THE CAPACITY FOR SKELETAL MUSCLE TO REPAIR AFTER EXERCISE-INDUCED MUSCLE DAMAGE IN YOUNG ADULTS WITH TYPE 1 DIABETES MELLITUS

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A Thesis Submitted to the School of Graduate Studies in

Partial Fulfillment of the Requirements for the Degree of Master of Science

McMaster University MASTER OF SCIENCE (2020), Hamilton, Ontario (Medical Sciences)

TITLE:	The Capacity for Skeletal Muscle to Repair after Exercise-Induced Muscle Damage in Young Adults with Type 1 Diabetes Mellitus
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NUMBER OF PAGES:	*ix*, 83

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor Dr. Thomas Hawke, for the support he has provided throughout my degree. I have been extremely fortunate to have a supervisor who not only cared about my research, but encouraged me to pursue my own interests—even if that meant taking a semester off to play varsity soccer!

I would also like to thank my committee, Dr. Gianni Parise and Dr. Geoff Werstuck, for generously offering their time, guidance, and goodwill throughout the preparation and completion of my thesis.

Thank you to my lab mates past and present—Irena, Athan, Cynthia, Molly, Mike, Ally and Maria. Completing this work would not have been possible without their continued support and friendship. I greatly look forward to having them as colleagues in the years ahead.

A special thanks to the Medical Sciences and Kinesiology graduate student community, and the McMaster Varsity Soccer Team, for providing a much needed escape from my studies and for helping keep things in perspective.

Last of all, thank you to my parents, Kent and Sue, my brother William, and my partner Christopher, for their unconditional love and support throughout my research journey.

LAY ABSTRACT

Type 1 diabetes mellitus (T1D) is a chronic disease where the body does not make enough insulin to control blood glucose levels. Overtime, unstable blood glucose levels can damage major organ systems, including skeletal muscle. Skeletal muscle plays a pivotal role in regulating our physical and metabolic capacities. In those with T1D, exercise-mediated improvements in muscle health have been shown to delay health complications. However, we do not know how diabetic skeletal muscle repairs from exercise in humans. In this thesis, we investigated the ability of skeletal muscle to recover from damaging exercise in young adults with T1D. For the first time, we showed that skeletal muscle repair was similar between otherwise healthy young adults with T1D and those without diabetes. Our findings suggest that persons with T1D can engage in high levels of physical activity without compromising their muscle health. Further studies are needed to understand how exercise type, intensity, and duration impact glycemic control in men and women with T1D.

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LIST OF ABBREVIATIONS

AGE	Advanced glycation end-products
ATP	Adenosine triphosphate
BMI	Body mass index
CON	Control
DAPI	4’,6-diamidino-2-phenylindole
ECM	Extracellular matrix
EDL	Extensor digitorum longus
EDTA	Ethylenediaminetetraacetic acid
EIMD	Exercise-induced muscle damage
eMyHc	Embryonic myosin heavy chain
FAP	Fibrogenic/adipogenic progenitors
FGF	Fibroblast growth factor
GLUT4	Glucose transporter type 4
HbA1c	Glycosylated hemoglobin
HGF	Hepatocyte growth factor
IGF-1	Insulin-like growth factor 1
Il-6	Interleukin 6
IPAQ	International Physical Activity Questionnaire
MET	Metabolic equivalent of task
MMP	Matrix metalloproteinase
MRF	Myogenic regulatory factor
MVC	Maximal voluntary isometric contraction
MyHc	Myosin heavy chain
NMJ	Neuromuscular junction
NSAID	Nonsteroidal anti-inflammatory drug
PAI-1	Plasminogen activator inhibitor 1
Pax	Paired box transcription factor
ROS	Reactive oxygen species
SC	Satellite cell
SD	Standard deviation
SEM	Standard error of the mean
STZ	Streptozotocin
T1D	Type 1 diabetes mellitus
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

LITERATURE REVIEW

1. TYPE 1 DIABETES MELLITUS

1.1. T1D at a glance

Type 1 diabetes mellitus (T1D) is a progressive autoimmune disease characterized by destruction of the insulin-producing pancreatic beta cells. As a result, those with untreated T1D are in a chronic state of hypoinsulinemia and hyperglycemia. Though T1D can occur at any age, it is most commonly diagnosed in adolescence around age 14[1]. T1D currently accounts for 5-10% of total cases of diabetes worldwide, with epidemiological studies reporting a 2-5% annual increase in the incidence rate of T1D globally[2,3]. Though significant efforts have been made to identify the cause of T1D, the exact etiology and pathogenesis of the disease remains unknown. In general, T1D appears to represent an incurable heterogenous disease, who's presentation is influenced by both genetic and environmental factors[4–6].

1.2. Therapeutic strategies

Exogenous insulin therapy is the mainstay of T1D management. Rapid or long-lasting insulin analogues are delivered subcutaneously through multiple daily injections or infused through an insulin pump. Achieving an optimal glycemic target while avoiding hypoglycemia can be challenging. Therefore, effective T1D management involves insulin therapy in combination with frequent blood glucose tests and careful dietary monitoring[7]. Exercise has proven beneficial for people of all ages living with T1D. Physical activity is associated with increased cardiovascular health, improved metabolic control, and enhanced psychological wellbeing[8].

Overall, maintaining normoglycemia and delaying diabetic complications requires balancing insulin delivery with diligent self-monitoring and healthy lifestyle habits.

1.3. Diabetic complications

The abovementioned therapeutic strategies, though vastly extending the life expectancy of those with diabetes (<3 years versus >70 years, post-diagnosis), come with considerable limitations[9,10]. These include longstanding recurrent bouts of dysglycemia, dyslipidemia, and insulin resistance, and the consequent development of health complications[11–13]. Generally, diabetes-related complications are separated into macrovascular (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (nephropathy, neuropathy, and retinopathy)[14]. These complications continue to be the leading cause of morbidity and mortality in persons with T1D[15].

Often unconsidered in these complications is the impact of T1D on skeletal muscle. Myopathy, damage to the skeletal muscle tissue, is observed in persons with T1D prior to other primary diabetic complications[12,16]. This thesis will highlight the importance of skeletal muscle and explore its ability to respond to injury in persons with T1D.

2. SKELETAL MUSCLE

2.1. Function

Skeletal muscle is the largest organ system in the human body, accounting for nearly 40% of a healthy person's body mass. Skeletal muscle is responsible for creating movement.

This not only includes our ability to walk and run, but perform routine tasks such as feeding ourselves and getting dressed. These movements are under conscious control of the peripheral nervous system. The basic unit of skeletal muscle is the muscle fiber. Muscle fibers utilize the body's fuel sources to produce energy (in the form of adenosine triphosphate (ATP)) to generate force. In addition to creating movement, skeletal muscle generates the force necessary for respiration, heat production, and joint stability. Thus, maintaining healthy skeletal muscle is essential to living a healthy active life.

2.2. Structure

Muscle consists of multiple tissues including skeletal muscle fibers, blood vessels, nerves, and connective tissue. Hundreds of individual muscle fibers are bundled together into fascicles. Separate connective tissue sheaths enclose each muscle fiber, fascicle, and subsequent whole muscle. The connective tissue layers provide structural support for the muscle during contraction, as well as provide pathways for incoming blood vessels and nerves.

Each skeletal muscle fiber is composed of a single muscle cell or myocyte. During development, mononucleated myocytes fuse together to form elongated multinucleated muscle fibers. The nuclei reside on the periphery of the muscle fiber beneath the sarcolemma. Each muscle fiber, in turn, contains multiple chains of contractile myofibrils. The sarcoplasmic reticulum, a membranous network containing calcium-rich fluid, surrounds each myofibril. Transverse tubules, invaginations of the sarcolemma, lie adjacent to the sarcoplasmic

reticulum. The close association between the sarcoplasmic reticulum and t-tubules is crucial for muscle contraction.

Myofibrils, themselves, are composed of repeating units of sarcomeres – highly organized arrangements of contractile actin and myosin filaments. The z-line is the junction between sarcomeres, and serves as an anchorage point for actin thin filaments. Myosin thick filaments are suspended between actin thin filaments. The alternating pattern of thin and thick filaments gives skeletal muscle its striated appearance. Other regions of the sarcomere include the central A- and H-band, surrounded on either side by the I-band (Fig. 1).

Actin contains binding sites for extending myosin cross-bridges. At rest, these binding sites are blocked by thin filament accessory proteins troponin and tropomyosin. When muscle receives a stimulus to contract, troponin and tropomyosin are displaced allowing actin and myosin to bind, the filaments to slide against one another, and the sarcomere to shorten.

Please refer to the review by Frontera and Ochala for a detailed description of skeletal muscle structure and function[17].

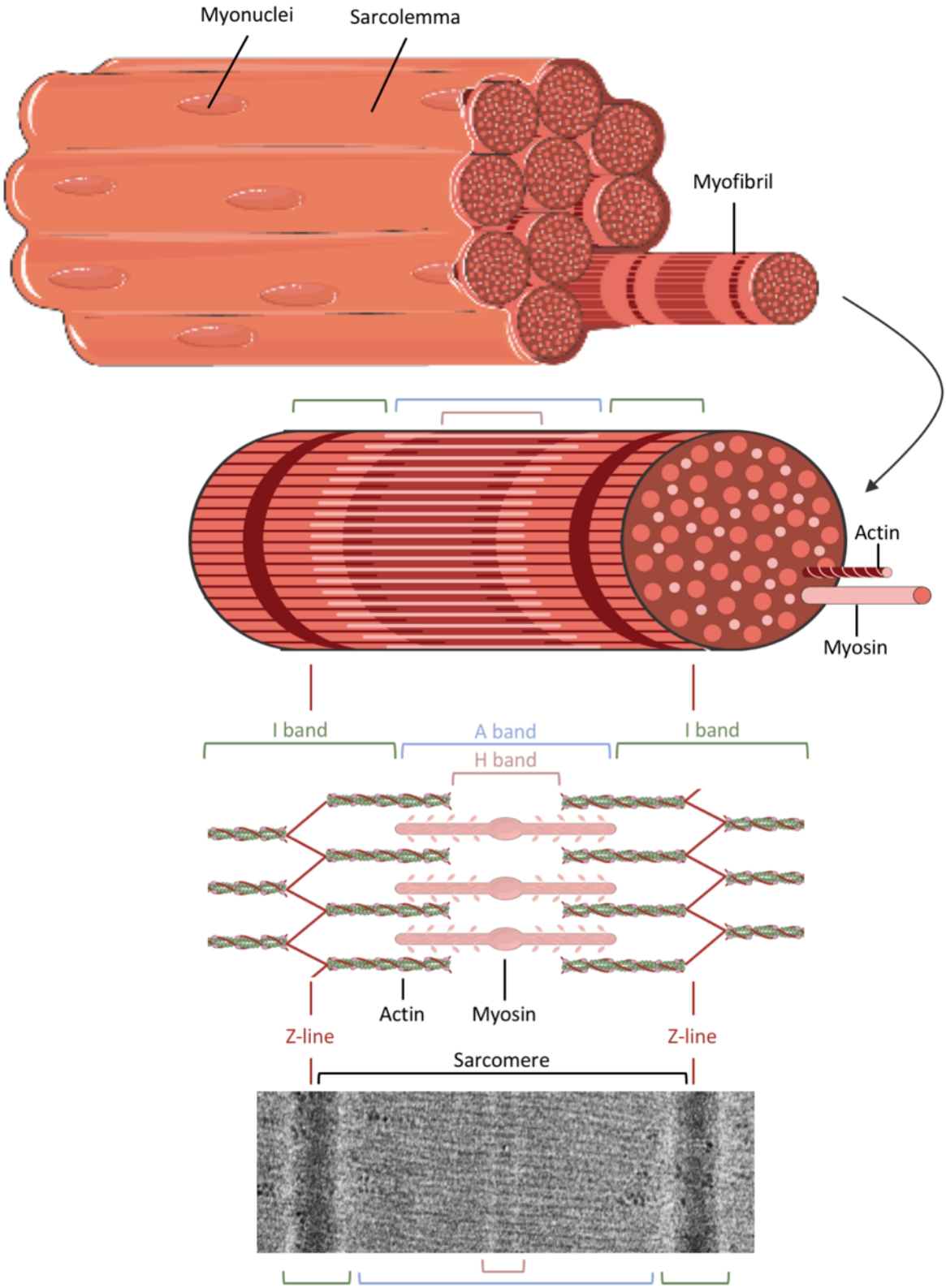
Myonuclei
Sarcolemma
Myofibril
Actin
Myosin
I band
A band
I band
H band
Actin
Myosin
Z-line
Z-line
Sarcomere

Figure 1. **Schematic representation of skeletal muscle, illustrating the muscle fascicle, myofibril and sarcomere.** The basic unit of skeletal muscle is the myofibril, which is composed of repeating units of contractile sarcomeres. The sarcomere itself is composed of actin thin filaments and myosin thick filaments. The z-line is the proteinous junction at the lateral border of the sarcomere, which anchors the actin thin filaments. The I-band surrounding the z-line contains only actin thin filaments, while the central H-band contains only myosin thick filaments. The A-band defines the entire length of the myosin thick filament. During muscle contraction, the I- and H-band shorten and the z-lines move closer together. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

2.3. Metabolism

Given its large volume, skeletal muscle has a substantial influence on whole-body metabolism. Skeletal muscle is a primary site for glucose uptake and storage, a regulator of lipid homeostasis, and a major protein reserve. In response to changing metabolic demands, muscle differentially utilizes these macronutrients (in addition to various intra- and extramuscular substrates) to produce the amount of ATP necessary for ongoing physical activity.

During high-intensity short duration exercise, muscle primarily relies on the anaerobic/glycolytic metabolism of glucose to lactate to produce energy. Whereas during prolonged low-intensity exercise, muscle relies of the aerobic/oxidative metabolism of glucose and lipids. GLUT4 is the major glucose transporter isoform expressed in skeletal muscle. During

exercise, muscle contraction stimulates GLUT4 translocation to the sarcolemma which promotes glucose uptake. Skeletal muscle metabolism is also under hormonal control. Muscle accounts for nearly 80% of insulin-mediated glucose utilization.

In humans, skeletal muscle is heterogenous. Muscle fibers are classified based on myosin ATPase content, myosin heavy chain isoform identification, as well as on the expression of enzymes that reflect the muscle fiber's metabolism (oxidative or glycolytic). Humans have three primary skeletal muscle fiber types: slow-twitch oxidative (type 1), fast-twitch oxidative glycolytic (type 2A), and fast-twitch glycolytic (type 2X). Skeletal muscle can adapt to changing metabolic requirements by altering muscle fiber type composition. For example, endurance exercise increases the proportion of type 1 muscle fibers, reflecting a concurrent increase in oxidative capacity. Certain health conditions can also alter muscle fiber types. These conditions include inhered myopathies such as muscular dystrophy, as well as acquired muscle-related disorders such as obesity and aging[18].

3. MUSCLE REGENERATION

Skeletal muscle is recognized for its dynamic ability to regenerate itself in response to injury. Injury may occur due to disease, exposure to myotoxic agents, extreme temperatures, or most commonly, the muscle's own contraction. Muscle has the capacity to regenerate up to a certain threshold[19]. Beyond this threshold, volumetric muscle loss leads to lasting functional impairments and ultimately, a reduced quality of life[20]. The phases of muscle regeneration recapitulate many aspects of embryonic muscle development[21,22]. Regeneration involves three

highly orchestrated, interdependent phases: the degenerative/inflammatory phase, the reparative phase, and the remodelling phase. The regenerative process is similar across different causes of injury; however, the extent of each phase varies with respect to the muscle damaged, the degree of damage, and the damage model used[23–25].

Table 1 summarizes the main events that occur during non-pathological skeletal muscle regeneration.

Table 1. Phases of skeletal muscle regeneration.

Phase	Main events
Degeneration/Inflammation	- Myofiber necrosis: ↑ permeability[26], ultrastructural alterations[27], ↑ serum levels of muscle proteins[28] - Inflammatory cells (neutrophils & macrophages) phagocytose debris & activate muscle stem cells[29–31]
Repair	- Revascularisation of the site of injury[32] - Satellite cells form new myofibers and/or replace injured myofibers[19]
Remodelling	- Creation of a protective fibrotic scar/scaffold[33] - Formation of NMJs & restoration of muscle contractility[34]

3.1. The degenerative/inflammatory phase

Myofiber necrosis is first observed after muscle injury. Necrosis is characterized by disruption of the myofiber sarcolemma, which increases myofiber permeability. The resulting influx of calcium ions leads to an abrupt decline in muscle strength[26]. Necrosis also involves a

loss of contractile material. Myofibrillar alterations, specifically streaming and dissolution of the z-line ultrastructure, appear immediately after mechanical damage[27]. As a consequence of the disturbance in myofibrillar integrity, molecules normally restricted to the muscle cytosol, such as creatine kinase, are liberated. Elevated serum levels of muscle proteins are commonly used as clinical biomarkers of rhabdomyolysis, as well as myocardial infarction and muscular dystrophy[28].

Necrotic cell death stimulates the inflammatory response. Complete muscle regeneration requires a balance of pro- and anti-inflammatory factors[35]. Neutrophils are the first inflammatory cell to invade the damaged myofiber. They peak in number 24-hours after injury and decline rapidly thereafter[29]. Neutrophils release high concentrations of reactive oxygen species and proteases to phagocytose cellular debris. They also secrete proinflammatory cytokines, such as interleukin-6 (Il-6), to recruit additional inflammatory cells to the site of damage, namely monocytes and macrophages[30]. By 48-hours after injury, macrophages are the predominant inflammatory cell type within the injured muscle fiber. Macrophages not only remove cellular and cytoskeletal debris, but secrete factors that activate the resident muscle stem cells[31]. Overall, the early inflammatory response both mediates muscle damage and activates the repair processes necessary for muscle recovery.

3.2. The reparative phase

The reparative phase is activated during muscle degeneration. Numerous intrinsic and extrinsic factors contribute to the repair process: muscle stem cells, muscle specific genes,

secreted factors, and the extracellular matrix (ECM). One of the first signs of muscle repair is the revascularization of the site of injury, which restores oxygen and nutrient delivery to the tissue[32]. Additional signs of muscle repair include centrally-located myonuclei and newly formed myofibers that express the embryonic isoform of myosin heavy chain (eMyHc)[36,37]. The hallmark of the reparative phase, however, is the activation of myogenic satellite cells. Satellite cells play a fundamental role in repairing and/or replacing the damaged muscle fibers.

3.2.1 Muscle stem cells

Satellite cells were first identified based on their anatomical position 'satellite' to the muscle fiber, between the basal lamina and sarcolemma[38]. They are morphologically distinct from adult muscle fibers, with abundant cytoplasm, reduced organelle content, and a small heterochromatin-rich nucleus[24]. Based on these features, it was theorized that satellite cells were resident muscle stem cells.

Isolated myofiber culture experiments revealed that satellite cells generate myoblasts[39,40]. Myoblasts were shown to fuse together and form new myotubes in both developing and regenerating skeletal muscle; however, the source of the myoblasts was previously not known[41]. Radiation and transplantation studies confirmed that grafted satellite cells could not only self-renew, but provide the myoblasts necessary for muscle regeneration *in-vivo*[42–44].

Satellite cells typically reside in a quiescent state. They are activated in response to physiological (e.g. exercise) and pathological stimuli (e.g. degenerative diseases) to generate

myoblasts[45]. Myoblasts can either fuse to each other or alternatively, fuse to an existing myofiber donating their nucleus. A small proportion of myoblasts re-enter quiescence to maintain the satellite cell pool.

It has been proposed that non-satellite cell populations can contribute to skeletal muscle repair. Progenitor cells isolated from bone marrow, neurons and other mesenchymal tissues have the capacity to differentiate into the myogenic lineage[19,46]. Though these stem cell populations have myogenic potential, experiments show that only Pax7+ satellite cells can aid in regeneration after injury and replenish the stem cell pool[47].

3.2.2 Pax7 and myogenic regulatory factors

It is well established that progression through the myogenic program relies on the expression of paired box transcription (Pax) factors and myogenic regulatory factors (MRFs).

Myogenesis is controlled by the paired-homeobox transcription factors Pax3 and Pax7. Pax3 is primarily involved in embryonic skeletal muscle development and is lethal if absent[48]. On the other hand, Pax7 is largely expressed by adult satellite cells which facilitate muscle fiber repair[49]. Quiescent satellite cells are identified by the expression of Pax7. The relationship between Pax7 and the MRFs influences myogenesis. Downregulation of Pax7 is necessary for myogenic differentiation[50].

The myogenic basic helix-loop-helix factors are MyoD, Myf5, Myogenin and MRF4. Activated satellite cells are characterized by high expression of Pax7, MyoD and/or Myf5. It is proposed that MyoD and Myf5 play distinct roles during muscle repair. MyoD is upregulated

within one day of injury and promotes satellite cell differentiation. In later stages of regeneration, MyoD is downregulated and increased expression of Myf5 and Pax7 promote satellite cell self-renewal[50,51]. Myogenin and subsequent MRF4 expression characterize myoblast terminal differentiation. Myogenin promotes fusion of myoblasts to new or injured muscle fibers. Myogenin then activates MRF4 expression to promote early differentiation and myotube formation. In maturing muscle fibers, MyoD and Myogenin expression is downregulated, while expression of MRF4 remains high[52,53].

Figure 2 illustrates myogenesis in response to an extrinsic stimulus.

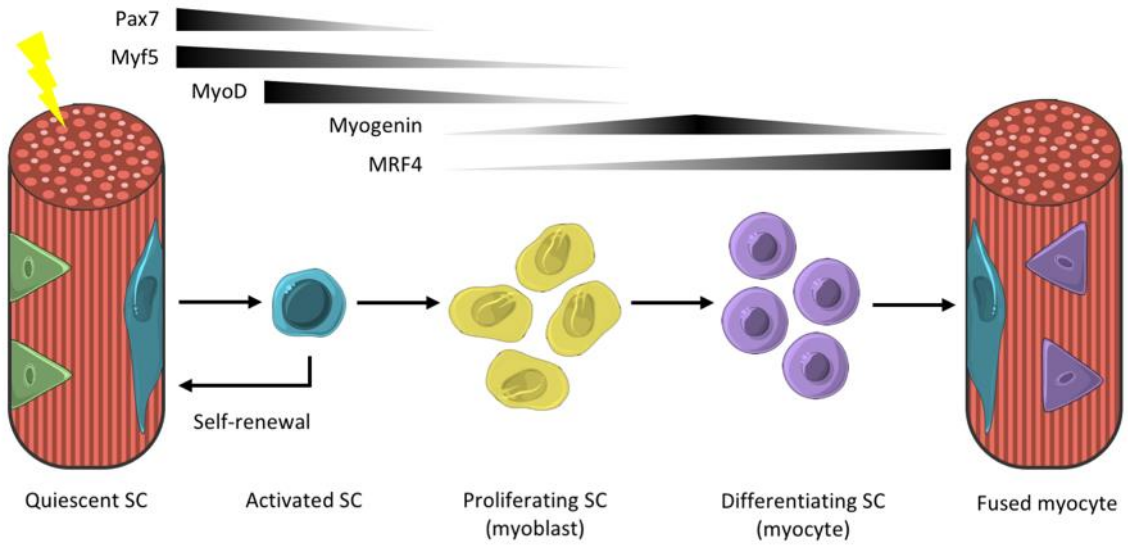

Figure 2. **Schematic illustration of myogenesis in response to muscle injury.** Satellite cells typically reside in a quiescent state beneath the sarcolemma and basal lamina of the muscle fiber. A stimulus (eg. mechanical injury) will activate the resident satellite cell population.

Activated satellite cells express the paired homeobox transcription factor, Pax7, and the myogenic regulator factors, Myf5 and MyoD. Sustained expression of MyoD and decreased expression of Pax7 promotes satellite cell proliferation and differentiation, respectively. While continued expression of Pax7 and Myf5 promotes satellite cell self-renewal. Upregulation of Myogenin and later, MRF4, promotes satellite cell differentiation. Sustained expression of MRF4 encourages differentiated satellite cells to fuse to each other or alternatively, fuse to an existing myofiber donating their nucleus. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

3.2.1 Regulators of myogenesis

Satellite cell behaviour is not only controlled by muscle specific genes, but by neighboring cells, secreted factors, and the surrounding ECM.

Endothelial cells promote satellite cell proliferation through the secretion of insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF). In turn, the differentiated myoblasts promote angiogenesis, which is essential for complete recovery of the damaged muscle[54]. Tumor necrosis factor alpha (TNF-α) is produced by activated leukocytes and stimulates muscle protein catabolism during the inflammatory response[55]. TNF-α has been shown to activate satellite cells, as well as enhance satellite cell proliferation[56]. Satellite cells are constantly surrounded by ECM components. Urokinase/plasmin can trigger a proteolytic cascade that

activates matrix-metalloproteinases (MMPs) during muscle repair. It has been proposed that MMP-9 remodels the ECM during the inflammatory phase to promote satellite cell activation[57,58].

The progressive loss of satellite cells with aging and other chronic diseases is attributed to pathological alterations to the satellite cell niche[59]. Evidently, maintenance of the stem cell niche is imperative for proper satellite cell activity and successful muscle repair.

Please refer to the review by Karalaki *et al.* for a detailed explanation of the role of regulatory factors in muscle regeneration[60].

3.3. The remodelling phase

The final regenerative phase encompasses maturation of the regenerated myofibers and restoration of the muscle's functional capacity. This stage is first characterized by production of ECM components, including: collagen, fibronectin, and laminin[34]. The initial fibrotic response strengthens and protects the site of injury. The resulting scar functions as a scaffold for new myofibers, as well as a guide for the formation of neuromuscular junctions (NMJs)[33]. Over time, the transformation of fibroblasts to myofibroblasts allows the scar tissue to contract. If the remodelling phase is prolonged, the overproduction of ECM components leads to significant scarring and the loss of muscle function.

Newly formed NMJs can be identified within two weeks of injury. Maturing neurons can directly impact protein and gene expression, which indirectly impacts satellite cell activity[61]. During this time, muscle fibers begin expressing the mature isoform of myosin heavy chain. This

is accompanied by an increase in muscle fiber size and migration of myonuclei to the fiber periphery. The remodelling phase is complete when muscle function is restored, and regenerated muscle is indistinguishable from undamaged muscle.

4. MYOPATHY IN HUMANS WITH T1D

Myopathy is a largely overlooked complication of T1D. There is strong evidence that skeletal muscle's physical and metabolic capabilities are impaired in persons with T1D. In the nondiabetic state, pancreatic insulin release into portal circulation allows the liver to be the primary regulator of glucose homeostasis. In T1D, peripheral insulin injections bypass the canonical 'liver first' pathway, forcing skeletal muscle to mediate glycemia. As a consequence, diabetic skeletal muscle is exposed to 3-5 times more glucose than normal[62,63]. For those with T1D, it is apparent that suboptimal skeletal muscle health would exacerbate dysglycemia, dyslipidemia, and the onset of insulin resistance. Therefore, preserving healthy skeletal muscle is crucial to maintaining metabolic control and mitigating the progression of diabetes-related complications.

The following sections summarize our current understanding of diabetic myopathy with respect to skeletal muscle function, structure and metabolism.

4.1. Function

Numerous studies report that skeletal muscle function is reduced in children, adolescents, and adults with T1D[64]. Children with T1D have lower aerobic fitness, maximal

oxygen consumption, and work capacity compared to their nondiabetic counterparts[16,65]. The decline in physical capacity is most pronounced in children with poor glycemic control[66]. Poor metabolic control is also associated with muscle impairments in adults with T1D. Adults with T1D have slower maximal isometric strength conduction velocity and increased muscle fatiguability compared to healthy nondiabetic controls[67]. These declines are worsened by peripheral neuropathy, a severe complication of longstanding diabetes[68,69]. Interestingly, neuropathy is not consistently correlated with reduced muscular endurance, suggesting that muscle impairments develop early in the course of diabetes[70].

Acute hyperglycemia also impairs aerobic capacity and maximal strength in those with T1D[71]. This finding highlights the difficulty of managing glycemia during exercise and may explain the variability seen in some exercise parameters. Physical activity has many health benefits for persons of all ages with T1D[72]. While exercise's impact on glycated hemoglobin levels (HbA1c, a measure of metabolic control) remain controversial, physical activity has been shown to decrease body mass index (BMI), improve insulin sensitivity, and delay health complications[73]. Despite these benefits, adults with T1D engage in less physical activity than their nondiabetic counterparts[74]. Current research is focused on understanding how different types of exercise affect glucose levels, such that persons with T1D can participate in physical activity without fear of severe hypoglycemia.

4.2. Structure

Decreased skeletal muscle mass and fiber size are strong indicators of unmanaged T1D[75]. Young, newly diagnosed persons with T1D demonstrate muscle fiber atrophy in absence of diabetic neuropathy[76]. Reske-Nielsen *et al.* examined changes to the skeletal muscle ultrastructure in those with recent onset, short-term, and long-term T1D[12]. Those with a shorter duration of diabetes had reduced muscle fiber diameters due to myofilament degradation. The sarcomere structure was also disrupted and abnormal mitochondria, lipid bodies, and glycogen granules accumulated between the myofibrils. These ultrastructural changes were exaggerated in the long-term group. Those with longstanding T1D also exhibited neurogenic alterations such as motor endplate sprouting and thickening of the capillary basement membranes. These are important findings as they emphasize the sensitivity of skeletal muscle to the T1D environment, prior to the onset of neuropathy.

4.3. Metabolism

The pathological changes to diabetic skeletal muscle function and structure are largely accompanied by alterations to metabolism. These include changes in the expression of muscle metabolic enzymes, notably increased lactate dehydrogenase activity, and decreased hexokinase and pyruvate dehydrogenase activity[77,78]. Similar metabolic impairments are seen with aging, albeit they appear in those with T1D at a much younger age[79]. Recent studies suggest that those with T1D have a greater reliance on anaerobic metabolism. Phosphorous-31 magnetic resonance spectroscopy measures taken during a bout of submaximal exercise, show

that those with T1D have decreased mitochondrial glycolytic flux and oxidative phosphorylation[80,81]. Moreover, these declines are strongly correlated with insulin resistance. The proposed dependence on anaerobic metabolism with T1D is consistent with an increase in the relative proportion of glycolytic muscle fibers and enzymes[82].

Mitochondrial dysfunction appears to be centrally implicated in the deterioration of diabetic skeletal muscle. Monaco *et al.* investigated mitochondrial bioenergetics in young adults with T1D[83,84]. They found that those with T1D had reduced oxidative capacities, specifically decreased complex II activity and increased production of mitochondrial reactive oxygen species. Furthermore, these alterations were more pronounced in T1D males. Future studies are needed to further elucidate the differential anaerobic shift in skeletal muscle metabolism observed in those with T1D.

5. MECHANISMS OF MYOPATHIC REGENERATION IN T1D

Skeletal muscle regeneration is a process critical to maintaining healthy muscle mass. Though muscle repair has not been directly investigated in humans with T1D, diabetic rodent models exhibit altered muscle regeneration after injury. Gulati and Swamy examined diabetic muscle repair by transplanting extensor digitorum longus (EDL) muscles from streptozotocin (STZ)-induced diabetic rats into nondiabetic hosts, and vice versa[85]. They found that regeneration was reduced in the diabetic hosts, irrespective of the source of the muscle. Moreover, diabetic and nondiabetic EDL muscles regenerated equally when transplanted into

nondiabetic hosts. Together, these findings suggest that poor regeneration is due to extrinsic changes in the diabetic environment, rather than intrinsic skeletal muscle dysfunction.

Alterations to the diabetic environment have been shown to negatively impact satellite cell behaviour. Following injury, satellite cells from diabetic mice fail to activate and myoblasts aberrantly fuse[86,87]. Only one study has examined satellite cells in humans with T1D. D'Souza *et al.* found that young adults with T1D (18-22 years old) had a small but significant decline in skeletal muscle satellite cell content compared to nondiabetic controls[88]. Based on mechanistic studies undertaken in Akita diabetic mice, the authors concluded that sustained activation of the Notch signaling pathway may prevent satellite cells from initiating proliferation. Given that childhood is a period of rapid muscle growth and development, altered satellite cell activity could result in chronic reductions in muscle repair and severe functional deficits by adulthood.

5.1. Oxidative stress

Mitochondrial dysfunction and oxidative stress negatively impact skeletal muscle health. A consequence of increased oxidative stress is altered satellite cell activity and concomitant impaired muscle regeneration[89,90]. Though oxidative stress is commonly associated with aged muscle, it has been shown to contribute to the progression of diabetic complications[13,91]. Hyperglycemia impairs skeletal muscle's oxidative balance, increasing free radical production and reducing antioxidant levels[92]. Satellite cells treated with ROS-inducing agents exhibit altered protein homeostasis, shortened lifespan, and reduced proliferative capacity[89]. Aragno *et al.* investigated the effects of hyperglycemia-induced oxidative stress on skeletal muscle in

STZ-diabetic rats[93]. They found that oxidative stress reduced the expression of critical muscle regulatory factors and impaired the synthesis of muscle creatine kinase. Combined, these findings suggest that diabetes-induced oxidative stress triggers a cascade of events that leads to altered satellite cell function and consequently, impaired muscle repair.

5.2. Chronic inflammation

Although the inflammatory response is required for complete skeletal muscle regeneration, chronic inflammation is associated with poor muscle repair. Chronic inflammation is present in both forms of diabetes and is likely caused by the formation of damaging advanced glycation end-products (AGE)[94]. AGEs accumulate with longstanding hyperglycemia and drive the progression of diabetes complications[95]. In skeletal muscle, chronic inflammation is linked to impaired function of immune cells, fibro/adipogenic progenitors (FAPs), and satellite cells[96]. Specifically, the imbalance of M1/M2 macrophages and increase in pro-fibrotic components creates an unfavourable environment for satellite cell activity and muscle repair[97,98].

Various chronic inflammatory factors influence satellite cell behaviour. Persons with T1D display elevated circulating levels of the pro-inflammatory cytokine, Il-6[99]. Chronic elevations in Il-6 impair satellite cell function, perhaps explaining the decrease in satellite cell content observed in those with T1D[100]. Similarly, the inflammatory mediator TNF-α is upregulated in T1D[101]. Cultured myoblasts treated with TNF-α are unable to properly differentiate, suggesting that diabetic skeletal muscle would display impaired satellite cell activity. Overall, these findings

suggest that chronic inflammation attenuates satellite cell behaviour and subsequent muscle repair in persons with T1D.

5.3. ECM Remodelling

The extracellular matrix plays an important role during muscle regeneration. After injury, degradation of the ECM releases growth factors that stimulate muscle repair[102]. The ECM is also necessary for satellite cell activity, muscle reinnervation, and the arrangement of newly regenerating muscle fibers[60,103]. In response to muscle damage, ECM remodelling allows satellite cells to properly migrate, fuse and form mature myofibers[104,105].

Collagen levels are elevated in diabetic skeletal muscle[106,107]. Plasminogen activator inhibitor 1 (PAI-1) is a critical inhibitor of the fibrinolytic system, which mediates collagen breakdown[57]. Krause *et al.* found that ECM remodelling was impaired in diabetic mice after injury, as a direct result of increased expression of PAI-1[107]. Moreover, inhibition of PAI-1 promoted macrophage and satellite cell infiltration which restored early impairments in skeletal muscle regeneration[108]. These results stress the importance of ECM remodelling in muscle regeneration, particularly in persons with T1D.

The proposed mechanisms of myopathic regeneration in persons with T1D are summarized in Figure 3.

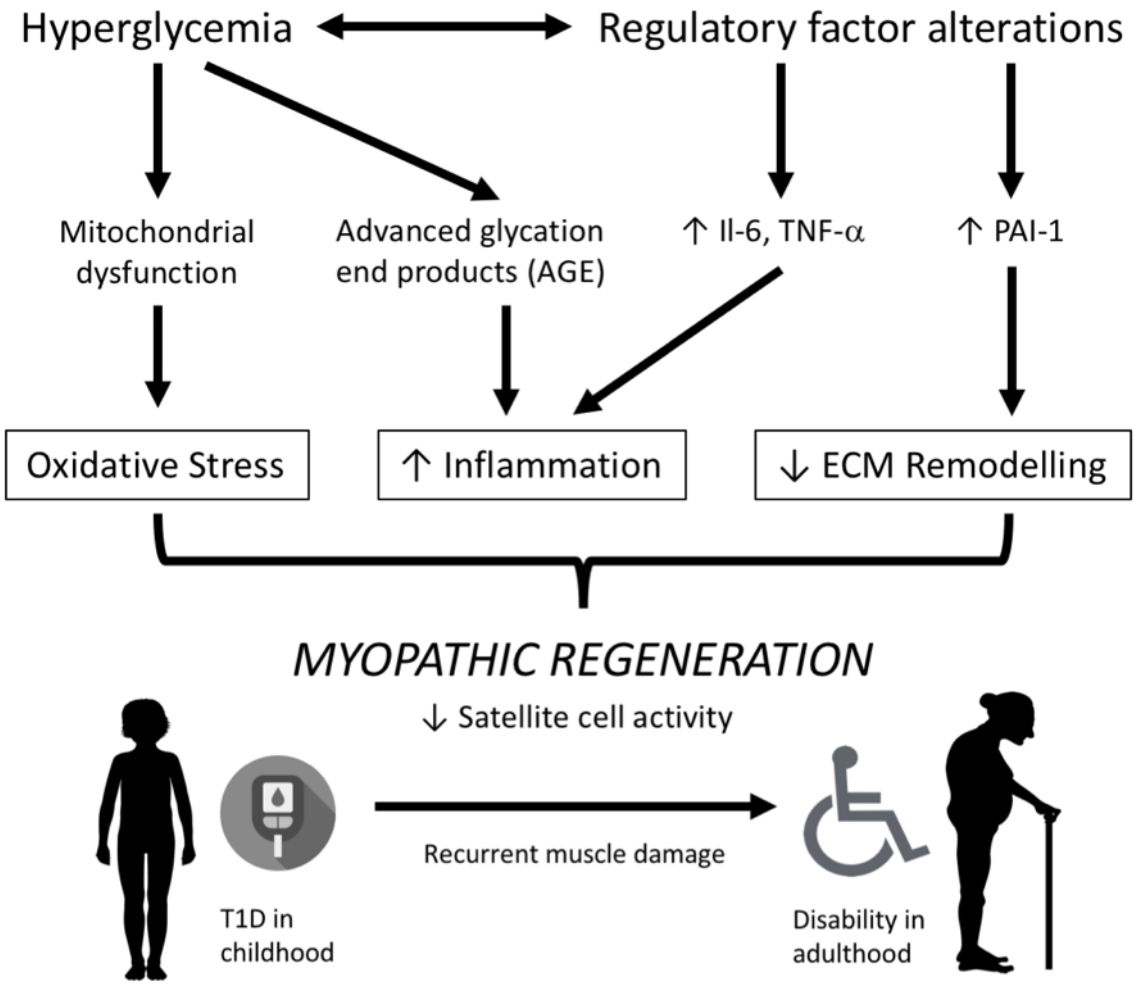

Figure 3. **Proposed mechanisms underlying myopathic regeneration in T1D**. Longstanding hyperglycemia and concurrent alterations in muscle regulatory factors contribute to diabetic myopathy. These changes lead to skeletal muscle mitochondrial impairments, the accumulation of advanced glycation end-products, and the overexpression of certain growth factors and cytokines. The consequent oxidative stress, inflammation, and ECM remodelling impairs satellite cell activity, and ultimately, skeletal muscle regeneration. T1D typically develops in childhood, a critical period of muscle growth. We theorize that recurrent attenuations in muscle

repair would account for the reduced functional capacity and premature institutionalization, which often characterizes those with T1D. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

6. STATEMENT OF PURPOSE

Declines in skeletal muscle health are observed early in the course of T1D, before other primary complications[12,16]. These impairments include mitochondrial dysfunction, decreased satellite cell content, and a progressive loss of muscle mass, power, and strength[64,109,110]. Muscle regeneration is a process critical to preserving healthy muscle mass. Unfortunately, muscle repair is consistently reduced in preclinical models of T1D[85,86,88,107,108]. As of yet, the impact of T1D on human skeletal muscle regeneration has not been established. Therefore, the purpose of this thesis is to investigate the capacity for skeletal muscle to repair after exercise-induced muscle damage in young adults with T1D.

7. MAJOR HYPOTHESIS

Young adults with T1D will display attenuated muscle repair following eccentric exercise-induced muscle damage relative to nondiabetic controls. Alterations in skeletal muscle repair on the cellular and molecular level will manifest functionally as an inability to recover force production.

8. REFERENCES

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THE CAPACITY FOR SKELETAL MUSCLE TO REPAIR AFTER EXERCISE-INDUCED MUSCLE DAMAGE IN YOUNG ADULTS WITH TYPE 1 DIABETES MELLITUS

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1. *Grace G. Grafham*: Designed the study, performed sample collection, data collection and data analysis, interpreted the results, and wrote the initial manuscript.
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ABSTRACT

There is strong evidence that skeletal muscle health is compromised in persons with type 1 diabetes mellitus (T1D). These impairments include reduced strength, mitochondrial dysfunction, and decreased satellite cell (SC) content. Maintaining healthy muscle requires successful muscle repair. Preclinical models of T1D consistently show impaired muscle regeneration. To date, the impact of T1D on human skeletal muscle repair has not been established; however, attenuated repair would account for the reduced functional capacity and premature institutionalization that often characterizes those with diabetes. The purpose of this study was to determine the impact of T1D on the recovery of skeletal muscle function, morphology, and ultrastructure after 300 unilateral eccentric contractions (90°/s) of the knee extensors. Eighteen men and women (18-30 years old) with (n=9) and without (n=9) T1D performed the exercise protocol. Pre-damage, and at 48- and 96-hours post-damage, subjects gave a blood sample and vastus lateralis biopsy, and performed a maximal isometric knee

extension. Given the sex-specific differences in muscle damage, control and T1D men and women were analyzed together and separately. Force production and recovery were comparable between control and T1D men and women. Exercise-related increases in creatine kinase activity and ultrastructural damage were also comparable between groups. There was a trend towards T1D men having more type 2 fast-twitch muscle fibers than T1D women ($p=0.055$). While baseline SC content was not different between groups, proliferating SC content was trending lower at 48-, and higher at 96-hours post-damage in T1D women compared to controls ($p=0.07$). In those with T1D, there was no correlation between muscle damage and HbA1c, but HbA1c was strongly correlated with vigorous physical activity ($\rho=0.881$, $p=0.002$). Contrary to preclinical studies, our data is the first to show that skeletal muscle repair is largely unaltered in otherwise healthy young adults with T1D. We attribute these differences to glycemic control and speculate that muscle repair is unaffected if individuals are optimally managing their diabetes. Considering the exercise-related dysglycemia seen in T1D, our results emphasize a need to define the dose of physical activity required for those with diabetes to properly regulate their blood glucose levels. We expect that this would in turn, improve skeletal muscle health and ultimately, extend the healthy lifespan of those living with T1D.

INTRODUCTION

Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by chronic hypoinsulinemia and hyperglycemia, in absence of exogenous insulin therapy. Insulin replacement therapy, though vastly extending the life expectancy of those with T1D, does not

represent a cure[1,2]. Over time, recurrent bouts of dysglycemia, dyslipidemia and the onset of insulin resistance, lead to the development of health complications[3–5].

One such complication is diabetic myopathy, a failure to maintain healthy skeletal muscle. Skeletal muscle is the largest organ system in the human body and as such, plays a pivotal role in regulating our physical and metabolic health. Diabetic muscle impairments (insulin insensitivity, mitochondrial dysfunction, and a loss of muscle mass, power and strength) appear early in the course of T1D[6–8]. Physical activity has proven beneficial for persons of all ages with T1D[9]. Therefore, understanding how diabetic skeletal muscle responds to exercise is crucial to optimizing muscle health and ultimately, mitigating the progression of diabetic complications.

Skeletal muscle regeneration is a highly orchestrated process whereby necrotic inflammation activates resident muscle stem cells (known as satellite cells) to repair and/or replace injured muscle fibers. Alterations to the diabetic environment namely, oxidative stress, chronic inflammation, and hormonal dysregulation, have been shown to impair satellite cell activity and subsequent muscle repair in T1D rodent models[7,10–14]. For example, increased expression of the hormone plasminogen activator inhibitor 1 (PAI-1) was found to prevent collagen remodelling and satellite cell migration in diabetic mice after injury[10].

To date, muscle repair has not been directly investigated in humans with T1D. Previously, we found that skeletal muscle from young adults with T1D displayed altered mitochondrial bioenergetics, including enhanced production of reactive oxygen species (ROS) which have been shown to impair human satellite cell proliferation *in-vitro*[17]. Our most notable

finding, however, was that young adults with T1D (18-22 years old) exhibited a marked reduction in skeletal muscle satellite content compared to nondiabetic controls[17]. So not only do young adults with T1D have a smaller population of satellite cells, but these cells may be ineffective in facilitating muscle repair.

Here, we hypothesized that skeletal muscle repair would be attenuated following injury in otherwise healthy young adults with T1D relative to those without diabetes. We predicted that alterations in repair on the cellular and molecular level would manifest functionally as an inability to recover force production.

METHODS

Participants

Eighteen subjects between 18-30 years old, with T1D (*male* n=4, *female* n=5) and without diabetes (*male* n=4, *female* n=5), were recruited to participate in this study. Subjects were matched on the basis of age, sex, body mass index (BMI), and level of physical activity (Table 1). Exclusion criteria included chronic health conditions with the exception of T1D, as well as significant orthopedic disability. Taking regular nonsteroidal anti-inflammatory drugs (NSAIDs), statin medication, or a history of smoking also warranted exclusion. Subjects with T1D were instructed to continue their habitual blood glucose management routine. Prior to inclusion in the study subjects gave their informed written consent. All experimental procedures were approved by the Hamilton Health Sciences Integrated Research Ethics Board (#5505).

Table 1. Participant characteristics

	Control (CON)		Type 1 diabetes (T1D)	
Characteristic	*Male*	*Female*	*Male*	*Female*
n	4	5	4	5
Age (years)	22 (2.16)	21.6 (3.91)	24 (4.62)	21 (3.94)
BMI (kg/m^2)	22.9 (0.718)	23.1 (0.955)	25.17 (3.38)	25.01 (3.44)
HbA1c (%)	5.5 (0.316)	5.24 (0.27)	7.05 (0.926)*	7.74 (2.13)*
Diabetes duration (years)	--	--	12.8 (3.60)	6.6 (6.67)
Physical Activity (MET minutes/week)[a]	3900 (1252)	4619 (3275)	6276 (3592)	3757 (2658)

Data are expressed as means (SD)

[a]Computed using the International Physical Activity Questionnaire (IPAQ)

*Significant difference from control group, $p<0.05$.

Study Design

The study design is illustrated in Figure 1. For each subject, one leg was randomly selected to perform the study protocols, described below. During an initial visit to the research clinic (Visit #1), eligible subjects performed a baseline maximal voluntary isometric contraction of the knee extensors, followed by a vastus lateralis muscle biopsy and venous blood draw. Subjects returned ten days later (Visit #2) and completed the eccentric exercise-induced muscle damage protocol, followed by a maximal isometric knee extension. 48- (Visit #3) and 96-hours (Visit #4) after the eccentric damage protocol, subjects performed another maximal isometric contraction, and gave a muscle biopsy and blood draw. Subjects were told to refrain from exercising and using NSAIDs throughout the duration of the study.

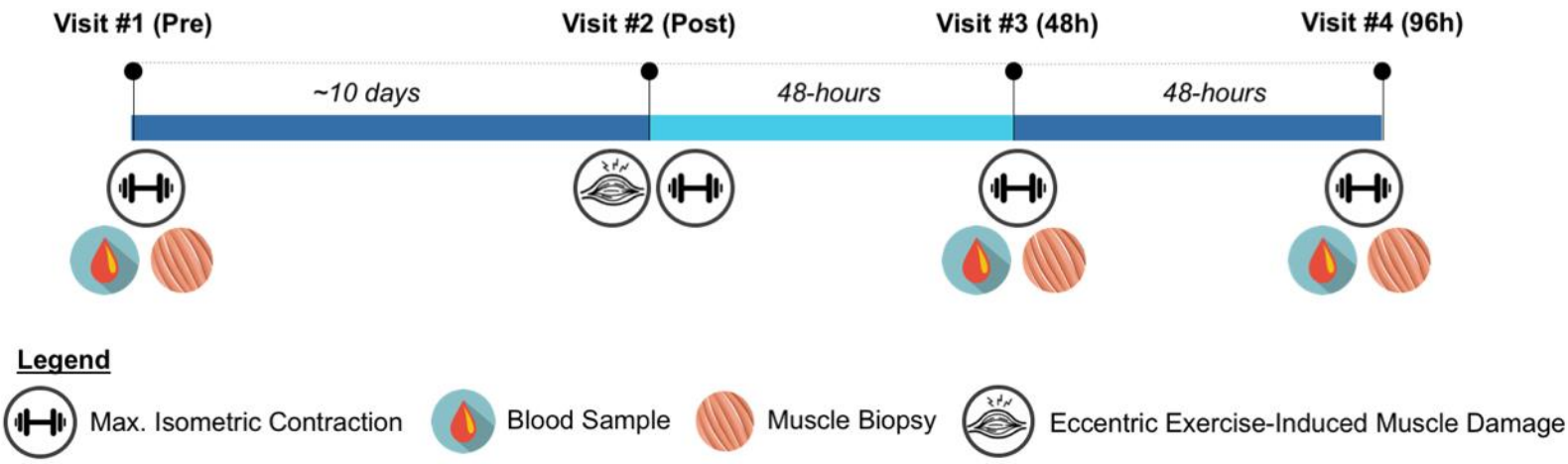

Figure 1. **Graphical illustration of study design.**

Muscle Biopsy

Percutaneous needle biopsies were collected by a trained physician from the midportion of the vastus lateralis using a 5 mm Bergstrom needle. The muscle biopsy procedure was performed at baseline, and repeated 48- and 96-hours following the eccentric damage protocol. Each repeated biopsy was spaced 3 cm apart to minimize any effect of the previous biopsies. Upon excision, muscle samples were divided and prepared for analysis.

Blood Collection

Blood samples were obtained from the antecubital vein immediately following the muscle biopsy procedure pre-damage, and at 48- and 96-hours post-damage. The blood collection was performed by a medically trained and certified member of the laboratory group. Blood samples (~12 mL) were collected in an EDTA-containing tube and a tube without anti-

coagulants. Samples were centrifuged at 2000 rpm for 15 minutes. Aliquots of plasma and serum were frozen in liquid nitrogen and stored at -80°C. An additional blood sample was collected pre-damage for HbA1c analysis. Analysis of HbA1c and serum creatine kinase activity were performed by the McMaster Medical Centre Core Laboratory.

Eccentric Exercise-Induced Muscle Damage

Three-hundred unilateral maximal isokinetic muscle-lengthening contractions were performed on the Biodex Dynamometer (Biodex-System 3). Each contraction began with the subject's leg positioned at 80° of knee flexion. As the subject forcefully extended their leg, the Biodex Dynamometer resisted the extension force causing the subject's muscle to lengthen as their knee was bent to 20° of flexion. Contractions were performed at a rate of 90°/s over a range of 60° of movement. The protocol was divided into 30 sets of 10 contractions, with 1 minute of rest between sets. Subjects were briefly familiarized with the equipment prior to the intervention. During each set, subjects received visual feedback on a computer screen and were verbally encouraged by the investigators. This protocol has been shown to elicit a substantial degree of skeletal muscle damage[18].

Maximal Voluntary Isometric Contraction

Maximal voluntary isometric knee extensions were performed on the Biodex Dynamometer (Biodex-System 4). The test consisted of three 5-second maximal knee extensions, with 30 seconds of rest between contractions. A knee angle of 90° was used for

each contraction. The peak recorded torque for the set was taken as the subject's maximal isometric strength. The assessment was performed prior to the muscle biopsy, immediately post, and at 48- and 96-hours after eccentric muscle damage.

Double Antibody Fluorescent Immunohistochemistry

Due to technical limitations, frozen and formalin-fixed paraffin-embedded skeletal muscle tissue samples were used for immunohistochemical analysis. Primary and secondary antibodies used for immunohistochemical staining are detailed in Table 2.

Frozen Muscle Sections

Fresh muscle samples were mounted in Tissue Tek optimal cutting temperature (OCT) compound and frozen in liquid nitrogen cooled 2-methylbutane. The OCT embedded samples were stored at -80°C prior to sectioning. Muscle cross-sections (6 μm thick) were prepared, allowed to air dry, and fixed in 4% paraformaldehyde. Sections were 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 1 hour at room temperature. Tissue sections were then incubated in the primary antibody against myosin heavy chain 1 (MyHc1) at 4°C overnight. Following primary antibody incubation, sections were incubated in the secondary antibody Alexa fluor 488 goat anti-mouse for 2 hours at room temperature. For double antibody staining, tissue sections were re-fixed, blocked, and incubated for 2 hours in the primary antibody against laminin. Last, sections were incubated in

the secondary antibody Alexa fluor 488 goat anti-rabbit for 2 hours. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Washes between incubations were performed using Tris-buffered saline.

Formalin-Fixed Paraffin-Embedded Muscle Sections

Fresh muscle samples were fixed in 4% paraformaldehyde at 4°C overnight and then stored in 70% ethanol. After embedding into paraffin wax, muscle cross-sections (6 μm thick) were prepared. Sections were deparaffinized, rehydrated, and subjected to citrate-mediated high-temperature antigen retrieval (0.01 M citrate buffer). Tissue sections were then incubated in 3% hydrogen peroxide for 30 minutes and blocked in Tris-NaCl blocking buffer (0.1 M Tris-HCl, 0.15 M NaCl, 0.5% Tyramide Signal Amplification Blocking Reagent in distilled water) for 1 hour. After which, sections were incubated in the primary antibody against Pax7 at 4°C overnight. A secondary biotinylated goat anti-mouse IgG1 antibody was then applied for 1 hour at room temperature. Following secondary antibody incubation, sections were incubated with Vectastain Elite ABC reagent for 2 hours (Vector) and the Tyramide Signal Amplification Kit (Akoya Biosciences; fluorescein) for 10 minutes. For double antibody staining, sections were re-blocked and incubated in the appropriate primary antibody (laminin or Ki67) at 4°C overnight. A secondary horseradish peroxidase (HRP) goat anti-rabbit IgG antibody was applied for 1 hour at room temperature, followed by the Tyramide Signal Amplification Kit (cyanine 3: laminin and Ki67) for 10 minutes. Last, nuclei were counterstained with DAPI, and sections were incubated

for 30 minutes in Sudan Black B to remove autofluorescence. Washes between incubations were performed using Tris-buffered saline.

Table 2. Details of primary and secondary antibodies used for immunohistochemical staining of frozen and formalin-fixed paraffin-embedded muscle sections.

Antibody	*Host Species*	*Source*	*Details*	*Dilution*
Primary				
Anti-Laminin	Rabbit	Abcam	Ab11575	1:500
Anti-MyHc1	Mouse	Developmental Studies Hybridoma Bank	A4.951 Slow isoforms	1:1
Anti-Pax7	Mouse	Developmental Studies Hybridoma Bank	Pax7-s	1:1
Anti-Ki67	Rabbit	Biocare Medical	CRM 325	1:300
Secondary				
Alexa Fluor® 488 Anti-Mouse IgG1	Goat	Invitrogen	A-21121	1:1000
Alexa Fluor® 488 Anti-Rabbit IgG	Goat	Invitrogen	A-21467	1:500
Biotin-SP Anti-Mouse IgG1	Goat	Jackson ImmunoResearch Laboratories	115-065-205	1:300
HRP Anti-Rabbit IgG	Goat	Abcam	Ab7171	1:300

Immunohistochemical Analysis

Fluorescent images were captured with a Nikon 90 Eclipse microscope (Nikon Inc., Melville, New York) and analysed using Nikon Elements AR software v4.6. At least ≥100 muscle

fibers per subject per time point were included in the analyses for minimum feret diameter, muscle fiber type, and total/proliferating satellite cell content. In resting muscle, laminin is in close proximity to the plasma membrane, and was therefore used as a surrogate plasma membrane marker for quantification of minimum feret diameter. Fiber type analysis was performed by quantifying the total number of MyHc1-positive and negative fibers. Satellite cells were identified via co-staining of Pax7 and DAPI, and localization beneath the basal lamina. Satellite cell content was expressed as a percentage of total muscle fibers analyzed. Proliferating satellite cells were identified via colocalization of Pax7, Ki67 and DAPI, and were expressed as a percentage of total satellite cell content. All immunohistochemical analyses were completed by one researcher in a blinded fashion.

Transmission Electron Microscopy

Fresh muscle samples were immediately fixed in 2% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, and processed as previously described[19]. Ultra-microtomy was performed; longitudinal sections (65 nm thick) were then mounted onto copper grids and contrasted with uranyl acetate and lead citrate. Electron micrographs were acquired using a JEOL JEM-1200EX transmission electron microscope (JEOL, Peabody, MA, USA). Z-line morphology was examined in a blinded fashion. Approximately 10-30 unique fibers were analyzed for muscle damage per subject (control, *male* n=4, *female* n=4; T1D, *male* n=4, *female* n=4), with the exclusion of hypercontracted fibers. The quantification for z-line streaming was performed based on work by Newham and Gibala[20,21]. A disrupted fiber constituted any disturbance to the typical

myofibrillar banding pattern. An area occupying 1-2 adjacent myofibrils and/or 1-2 continuous sarcomeres was categorized as 'focal' disruption (Fig. 2B). An area occupying 3-10 adjacent myofibrils, 3-10 continuous sarcomeres, and/or ≥10 areas of focal disruption was categorized as 'moderate' disruption (Fig. 2C). An area occupying ≥10 adjacent myofibrils, ≥10 continuous sarcomeres, and/or ≥10 areas of moderate disruption was categorized as 'extreme' disruption (Fig. 2D). Fibers were categorized based on the greatest level of disruption observed. For statistical analysis, fiber disruption was quantified as a weighted percentage of both the degree of damage (focal, moderate, extreme) and the total number of disrupted fibers.

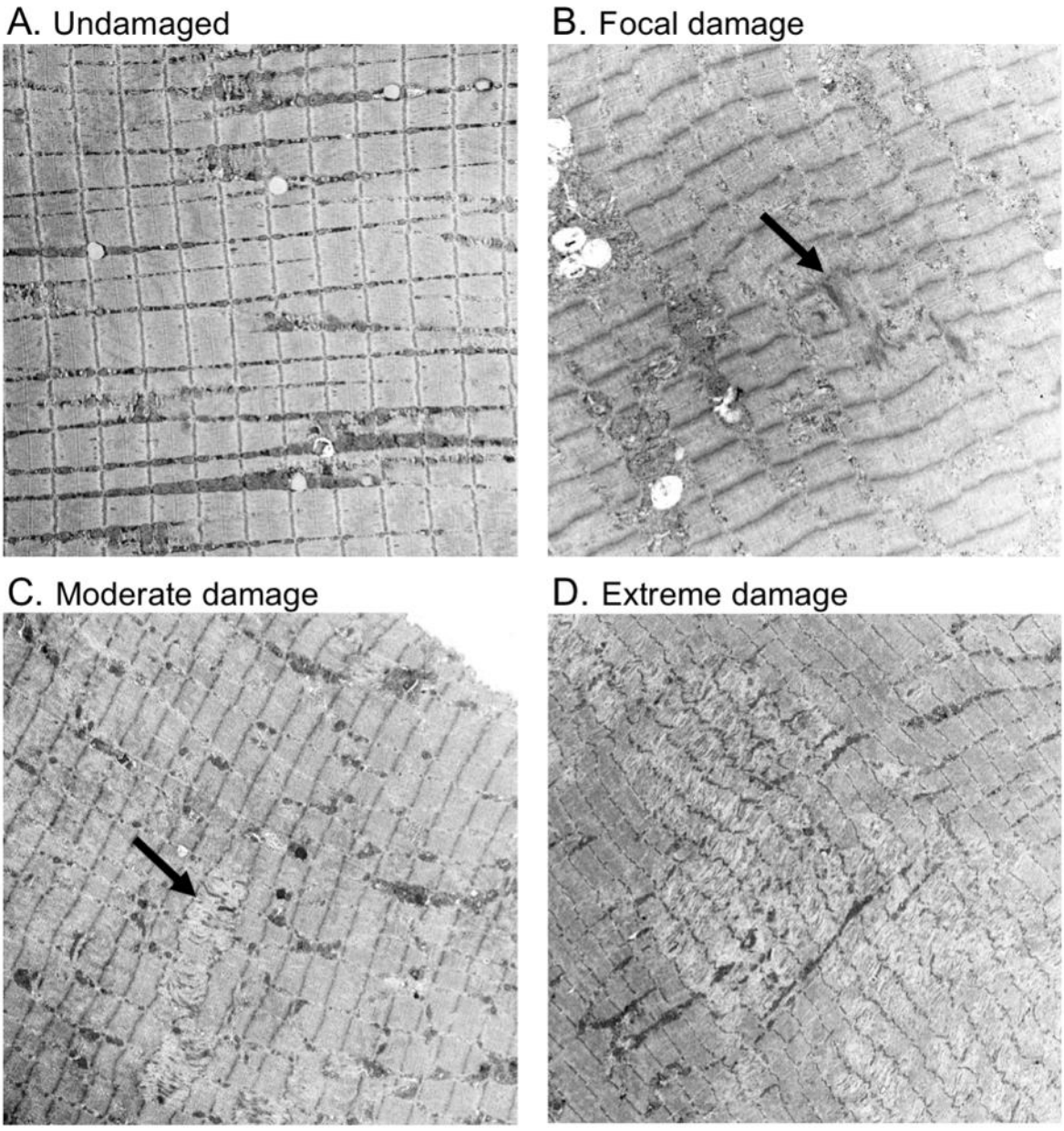

Figure 2. **Sample electron micrographs illustrating the levels of myofibrillar disruption.** (A) Undamaged muscle (x7,500). (B) Focal damage, black arrow (x10,000). (C) Moderate damage, black arrow (x6,000). (D) Extreme damage (x4,000).

Sample Size Calculation

The sample size for this study was calculated by power analysis with skeletal muscle satellite cell content as the primary outcome. Due to the novelty of the current study, we found no past work—in type 1 or type 2 diabetics—that matched our prospective criteria. Therefore, our sample size was calculated based on a previous study from Nederveen *et al.,* which measured satellite cell content following eccentric damage of the knee extensors in young men with high and low skeletal muscle capillarization[22]. At 24-hours post-damage, satellite cell content increased by 7.94% (high) and 0.91% (low), with a pooled standard deviation of 4.975%. Based on these results, we estimated that 8 subjects per group were needed to achieve statistical significance (α=0.05, β=0.8) using a two-way repeated measures ANOVA.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism v7.0 and IBM SPSS v23.0. Subject characteristics and baseline muscle measures were analyzed using parametric or nonparametric independent sample t-tests, where appropriate. Analysis of serum creatine kinase activity was performed on log transformed data. Post-damage muscle measures were compared between groups using a two-way repeated measures ANOVA with Sidak's post-hoc test, when necessary. A series of spearman's rho associations were performed for subjects with T1D. Significance was set at $p<0.05$, and is represented as significantly different from *control group, #males, †same sex control group, ‡opposite sex of the same group, §pre-damage value. Data are presented as means $\pm$ standard error of the mean (SEM), unless otherwise stated.

RESULTS

Results are organized into three sections: baseline, overall, and response. 'Baseline' comparisons were made between groups prior to eccentric damage. 'Overall' comparisons were made between timepoints following eccentric damage, irrespective of sex or diabetes status. 'Response' comparisons were made between groups and timepoints following eccentric damage, accounting for sex and diabetes status.

Participant Characteristics

Subjects' anthropometric and metabolic characteristics are summarized in Table 1.

Baseline. Control and T1D subjects were similar in age, BMI and self-reported physical activity level.

Skeletal Muscle Fiber Characteristics

Pathological changes to the skeletal muscle architecture are often associated with declines in physical performance. To determine whether structural differences were present before muscle damage, we first compared subjects' fiber type distribution (Fig. 3B) and average fiber size (Fig. 3C) using immunohistochemistry. Based on previous literature, we hypothesized that those with T1D would have a greater proportion of type 2 muscle fibers relative to matched controls.

Baseline. All subjects displayed a greater proportion of type 2 muscle fibers compared to type 1 ($p<0.001$; Fig. 3B). Contrary to our hypothesis, fiber type proportions were similar

between control and T1D subjects, collectively (p=0.737; Fig. 3B, *right*). Fiber type proportions were also similar between men and women (p=0.145; *left, center*); however, men had significantly larger muscle fibers than women, irrespective of diabetes status (p=0.0018; Fig. 3C). There were no significant differences in muscle fiber type proportion or fiber size between control and T1D men (p=0.296, p=0.636; Fig. 3B-C, *left*), and control and T1D women (p=0.546, p=0.405; Fig. 3B-C, *center*). Interestingly, the proportion of type 2 muscle fibers was trending higher in T1D men compared to T1D women (p=0.055; Fig. 3B, *left*, *center*).

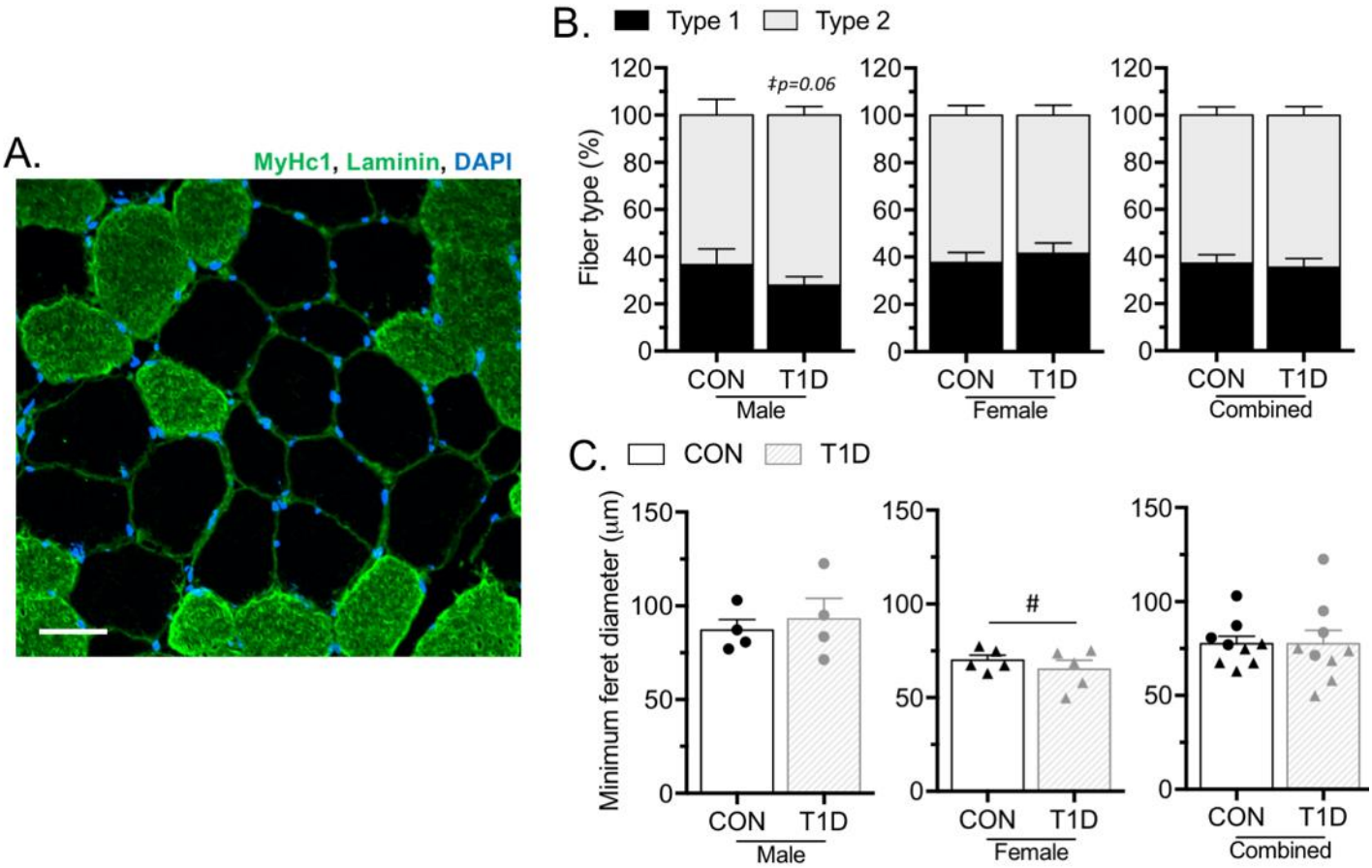

Figure 3. **Baseline myofiber architecture**. (A) Representative image of a MyHc1/laminin/DAPI stained skeletal muscle cross-section. Characterization of skeletal muscle (B) fiber type distribution and (C) fiber size in control (CON) and type 1 diabetes (T1D) men and women. Panels of (B) and (C) show comparisons between (left) males, (center) females, and (right) both sexes combined. Data are expressed as means ± SEM. ‡Significant difference from the opposite sex of the same group, $p<0.05$. #Significant difference from males, $p<0.05$. Scale bar=50μm.

Maximal Isometric Strength Production and Recovery

In addition to structural alterations, the progressive loss of muscle strength has been shown to contribute to physical disability. To assess subjects' functional capacities, we compared their baseline quadriceps force production and ability to regain isometric strength after eccentric contractions (Fig. 4).

Baseline. Prior to muscle damage, men were collectively stronger than women ($p=0.001$; Fig. 4A-B, *left*). Maximal isometric strength was not significantly different in control men compared to T1D men ($p=0.354$; Fig. 4A, *left*), and control women compared to T1D women ($p=0.872$; Fig. 4B, *left*).

Overall. Maximal isometric strength was significantly reduced immediately post, and at 48- and 96-hours post-damage, as compared to pre-damage ($p<0.0001$; Fig. 4C, *right*). There was no change in strength recovery between 48- and 96-hours post-damage ($p=0.986$).

Response. Strength recovery was similar between collective groups: male versus female ($p=0.173$; Fig. 4A-B, *right*) and control versus T1D ($p=0.235$; Fig. 4C, *right*). Similarly, there was

no significant difference in strength recovery between control and T1D men (p=0.203; Fig. 4A, *right*), control and T1D women (p=0.872; Fig. 4B, *right*), control men and women (p=0.267; Fig. 4A-B, *right*), and T1D men and women (p=0.607; 4A-B, *right*).

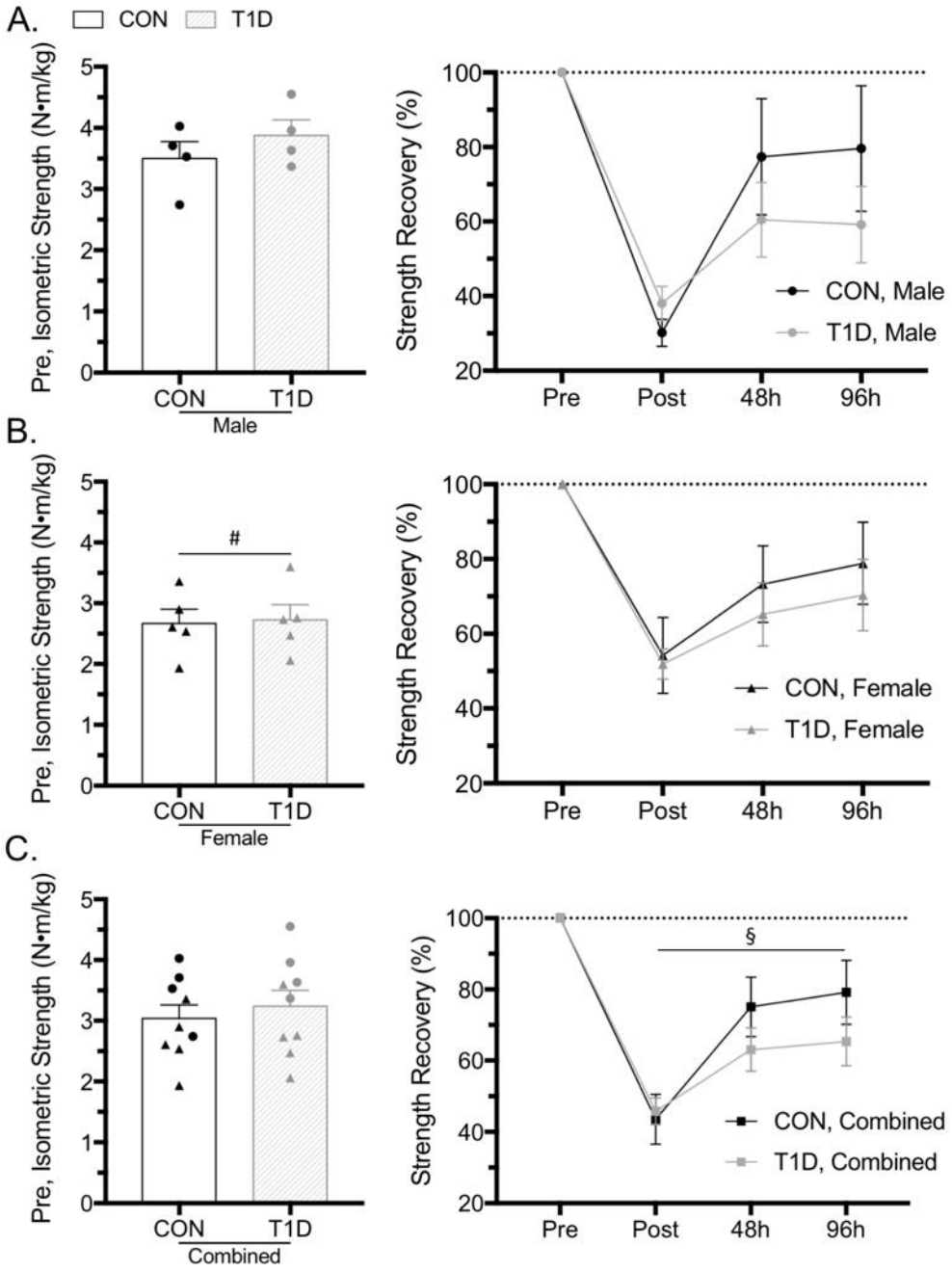

Figure 4. **Force production and recovery from eccentric damage**. (A-C, left panel) Pre-damage maximal isometric strength normalized to body weight in control (CON) and type 1 diabetes (T1D) men and women. Left panels show comparisons between (A, •) males, (B, ▲) females and (C) both sexes combined. (A-C, right panel) Characterization of strength recovery from baseline (Pre, 100%), immediately (Post), and at 48- and 96-hours after eccentric damage in CON and T1D men and women. Right panels show comparisons between (A, •) males, (B, ▲) females and (C, ■) both sexes combined. Data are expressed as means ± SEM. #Significant difference from males, $p<0.05$. §Significant difference from pre-damage, $p<0.05$.

Serum Creatine Kinase Activity

The appearance of creatine kinase in the blood frequently serves as an indirect marker of muscle injury. Therefore, to evaluate muscle damage indirectly we compared subjects' serum creatine kinase levels prior to and following eccentric contractions (Fig. 5, Table 3).

Baseline. Prior to the intervention, serum creatine kinase levels were similar between groups: male versus female ($p=0.426$; Fig. 5, *left*, *center*) and control versus T1D ($p=0.118$; Fig. 5, *right*). Serum creatine kinase activity was also comparable between control and T1D men ($p=0.135$; Fig. 5, *left*), control and T1D women ($p=0.590$; Fig. 5, *center*), control men and women ($p=0.837$; Fig. 5, *left*, *center*), and T1D men and women ($p=0.316$; Fig. 5, *left*, *center*).

Overall. Serum creatine kinase levels were significantly increased at 48- ($p<0.001$) and 96-hours ($p<0.0001$) after eccentric damage, as compared to pre-damage (Fig. 5, *right*). Serum creatine kinase activity peaked at 96-hours post-damage (versus 48-hours, $p=0.003$).

Response. Collectively, men had significantly higher serum creatine kinase levels than women at 48- (p=0.0001) and 96-hours (p=0.0011) after muscle damage (Fig. 5, *left*, *center*). However, creatine kinase levels were not significantly different between control and T1D men (p=0.507; Fig. 5, *left*), and control and T1D women (p=0.266; Fig. 5, *center*).

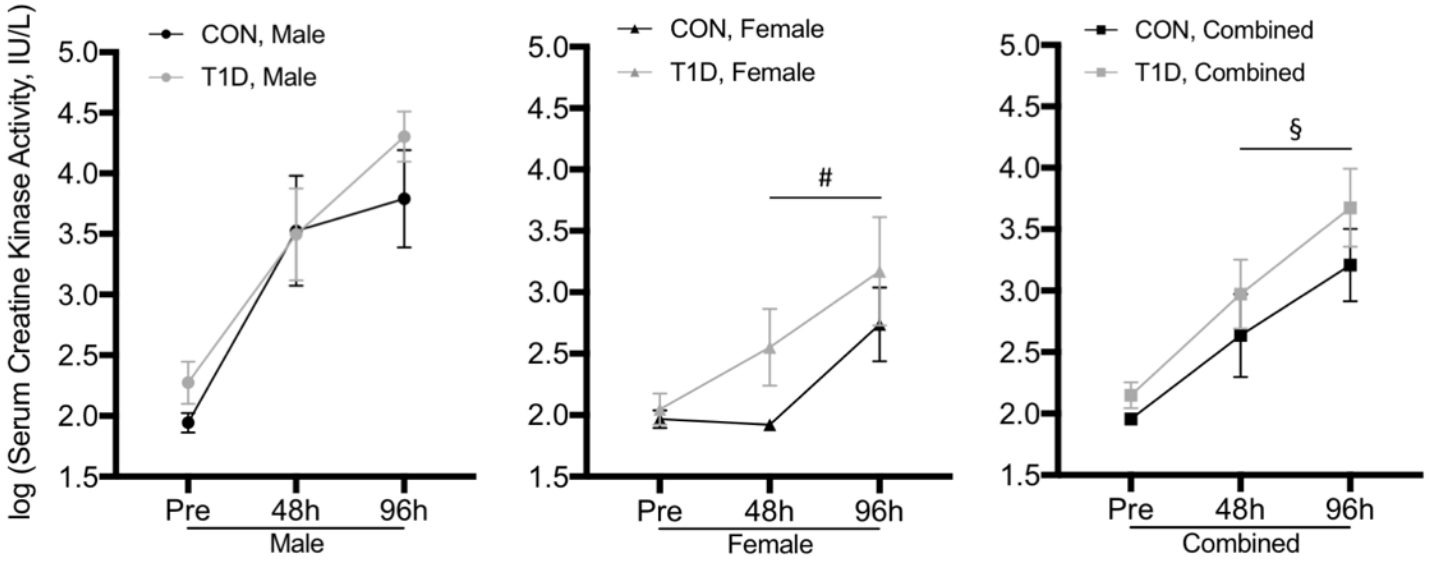

Figure 5. **Serum creatine kinase responses to eccentric damage**. Characterization of serum creatine kinase activity in control (CON) and type 1 diabetes (T1D) men and women, pre-, and at 48- and 96-hours post-eccentric muscle damage. Comparisons between (left panel, •) males, (center panel, ▲) females, and (right panel, ■) both sexes combined. Log transformed data are shown. Data are expressed as means ± SEM. #Significant difference from males, p<0.05. §Significant difference from pre-damage, p<0.05.

Table 3. Serum creatine kinase levels (IU/L) for males and females with and without T1D at baseline, and 48- and 96-hours after eccentric muscle damage.

		Baseline	48-hours	96-hours
CON	*Male*	92 (15)	11,377 (8,174)	15,391 (8,645)
	Female	98 (17)	85 (9)[#]	1,498 (1,082)[#]
T1D	*Male*	241 (105)	8,212 (5,851)	28,377 (13,588)
	Female	132 (40)	835 (463)[#]	5,256 (2,691)[#]

Data are expressed as means (SEM). #Significant difference from males, $p<0.05$. Main effect of time, $p<0.05$.

Total Satellite Cell Response

Satellite cells are essential for successful muscle repair and were therefore a primary focus of the current study. To assess subjects' satellite cell population, we used immunohistochemistry to measure total satellite cell content at baseline and following eccentric muscle damage (Fig. 6). Based on previous findings, we hypothesized that those with T1D would have less satellite cells relative to matched controls.

Baseline. Surprisingly, baseline satellite cell content was similar between control and T1D subjects (p=0.140; Fig. 6F, *right*). Satellite cell content was also similar between men and women collectively (p=0.152; Fig. 6F, *left*, *center*), as well as between control and T1D men (p=0.686; Fig. 6F, *left*), control and T1D women (p=0.332; Fig. 6F, *center*), control men and women (p=0.417; Fig. 6F, *left*, *center*), and T1D men and women (p=0.413; Fig. 6F, *left*, *center*).

Overall. Satellite cell content was significantly increased at 48- and 96-hours after eccentric damage, as compared to pre-damage ($p<0.001$; Fig. 6F, *right*). There was no change in satellite cell content between 48- and 96-hours post-damage ($p>0.999$).

Response. Following eccentric damage, satellite cell content was similar between groups: male versus female ($p=0.630$; Fig. 6F, *left*, *center*) and control versus T1D ($p=0.641$; Fig. 6F, *right*). Likewise, post-damage satellite cell content was comparable between control and T1D men ($p=0.434$; Fig. 6F, *left*), control and T1D women ($p=0.978$; Fig. 6F, *center*), control men and women ($p=0.404$; Fig. 6F, *left*, *center*), and T1D men and women ($p=0.928$; Fig. 6F, *left*, *center*).

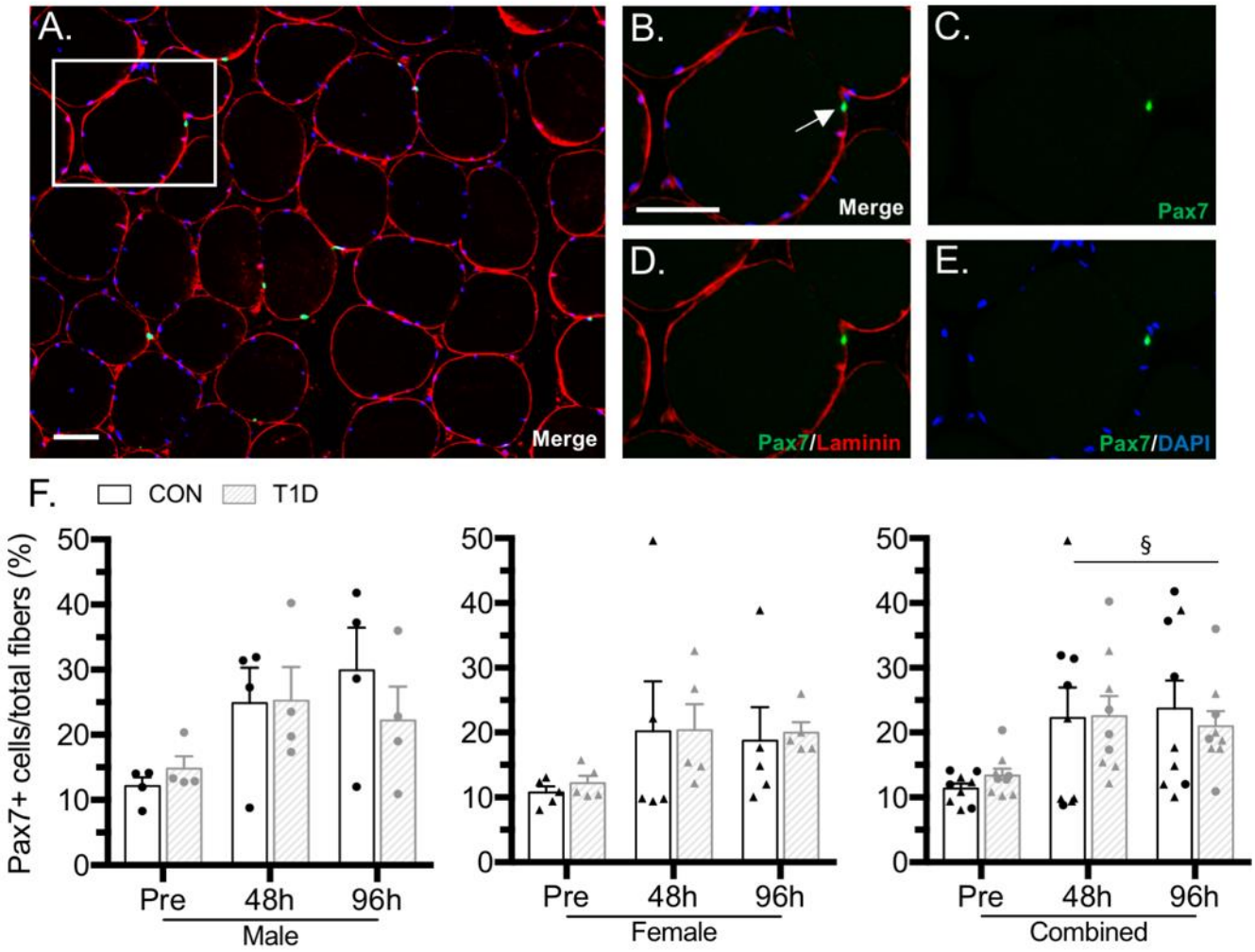

Figure 6. **Skeletal muscle satellite cell response to eccentric exercise.** (A) Representative image of a Pax7/laminin/DAPI stained skeletal muscle cross-section. Channel views of (B) merge, (C) Pax7, (D) Pax7/laminin, and (E) Pax7/DAPI. White arrow indicates a satellite cell expressing Pax7 and DAPI, and localized beneath the basal lamina. (F) Characterization of total skeletal muscle satellite cell content in control (CON) and type 1 diabetes (T1D) men and women pre-, and at 48- and 96-hours post-eccentric muscle damage. Comparisons between (left panel, •) males, (center panel, ▲) females, and (right panel) both sexes combined. Data are expressed as means

± SEM. #Significant difference from males, p<0.05. §Significant difference from pre-damage, p<0.05. Scale bar=50μm.

Satellite Cell Proliferation

Muscle repair is also dependent on satellite cells' ability to properly activate and progress through the myogenic program. Though we observed no notable differences in total satellite cell content, we were interested in determining whether there were differences in satellite cell proliferation (Fig. 7).

Baseline. Prior to eccentric damage, there were few proliferating satellite cells (Fig. 7F). Baseline satellite cell proliferation was similar between collective (sex, diabetes status) and sex/diabetes status-separated groups (p>0.05; Fig. 7F).

Overall. Proliferating satellite cell content was significantly increased at 48- and 96-hours after muscle damage, as compared to pre-damage (p<0.01; Fig. 7F, *right*). There was no significant change in satellite cell proliferation between 48- and 96-hours post-damage (p>0.999).

Response. Following eccentric contractions, satellite cell proliferation was comparable between control and T1D subjects (p=0.211; Fig. 7F, *right*). Women had collectively fewer proliferating satellite cells than men at 48- (p=0.015), but not 96-hours (p=0.463) post-damage (Fig. 7F, *left*, *center*). Satellite cell proliferation was similar between control and T1D men (p=0.735; Fig. 7F, *left*). Interestingly, satellite cell proliferation was trending higher at 48-hours

and lower at 96-hours post-damage, in T1D women as compared to control women (p=0.07; Fig. 7F, *center*).

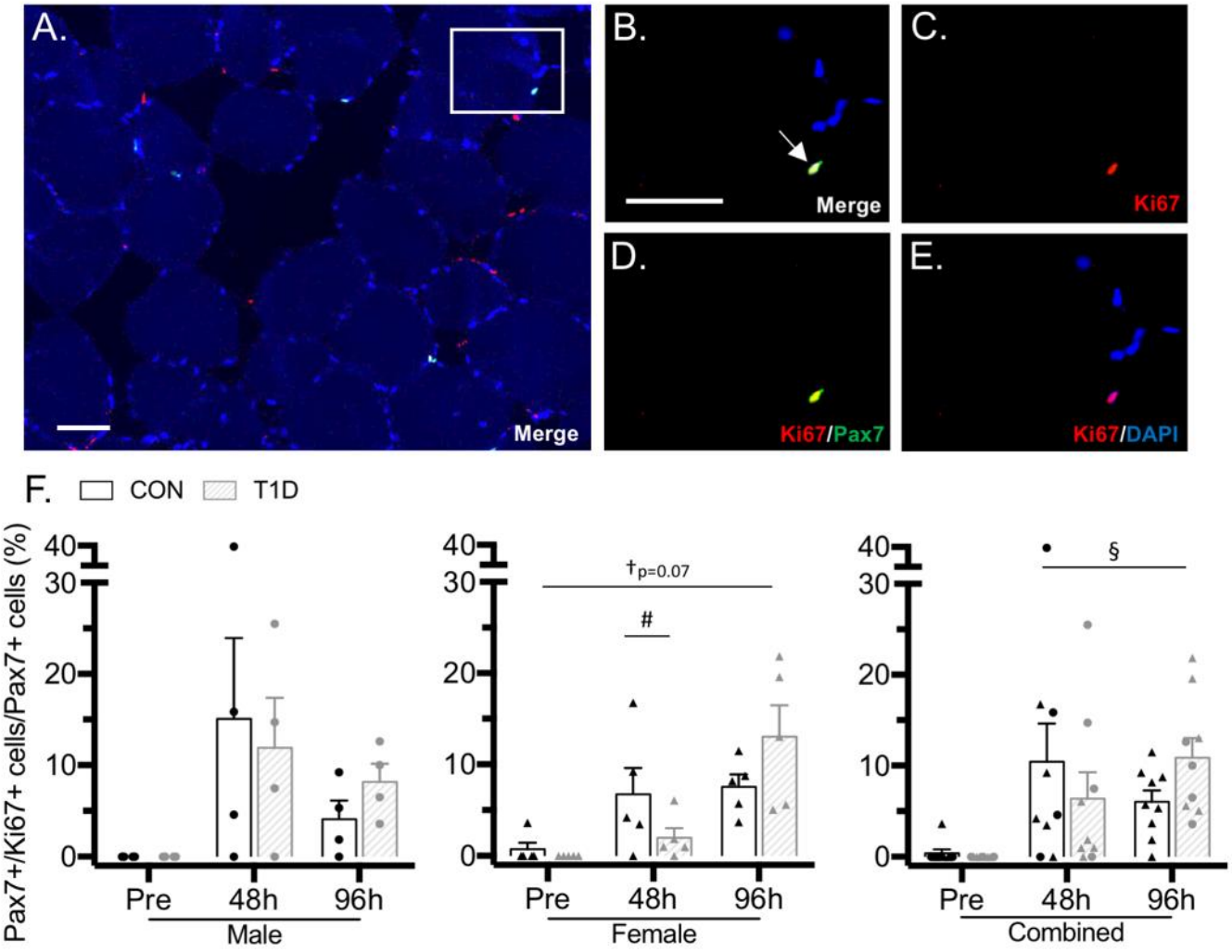

Figure 7. **Satellite cell proliferation following muscle damage**. (A) Representative image of a Ki67/Pax7/DAPI stained skeletal muscle cross-section. Channel views of (B) merge, (C) Ki67, (D) Ki67/Pax7, and (E) Ki67/DAPI. White arrow indicates a proliferating satellite cell expressing Ki67, Pax7 and DAPI. (F) Characterization of proliferating satellite cell content in control (CON)

and type 1 diabetes (T1D) men and women, pre- and at 48- and 96-hours post-eccentric muscle damage. Comparisons between (left panel, •) males, (center panel, ▲) females, and (right panel) both sexes combined. Data are expressed as means ± SEM. #Significant difference from males, $p<0.05$. §Significant difference from pre-damage, $p<0.05$. †Significant difference from control group of the same sex. Scale bar=50μm.

Ultrastructural Disruption

Muscle damage also results in dissolution of the z-line ultrastructure. We next used transmission electron microscopy to investigate ultrastructural muscle alterations. We compared the weighted percentage of myofiber disruption between subjects prior to and following eccentric damage (Fig. 8).

Baseline. Prior to the intervention, there were few subjects with ultrastructural myofibrillar disruption (Fig. 8). The weighted percentage of myofibrillar disruption was similar between collective (sex, diabetes status) and sex/diabetes status-separated groups ($p>0.05$; Fig. 8).

Overall. Weighted myofibrillar disruption increased at 48- and 96-hours post-damage, as compared to pre-damage ($p<0.01$; Fig. 8, *right*). There was no change in ultrastructural disruption between 48- and 96-hours after eccentric damage ($p>0.05$).

Response. Following eccentric contractions, weighted myofibrillar disruption was similar between groups: male versus female ($p=0.567$; Fig. 8, *left, center*) and control versus T1D ($p=0.278$; Fig. 8, *right*). Myofibrillar disruption was also comparable between control and T1D

men (p=0.579; Fig. 8, *left*), control and T1D women (p=0.386; Fig. 8, *center*), control men and women (p=0.907; Fig. 8, *left, center*), and T1D men and women (p=0.450; Fig. 8, *left, center*).

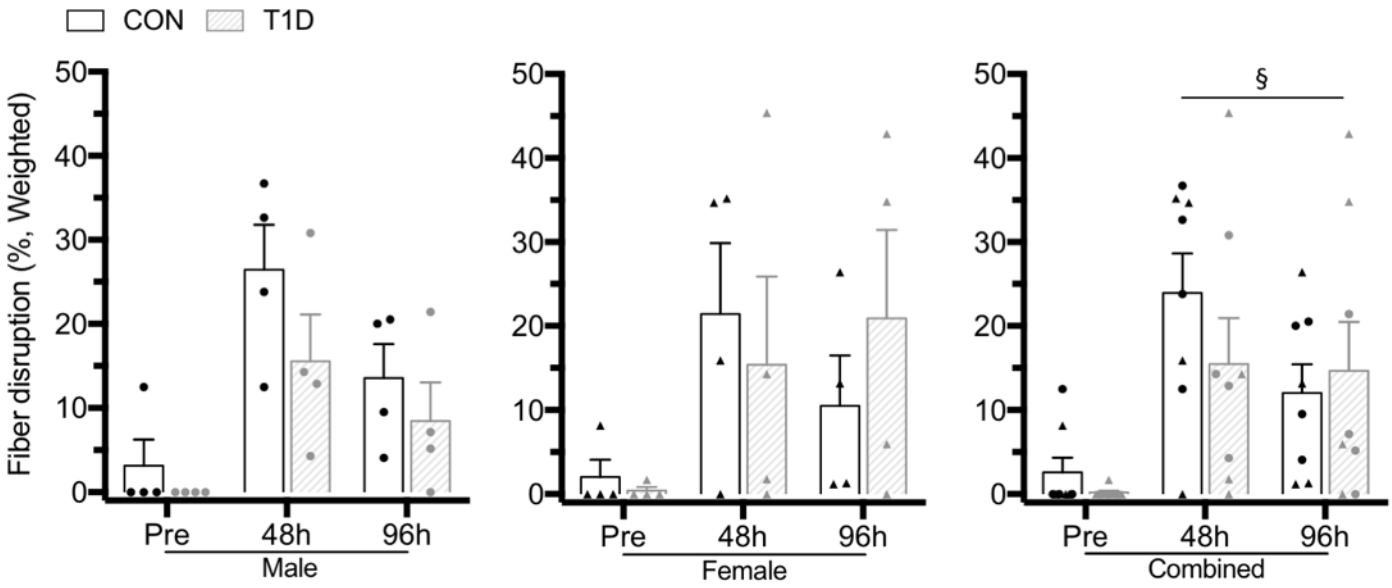

Figure 8. **Exercise-mediated disruption of the skeletal muscle ultrastructure.** Characterization of ultrastructural disruption based on the number and severity of damaged myofibers in control (CON) and type 1 diabetes (T1D) men and women, pre- and at 48- and 96-hours post-eccentric muscle damage. Comparisons between (left panel, •) males, (center panel, ▲) females, and (right panel) both sexes combined. Data are expressed as means ± SEM. §Significant difference from pre-damage, $p<0.05$.

Correlation Analysis

Last, to discern potential physiological influences on muscle repair, we aimed to associate T1D subjects' anthropometric and metabolic characteristics with the various indices

of muscle damage. Because the 48- and 96-hour timepoints were similar between most damage markers (with the exception of serum creatine kinase activity), the 48-hour timepoint was selected for the associations. Relevant spearman's rho associations are summarized in Table 4.

In those with T1D, HbA1c was strongly positively correlated with vigorous physical activity (ρ=0.881, p=0.002), but not with any of the selected markers of muscle damage. At 48-hours after eccentric damage, weighted ultrastructural disruption was highly positively correlated with both the percent change in total satellite cell content (ρ=0.857, p=0.007) and the number of regenerating myofibers (ρ=0.805, p=0.016; Fig. S1). Subjects' isometric strength immediately after eccentric damage was strongly negatively correlated with satellite cell proliferation at 48-hours (ρ=-0.728, p=0.026) and serum creatine kinase activity at 96-hours (ρ=-0.867, p=0.002) post-damage.

Table 4. Spearman's rho associations between T1D subjects' characteristics and indices of muscle damage.

Variables	**Hba1c (%)**	**Vigorous physical activity (MET min./ week)**	**SC content (ΔPre-48h)**	**Proliferating SC content (%, 48h)**	**eMyHc+ fibers (%, 48h)**	**Fiber disruption (%, 48h)**	**Peak serum creatine kinase (IU/L)**
Vigorous physical activity (MET min./week)	**0.881*** ***0.002***						
SC content (ΔPre-48h)	-0.31 *0.417*	-0.194 *0.617*					
Proliferating SC content (%, 48h)	0.109 *0.78*	0.352 *0.353*	0.192 *0.62*				
eMyHc+ fibers (%, 48h)	-0.255 *0.507*	-0.069 *0.861*	0.61 *0.081*	0.34 *0.37*			
Fiber disruption (%, 48h)	-0.31 *0.456*	-0.12 *0.776*	**0.857*** ***0.007***	0.383 *0.349*	**0.805*** ***0.016***		
Peak serum creatine kinase (IU/L)	-0.285 *0.458*	-0.042 *0.914*	0.45 *0.224*	0.444 *0.232*	0.153 *0.695*	0.476 *0.233*	
Isometric strength (% of Pre, Post)	0.293 *0.444*	0.025 *0.948*	-0.35 *0.356*	**-0.728*** ***0.026***	-0.22 *0.569*	-0.381 *0.352*	**-0.867*** ***0.002***

The first number is the spearman's rho for the appropriate pair, with the associated p-value shown in italics beneath. *Significant associations ($p<0.05$) are shown in bold type.

DISCUSSION

There is strong evidence that skeletal muscle health is compromised early in the course of T1D[7,23]. Considering the importance of skeletal muscle in maintaining metabolic control, it is apparent that suboptimal muscle health would impair glucose and lipid homeostasis, leading to insulin resistance and the onset of diabetic complications. In those with T1D, physical activity improves insulin sensitivity and delays health complications[9]. However, the capacity for humans with T1D to recover from damaging exercise has not been determined. For the first time, we show that muscle repair in young adults with moderate-well controlled T1D is comparable to those without diabetes. Contrary to preclinical studies, we observed no robust declines in the recovery of diabetic muscle function, morphology, and ultrastructure after muscle injury. These findings not only expand our understanding of diabetic muscle repair, but emphasize the importance of exercise as a therapeutic tool for managing T1D.

The following sections will discuss subjects' 1) collective, 2) sex-specific, and 3) T1D-specific responses to eccentric damage.

Collective Response to Eccentric Damage

It is well established that a single bout of intensive unaccustomed eccentric exercise can induce substantial muscle damage. Symptoms of eccentric damage include a marked decline in muscle strength, concurrent with the loss of myofibrillar integrity, liberation of muscle proteins, and initiation of muscle repair[24]. Collectively, our findings parallel past reports on eccentric exercise-induced muscle damage in humans[21,25–28]. We found that maximal isometric strength

was lowest immediately after eccentric damage; strength improved in the proceeding 48- and 96-hours but did not return to baseline. Serum creatine kinase activity increased at 48-hours and peaked at 96-hours post-damage. Skeletal muscle satellite cell content also increased following eccentric damage, as did satellite cell proliferation and ultrastructural disruption.

Unexpectedly, we observed few changes in muscle damage between the 48- and 96-hour timepoints. Multiple factors influence subjects' responses to eccentric damage, including their age and training status, as well as the type and intensity of the exercise protocol[29–31]. Given the diversity of subjects in the current study, we reason that their responses to eccentric damage were too variable to discern significant differences between the timepoints.

Sex-Specific Response to Eccentric Damage

Sex-specific differences in exercise-induced muscle damage have been frequently reported in humans and animal models. These differences are generally attributed to the female sex hormone 17β-estradiol and the male sex hormone testosterone.

Muscle strength is associated with muscle fiber size, a correlate of muscle mass[32,33]. Consistent with the literature, we found that men were collectively stronger and had larger muscle fibers than women[34]. This is likely due to the anabolic effects of testosterone which upregulate protein synthesis and cause muscle fiber hypertrophy[35]. Males also tend to have a lower proportion of type 1 muscle fibers than females; however, we did not observe any sex-specific differences in MyHc isoform expression in the current study[36]. It is important to acknowledge that our fiber typing protocol only allowed for comparisons between type 1 and

type 2 MyHc isoforms. A full muscle fiber type analysis, including hybrid fibers, may yield results consistent to what is commonly reported.

Sex-specific differences in creatine kinase activity are controversial. We found that men had greater elevations in serum creatine kinase activity than women post-damage, but not pre-damage. Consistent with our findings, attenuated creatine kinase responses have been reported in female rats after electrical muscle stimulation[37]. Researchers speculate that 17β-estradiol stabilizes the sarcolemma during force production, protecting it from mechanical damage[38]. Although males had greater creatine kinase levels than females, strength recovery was comparable between sexes. This discrepancy has also been reported in the literature, where creatine kinase levels are not representative of declines in isometric strength[39–41].

Consistent with the literature, we found that males had more proliferating satellite cells than females 48-hours post-damage[42]. The greater proliferative capacity of male satellite cells is attributed to testosterone-mediated increases in insulin-like growth factor-1 (IGF-1) and transforming growth factor-beta (TGF-β) signaling[43–45]. In terms of skeletal muscle ultrastructure, men and women experienced a similar degree of myofiber disruption. Sex-specific differences in ultrastructural muscle damage have not been previously described; however, baseline ultrastructure appears similar between sexes[46].

Together, our findings indicate that responses to exercise-induced muscle damage differ between sexes. As such, we chose to compare muscle damage between control and T1D subjects together and in a sex-specific manner.

Diabetes-Related Response to Eccentric Damage

The primary focus of the current study was to evaluate the capacity for skeletal muscle to repair following eccentric damage in otherwise healthy young adults with T1D. We hypothesized that skeletal muscle repair would be attenuated in those with T1D relative to those without diabetes. Moreover, we predicted that alterations in repair on the cellular and molecular level would manifest functionally as an inability to recover force production.

Changes in skeletal muscle oxidative capacity are often reflected by shifts in MyHc isoform expression and concomitant shifts in metabolic enzyme activity[47,48]. Fritzsche *et al.* previously showed that persons with T1D have greater type 2X fast glycolytic muscle fibers, and fewer type 1 slow oxidative muscle fibers relative to nondiabetic controls[49]. Others have reported similar increases in glycolytic enzyme activity in T1D, indicative of an overall increase in glycolytic muscle fibers and a shift towards glycolytic metabolism[50]. Contrary to these findings, we did not observe any differences in muscle fiber MyHc isoform expression between control and T1D subjects, collectively. There was however, a trend towards greater type 2 fast-twitch muscle fibers in T1D men compared to women. Recently, Monaco *et al.* found that T1D alters skeletal muscle mitochondrial respiration to a larger extent in men than women. Specifically, T1D men had increased complex I sensitivity but decreased complex II sensitivity and capacity[51]. The sex-specific differences in mitochondrial respiration mirror the increase in type 2 muscle fibers seen in T1D men, but not women. Albeit, this relationship is purely speculative, considering we did not analyze myofiber metabolic enzyme activity in the current

study. Nonetheless, these observations suggest that men and women with T1D exhibit differential responses in skeletal muscle metabolism.

Myofiber atrophy and decreased force production are observed in persons with untreated T1D, as well as those with longstanding diabetes and consequent neuropathy[52–54]. Force production is also decreased in persons with T1D following acute hyperglycemia[55]. We found that muscle fiber size, force production, and strength recovery were not significantly different between control and T1D men, and control and T1D women. Almeida *et al.* similarly showed that baseline maximal isometric strength was comparable between adults with and without T1D[56]. Albeit, diabetic skeletal muscle fibers were slower conducting and more fatigable. Interestingly, the researchers did not observe the latter findings in T1D subjects with good glycemic control (HbA1c=6.67±0.79%). This is in agreement with previous observations showing that hyperglycemia induces ROS and nitrous oxide (NO) production, leading to impaired muscle contractility in diabetic rodents[57,58]. Those with T1D in the current study had moderate-well controlled diabetes (HbA1c=7.43±1.65%) and did not exhibit symptoms of peripheral neuropathy. Therefore, isometric strength appears to be unaffected if individuals are managing their diabetes well and are euglycemic at the time of the assessment.

In line with muscle fiber composition and force production, we observed no differences in baseline satellite cell content between men and women with and without T1D. This is in contrast to previous work by D'Souza *et al*. which showed that young adults with T1D had fewer skeletal muscle satellite cells relative to those without diabetes[17]. This discrepancy may be due to differences in glycemic control, with subjects in the current study exhibiting notably lower

glycosylated hemoglobin levels (HbA1c=7.43±1.65% versus 8.40±0.27%). Hyperglycemia-induced changes to the muscle stem cell niche, namely oxidative stress and chronic inflammation, have been shown to impair satellite cell behaviour[12,55]. When exposed to a high glucose environment, satellite cells were found to differentiate into adipocytes, depleting the skeletal muscle stem cell pool[59]. Therefore, it is plausible that satellite cell content is unaltered if persons with T1D are regulating their blood glucose levels.

The failure of satellite cells to progress through myogenesis and return to quiescence may also deplete the satellite cell pool[60]. While satellite cell content was normal at rest, we speculated that those with T1D may display altered satellite cell activity after muscle injury, akin to diabetic rodents[10]. Contrary to our hypothesis, we observed no significant differences in total or proliferating satellite cell content between control and T1D subjects, post-damage. Previously, our laboratory and others have demonstrated that aberrant cell signaling (Notch, Myostatin, TGF-β, Smad3) inhibits satellite cell activation in diabetic mice after injury[13,17]. But, treatment of diabetic mice with insulin rescues satellite cell function and mediates the abovementioned signaling pathways[13]. Though insulin appears to restore muscle repair, it is possible that alternative regenerative processes may still be impacted. For example, excessive collagen accumulation has been shown to impair macrophage infiltration in diabetic mice after muscle injury[11]. Furthermore, alterations in angiogenesis have been found to hinder tendon repair in diabetic rats[61]. Future studies should investigate alternative muscle repair processes in humans with T1D.

Though satellite cell proliferation was comparable between control and T1D subjects collectively, there was a trend for T1D women to have less proliferating satellite cells than control women, post-damage. This observation indicates that women with T1D may experience delayed satellite cell proliferation, perhaps due to hormonal dysregulation. For instance, the hormone IGF-1 enhances satellite proliferation *in-vitro,* yet circulating IGF-1 is largely decreased in persons with T1D[62,63]. Hormonal contraceptives, of which 4/5 T1D women reported using, have also been shown to inhibit IGF-1 expression[64,65]. We reason that a cumulative reduction in circulating IGF-1, due to the combined effects of diabetes, female sex, and hormonal contraceptives, may explain the delay in satellite proliferation in women with T1D. Though IGF-1 was not directly investigated in the current study, this finding nonetheless highlights the importance of taking a sex-specific approach to diabetes care.

In response to metabolic and mechanical disruption the permeability of the muscle fiber plasma membrane increases, allowing for the loss of myofibrillar proteins into circulation. Howard *et al.* found that diabetic myocytes displayed poor plasma membrane integrity, leading to a higher level of membrane repair failure in diabetic mice after injury[66]. Consistent with these findings, Jevrić-Čaušević *et al.* reported that humans with T1D expressed higher baseline serum creatine kinase levels than nondiabetic controls. Albeit, diabetic creatine kinase levels were within the normal range (T1D=132.90±17.90 U/L, normal=22-198 U/L)[67]. Frank *et al.* attributed the aforementioned elevations in creatine kinase activity to diabetic peripheral neuropathy, which Jevrić-Čaušević *et al.* did not investigate[68]. In line with the latter, we observed no differences in pre- or post-damage creatine kinase activity between otherwise

healthy young adults with T1D and those without diabetes. In general, it appears that male sex and peripheral neuropathy have a greater influence on creatine kinase activity than diabetes alone.

Congruent with serum creatine kinase activity, we observed no differences in ultrastructural disruption between control and T1D subjects. In 1977, Reske-Nielsen *et al.* showed that persons with short-term T1D displayed ultrastructural skeletal muscle damage: myofilament degradation, abnormal mitochondria, and the accumulation of lipids and glycogen[4]. Moreover, these alterations were exacerbated in those with longstanding T1D and consequent neurological impairments. Considering the major advances in insulin therapy and diabetes care since 1977, persons with T1D in the current study were likely able to better manage their disease. New diabetes technologies like real-time continuous glucose monitoring devices have been shown to reduce glycosylated hemoglobin levels, as well as increase the time persons with T1D spend in euglycemia. Our results highlight the efficacy of novel insulin therapy and further suggest that skeletal muscle health is unaltered if diabetes is properly managed.

A common theme in this discussion is the importance of glycemic control. It is well established that good glycemic control prevents diabetic complications, including myopathy. HbA1c is the gold standard for monitoring glycemic control and is a dominant predictor of all-cause mortality[69]. While individual markers of muscle damage were similar between control and T1D subjects, we aimed to evaluate the impact of glycemic control on skeletal muscle repair. Surprisingly, we found that HbA1c (and likewise, diabetes duration) was not correlated

with any index of muscle damage. Diabetes Canada defines poor glycemic control as having an HbA1c $\geq$ 7.5%, yet only 3 subjects in the current study exceeded this value[9]. Considering our T1D population was 1) physically active and 2) had generally well controlled diabetes, we reason that they were unlikely to exhibit attenuated muscle repair following eccentric exercise.

Our study also highlights a widely-recognized concept – that animal research may be a poor predictor of human responses. Animal models of T1D have contributed enormously to our understanding of diabetes pathophysiology and continue to be the first step in investigating novel therapies. Researchers have consistently shown that skeletal muscle repair is attenuated in rodent models of T1D[10,14,70]. The majority of these experiments, however, were performed on uninsulinized rodents, which does not reflect the current standard of care for humans with T1D. Contrary to past rodent work, our data suggests that muscle repair is unaltered in humans with moderate-well controlled diabetes. Our early findings emphasize a need to examine muscle regeneration in insulin-treated diabetic animal models, and to continue to validate animal research in humans with T1D when possible.

CLINICAL CONSIDERATIONS

Exercise bestows many health benefits on persons with T1D. These benefits range from increased cardiovascular fitness to lowered insulin requirements, improved endothelial function, and ultimately, an enhanced quality of life[71]. To achieve these benefits, healthcare professionals recommend that adults (with and without diabetes) engage in at least 150 minutes of moderate- to vigorous-intensity aerobic physical activity per week, with at least 2

days per week of resistance training[72]. More exercise bestows even greater health benefits. Thus, our study demonstrates that young adults with moderate-well controlled T1D can engage in higher levels of physical activity without compromising their skeletal muscle health.

Despite the undeniable benefits of regular exercise, more than 60% of persons with T1D remain sedentary[73]. The reasons for this are multifaceted, but largely include fear of severe hypoglycemia and inadequate knowledge around exercise management[74]. It is well established that exercise type, intensity and duration have disparate effects on blood glucose in those with T1D[75,76]. These glycemic responses are further influenced by counterregulatory hormones, food intake, and one's insulin regimen[77–79]. The dysglycemic responses to exercise in T1D were apparent in the current study, where those with higher levels of vigorous physical activity exhibited poorer glycemic control.

Unfortunately, the appropriate dose of exercise needed for improved metabolic control in those with T1D remains unclear[80]. Small clinical trials suggest that resistance training in adults with T1D improves glycemic control (through moderate reductions in HbA1c), enhances insulin sensitivity, and increases muscular strength[80]. Compared to aerobic exercise, Yardley *et al.* found that resistance training mitigated T1D dysglycemic responses both during and after exercise[81,82]. The eccentric damage protocol in the current study was similar to a bout of resistance training—high-intensity activity performed in relatively short-duration intervals. Considering the positive effect of resistance training on metabolic control, our results suggest that those with T1D can safely engage in resistance exercise beyond the recommended 2 days per week.

In those with T1D, exercise-mediated improvements in skeletal muscle health can improve glycemic control, and vice versa. But, overall glycemic control is not enhanced without appropriate nutritional and insulin management[83]. Future studies are necessary to understand the impact of exercise type, intensity and duration on glycemic responses in persons with T1D.

LIMITATIONS AND FUTURE DIRECTIONS

A major limitation of our study is the wide inter- and intra-subject variability. The variability is most apparent in subjects' responses to eccentric muscle damage, particularly serum creatine kinase activity. Previous studies have shown a similar spread in subjects' responses to eccentric exercise; however, the origin of this variability is unclear[31]. Responses appear to be dependent on personal characteristics (age, sex, training status, muscle composition), as well as on the exercise protocol (type, intensity, duration)[29,30,84]. In the current study, we chose to include subjects of both sexes and of various training backgrounds. Furthermore, we selected an intense exercise protocol that elicited muscle damage beyond a typical bout of physical activity. We reason that these choices contributed to the wide variability in subjects' responses to eccentric damage. In future, we hope to explore of how different subgroups (eg. sedentary versus resistance trained) respond to lower intensities of eccentric exercise.

The muscle biopsy itself may represent a source of variation. While muscle biopsy is the gold standard for diagnosing myopathies, it may not be sensitive to milder conditions like exercise-induced muscle damage. In patients with inflammatory myopathy, Diaz *et al.* found

that muscle biopsies were not homogenous and serial sections from different levels were required for accurate diagnosis[85]. Another limitation could be the location of muscle biopsy. Skeletal muscle damage involves the rupture of myofibrils near the myotendinous junction[86]. It is unsafe to biopsy the myotendinous junction in humans, therefore, muscle biopsies are taken from the midportion of the vastus lateralis. The heterogeneity and suboptimal location of the muscle biopsy may explain the variability in subjects' responses. Alternatively, ultrasound or magnetic resonance imaging can be used to characterize muscle injury[87]. These techniques are non-invasive and in conjunction with muscle biopsy, would allow for improved characterization of exercise-induced skeletal muscle damage.

A final limitation of our study is the sample size (control, *male* n=4, *female* n=5; T1D, *male* n=4, *female* n=5). Our small sample size likely exacerbated the variability in subjects' responses to eccentric damage. Nonetheless, this study is the first to directly investigate skeletal muscle repair in humans with T1D. We speculate that current trends in the data may become more apparent with a larger cohort of subjects. For example, given the trend in satellite cell proliferation between control and T1D women, we have estimated that 34 women per group are required to achieve statistical significance (α=0.05, β=0.8, η_p^2=0.283). It may challenging, however, to obtain repeated muscle biopsies from 60+ individuals. Thus, while we are limited by our sample size our novel findings should not be discounted. In future, a larger cohort of subjects is necessary to not only validate our results, but to explore how variables such as sex and glycemic control impact muscle repair in young adults with T1D.

CONCLUSION

In summary, our data is the first to show that skeletal muscle repair, in terms of muscle function, morphology, and ultrastructure, is largely unaltered in otherwise healthy young adults with T1D. These novel findings are in contrast to previous work from our lab, which showed that muscle repair was attenuated in untreated diabetic mice and that baseline satellite cell content was reduced in young adults with T1D[11,17]. We attribute these inconsistencies to differences in glycemic control, and speculate that skeletal muscle repair is generally unaffected if those with T1D are optimally managing their blood glucose levels. Exercise has many health benefits for persons with T1D, yet in the current study those with the highest levels of vigorous physical activity exhibited the poorest glycemic control. Considering the metabolic impairments seen in T1D, our results emphasize a need to define the duration, intensity, and type of activity required for persons with T1D to maintain euglycemia during exercise. We expect that this would in turn, improve skeletal muscle health and ultimately, extend the healthy lifespan of those living with T1D.

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APPENDIX

SUPPLEMENTAL METHODS

Immunohistochemical Analysis of Myofiber Regeneration

Formalin-fixed paraffin embedded tissue sections were prepared as previously described. Tissue sections were incubated in the primary antibody against embryonic myosin heavy chain (F1.652, Developmental Studies Hybridoma Bank, 1:1) at 4°C overnight. A secondary biotinylated goat anti-mouse IgG1 antibody was then applied for 1 hour at room temperature. Following secondary antibody incubation, sections were incubated for 2 hours with Vectastain Elite ABC reagent and the Tyramide Signal Amplification Kit (cyanine 3) for 10 minutes. For double antibody staining, sections were re-blocked and incubated in the primary antibody against dystrophin (ab15277, Abcam, 1:300) at 4°C overnight. A secondary goat anti-rabbit peroxidase IgG antibody was applied for 1 hour at room temperature, followed by the Tyramide Signal Amplification Kit (fluorescein) for 10 minutes. Last, nuclei were counterstained with DAPI, and sections were incubated in Sudan Black B for 30 minutes to remove autofluorescence. Washes between incubations were performed using Tris-buffered saline. The number of subjects with eMyHc-positive fibers were quantified at each time point. A chi-square test for independence was used to compare ordinal data between groups.

SUPPLEMENTAL FIGURES

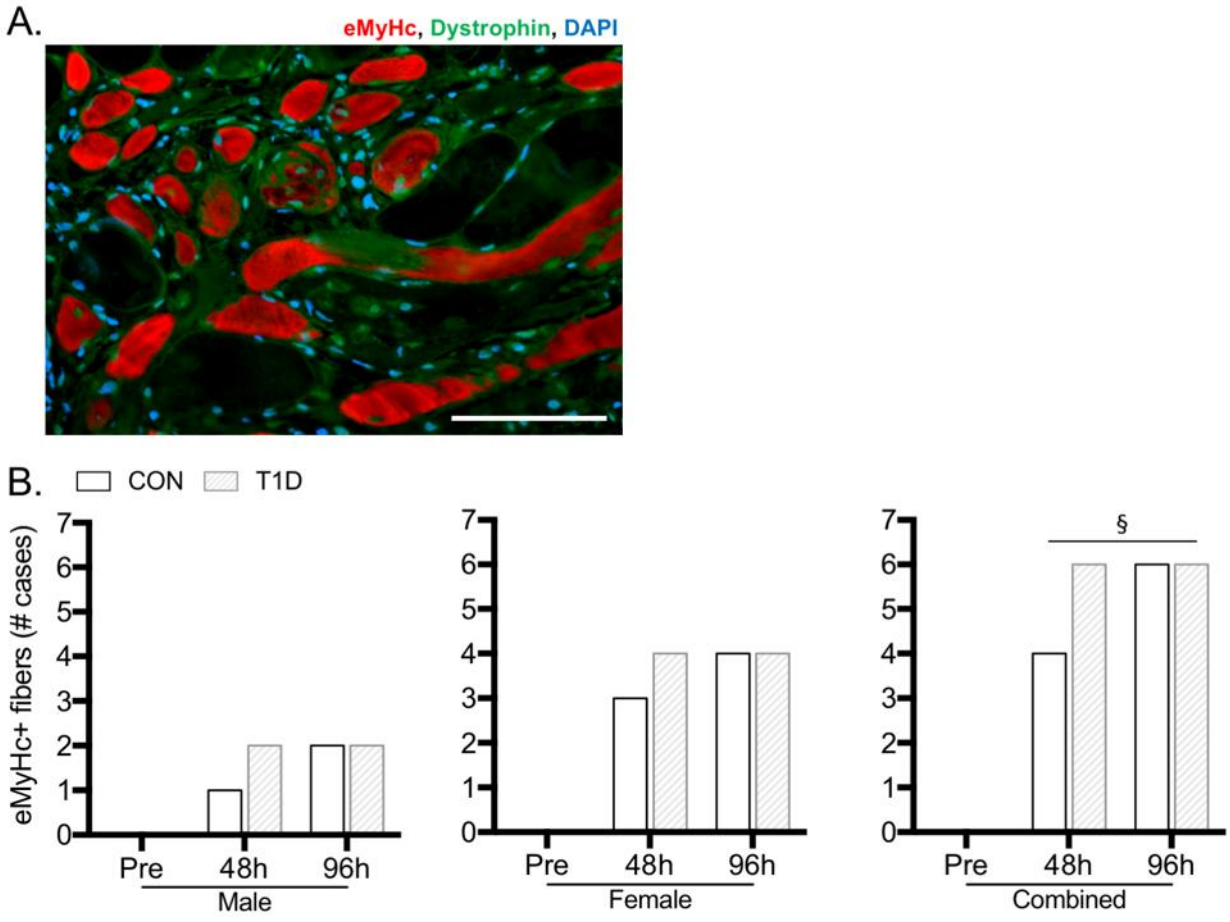

Figure S1. **Muscle fiber regeneration following eccentric contractions.** (A) Representative image of a eMyHc/Dystrophin/DAPI stained muscle cross-section. Regenerating muscle fibers express eMyHc. (B) Number of control (CON) and type 1 diabetes (T1D) men and women presenting with eMyHc-positive muscle fibers pre-, and at 48- and 96-hours post-eccentric muscle damage. Comparisons between (left panel) males, (center panel) females, and (right panel) both sexes combined. Log transformed data are shown. Data are expressed as number of cases. §Significant difference from pre-damage, $p<0.05$. Scale bar=100μm.