riddell, M. C. (2013). Exercise and type 1 diabetes (T1DM). *Comprehensive Physiology*, *3*, 28.

Gallagher, I. J., Scheele, C., Keller, P., Nielsen, A. R., Remenyi, J., Fischer, C. P., Roder, K., Babraj, J., Wahlestedt, C., Hutvagner, G., Pedersen, B. K., & Timmons, J. A. (2010). Integration of microRNA changes in vivo identifies novel molecular features of muscle insulin resistance in type 2 diabetes. *Genome Medicine*, *2*(2), 9. https://doi.org/10.1186/gm130

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I.-M., Nieman, D. C., & Swain, D. P. (2011). Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *43*(7), 1334–1359. https://doi.org/10.7916/D8CR5T2R

Goodpaster, B. H., Thaete, F. L., Simoneau, J. A., & Kelley, D. E. (1997). Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes*, *46*(10), 1579–1585. https://doi.org/10.2337/diacare.46.10.1579

Gordin, D., Forsblom, C., Ronnback, M., Parkkonen, M., Waden, J., Hietala, K., & Groop, P.-H. (2008). Acute hyperglycaemia induces an inflammatory response in young patients with type 1 diabetes. *Annals of Medicine*, *40*(8), 627–633. https://doi.org/10.1080/07853890802126547

Greenbaum, C. J. (2002). Insulin resistance in type 1 diabetes. *Diabetes/Metabolism Research and Reviews*, *18*(3), 192–200. https://doi.org/10.1002/dmrr.291

He, J., Watkins, S., & Kelley, D. E. (2001). Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. *Diabetes*, *50*(4), 817–823. https://doi.org/10.2337/diabetes.50.4.817

Hendrickse, P., Galinska, M., Hodson-Tole, E., & Degens, H. (2018). An evaluation of common markers of muscle denervation in denervated young-adult and old rat gastrocnemius muscle. *Experimental Gerontology*, *106*, 159–164. https://doi.org/10.1016/j.exger.2018.03.007

Hepple, R. T., & Rice, C. L. (2016). Innervation and neuromuscular control in ageing skeletal muscle. *The Journal of Physiology*, *594*(8), 1965–1978. https://doi.org/10.1113/JP270561

Hickey, M. S., Carey, J. O., Azevedo, J. L., Houmard, J. A., Pories, W. J., Israel, R. G., & Dohm, G. L. (1995). Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. *The American Journal of Physiology*, *268*(3 Pt 1), E453-457. https://doi.org/10.1152/ajpendo.1995.268.3.E453

Hittel, D. S., Berggren, J. R., Shearer, J., Boyle, K., & Houmard, J. A. (2009). Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. *Diabetes*, *58*(1), 30–38. https://doi.org/10.2337/db08-0943

Iscoe, K. E., & Riddell, M. C. (2011). Continuous moderate-intensity exercise with or without intermittent high-intensity work: Effects on acute and late glycaemia in athletes with type 1 diabetes mellitus. *Diabetic Medicine*, *28*(7), 824–832. https://doi.org/10.1111/j.1464-5491.2011.03274.x

Jevric-Causevic, A., Malenica, M., & Dujic, T. (2006). Creatine kinase activity in patients with diabetes mellitus type I and type II. *Bosnian Journal of Basic Medical Sciences*, *6*(3), 5–9. https://doi.org/10.17305/bjbms.2006.3135

Kalyani, R. R., Saudek, C. D., Brancati, F. L., & Selvin, E. (2010). Association of diabetes, comorbidities, and a1c with functional disability in older adults: Results from the National Health and Nutrition Examination Survey (NHANES), 1999–2006. *Diabetes Care*, *33*(5), 1055–1060. https://doi.org/10.2337/dc09-1597

Kanda, K., & Hashizume, K. (1989). Changes in properties of the medial gastrocnemius motor units in aging rats. *Journal of Neurophysiology*, *61*(4), 737–746. https://doi.org/10.1152/jn.1989.61.4.737

Kelley, D. E., Goodpaster, B., Wing, R. R., & Simoneau, J. A. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *The American Journal of Physiology*, *277*(6), E1130-1141. https://doi.org/10.1152/ajpendo.1999.277.6.E1130

Kelly, N. A., Hammond, K. G., Bickel, C. S., Windham, S. T., Tuggle, S. C., & Bamman, M. M. (2017). Effects of aging and Parkinson's disease on motor unit remodeling: Influence of resistance exercise training. *Journal of Applied Physiology*, *124*(4), 888–898. https://doi.org/10.1152/japplphysiol.00563.2017

Kelly, N. A., Hammond, K. G., Stec, M. J., Bickel, C. S., Windham, S. T., Tuggle, S. C., & Bamman, M. M. (2018). Quantification and characterization of grouped type 1 myofibers in human aging. *Muscle & Nerve*, *57*(1), E52–E59. https://doi.org/10.1002/mus.25711

Kennedy, A., Nirantharakumar, K., Chimen, M., Pang, T. T., Hemming, K., Andrews, R. C., & Narendran, P. (2013). Does exercise improve glycaemic control in type 1

diabetes? A systematic review and meta-analysis. *PLOS ONE*, *8*(3), e58861. https://doi.org/10.1371/journal.pone.0058861

Lexell, J., & Downham, D. Y. (1991). The occurrence of fibre-type grouping in healthy human muscle: A quantitative study of cross-sections of whole vastus lateralis from men between 15 and 83 years. *Acta Neuropathologica*, *81*(4), 377–381. https://doi.org/10.1007/BF00293457

Macaluso, F., Isaacs, A. W., & Myburgh, K. H. (2012). Preferential type II muscle fiber damage from plyometric exercise. *Journal of Athletic Training*, *47*(4), 414–420. https://doi.org/10.4085/1062-6050-47.4.13

Meredith, C. N., Frontera, W. R., Fisher, E. C., Hughes, V. A., Herland, J. C., Edwards, J., & Evans, W. J. (1989). Peripheral effects of endurance training in young and old subjects. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *66*(6), 2844–2849. https://doi.org/10.1152/jappl.1989.66.6.2844

Meyer, C., Dostou, J. M., Welle, S. L., & Gerich, J. E. (2002). Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *American Journal of Physiology-Endocrinology and Metabolism*, *282*(2), E419–E427. https://doi.org/10.1152/ajpendo.00032.2001

Monaco, C. M. F., Bellissimo, C. A., Hughes, M. C., Ramos, S. V., Laham, R., Perry, C. G. R., & Hawke, T. J. (2019a). Sexual dimorphism in human skeletal muscle mitochondrial bioenergetics in response to type 1 diabetes. *American Journal of Physiology-Endocrinology and Metabolism*. https://doi.org/10.1152/ajpendo.00411.2019

Monaco, C. M. F., Bellissimo, C. A., Hughes, M. C., Ramos, S. V, Laham, R., Perry, C. G. R., & Hawke, T. J. (2019b). Sexual dimorphism in human skeletal muscle mitochondrial bioenergetics in response to type 1 diabetes. *American Journal of Physiology-Endocrinology and Metabolism*, *318*(1), E44–E51. https://doi.org/10.1152/ajpendo.00411.2019

Monaco, C. M. F., Gingrich, M. A., & Hawke, T. J. (2019). Considering type 1 diabetes as a form of accelerated muscle aging. *Exercise and Sport Sciences Reviews*, *47*(2), 98–107. https://doi.org/10.1249/JES.0000000000000184

Monaco, C. M. F., Hughes, M. C., Ramos, S. V, Varah, N. E., Lamberz, C., Rahman, F. A., McGlory, C., Tarnopolsky, M. A., Krause, M. P., Laham, R., Hawke, T. J., & Perry, C. G. R. (2018). Altered mitochondrial bioenergetics and ultrastructure in the skeletal muscle of young adults with type 1 diabetes. *Diabetologia*, *61*(6), 1411–1423. https://doi.org/10.1007/s00125-018-4602-6

Morley, J. E. (2008). Diabetes and aging: Epidemiologic overview. *Clinics in Geriatric Medicine*, *24*(3), 395–405. https://doi.org/10.1016/j.cger.2008.03.005

Nair, K. S., Garrow, J. S., Ford, C., Mahler, R. F., & Halliday, D. (1983). Effect of poor diabetic control and obesity on whole body protein metabolism in man. *Diabetologia*, *25*(5), 400–403. https://doi.org/10.1007/BF00282518

Nederveen, J. P., Joanisse, S., Snijders, T., Thomas, A. C. Q., Kumbhare, D., & Parise, G. (2018). The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men. *The Journal of Physiology*, *596*(6), 1063–1078. https://doi.org/10.1113/JP275155

Nishimune, H., & Shigemoto, K. (2018). Practical anatomy of the neuromuscular junction in health and disease. *Neurologic Clinics*, *36*(2), 231–240. https://doi.org/10.1016/j.ncl.2018.01.009

Petersen, K. F., & Shulman, G. I. (2002). Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The American Journal of Cardiology*, *90*(5, Supplement 1), 11–18. https://doi.org/10.1016/S0002-9149(02)02554-7

Rewers, M. J., Pillay, K., De Beaufort, C., Craig, M. E., Hanas, R., Acerini, C. L., & Maahs, D. M. (2014). Assessment and monitoring of glycemic control in children and adolescents with diabetes. *Pediatric Diabetes*, *15 Suppl 20*. https://doi.org/10.1111/pedi.12190

Richter, E. A., & Hargreaves, M. (2013). Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological Reviews*, *93*(3), 993–1017. https://doi.org/10.1152/physrev.00038.2012

Riddell, M. C., & Perkins, B. A. (2006). Type 1 diabetes and vigorous exercise: applications of exercise physiology to patient management. *Canadian Journal of Diabetes*, *30*(1), 63–71. https://doi.org/10.1016/S1499-2671(06)01010-0

Rowan, S. L., Rygiel, K., Purves-Smith, F. M., Solbak, N. M., Turnbull, D. M., & Hepple, R. T. (2012). Denervation causes fiber atrophy and myosin heavy chain co-expression in senescent skeletal muscle. *PLoS ONE*, *7*(1). https://doi.org/10.1371/journal.pone.0029082

Sayer, A. A., Dennison, E. M., Syddall, H. E., Gilbody, H. J., Phillips, D. I. W., & Cooper, C. (2005). Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? *Diabetes Care*, *28*(10), 2541–2542. https://doi.org/10.2337/diacare.28.10.2541

Simoneau, J. A., Veerkamp, J. H., Turcotte, L. P., & Kelley, D. E. (1999). Markers of capacity to utilize fatty acids in human skeletal muscle: Relation to insulin resistance and obesity and effects of weight loss. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *13*(14), 2051–2060. https://doi.org/10.1096/fasebj.13.14.2051

Simoneau, J.-A., & Kelley, D. E. (1997). Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. *Journal of Applied Physiology*, *83*(1), 166–171. https://doi.org/10.1152/jappl.1997.83.1.166

Singh, B., & Saxena, A. (2010). Surrogate markers of insulin resistance: A review. *World Journal of Diabetes*, *1*(2), 36–47. https://doi.org/10.4239/wjd.v1.i2.36

Sinha, I., Sakthivel, D., & Varon, D. E. (2017). Systemic regulators of skeletal muscle regeneration in obesity. *Frontiers in Endocrinology*, *8*, 29. https://doi.org/10.3389/fendo.2017.00029

Sonjak, V., Jacob, K., Morais, J. A., Rivera-Zengotita, M., Spendiff, S., Spake, C., Taivassalo, T., Chevalier, S., & Hepple, R. T. (2019). Fidelity of muscle fibre reinnervation modulates ageing muscle impact in elderly women. *The Journal of Physiology*, *597*(19), 5009–5023. https://doi.org/10.1113/JP278261

Spendiff, S., Vuda, M., Gouspillou, G., Aare, S., Perez, A., Morais, J. A., Jagoe, R. T., Filion, M.-E., Glicksman, R., Kapchinsky, S., MacMillan, N. J., Pion, C. H., Aubertin-Leheudre, M., Hettwer, S., Correa, J. A., Taivassalo, T., & Hepple, R. T. (2016). Denervation drives mitochondrial dysfunction in skeletal muscle of octogenarians. *The Journal of Physiology*, *594*(24), 7361–7379. https://doi.org/10.1113/JP272487

Williams, K. V., Erbey, J. R., Becker, D., Arslanian, S., & Orchard, T. J. (2000). Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes*, *49*(4), 626–632. https://doi.org/10.2337/diabetes.49.4.626

Wong, E., Backholer, K., Gearon, E., Harding, J., Freak-Poli, R., Stevenson, C., & Peeters, A. (2013). Diabetes and risk of physical disability in adults: A systematic review and meta-analysis. *The Lancet Diabetes & Endocrinology*, *1*(2), 106–114. https://doi.org/10.1016/S2213-8587(13)70046-9

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