Alterations in mitochondrial functions and morphology in muscle and non-muscle tissues in type 1 diabetes: implications for metabolic health

Cynthia M.F. Monaco¹, Christopher G.R. Perry², and Thomas J. Hawke¹

¹Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

²School of Kinesiology and Health Science, Muscle Health Research Centre, York University, Toronto, Ontario, Canada

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NEW FINDINGS

What is the topic of this review?

This symposium review provides an overview of the evidence of impaired mitochondrial functions and/or morphology in people with type 1 diabetes (T1D) across various organ systems.
What advances does it highlight?

Impairments to mitochondria functions and morphology may be a primary mechanism underlying the pathophysiology of various complications in people with T1D.

Abstract (127 words)

We recently made the observation that there are significant alterations to the ultrastructure and functions of mitochondria in skeletal muscle of people with type 1 diabetes (T1D). These alterations are proposed to lead to decreased energy production in skeletal muscle during exercise and thus may contribute to the impaired aerobic exercise capacity reported in some people with T1D. This Symposium Review has two aims. The first is to summarise the evidence that similar alterations also occur in the mitochondria present in organ systems outside skeletal muscle in people with T1D. The second aim is to summarise the evidence that these alterations in these extramuscular organ systems may contribute to the development and progression of the known complications of T1D, which eventually lead to the reported premature mortality.
Abbreviations

**DN**, diabetic neuropathy; **ROS**, reactive oxygen species; **T1D**, type 1 diabetes; **31P-MRS**, 31-phosphorus magnetic resonance spectroscopy

Introduction

Type 1 diabetes (T1D) is a complex, autoimmune-mediated metabolic disease characterized by hyperglycemia and insulin deficiency. Millions of people worldwide are afflicted with T1D (Xu et al., 2018), and the prevalence has consistently risen over the last decade (You & Henneberg, 2016). Insulin therapy continues to be the primary treatment option for people with T1D, and while advances in technology (e.g. insulin pumps and continuous glucose monitors) have facilitated the clinical management of this disease, the unfortunate reality is that diabetes complications (e.g. cardiomyopathy, neuropathy, nephropathy, retinopathy) still develop and remain the major cause of morbidity and premature mortality in this population (Mameli et al., 2015). Thus, it is clear that insulin therapy is not a cure, and if we are to improve the healthy lifespan of people living with T1D, greater research efforts are urgently needed towards unraveling the mechanisms underlying the development of diabetes complications in multiple organs and tissues.
Mitochondria are unique and highly dynamic organelles that regulate a variety of cellular functions and processes in nearly all cell types. These include energy supply to the cell in the form of adenosine triphosphate (ATP), production and emission of reactive oxygen species (ROS), Ca\(^{2+}\) homeostasis, and cell death. Mitochondria also demonstrate tissue-specificity due to the fact that the vast majority of mitochondrial proteins are derived from the nuclear genome and most tissues inherently have different metabolic and energetic demands (Ventura-Clapier et al., 1998). It is thus not surprising that alterations in mitochondrial morphology and functions, commonly termed mitochondrial dysfunction, accompany various human diseases, including myopathies, neurodegeneration, aging, and diabetes (Wallace, 1999). However, it’s important to note that the term mitochondrial dysfunction is ill-defined as it remains unclear whether the changes in mitochondria and hence, cellular function, that accompany diseases are simply a physiological adaptation or a pathological maladaptation of the mitochondria. These include impairments in electron transport chain (ETC) activity, decreased ATP synthesis, increased/excessive ROS, shifts in metabolic substrate utilization, defects in mitochondrial dynamics (e.g. mitochondrial shape/size) and loss of cristae density just to name a few. Maintaining mitochondrial homeostasis is thus critical for cell survival, which
largely depends on the balance between mitochondrial biogenesis, fission and fusion, and mitophagy. This symposium review gives an overview of the evidence of impaired mitochondrial structure and/or functions in people with T1D across various organ systems, which may be a key mechanism in the pathophysiology of diabetes complications.

**TYPE 1 DIABETES: IMPACT ON SKELETAL MUSCLE AND MITOCHONDRIA**

In rodents, T1D is known to lead to abnormalities in skeletal muscle morphology/ultrastructure, functions and metabolic health (i.e. insulin resistance) (Krause et al., 2011). While less understood, studies in humans with T1D also suggest impairments in skeletal muscle mass, function and metabolism [see previous reviews: (Krause et al., 2011; Monaco et al., 2017, 2019)]. Although these impairments in skeletal muscle are not considered life-threatening (and as a result are often overlooked clinically), diabetic myopathy is likely the primary reason underlying the greater risk of sarcopenia, exercise intolerance, mobility limitations, physical disability and frailty that has been reported, especially with increasing age, in those with diabetes relative to non-diabetes (Yanase et al., 2018). In fact, we recently postulated that T1D recapitulates a condition of accelerated muscle aging; with many of the deficiencies that occur in aged
muscles already being present in individuals with T1D but at a significantly earlier age (Monaco et al., 2019). Indeed, while the underlying mechanisms are undoubtedly multifactorial, there is evidence to suggest that similar to aging, alterations in mitochondria are associated with the observed reductions in aerobic exercise capacity and overall skeletal muscle metabolic health that occurs in some people with T1D (Monaco et al., 2019).

Along these lines, we have recently discovered that skeletal muscle mitochondria in young adults with T1D have irregularly organized cristae at the ultrastructural level, decreased ability to produce ATP in response to increasing energy demand (ADP), increased complex III-derived ROS emission and increased susceptibility to opening of the mitochondrial permeability transition pore (mPTP) in permeabilized muscle fiber bundles (Monaco et al., 2018). Other groups, but not all [see (Monaco et al., 2019)], have also found reductions in skeletal muscle mitochondrial oxidative phosphorylation in adolescents and adults with T1D utilising 31-phosphorus magnetic resonance spectroscopy (31P-MRS), and this may be linked to insulin resistance, potentially through altered redox signaling (Fisher-Wellman & Neufer, 2012). Insulin resistance will invariably impact muscle mass and function through an imbalance between protein synthesis and
degradation (Hebert & Nair, 2010). Moreover, in aging rodent studies, increases in ROS have been associated with accumulation of oxidized proteins and altered proteostasis, leading to denervation of motor units, and consequently, loss of muscle innervation, muscle fiber atrophy and loss of muscle function [discussed in more detail in (Vasilaki et al., 2017)]. Opening of the mPTP plays an important role in the regulation of programmed cell death, and hence muscle fiber degeneration (i.e. muscle mass/size), via the release of pro-apoptotic factors from mitochondria. Altogether, it remains to be investigated whether these mechanisms also operate in people with T1D.

**TYPE 1 DIABETES: BEYOND SKELETAL MUSCLE**

**CARDIAC TISSUE MITOCHONDRIA**

The cardiac muscle is one of the most oxidative and energetically demanding tissues in the body. To put this into perspective: mitochondria occupy nearly 30-40% of the cell volume in the heart, compared to <10% in skeletal muscle, and they rely mainly on fatty acids to generate and supply the large amounts of ATP required for the beating heart (i.e. fatty acid oxidation) (Ponsot et al., 2005). In other words, cardiac mitochondria are highly adapted for the continuous provision of ATP to sustain daily contractile...
activity. In T1D, studies have shown that some individuals have abnormal left ventricle (LV) function despite the absence of major coronary artery disease or hypertension (Shivu et al., 2010). This indicates that the T1D environment itself may directly impact the cardiac tissue (through mechanisms such as unrecognized microvascular disease, autonomic neuropathy, oxidative stress and impaired cardiac energetics).

With respect to this review, two studies, to the best of our knowledge, have interrogated cardiac energetics in humans with T1D. Utilizing $^{31}$P-MRS imaging to quantify the high-energy phosphates of the LV, Metzler et al. (2002) found that compared to age-matched healthy male controls, men with T1D (36 years old; HbA$_{1c}$ 7.6%; 4-42 years history of T1D) have a 13% reduction in the phosphocreatine (PCr) over β-adenosine-triphosphate concentration (PCr/ATP) ratios; a commonly used measure to characterize the in vivo energy status of the heart. Shivu et al. (2010) found that newly diagnosed men and women with T1D (32 years old; HbA$_{1c}$ 7.4%; 0-6 years history of T1D) have a 31% reduction in PCr/ATP ratios whereas men and women with longer duration of T1D (33 years old; HbA$_{1c}$ 8.3%; 11-25 years history of T1D) have a 41% reduction compared to matched controls. We acknowledge that many factors are at play in determining the PCr/ATP equilibrium, including rates of mitochondrial oxidative...
phosphorylation, microvascular dysfunction and the presence of cardiac fibrosis. The latter refers to the fact that people with T1D are known to have increased myocardial fibrosis (Armstrong et al., 2017); an observation that would imply that their cardiomyocytes have to work at a higher workload per cell to compensate for the smaller LV volume fraction. This would not only lead to an inability in maintaining the PCr/ATP ratio, but also a lower maximal cardiac output and VO$_2$max. Nonetheless, without further experiments and studies, these assessments, for the moment, provide indirect evidence for the concept of impaired cardiac mitochondrial bioenergetics in vivo in T1D.

It’s interesting to note that ischemic preconditioning does not appear to protect atrial samples of adults with T1D from ischemia/reperfusion injury compared to those without diabetes (Ghosh et al., 2001; Hassouna et al., 2006). These studies suggest that the mechanism underlying this failure to precondition may involve mitochondrial dysfunctions; specifically, dysfunction of the mitochondrial-$K_{ATP}$ channels as pharmacological manipulation of the mitochondrial-$K_{ATP}$ channels failed to protect the T1D samples as opposed to the non-diabetic samples from ischemia/reperfusion injury. It is thought that this leads to mitochondrial depolarization, superoxide production and
ultimately, an inability of the cardiomyocytes to respond to preconditioning. Clearly there remains tremendous

**NERVOUS TISSUE MITOCHONDRIA**

The central nervous system also has an immense metabolic demand. Similar to cardiac muscle, neurons rely heavily on oxidative metabolism for high energy supply. In a resting state (i.e. non-stimulated condition), approximately 60% of mitochondrial-derived ATP is expended on the maintenance of ionic gradients/signaling processes across the cell membranes (Silver & Erecińska, 1998). During enhanced neural activity, this percentage increases even more. In T1D, there is a growing appreciation for an underlying role of impairments in mitochondria in cells of the nervous system (neurons, glial and Schwann cells). However, the evidence to date has been in rodent models or cell culture models of hyperglycemia/T1D. With this caveat in mind, there are reports that mitochondrial trafficking and functions may be abnormal, particularly within distal axons. A number of studies supporting mitochondria's role in diabetic neuropathy (DN) have come from Feldman’s group, where they have observed that hyperglycaemia induced mitochondrial biogenesis and an increase in mitochondrial fission, thereby creating numerous small, damaged mitochondria within neurons (Feldman et al., 2017).
More recently, an emerging idea in the field of DN has been proposed: an imbalance in the mitochondrial redox state as a result of disorders in transferring energy between axons and their supporting glia cells (see Feldman et al., 2017 for more details). The interplay and inter-dependence of multiple cell types within the nervous system for survival is well appreciated and it is the hope that future studies will uncover a strong relationship between deficits in energy metabolism in supporting cell types and axonal loss in the nervous system.

Within the context of human T1D neuropathy studies, mitochondrial ultrastructure has been undertaken and mitochondrial dysfunctions inferred from these observations (Schmidt et al., 1997; Kalichman et al., 1998). Importantly, the majority of these ultrastructure studies were done in T1D patients with clinically diagnosed neuropathy so the relevance of this work (with respect to mitochondrial dysfunctions as a mediator of neuropathy) is limited. Some early work by Reske-Nielsen et al. (1977) included 1 newly-diagnosed adult (3 weeks of diabetes), and 3 adults with longer duration diabetes (24-32 years of diabetes). In the motor plate of the newly diagnosed adult, no abnormalities in mitochondria were reported in the Schwann cells, axon, or synaptic clefts. However, the motor end plates exhibited an increased presence of lipid bodies/lipofuscin which may
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be indicative of an early mitochondrial impairment (e.g. reductions in mitochondrial respiration causing accumulation of lipids in the cells). In contrast to newly diagnosed, the longer duration group exhibited reductions (and in one case, a loss) in mitochondrial content within the sarcoplasm around the synaptic clefts consistent with the aforementioned changes in mitochondrial biogenesis. Sural nerve biopsies from T1D individuals with neuropathy revealed enlarged and swollen mitochondria and cristae effacement in the Schwann cells as well as tightly aggregated mitochondria in post-synaptic dendrites (Schmidt et al., 1997; Kalichman et al., 1998). To the best of our knowledge, whether mitochondrial ATP production and ROS are altered in neural tissue of people with T1D remains to be established.

**KIDNEY TISSUE MITOCHONDRIA**

Though it may be underappreciated, renal tissue is second only to cardiac tissue with respect to energy demands and mitochondrial content (Bhargava & Schnellmann, 2017). This largely stems from the fact that many of the kidney’s primary functions, including waste removal from blood, nutrient reabsorption, and electrolyte and fluid balance, rely on ion gradients generated by the Na\(^+\)-K\(^+\)-ATPase. The abundance of normal mitochondria in renal tissue therefore ensures sufficient ATP supply for the ion
gradients, and hence, normal kidney function. Ultimately, impairments in the renal mitochondria with T1D should result in obvious, measurable deficits. While evidence of a mitochondrially-mediated basis for diabetic nephropathy has only recently been reported in humans (discussed below), the findings in many ways are consistent with the body of evidence in T1D rodent models. In these rodent studies, isolated kidney mitochondria displayed gradual and progressive reductions in ATP, reductions in the individual expression and activity of the electron transport chain proteins, increased fragmentation (i.e. defective mitochondrial dynamics), and increased capacity for opening of the mPTP, which correlated with mitochondrial H$_2$O$_2$ emission (Dugan et al., 2013; Coughlan et al., 2016). Importantly, some of these changes preceded the development of renal lesions, suggesting that alterations in mitochondrial phenotype is a primary cause of diabetic kidney disease, at least in T1D rodents.

Similar to rodent studies, mesangial cells from human kidney (HMCs) cultured in high glucose (25mM) display gradual and progressive reductions in mitochondrial respiration (ATP), increased cellular ROS (assay used was not mitochondrial-specific), and reduced cell viability (indirectly suggests increased susceptibility to opening of mPTP) (Czajka et al., 2015). Interestingly, the increase in ROS occurs in parallel with an increase
in mtDNA, suggesting an increase in mitochondrial content; however, further analysis in this study revealed a reduction in mtDNA transcription, increased mtDNA damage, and reduced mitophagy. In addition, mitochondria became more rounded and fragmented with increasing exposure time to high glucose. Thus, alterations in renal mitochondrial functions and morphology appears to occur prior to renal damage and as a direct result of continuous exposure to hyperglycemia in human cells.

Similar findings have also been reported in another study albeit in proximal tubular biopsies obtained from people with diabetes (pooled T1D and T2D) and diabetic nephropathy (Zhan et al., 2018). In particular, this study found an increase in superoxide (DHE staining), cytochrome c release (indirectly indicates increased opening of mPTP), and perturbations in mitochondrial dynamics (decreased expression of protein markers of mitochondrial fusion, increased expression of protein markers of mitochondrial fission, increased and scattered mitochondrial fragments, and remodeled mitochondrial cristae). Mitochondrial ATP production was not interrogated in this study. Interestingly, in surrogate cells for kidney tissue (peripheral blood mononuclear cells), basal and maximal mitochondrial respiration is decreased in people with diabetes (pooled T1D and T2D) with diabetic nephropathy compared to those without diabetic nephropathy but is not
different in people with diabetes without diabetic nephropathy compared to controls (Czajka et al., 2015). Consequently, without renal biopsies from people with T1D without diabetic nephropathy, the cause-effect relationship between changes in mitochondrial content/morphology and bioenergetics (respiration, ROS, mPTP) must still be defined. This will be essential in unraveling the contributions of mitochondrial dysfunctions to diabetic nephropathy development and progression in humans.

Lastly, a metabolomics study of urine from patients with and without diabetic kidney disease has also been undertaken (Sharma et al., 2013). Indeed, while not a direct assessment of mitochondria metabolism in kidney tissue, urine metabolomics permit the measurement of a wide range of metabolites in which the kidney is responsible for concentrating and excreting them in the urine. Thus, urine metabolomics allows for indirect insights into biochemical pathways linked to kidney dysfunction. In this particular study, the researchers found that in people with diabetes (both T1D and T2D), a number of metabolites were linked together in a large network and virtually all of these metabolites were produced by enzymes localized to the mitochondria. It was further reported that a significant reduction of these metabolites occurred in the urine of people with diabetes (both T1D and T2D) with and without diabetic kidney disease. The

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reduction in these metabolites suggest that the mitochondrial enzymes responsible for producing them might also be attenuated, thereby suggesting an overall reduction in mitochondrial function in kidneys from people with diabetes.

SUMMARY AND PERSPECTIVES

The intention of this Symposium Review article was to spark discussion and research ideas regarding the concept of mitochondrial ‘dysfunction’ as a primary mechanism in diabetes complications development and/or progression, and to summarize the limited human literature in T1D as it pertains to abnormalities in mitochondrial structure and functions within a number of tissues. This idea was borne out of: (1) our previous work in skeletal muscle, which demonstrated the presence of clear mitochondrial deficiencies, both in vivo and in vitro, in adults with T1D prior to any notable impairments in functionality [reviewed in (Monaco et al., 2019)]; and (2) the pathways in which Brownlee’s “unified theory” of diabetes complications (Brownlee, 2005) identified as ‘damaging pathways’ may be expandable to more tissues, with the concept that the mitochondrial-derived superoxide may be the result of hyperglycemia or from impairments to mitochondrial bioenergetics (by mechanisms yet to be elucidated). We do acknowledge the limitations that are currently present in the data supporting this
expansion of the unified theory, particularly in human studies investigating the mechanisms leading to cardiovascular disease, nephropathy and neuropathy in T1D, as there are ethical limitations to in vivo tissue sampling (biopsies). However, recent advances with non-invasive methods such as $^{31}$P-MRS are expected to open opportunities to further test this line of enquiry as the need for clinically attenuating the development and progression of diabetes complications cannot be understated.

CONCLUSIONS

The research described in this review suggests that mitochondria are negatively impacted by the T1D environment in skeletal, cardiac, nervous and kidney tissues in people with T1D through mechanisms yet to be fully elucidated. A consistent finding across the majority of these tissues is the impairment in mitochondrial dynamics (e.g. increased fragmented mitochondria) and bioenergetics. Specifically, reductions in mitochondrial respiration (i.e. ATP production) and increases in ROS, which are worsened by the presence of diabetes complications (Figure 1). As such, the authors expect that the widespread energy depletion (potentially a result of altered mitochondrial dynamics) likely goes hand-in-hand with elevated mitochondrial ROS, especially during repeated...
bouts of hyperglycemia. This likely culminates in oxidative stress and damage to cells and consequently, development of diabetes complications across a variety of tissues.

COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

C.M.F.M, C.G.R.P, T.J.H: conception and design of research, drafting, editing and revision of manuscript. T.J.H. approved the final version of the manuscript and is the guarantor of this work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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REFERENCES


Figure 1  Our current understanding of the impact of T1D on mitochondrial functions and morphology across various tissues in people. Dyslipidemia, dysglycemia, hyperinsulinemia (from subcutaneous insulin injections), and insulin resistance are characteristic of T1D and are considered to be the primary contributors in the development of diabetes complications. At the cellular level, these characteristics can lead to substrate overload (e.g. glucose toxicity) and decreased/poor insulin signaling, all of which can directly or indirectly impact mitochondria. Alterations in mitochondrial
functions and morphology accompany various human diseases, including myopathies, neurodegeneration, aging, and diabetes (Wallace, 1999). It is thus our working hypothesis that impairments to mitochondria may be a primary cellular mechanism underlying the pathophysiology of various complications in people with T1D. Unfortunately, there exists a very finite amount of human literature on the impact of T1D on mitochondria in skeletal muscle, heart, nervous system, and kidneys. What is currently known is listed under each respective tissue. The question mark indicates that no studies to date have interrogated this measure in people with T1D. The inclusion of multiple arrows and ‘normal’ means that this measure differed across various studies or differed due to disease status and presence of secondary complications.