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Considering Type 1 Diabetes as a Form of Accelerated Muscle Aging

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Considering Type 1 Diabetes as a Form of Accelerated Muscle Aging

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Abstract

Recent evidence reveals impairments to skeletal muscle health in adolescent/young adults with type 1 diabetes (T1D). Interestingly, the observed changes in T1D are not unlike aged muscle; particularly, the alterations to mitochondria. Thus, we put forth the novel hypothesis that T1D may be considered a condition of accelerated muscle aging and that, similar to aging, mitochondrial dysfunction is a primary contributor to this complication.

Key Points

- Emerging evidence in humans indicates that type 1 diabetes (T1D) impairs skeletal muscle health (e.g., mass, function, metabolism)
- Impairments to skeletal muscle health in T1D are, in many ways, similar to that observed in the muscle of aged individuals, but are occurring at a younger age in T1D
- Mitochondrial dysfunction has been implicated in the deterioration of skeletal muscle health in aging healthy adults
- Adolescent/young adults with T1D display dysfunctional skeletal muscle mitochondria despite being recreationally-active and having moderately well-controlled glycemia
- We hypothesize that mitochondrial dysfunction is a primary mediator of the accelerated muscle aging phenotype in those with T1D

Key Words: Type 1 diabetes; aging; skeletal muscle health; atrophy; metabolism; muscle strength; mitochondrial dysfunction

INTRODUCTION

Type 1 diabetes (T1D) is a metabolic disease caused by the autoimmune-mediated destruction of the insulin-producing pancreatic beta cells, resulting in little to no insulin production. Approximately 1.5 million North Americans currently live with T1D and evidence indicates that T1D prevalence is on the rise worldwide [1]. Multiple daily subcutaneous insulin injections/insulin pump therapy, frequent blood glucose tests and careful dietary monitoring (e.g. carbohydrate counting) are currently the standard of care for those with T1D. While these therapeutic strategies allow for treatment of T1D, they are not a cure, and the limitations of exogenous insulin therapy lead to chronic, recurrent bouts of dysglycemia, dyslipidemia and, ultimately, insulin resistance. These three factors have been identified as the major contributors to the development of long-term diabetic complications, including neuropathy, nephropathy and cardiovascular disease [2].

Surprisingly, the impact of T1D on skeletal muscle has received little clinical attention despite the importance of skeletal muscle to our physical and metabolic well-being. There is, however, accumulating evidence of structural, functional and metabolic alterations to the skeletal muscle of both rodent models and humans with T1D; changes that appear to present prior to the clinical onset of many other diabetic complications (see [3] and [4] for more detailed and extensive reviews). In many ways, these alterations to the 'health' of skeletal muscle are consistent with alterations observed in aged muscle including declines in muscle mass and strength, as well as dysregulation of glucose, lipid and protein metabolism, albeit at a considerably younger age. Particularly noteworthy, at the cellular level, are the similarities in the structural and metabolic alterations to mitochondria in both conditions whereby the mitochondria appear to have a reduced oxidative capacity [5–10], increased capacity to produce reactive

oxygen species (ROS) [10,11] and an increased susceptibility to opening of the mitochondrial permeability transition pore (mPTP) [10,12]. Of clinical significance though is the fact that these cellular changes are occurring at a younger age in those with T1D (<30 years-old in T1D versus ~50-80 years-old in otherwise healthy individuals). With this as our backdrop, we put forth the novel hypothesis that T1D recapitulates a condition of accelerated skeletal muscle aging and that, similar to the decline in muscle health with increasing age, mitochondria are a primary mediator of this accelerated muscle aging phenotype. It is thus the purpose of this review to discuss the recent findings that support this novel hypothesis and identify the gaps in the literature that may be used to direct future studies in order to improve the healthy lifespan of those living with T1D.

SIMILARITIES BETWEEN AGED AND T1D SKELETAL MUSCLE

Muscle Mass

As skeletal muscle ages, there is a progressive reduction in mass and function (sarcopenia). The loss of skeletal muscle mass typically begins around 50 years of age and continues to gradually decline by ~2% each year [13]. This leads to an increased risk of frailty, physical disability, chronic metabolic disease and mortality [14]. Aged skeletal muscles have been associated with a decline in myofiber number and size (atrophy) and studies have reported that the decrease in myofiber cross-sectional area is fiber-type specific, with type II muscle fibers being on average 10-40% smaller in the elderly compared with young adults [15]. In contrast, type I muscle fibers appear to remain largely unaffected [16]. These progressive changes to aging skeletal muscle are further exacerbated by: (1) the “anabolic resistance” observed in elderly people, where muscle protein synthesis has a blunted responsiveness to nutritional (e.g. protein-rich diet) and exercise interventions (e.g. resistance exercise), causing a loss in the ability

of muscle to maintain its protein mass ([17]); and (2) the impairments to muscle regeneration that exist in the elderly, with considerable evidence supporting reductions in muscle stem (satellite) cell content and activation in this population (for review see [18]). As a result, these alterations make it considerably more difficult for the elderly to recover from insults to the muscle (e.g. prolonged periods of disuse, injury, and/or reduced daily step counts), and exacerbates the loss of muscle mass with increasing age.

A loss of muscle mass has also been reported in those with T1D. Prior to the discovery of insulin, patients with T1D were cachectic and only lived 1 to 2 years following diagnosis. While the introduction of insulin therapy has rescued T1D patients from this debilitating syndrome and early mortality, the few studies conducted to date have reported an overall reduction of myofiber size/muscle volume/cross-sectional area in those newly diagnosed ([19]) and in middle-aged (35-50 years old) persons with T1D compared to controls (Andersen's work reviewed in [4]). Interestingly, this deficit in muscle volume/cross-sectional area in adults with T1D was present even in those without neuropathy, but was amplified with presence of neuropathy [4].

Where the evidence is less equivocal is in children and adolescents with T1D following a period of insulin therapy. Many studies report no differences in lean body mass (measured by either dual energy X-ray absorptiometry or magnetic resonance imaging) compared to matched controls or normative values [9,20–22]. However, evidence is accumulating that this maintenance of muscle mass is evident only in those with 'good' glycemic control [23,24]; an observation consistent with insulin's suppression of protein degradation pathways and up-regulation of anabolic pathways, as well as the reported aberrations in the growth hormone-insulin-like growth-factor-I (GH-IGF-I) axis correlated to poor glycemic control [25]. Given that

those with T1D are not euglycemic all day – everyday, we would speculate that periods of disrupted protein balance are impacting the ability to achieve optimal muscle mass though further study in this area is greatly needed. The importance of a healthy and optimal muscle mass with increasing age cannot be overstated [17]. As mentioned above, our limited understanding of the temporal impact of T1D on skeletal muscle mass is an area where future studies are necessary.

Whether fiber-type specific attenuations in fiber cross-sectional area may exist in the muscles of patients with T1D is also inconclusive. To the best of our knowledge, only two studies have examined this in detail following the introduction of the more intensive insulin therapies still in use today. Specifically, Andreassen *et al.* [26] observed a greater frequency of type II (glycolytic) fibers and larger fiber diameters (both type I and type II) in distal muscles (gastrocnemius) of 16 T1D adults (10 with neuropathy, 6 without) in comparison to patients with type 2 diabetes (T2D) and controls. Interestingly, no relationship between neuropathy and fiber diameter/fiber-type proportion was found, and no difference in the number of capillaries per fiber was observed, suggesting intrinsic impairments as a result of T1D (rather than the result of capillary rarefaction, for example). Fritzsche *et al.* [27] also reported greater frequency of type II fibers, albeit in young adults with T1D, muscle fiber cross-sectional area was not measured. Thus, it is possible that the increased number of glycolytic fibres resulted from increased atrophy, leading to an increased number of smaller fibers rather than an expansion in the number of functional type II fibres, affecting quantification.

As mentioned above, an inability to properly repair from muscle damage has been reported in older adults and has been linked to reductions in muscle size/mass [28]. A loss of muscle satellite cells in aged muscle has also been identified as a contributing factor to the

decline in muscle mass with advancing age [28]. While no muscle regeneration studies have been undertaken in humans with T1D, rodent models of T1D indicate that their muscles are more prone to damage [29] and exhibit delayed regeneration [30]. What is known however, is that both T1D human (~18-21 years-old) and mouse skeletal muscles demonstrate a significant reduction in satellite cell content [29] consistent with aged skeletal muscle.

Muscle Function

In otherwise healthy adults, the age-related changes to muscle eventually manifests in functional limitations and disability; adversely impacting activities of daily living and quality of life. Decreased muscle strength (maximal muscle force) and power (product of force and velocity of muscle contraction) are common features seen in aging and sarcopenia, with the rate of loss of muscle isometric force being ~1-2% per year and muscle power being 3-4% per year [31]. Considering that the majority of activities of daily living rely on muscle power (e.g. raising from a chair or climbing stairs) versus muscle strength, the greater power decline that occurs with advanced age is likely the fundamental cause of increased risk of falls and vulnerability to injury reported in the older population [32].

Similar to aged muscle, T1D has been demonstrated to compromise skeletal muscle function. In fact, the decline in function appears to begin early in life (and disease progression) as multiple studies performed in children with T1D have observed reduced muscle strength, power and increased fatigability compared with their counterparts [33–36]; however, this is not universally reported (reviewed in [4]). Nonetheless, this remains an important observation for two reasons: (1) the impairments in muscle function in youth indicate the skeletal muscle dysfunction is a primary diabetic complication (rather than secondary to other complications such as neuropathy), and (2) as T1D is a chronic disease, the manifestation of impaired muscle

health early in T1D could mean an earlier development of sarcopenia in those with this disease. Support for this comes from studies in adults with T1D which demonstrate that both isometric and isotonic maximal force production are decreased compared to controls [4,37]. With advancing age, the reductions in skeletal muscle strength become more closely associated with the severity of neuropathy in those with T1D [4,26,37]. Therefore, similar to the loss of function seen in aging muscle, muscle strength/power decreases with increasing T1D duration, however, this decline becomes more accelerated with the development of neuropathy (particularly in the distal limb musculature). Akin to the age-related declines in functionality, over 70% of people with diabetes (both T1D and T2D) report difficulty with routine physical activities and diabetes alone was associated with 2-3 times increased odds of suffering from disability [38].

Muscle Metabolism

The changes in skeletal muscle mass and function that occur with increasing age are also accompanied by deteriorations in metabolism, including dysregulation in glucose, lipid and protein metabolism.

The evidence to date generally supports age-related reductions in the protein synthesis pathways, specifically decreases in Akt/PKB-mTORC signaling, as well as age-related increases in the protein degradation pathways (e.g. ubiquitin proteasome system), all of which inevitably impact skeletal muscle mass and promote wasting (reviewed in [39,40]). In T1D, our understanding of changes in protein metabolism is extremely limited. To the best of our knowledge, no studies to date have investigated the protein synthesis (e.g. mTORC) and degradation (e.g. ubiquitin) pathways at the molecular level in those with T1D. Thus, it is unknown if the changes seen in protein signaling in older, nondiabetic adults are comparable to adults with T1D. Our limited understanding of protein metabolism in T1D stems largely from the

whole-body level studies conducted in adults (~30 years old) by Nair and colleagues [41–43]. These researchers found that protein metabolism in T1D appears to be largely dependent on insulin therapy and glycemic control. For instance, poor T1D management has been shown to cause an increase in both whole-body protein breakdown and protein synthesis, with breakdown rates far exceeding synthesis rates, resulting in a net protein loss. More recent work revealed that this loss largely stems from proteins within skeletal muscle [44]. In contrast, good glycemic control and adequate insulin injections seems to only allow for protein conservation (via reductions in protein breakdown) [43]. Thus, similar to aging muscles, T1D appears to negatively impact protein metabolism. Taken together, it is clear that studies are urgently warranted to not only elucidate the impact of T1D on protein metabolism in youth and elderly T1D individuals, but also whether aging adults with T1D develop anabolic resistance similar to non-T1D aging adults, and if so, at what age and timepoint in disease duration.

With respect to fuel metabolism, key enzymes in glucose metabolism, including hexokinase (HK), lactate dehydrogenase (LDH) and citrate synthase (CS) have all been shown to have lower activity with advanced age [45]. Additionally, aging appears to downregulate transcripts important for lipid transport and oxidation in human skeletal muscle [46], which likely explains the increased presence of intramyocellular lipids (IMCL) in older adults [46]. Importantly, the combination of decreased fat oxidation and decreased mitochondrial oxidative phosphorylation (discussed in detail below) seen in aging muscle can cause incomplete oxidation of fatty acids, which in turn can lead to increased ROS production and the accumulation of toxic lipid metabolites in the muscle cells, and consequently, interference with muscle contraction (discussed above) and the insulin signaling pathway [46,47]. Not surprisingly, older individuals

are more likely to develop insulin resistance, resulting in a further impact on the metabolic capacities of skeletal muscle, including muscle protein turnover.

While T1D is inherently a metabolic disease characterized by impaired glucose handling, some of the aforementioned impairments with aging are also seen within the muscle of individuals with T1D, but at a much younger age. In particular, early studies have reported lower HK activity in adults with T1D (~29 and 32 years-old) compared to non-T1D controls [48,49]. In contrast, younger adults with T1D (~21 years-old) appear to have normal HK activity [50], suggesting there may be a sensitive threshold for the onset of glycolytic impairments in T1D muscle. LDH activity has also been reported to be elevated in T1D muscle [48,49] and this may be a result of decreased pyruvate dehydrogenase activity [48] and/or mitochondrial dysfunction. To the best of our knowledge, only one study has measured CS in humans with T1D (~32 years-old), and no differences was reported [49]. While there are disparities in which key metabolic enzymes are affected with aging and T1D, insulin resistance still develops in those with T1D and importantly, this appears to occur early in disease progression (e.g. adolescence) [9]. Consequently, the mechanisms that lead to declines in insulin sensitivity are not fully understood in T1D, but similar to muscles of older adults, the muscles of those with T1D have been reported to exhibit greater IMCL content [51], particularly in those who are greater than 30 years old and in poor glycemic control [20,52]. The increased IMCL content is very likely the result of a reduced mitochondrial oxidative capacity [7–10], similar to aging, in association with increased FFA delivery resultant from adipose tissue insulin resistance, which has been well characterized in those with this disease [20,23,53,54]. We therefore speculate that muscles in those with T1D exhibit an increased presence of toxic lipid metabolites, similar to aged skeletal muscle, resulting

in impaired insulin signaling and ultimately impaired muscle metabolism, including the muscle protein synthesis and degradation pathways.

MITOCHONDRIAL DYSFUNCTION AS THE UNDERLYING MECHANISM FOR ACCELERATED MUSCLE AGING IN T1D: A “MITO-CENTRIC” HYPOTHESIS

Mitochondria are commonly referred as the “powerhouse of the cell” due to their essential role in the production of the high-energy molecule adenosine triphosphate (ATP). However, mitochondria have many other vital roles in muscle cells including ROS production/signaling, Ca²⁺ homeostasis and the regulation of programmed cell death (apoptosis). This makes them indispensable organelles for the regulation of skeletal muscle mass, function and metabolism. It is thus not surprising that mitochondrial dysfunction has long been considered to be centrally implicated in the aging process and age-related deteriorations to skeletal muscle health. While we acknowledge that there is still some areas of debate within this highly complex area, owing in part to the nature of methodologies employed for interrogating mitochondrial function, as well as the age and level of physical activity of subjects studied (we refer the readers to [55] for more detail), the weight of evidence in aged human muscle supports reductions in mitochondrial oxidative capacity [5,6], reductions in mitochondrial respiration at physiological ADP concentrations [11], increased mitochondrial ROS production [11] and increased sensitization of the mPTP [12]. Thus, mitochondrial dysfunction appears to be strongly implicated in the continuum of age-induced skeletal muscle dysfunction. The following sections will concisely discuss basic mitochondrial physiology, how mitochondrial dysfunction is implicated in deteriorations to skeletal muscle health and the evidence supporting our novel

hypothesis that mitochondria are a primary mediator of accelerated muscle aging in T1D (Figure 1).

Brief Overview of Mitochondrial Physiology

Mitochondrial Respiration

Mitochondria produce ATP via the mechanism of oxidative phosphorylation (also referred to as mitochondrial respiration). As depicted in Figure 2, a series of specific proteins (Complexes I through V) located in the inner mitochondrial membrane, termed the electron transport chain (ETC), catalyze the oxidation of respiratory substrates (electron carriers) NADH and FADH₂, generated during the metabolism of carbohydrates (glycolysis) and lipids (β-oxidation). Electrons are then transferred into the ETC and, through a series of redox reactions, are passed down from Complex I or Complex II all the way to Complex IV until they reach the final electron acceptor, oxygen. Free energy is released during these chemical reactions which is captured and used to “pump” protons across the mitochondrial inner membrane and into the intermembrane space against an electrochemical gradient. This creates a proton-motive force, also known as the mitochondrial membrane potential ($\Delta\Psi$), that in turn drives protons back into the matrix through the ATP synthase (Complex V), rotating a part of the enzyme that drives the phosphorylation of ADP into ATP.

Mitochondrial ROS Production

Approximately 2% of the total oxygen consumed during ‘normal’ oxidative phosphorylation results in the production of ROS due to natural electron leakage from the ETC. These leaked electrons are highly unstable and react quickly with nearby oxygen to produce oxygen free radicals, such as the superoxide radical (O₂^{•-}) and hydroxyl radical (•OH), as well as non-radicals, such as hydrogen peroxide (H₂O₂). The mitochondrial matrix and cytoplasm are

equipped with enzymatic (e.g. glutathione, superoxide dismutase and catalase) and non-enzymatic (e.g. vitamin E) antioxidant defenses to counterbalance these oxidants. At low, physiological levels, ROS acts as an important signaling molecule for various processes, including muscle contraction, cell proliferation and cell adaptation to exercise training. However, when ROS levels exceed antioxidant capacity (as may occur with excess substrate delivery, dysfunctional mitochondria and/or reduced antioxidant defences), it becomes pathological and causes oxidative stress.

Mitochondrial Ca²⁺ Uptake

Mitochondria also contain a Ca²⁺ uniporter that allows Ca²⁺ flux into the matrix, a process that is essential for ATP production and for regulating muscle contractile function and programmed cell death. The influx of Ca²⁺ into the matrix not only contributes to shaping of the sarcoplasmic Ca²⁺ transients but also simultaneously stimulates mitochondrial energy production by activating select Ca²⁺-sensitive mitochondrial dehydrogenases [56]. This ensures that adequate energy is provided to support muscle contraction. Moreover, mitochondria can aid in the buffering of cytoplasmic Ca²⁺ by chelating excess levels of this ion with inorganic phosphate in its matrix. However, Ca²⁺ overload and/or the combination of excess Ca²⁺ and damaged mitochondria can induce opening of the mPTP. mPTP opening collapses the $\Delta\Psi$ and leads to decreased ATP production, disruption of ionic homeostasis and swelling. As the mitochondrial swells, the cristae of the inner mitochondrial membrane begin to unfold and the increased pressure on the outer mitochondrial membrane causes it to rupture and releases pro-apoptotic factors that activate caspase cascades in the cytosol to initiate cellular fragmentation and ultimately, cell death.

Mitochondrial Dysfunction and the Impact on Skeletal Muscle Health

At this point in time, the term mitochondrial dysfunction is ill-defined owing to the multiplicity of mitochondrial functions in a cell, as described above, as well as the fact that it remains unclear as to whether altered mitochondrial function reflects a physiological adaptation, a pathological maladaptation, or simply a pathological phenomenon. For the purpose of this review, mitochondrial dysfunction will entail any abnormality in the key physiological roles (e.g. respiration, ROS, Ca²⁺ uptake) of a mitochondrion (i.e. intrinsic mitochondrial function).

Depending on the metabolic state of the cell, mitochondria regulate skeletal muscle mass, function and metabolism via either the activation of anabolic or catabolic signaling pathways, some of which feed-forward to myonuclei to either upregulate or downregulate the expression of genes important for muscle protein synthesis/degradation, muscle contraction and substrate oxidation (reviewed in [57]). For example, excess ROS production, caused by either (i) nutrient excess, (ii) a combination of nutrient excess and reduced mitochondrial respiration, (iii) damaged mitochondria, (iv) depleted antioxidant defenses or (v) a combination of these factors, can induce the expression of atrogenes via activation of the JNK/FoxO signaling pathway as well as the activation of endoplasmic reticulum stress, which in turn suppress protein synthesis and promote protein degradation, and hence, muscle atrophy [57]. Excess mitochondrial ROS can also lead to opening of the mPTP and thus increase apoptotic potential. Opening of the mPTP can also be triggered by impairments in the ability of the mitochondria to retain excess levels of Ca²⁺ due to failure of cytosolic Ca²⁺ homeostasis and ionic disturbances. Irrespective of the cause, the resultant increase in apoptosis invariably promotes muscle atrophy. Increased mitochondrial ROS can also lead to nuclear and mtDNA mutations/deletions, protein damage (including ETC enzymes) and lipid peroxidation – in other words, oxidative stress – all of which can directly and

indirectly impact muscle mass, function and metabolism. For instance, damage to enzymes of the ETC caused by ROS can lead to even greater ROS production/damage, creating a vicious cycle that perpetuates mitochondrial dysfunction and apoptotic cell death. Mitochondrial dysfunction, specifically impaired mitochondrial respiration, can also lead to the accumulation of incompletely oxidized substrates and toxic metabolites, which have been implicated in impaired muscle contraction [57]. Furthermore, impaired mitochondrial respiration not only drives mitochondrial ROS production, but also increases the AMP/ATP ratio, which in turn leads to the activation of the energy sensor molecule AMP-activated protein kinase (AMPK). Increased AMPK activity inhibits anabolic pathways, including muscle protein synthesis by inhibiting mTOR and directly phosphorylating FoxO3.

Mitochondrial Dysfunction in Skeletal Muscle of Individuals with T1D

Mitochondrial Energy Production

While dramatic efforts have been put towards interrogating mitochondrial function in T2D over the past two decades, surprisingly little efforts have been made in the area of T1D. To our knowledge, the first assessment of mitochondrial function in individuals with T1D was undertaken in 2003. Crowther *et al.* [7] used the non-invasive *in vivo* technique ³¹Phosphorous-magnetic resonance spectroscopy (³¹P-MRS) to measure the rate constant of phosphocreatine (PCr) re-synthesis, a measure that infers *in vivo* mitochondrial oxidative capacity, in ~36 year-old men with an HbA1c of <7.0% (i.e. well-controlled T1D) and disease duration of ~18 years (no neuropathy reported). They observed a slower rate of PCr recovery following a 30 sec bout of isometric contraction [70% of maximal voluntary contraction (MVC)] compared to matched controls. Importantly, this occurred irrespective of level of physical activity in the T1D individuals (4 sedentary and 3 recreationally-active), indicating: (1) men with T1D in their

thirties already have a reduced muscle mitochondrial oxidative capacity despite having well-controlled glycemia and (2) being recreationally-active with T1D was not sufficient to prevent attenuations in muscle mitochondrial respiratory function. Despite these important findings, the next studies to interrogate mitochondrial function in T1D was not until 8 years later. Using the same technique, Kacerovsky *et al.* [8] interrogated the ability of insulin to stimulate flux through mitochondrial ATP synthase (fATP) during a hyperinsulinemic-euglycemic clamp in ~36 year-old men and women with well-controlled T1D (HbA1c 6.8%) and disease duration of ~17 years. They found that insulin failed to stimulate fATP in the T1D group compared to matched controls, suggesting abnormal mitochondrial oxidative metabolism. However, this may not have been an intrinsic mitochondrial defect per se as these participants also had an ~50% lower whole-body glucose disposal compared to controls, indicating the presence of insulin resistance, which could account for the attenuation in fATP. In contrast, Item *et al.* [58] reported no differences in the rate of PCr recovery following a 30 sec bout of isometric contraction (85% of MVC) in untrained ~27 year-old women with an HbA1c of 7.6% and disease duration of 13 years. Taken together, these studies indicate that the degree of muscle mitochondrial dysfunction in T1D may depend on age and sex. However more recent work contradicts these findings. Cree-Green *et al.* [9] interrogated mitochondrial oxidative capacity and the rates of mitochondrial oxidative phosphorylation (i.e. time taken to convert ADP to ATP) in sedentary/recreationally-active adolescents (~15 years-old) with an HbA1c of ~8.2% (i.e. moderately-controlled for adolescents with T1D) using ³¹P-MRS. Following a 90 sec bout of isometric contraction (70% of MVC), they found mitochondrial oxidative capacity was significantly lower in adolescents with T1D compared to matched controls, similar to Crowther *et al.*'s [7] findings, and they also found a delay in the recovery of ADP (i.e. slower rates of mitochondrial oxidative phosphorylation).

Therefore, the evidence to date, albeit limited, highlights skeletal muscle mitochondrial dysfunction in individuals with T1D; a complication that appears to occur early in diabetes progression.

While ^{31}P -MRS is undoubtedly advantageous for capturing *in vivo* ATP kinetics under physiological conditions, a limitation of this technique is the lack of resolution into the underlying changes responsible for impairments in mitochondrial function. This limitation can be overcome using *in vitro* techniques such as high-resolution respirometry and permeabilized myofiber bundles. We recently utilized this technique to interrogate mitochondrial function in young adults with T1D under moderate glycemic control (HbA1c 7.9%) [10]. Specifically, it was found that young, physically-active men and women (~26 years-old) with T1D (duration ~15 years) had an ~20% reduction in mitochondrial oxidative capacity compared to matched controls; consistent with the aforementioned *in vivo* work by Crowther [7] and Cree-Green [9]. In addition, significant attenuations in the sensitivity and respiratory capacity of Complex II of the ETC, but not Complex I, revealing for the first time, site-specific deficiencies in the mitochondrial ETC from muscle of young adults with T1D. Importantly, these impairments in mitochondrial respiratory function occurred in the absence of changes in mitochondrial content (measured by electron microscopy and Western blot) and capillary density, implying these mitochondrial impairments were intrinsic to the mitochondria and not secondary to oxygen/energy supply or mitochondrial content.

Mitochondrial ROS Production / Oxidative Stress

Early work by Brownlee *et al.* (reviewed in [59]) demonstrated that hyperglycemia leads to enhanced mitochondrial ROS production in tissues that cannot efficiently regulate glucose uptake, including endothelial, mesangial and Schwann cells, thereby increasing the susceptibility

to oxidative stress and ultimately cellular damage/complications. Since then, it has largely been thought that tissues that can regulate glucose transport (i.e. the insulin-sensitive tissues: skeletal muscle, adipose tissue and liver) are not susceptible to damage inflicted by hyperglycemia in T1D. In fact, until recently, it remained unknown whether T1D impacts mitochondrial ROS production/oxidative stress in these insulin-sensitive tissues in humans. Using permeabilized myofibers, the capacity of 5 different sites within mitochondria to emit H_2O_2 in the skeletal muscle of young men and women with and without T1D was assessed [10]. It was found that mitochondrial H_2O_2 emission (production minus scavenging) was significantly elevated at Complex III in T1D compared to matched controls. While only 1 site of enhanced ROS emission was detected, Complex III has been demonstrated to be the main producer of ROS within the ETC [60], and thus further study is needed to fully elucidate the importance of elevated ROS from Complex III and whether this manifests in an oxidative stress within the skeletal muscle of those with T1D. Furthermore, establishing whether the increase in mitochondrial H_2O_2 emission potential in T1D muscle is the result of attenuations in anti-oxidant defenses (as has been reported in aged muscle [57]) or dysfunction at Complex III is necessary. In particular, a critical outcome of excess ROS/oxidative stress includes DNA and protein damage and apoptotic cell death, as previously mentioned, which can lead to a decrease in muscle function by damaging proteins critical for muscle contraction as well as a decrease in muscle size by reducing fiber number and selectively removing individual targeted myonuclei [57].

Mitochondrial Ca^{2+} Uptake

Opening of the mPTP is favored by mitochondrial Ca^{2+} overload and high concentrations of ROS leading to programmed cell death. An increased susceptibility to opening of the mPTP has been documented in aged muscle [12]. To date, we [10] are the only group that demonstrated

that skeletal muscle mitochondria in those with T1D have significant reductions in Ca^{2+} retention capacity (CRC) compared to matched controls; highlighting increased apoptotic potential and mitochondrial Ca^{2+} overload in human T1D skeletal muscle, and further supporting the mitochondrial dysfunction in this tissue with T1D. Clearly future work in this area is needed as the role of mitochondria in intracellular Ca^{2+} handling continues to gain appreciation.

SUMMARY OF EVIDENCE SUPPORTING ACCELERATED AGING IN THE MUSCLES OF THOSE WITH T1D

This article discussed how the ‘health’ of aged muscle, including decreased muscle mass and function and dysregulated metabolism, in otherwise healthy older adults is in many ways similar to that of the muscle from individuals with T1D of a younger age. Specifically, the loss of muscle mass has been observed in young, newly diagnosed patients with T1D [19], in adolescents with T1D that are in poor glycemic control [61] and in individuals with T1D in their 5th decade of life (age 40 to 49) [4,37]; declines which typically begin in the 6th decade (age 50 to 59) in otherwise healthy individuals. The presence of neuropathy has been shown to amplify this difference [4,26,37]. Similarly, decrements in muscle strength with T1D have been reported to occur in childhood [34], continue into adolescence [33–36] and appear to remain significantly lower by middle-age (~50 years old) even in the absence of secondary complications (e.g. neuropathy), compared to matched counterparts [4,37]. Metabolic dysregulation is inherent to T1D and thus it is not surprising that some of the metabolic changes observed in aged muscle, including altered HK activity [45,48,49] and reduced mitochondrial oxidative phosphorylation [5–11], also occurs early in T1D (less than 36 years-old versus greater than 50 years-old).

Altogether, the evidence in the human T1D literature (albeit incomplete), led us to postulate that T1D is a disease that accelerates the aging of skeletal muscle.

While the pathogenesis behind this accelerated skeletal muscle dysfunction is undoubtedly multifactorial, the combination of the vital role of mitochondria for skeletal muscle health and the work of us and others [7–9,10] led us to consider impairments to mitochondria as a primary mediator of this phenotype similar to that reported in aging muscle [5,6,11,12]. Specifically, the age-related attenuations in mitochondrial respiration [11] and oxidative capacity [5,6], increased mitochondrial ROS production [11] and impaired mitochondrial Ca^{2+} handling [12] reported in older adult muscle mimics that which we recently observed in ~26 year old, physically-active adults with T1D. These early deficits to skeletal muscle mitochondria in T1D adults also occurred in the absence of changes in mitochondrial content and vascularity, suggesting an intrinsic dysfunction as a result of T1D. However, we acknowledge that while both aging and T1D share similar skeletal muscle characteristics, it is likely that the underlying etiology of mitochondrial dysfunction remains different between both conditions. For instance, there is a strong genetic component to the aging process including accumulation of DNA mutations as well as an inability to repair DNA and to properly synthesize proteins [55]. Whether this occurs in T1D muscle is still unknown (to our knowledge). In contrast, T1D is a disease characterized by a hyperglycemic and hypoinsulinemic *milieu*, and thus it seems plausible that substrate overload (both glucose and lipids) as a result of repeated subcutaneous insulin injections, which bypass the typical liver-first canonical pathway (reviewed in [3]), is leading to increased glycation and post-translational modifications of skeletal muscle proteins, thereby impacting function. It is clear that there remains considerable work to be done in this area to prove our mito-centric hypothesis of accelerated muscle aging in T1D, particularly if we are to

develop adjuvant therapies for improving the quality of life and healthy lifespan of those currently living with this chronic disease.

FUTURE PERSPECTIVES: WHY IT IS TIME FOR T1D-SPECIFIC EXERCISE GUIDELINES

The systemic benefits of exercise training cannot be disputed. Improvements (adaptations) to skeletal muscle have largely been considered to be the primary mediator of the positive whole-body effects of exercise. Of particular relevance with respect to this article is the potent ability of exercise training to significantly improve skeletal muscle health in older adults. Indeed, resistance training has garnered considerable attention in the past decade due to accumulating evidence in both older men and women showing that resistance exercise increases muscle mass [62] and myofiber size [63], improves muscle quality and functional abilities [62], reduces age-related attenuations in muscle strength and increases in muscle fat infiltration [64], as well as prevents muscle insulin resistance [65].

While the cellular mechanisms responsible for the adaptations of skeletal muscle to exercise training are multifactorial and remain to be fully elucidated, it has long been known that exercise induces mitochondrial biogenesis [66]. An increase in the generation of new mitochondria results in the muscle not only becoming more efficient at utilizing substrates to synthesize ATP, and hence more resistant to fatigue, but also at maintaining cellular homeostasis. In more recent years, there's become a greater appreciation and interest in exercise-mediated degradation of damaged/dysfunctional mitochondria, and hence, mitochondrial turnover, a process that is crucial for maintaining a healthy mitochondrial pool and ultimately homeostasis of cellular processes [67]. Consequently, few studies to date have sought to

interrogate this in aged human muscle. Although contradictory findings exist in aging-associated impairments in mitochondrial biogenesis and degradation, both aerobic and resistance exercise have been suggested to induce mitophagy in aged muscle (reviewed in [67]). As such, exercise training is most likely an effective means of stimulating mitochondrial turnover, and hence, maintaining a healthy mitochondrial pool (i.e. improving mitochondrial function) in aged skeletal muscle.

Despite the fact that evidence has existed as early as 1977 depicting aberrant mitochondria at the ultrastructural level in the muscle of adults with T1D [68], and that since 2003 there has been evidence for mitochondrial functional deficits in ~30 year-old T1D men [7], no studies to date have sought to investigate whether impairments to mitochondrial biogenesis/mitophagy exist in T1D muscle. As previously mentioned, we [10] recently demonstrated for the first time that skeletal muscle mitochondrial content is not different in physically active, young men and women with T1D compared to matched counterparts, suggesting mitochondrial biogenesis is not impaired in T1D. We did, however, observe ultrastructural abnormalities, similar to those reported in 1977, that would imply impairments in mitochondrial turnover in T1D [10] even with the use of more intensive insulin therapies. Specifically, electron tomography analysis revealed not only mitochondria with morphological defects, including both a loss of cristae and/or abnormal organization of remaining cristae, but also an increased presence of autophagic debris/remnants in both the subsarcolemmal and intermyofibrillar regions of the muscle (Figure 3), suggestive of impairments in clearance of damaged organelles. This is of utmost concern and of clinical significance because, as mentioned earlier, these participants were young, physically-active adults (~26 years old) whose moderate to vigorous activity levels exceeded current exercise guidelines set forth by most major national

diabetes associations (which are similar to the exercise guidelines of those without T1D). Thus, despite exceeding current exercise guidelines, this was not sufficient to fully maintain a healthy skeletal muscle mitochondrial pool.

Taken together, if our proposed novel hypothesis holds true, then increasing the balance of time spent resistance-training may prove fundamentally important in T1D, if we are to consider how impactful these activities are for improving the skeletal muscle health in older adults, as discussed above. With that said, future studies are clearly needed to define the duration, intensities and types of exercise (e.g. high-intensity interval training) necessary for those with T1D in order to optimize their skeletal muscle health. It is expected that this in turn would reduce the development of secondary complications, disability and ultimately premature mortality.

CONCLUSION

Despite the limited studies on the impact of T1D on human skeletal muscle health, the recent work from our lab [10,29] and others [7–9,48–50] has demonstrated structural and metabolic impairments in the muscle of individuals with T1D, at both the tissue and cellular levels, across all age groups studied – adolescence to middle-age – and suggest that glycemia and duration of diabetes is not a major determinant of these deficiencies. Importantly, the bulk of these studies were conducted in recreationally-active T1D individuals who met/exceeded current exercise guidelines, emphasizing the urgent need to coordinate muscle analysis with various exercise training regiments in those with T1D with the goal to correct, and ideally improve, muscle's metabolic health and ultimately, quality of life and lifespan.

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FIGURE LEGENDS

Figure 1. Proposed mechanism by which accelerated muscle aging occurs in adolescent/young adults with type 1 diabetes (T1D). Similar to aging muscle, mitochondrial dysfunction in 15-36 year-old individuals with T1D results in increased mitochondrial reactive oxygen species (ROS) emission potential, decreased mitochondrial energy production (ATP) and decreased mitochondrial calcium retention capacity (CRC). The increased ROS emission potential can directly impact mitochondrial and nuclear DNA and cause oxidative stress as well as indirectly enhance the expression of atrogenes, which in turn impact protein turnover (specifically net protein breakdown), and ultimately skeletal health (i.e. mass, function and metabolism). Similarly, the health of skeletal muscle is also directly impacted by a decrease in ATP production and by a decrease in mitochondrial CRC, where the latter increases the potential for cell death via apoptosis. Thus, mitochondrial dysfunction is a primary contributor to impaired skeletal muscle health in those with T1D.

[option 2 caption is different]

Figure 2. Schematic representation of mitochondrial oxidative phosphorylation. The electron carriers NADH and FADH₂, derived from the metabolism of carbohydrates and lipids, donate electrons (e⁻) to Complex I and Complex II, respectively. The electrons are then passed down in a series of redox reactions until they reach the final electron acceptor, oxygen (O₂), producing water (H₂O). Free energy is released during the transfer of electrons which is captured and used by Complex I, III, and IV to pump protons (H⁺) from the matrix into the intermembrane space (IMS) against an electrochemical gradient to create the protonmotive force (the “energy supply”). The protonmotive force, or membrane potential, in turn can drive the production of

ATP from ADP (the “energy demand”) and inorganic phosphate (P_i) by driving protons back into the matrix through Complex V.

Figure 3. Representative electron tomography images of skeletal muscle from young adults with type 1 diabetes (T1D) and without (control). An increased presence of autophagic remnants (highlighted in magenta) were more frequently observed in both the subsarcolemmal and intermyofibrillar regions of the T1D muscle compared to control in addition to irregularities in the organization of the mitochondrial cristae (highlighted in red). Green highlighting, lipid droplets; light green highlighting, sarcolemma; yellow highlighting, triads/sarcoplasmic reticulum; cyan highlighting, nucleus. Scale bar, 200 nm.

Figure 1

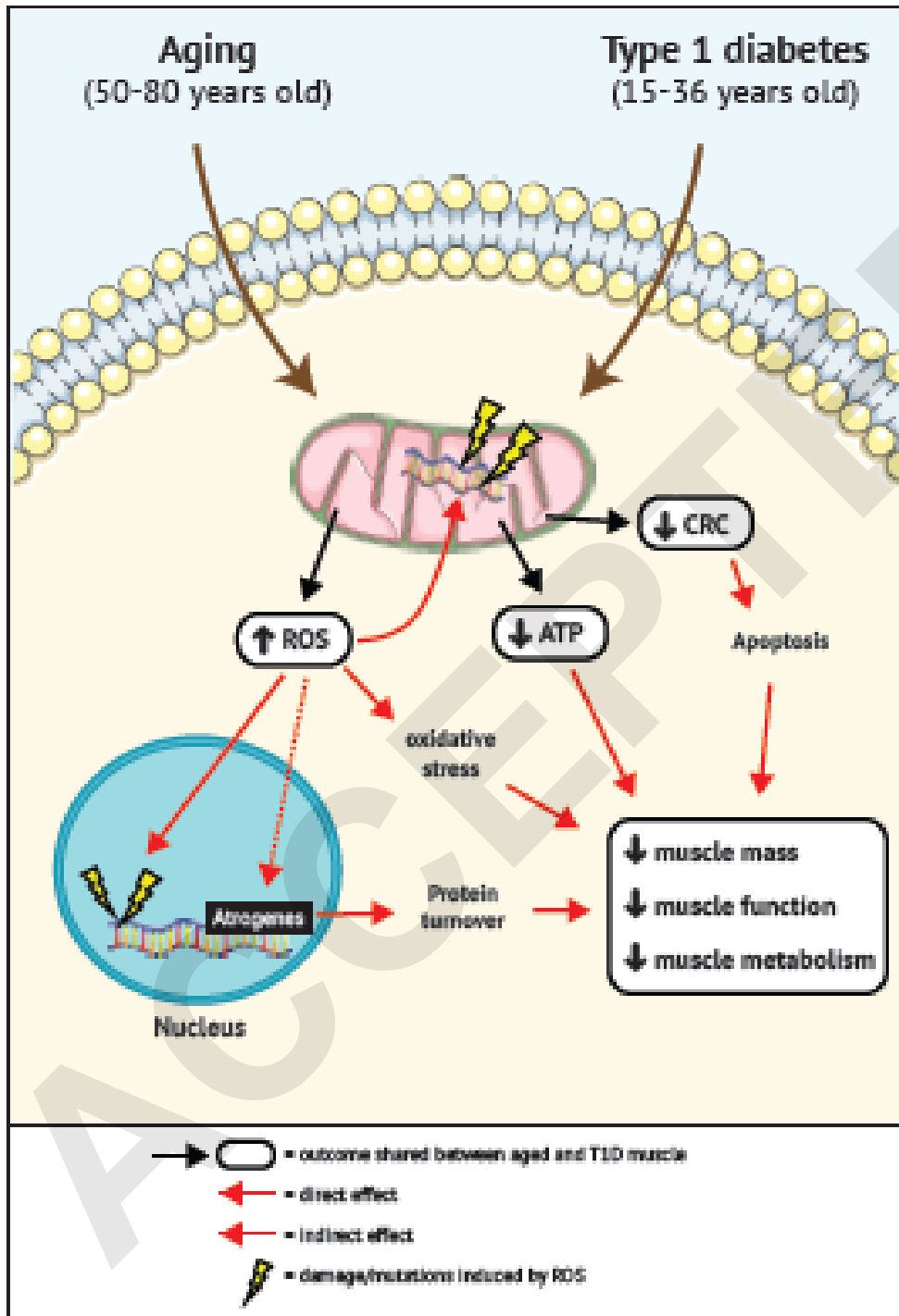


Figure 2

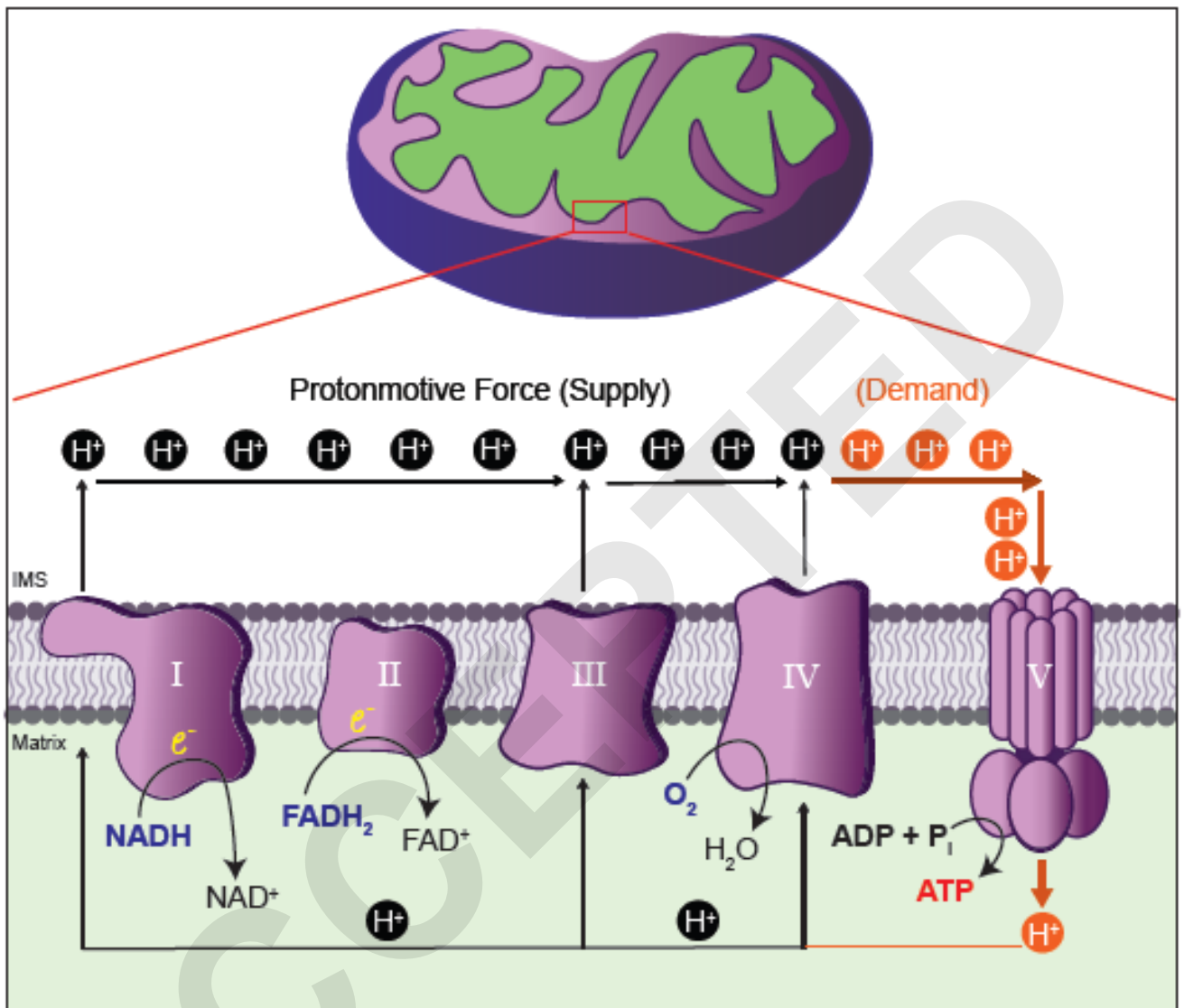
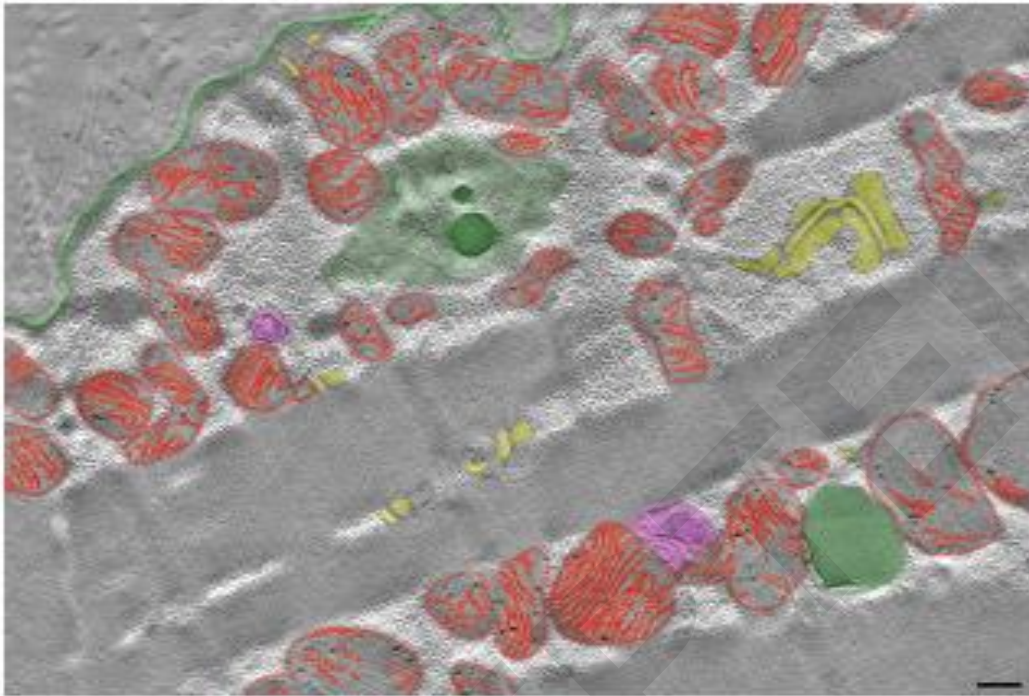
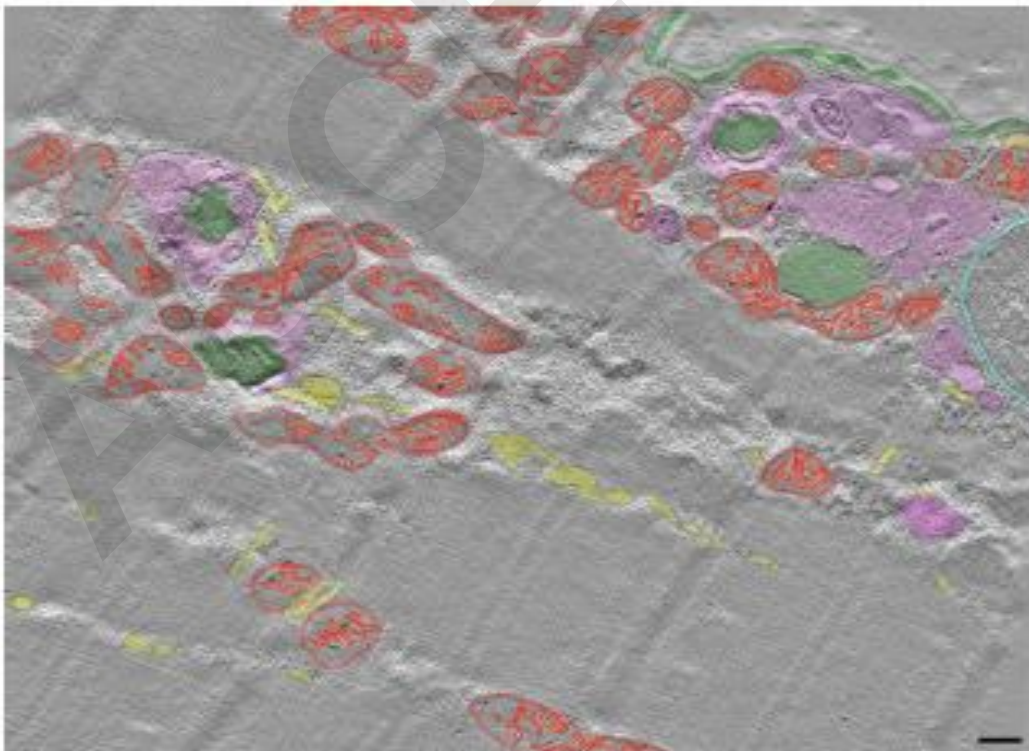


Figure 3

CONTROL



TYPE 1 DIABETES



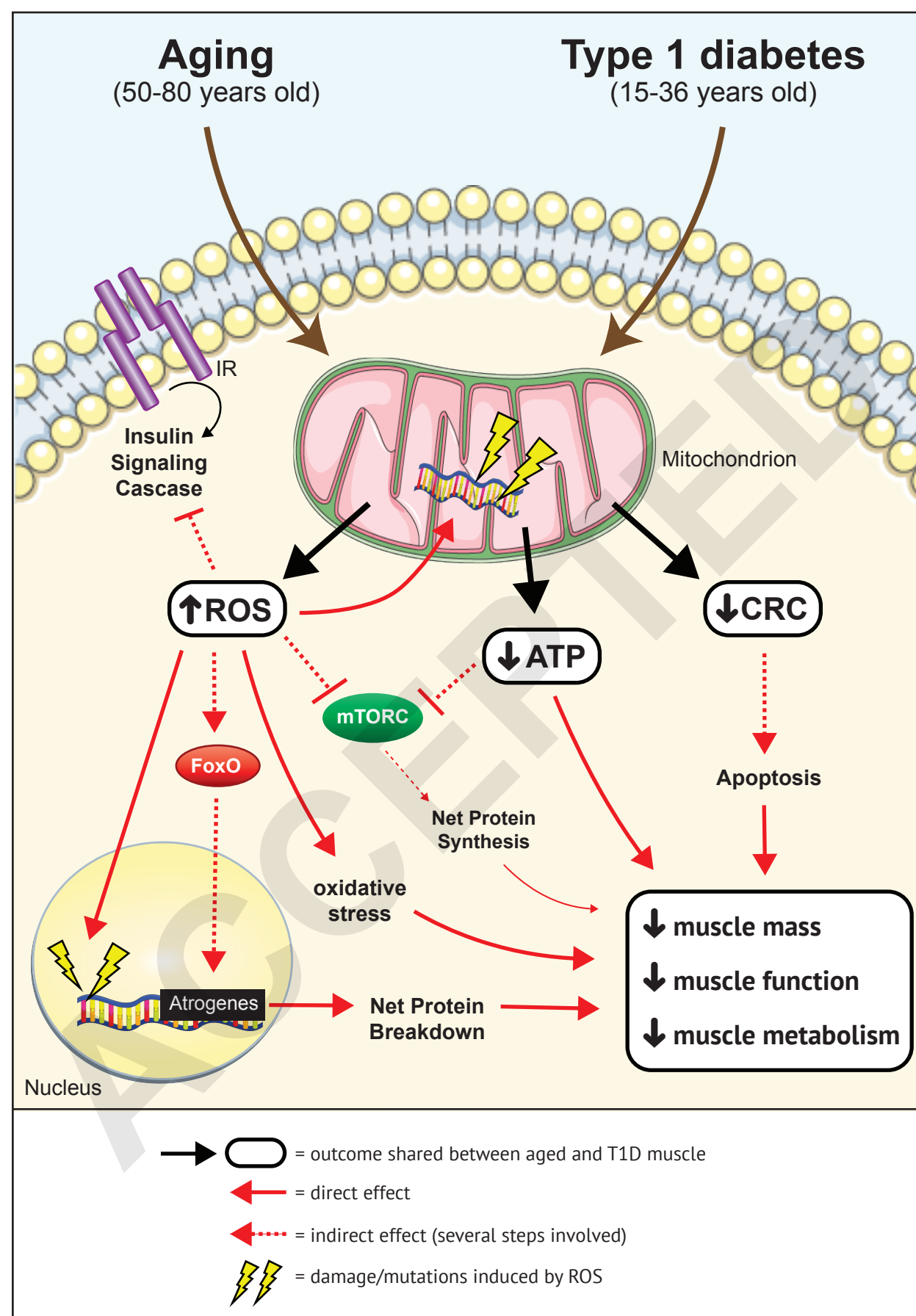


Figure 1. Proposed mechanism by which accelerated muscle aging occurs in adolescent/young adults with type 1 diabetes (T1D). Similar to aging muscle, mitochondrial dysfunction in 15-36 year-old individuals with T1D results in increased mitochondrial reactive oxygen species (ROS) emission potential, decreased mitochondrial energy production (ATP) and decreased mitochondrial calcium retention capacity (CRC). The increased ROS emission potential can directly impact mitochondrial and nuclear DNA and cause oxidative stress. It can also indirectly enhance the expression of atrogenes via activation of FoxO and reduce rates of protein synthesis indirectly via inhibition of mTORC. Increased ROS emission potential has also been shown to interfere with the insulin signaling cascade and induce insulin resistance. Interference with the insulin signaling cascade, and reductions in mitochondrial ATP, can both cause reductions in mTORC, further impacting muscle protein synthesis. Additionally, reductions in ATP can lead to reduced muscle function and inevitably impair muscle metabolism. Reductions in CRC increases the potential for cell death via apoptosis. Thus, mitochondrial dysfunction is a primary contributor to impaired skeletal muscle health in those with T1D. IR, insulin receptor; FoxO, forkhead box; mTORC, mammalian target of rapamycin complex.