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REVIEW | *Mitochondria Dysfunction in Aging and Metabolic Diseases*

Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis

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Pinti MV, Fink GK, Hathaway QA, Durr AJ, Kunovac A, Hollander JM. Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis. *Am J Physiol Endocrinol Metab* 316: E268–E285, 2019. First published January 2, 2019; doi:10.1152/ajpendo.00314.2018.—Type 2 diabetes mellitus (T2DM) is a systemic disease characterized by hyperglycemia, hyperlipidemia, and organismic insulin resistance. This pathological shift in both circulating fuel levels and energy substrate utilization by central and peripheral tissues contributes to mitochondrial dysfunction across organ systems. The mitochondrion lies at the intersection of critical cellular pathways such as energy substrate metabolism, reactive oxygen species (ROS) generation, and apoptosis. It is the disequilibrium of these processes in T2DM that results in downstream deficits in vital functions, including hepatocyte metabolism, cardiac output, skeletal muscle contraction, β-cell insulin production, and neuronal health. Although mitochondria are known to be susceptible to a variety of genetic and environmental insults, the accumulation of mitochondrial DNA (mtDNA) mutations and mtDNA copy number depletion is helping to explain the prevalence of mitochondrial-related diseases such as T2DM. Recent work has uncovered novel mitochondrial biology implicated in disease progressions such as mtDNA heteroplasmy, noncoding RNA (ncRNA), epigenetic modification of the mitochondrial genome, and epitranscriptomic regulation of the mtDNA-encoded mitochondrial transcriptome. The goal of this review is to highlight mitochondrial dysfunction observed throughout major organ systems in the context of T2DM and to present new ideas for future research directions based on novel experimental and technological innovations in mitochondrial biology. Finally, the field of mitochondria-targeted therapeutics is discussed, with an emphasis on novel therapeutic strategies to restore mitochondrial homeostasis in the setting of T2DM.

diabetes mellitus; mitochondria dysfunction

INTRODUCTION

The International Diabetes Federation reports the number of adults 18–99 yr old with type 2 diabetes mellitus (T2DM) worldwide to be 451 million and predicts this figure will rise to 693 million by 2045 if trends continue (57). The increased risk of developing T2DM in both aging and obese populations, coupled with epidemiological data revealing rises in aged and obese demographics, necessitates deep understanding of the molecular changes underlying these links (99). Many have identified the mitochondrion as a locus of convergence for the host of dysregulated pathways in aging, obesity, and T2DM. Aging, for example, has been implicated in both mitochondria-intrinsic impairment such as mtDNA mutation and depletion and in mitochondria-related processes like apoptosis (16, 41, 92, 197). Obesity is characterized by an increased circulating free fatty acid concentration and accumulation of triacylglycerol in peripheral tissues that contribute to mitochondrial alterations, including increased lipotoxicity, elevated oxidative stress, and impaired energy substrate metabolism and oxidative phosphorylation (OXPHOS) (21, 33, 66, 94, 203). The role of mitochondrial dysfunction is of particular importance in T2DM due to its established association with insulin resistance, as previously reviewed (58, 105, 116, 152, 190). However, an up-to-date synthesis and organization of the many mitochondrial alterations in T2DM using an organ-based approach is urgently needed. The following sections will provide in intimate detail the structural, functional, and molecular changes

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accompanying mitochondrial dysfunction in T2DM across organ systems (Fig. 1). The organization of sections represents primary effector organs of T2DM pathogenesis with adaptations observed early on at the beginning to secondarily impacted organs that may not reach significant dysfunction until late in T2DM progression.

MITOCHONDRIAL DYSFUNCTION IN THE T2DM DIGESTIVE SYSTEM

Mitochondrial Dysfunction in the T2DM Liver

Hepatic dysfunction in the form of nonalcoholic fatty liver disease (NAFLD) is commonly observed in patients with T2DM (107, 137, 150, 177, 183). Whether NAFLD is a cause or consequence of the diabetic pathology remains a topic of contention; however, the alterations in hepatic energy substrate metabolism and mitochondrial function in T2DM patients with NAFLD are well characterized. Decreased insulin sensitivity of the liver accompanied by increased hepatic fat storage are two such major metabolic changes found in the diabetic patient (87, 88, 137, 160). Mitochondria-intrinsic perturbations in obese, insulin-resistant patients with nonalcoholic steatohepatitis (NASH) include lower maximal respiration, increased mitochondrial uncoupling, and increased proton leak (85). These findings are further strengthened by the observation of decreased ATP content and turnover in the T2DM liver (160, 175).

Calcium homeostasis has also been shown to be an important contributor to mitochondrial function and insulin sensitivity in the liver (97, 146, 194). Notably, the endoplasmic reticulum (ER) has been shown to have functional association with mitochondria via mitofusin 2 tethering (49). These interactions at the mitochondria-associated ER membranes (MAMs) have been found to play a major role in insulin signaling, although whether the coupling of these organelles is increased or decreased in the insulin-resistant liver is debated (9, 185). Recent findings indicate that MAM disruption is observed in skeletal muscle of T2DM humans and mice (184). The influence of ER-mitochondria interactions on all other insulin-resistant organs in T2DM is currently unknown.

Mechanistic studies have uncovered a variety of potential molecular mediators to help explain changes in metabolism and mitochondrial dynamics in the diabetic liver. Members of the forkhead box transcription factor family have been shown to be among those mediators most prominently involved. It was found that Foxa2 is sequestered in the hepatocyte cytoplasm of insulin-resistant mice but that adenoviral introduction of a nuclear constitutively active form reverses many of the metabolic alterations in the T2DM liver described above (200). Conversely, Foxo1 knockout (KO) has been shown to reverse mitochondrial dysfunction and improve hepatic metabolism in insulin receptor substrate-1 and insulin receptor substrate-2 double-knockout (DKO) mice (35). Transcription factors of the PGC family have also been implicated in metabolic dysfunc-

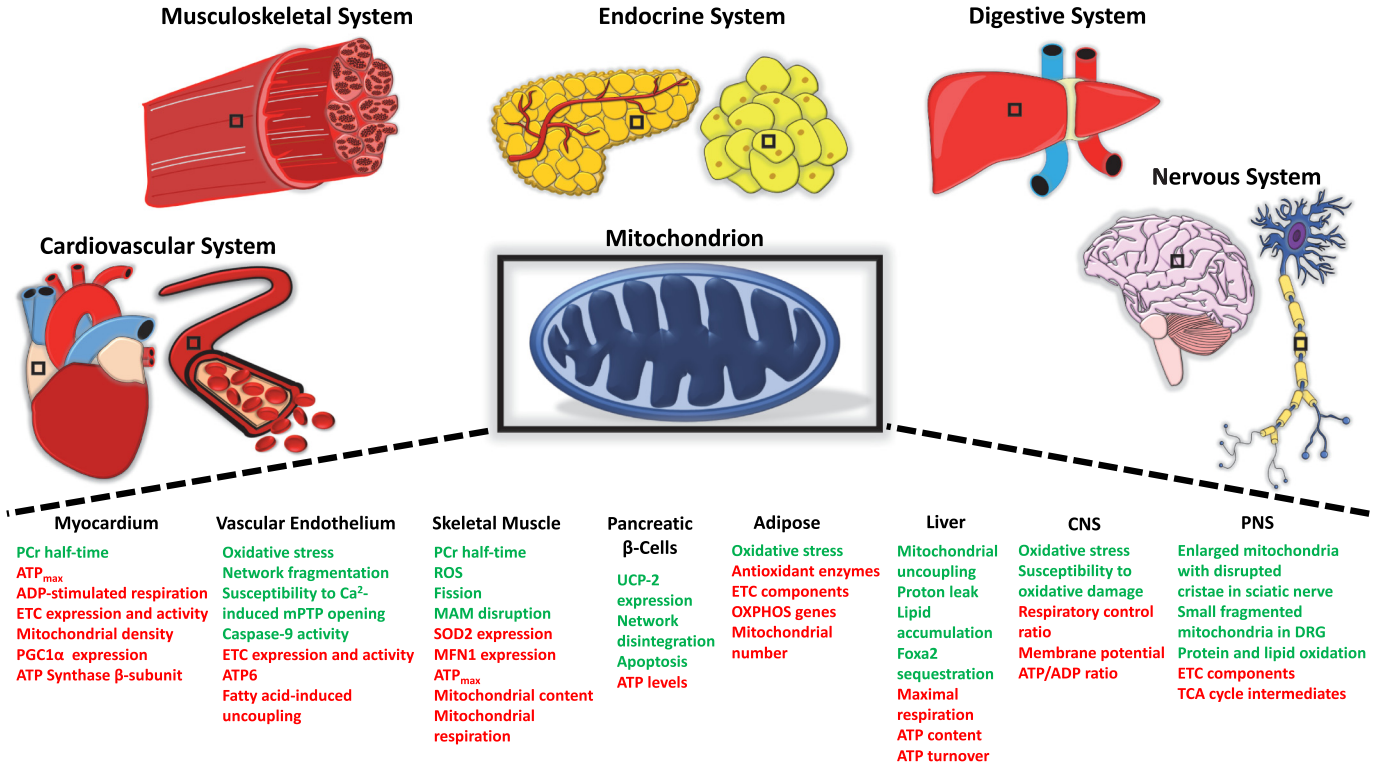

Fig. 1. Mitochondria dysfunction across organ systems in type 2 diabetes mellitus. Structural, functional, and molecular changes to mitochondria in multiple diabetic models are displayed. Green font indicates an increase in the change, whereas red font indicates a decrease in the change. ATP_{max}, maximal ATP synthesis rate; CNS, central nervous system; DRG, dorsal root ganglia; ETC, electron transport chain; MAM, mitochondria-associated ER membrane; MFN1, mitofusin 1; mPTP, mitochondrial morphology and permeability transition pore; OXPHOS, oxidative phosphorylation; PCr, phosphocreatine; PGC-1α, peroxisomal proliferator activator receptor-γ coactivator-1α; PNS, peripheral nervous system; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; UCP2, uncoupling protein 2.

tion of the diabetic liver (72, 86, 191). To highlight the important role of mitochondrial constituents in the diabetic liver, it was shown that long-chain acyl-CoA dehydrogenase-KO mice exhibit hepatic insulin resistance accompanied by hepatic steatosis (209). Furthermore, mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1-KO mice were protected from hepatic triacylglycerol and diacylglycerol accumulation and insulin resistance following high-fat feeding, indicating its vital role in the development of diabetic liver metabolic dysfunction (122). Interestingly, liver-specific promoter methylation of important transcription factors, including PPARGC1A and mitochondrial transcription factor A, as well as mtDNA/nDNA ratio, have been reported to be associated with insulin levels and liver PPARGC1A mRNA levels in NAFLD (170). Further investigation of mitochondrial dynamics in the diabetic liver using techniques of epigenetics and epitranscriptomics may provide a clearer explanation of the altered transcriptomic and proteomic profiles associated with impaired mitochondrial function.

MITOCHONDRIAL DYSFUNCTION IN THE T2DM MUSCULOSKELETAL SYSTEM

Mitochondrial Dysfunction in T2DM Skeletal Muscle

Skeletal muscle insulin resistance is a central factor in the pathogenesis of T2DM. Because of their major role in systemic metabolism, skeletal muscle mitochondria in the T2DM setting have been thoroughly investigated. Many groups have come to an agreement on skeletal muscle mitochondrial dysfunction in the context of T2DM, although whether this is a cause or consequence of T2DM is still debated (10, 11, 22, 81, 113, 116, 138, 147, 148, 161, 206). This dysfunction has been characterized by a variety of methods both in vivo and ex vivo. In a study of T2DM patients and BMI-matched controls, the phosphocreatine (PCr) recovery half-time of vastus lateralis muscle from diabetic patients was 45% longer than that of controls (161). These results are further strengthened by a separate group's finding of lower maximal ATP synthesis rate (ATP_{max}), as assessed by PCr recovery in vastus lateralis of T2DM patients (11). Phielix et al. (138) also found increased PCr half-time in diabetic vastus lateralis muscle and showed that ADP-stimulated basal respiration and FCCP-stimulated maximal respiration were decreased in mitochondria from the diabetic patient cohort. This decrease in mitochondrial respiratory capacity in diabetic patients is supported by the observation of decreased maximal ADP-stimulated respiration with pyruvate-malate combination in vastus lateralis mitochondria of diabetic patients (113). These functional impairments in mitochondria from diabetic skeletal muscle may be partly attributed to decreased subsarcolemmal mitochondria (SSM) electron transport chain (ETC) activity in vastus lateralis of T2DM patients compared with both obese and lean nondiabetic patients (148). Interestingly, many of these same mitochondrial abnormalities in hindlimb skeletal muscle of diabetic high-fat diet-fed mice, including decreased ADP-stimulated respiration, ETC complex I and III activities, and mitochondrial density, have also been reported (206). Whether mitochondrial dysfunction is the underlying cause or consequence of T2DM, the latter has been suggested based on findings of glucose intolerance preceding mitochondrial impairment in a diet-induced diabetic mouse model (22). In opposition to the above findings, some have been unable to find evidence of skeletal muscle mitochondrial dysfunction in the context of T2DM (24, 50). Although most studies focused on mitochondrial function in skeletal muscle have found some degree of dysfunction in T2DM, the ability of exercise interventions to prevent or reverse some aspects of this dysfunction is an area of active investigation and is addressed in a subsequent section.

It is important to consider the experimental techniques and normalization methods used to measure and interpret in vivo and ex vivo mitochondrial function. PCr half-time and ATP_{max} are in vivo assessments of mitochondrial function derived from phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) data obtained by fixing a surface coil in the middle of the vastus lateralis muscle and recording pre-exercise, during exercise, and postexercise (11, 50, 138, 161). Because of the inability to quantify the mitochondrial density of the precise muscle tissue providing the ^{31}P-MRS values, these measures cannot distinguish whether increased PCr half-time or decreased ATP_{max} is due to decreased mitochondrial number or impaired mitochondrial function or exactly how much each may contribute. One method of determining ex vivo mitochondrial function is measuring oxygen consumption polarographically (22, 24, 113, 115, 138, 206). To ensure mitochondrial function rather than density is represented by using this technique, a variety of normalization methods have been employed to normalize for mitochondria content including citrate synthase activity, mtDNA copy number, isolated mitochondria protein concentration, or a combination of these parameters (24, 113, 138, 206). Another ex vivo analysis of mitochondria function can be found in the measurement of ETC complex activities following mitochondrial permeabilization. Optimal normalization of ETC complex activity data follows suit with those methods used for normalizing mitochondrial respiration data (113, 115, 148, 206). Whereas in vivo mitochondrial analysis provides a more physiologically relevant representation of mitochondrial function within its network and environment, ex vivo mitochondrial assessment allows one to control for mitochondrial density. A combination of in vivo and ex vivo mitochondrial analysis in the model being studied is ideal to provide the greatest insight into mitochondrial function. It is essential to consider both the technique to assess mitochondrial function and the method(s) of normalization when interpreting results of mitochondria function in all tissue types.

The shift in energy substrate availability for insulin-resistant skeletal muscle of T2DM patients and animal models necessitates an alteration in mitochondrial metabolism and is also associated with decreased expression of antioxidant genes in concert with increased oxidative stress (25). Multiple groups have converged on the important role peroxisomal proliferator activator receptor-γ coactivator-1α (PGC-1α) plays in mediating this metabolic change (117, 132). PGC-1α, a major promoter of oxidative metabolism and mitochondrial biogenesis, was found to be downregulated in vastus lateralis of T2DM patients when compared with controls with no family history of diabetes mellitus (132). Decreases in expression of PGC-1α-responsive genes involved in fatty acid oxidation (FAO), glycolysis, the tricarboxylic acid (TCA) cycle, and the ETC were also reported (132). The precise regulation of upstream and downstream pathways of the PGC-1 family of coactivators

in health and metabolic disease has been reviewed previously (98). Gene Set Enrichment Analysis identified the OXPHOS gene set to have the most significantly changed expression in DNA microarray data from vastus lateralis samples of patients with T2DM relative to controls (117). In agreement with these findings, others have found downregulation of the ATP synthase gene transcript in skeletal muscle of T2DM subjects (172). Decreased FAO in vastus lateralis muscle of non-insulin-dependent diabetes mellitus patients has been observed by others; however, a concomitant increase in glucose oxidation was also reported (82). In addition to changes in the expression of oxidative metabolism components in the T2DM phenotype, it has been reported that the levels of phosphorylated ATP synthase β-subunit are significantly lower in T2DM (71). Others have reported increased expression of mtDNA-encoded cytochrome oxidase I, cytochrome oxidase III, and NADH dehydrogenase IV transcripts in T2DM skeletal muscle when normalized to mtDNA copy number, although this does not necessarily indicate the increased protein expression of these genes (8). Potential factors that may account for the increase in these gene transcripts important in OXPHOS that is contradictory to the above studies in skeletal muscle include the lack of controlling for age, sex, and other important patient characteristics between nondiabetic and T2DM groups as well as skeletal muscle samples being taken from either gastrocnemius or quadriceps instead of a single location in all subjects (8). Additional factors that may account for differences in mitochondrial function and gene expression between studies comparing skeletal muscle of nondiabetic and T2DM subjects include normalization methods and differences in fitness level as well as medication history of participants. The global decrease in expression of genes involved in oxidative metabolism of skeletal muscle mitochondria found in most studies investigating T2DM pathology may help explain the decreased basal and maximal respiration in this population described above.

It will be of great value to investigate additional mitochondrial dynamics, including calcium homeostasis, fission/fusion balance, network formation, and mitochondrial reticulum alterations in the setting of T2DM to determine how changes in these processes may influence metabolic dysregulation in skeletal muscle mitochondria. To this end, it was shown that right atrial tissue of T2DM patients displayed markedly reduced intrinsic contraction, increased oxidative stress, and mitochondria dysfunction associated with observations of mitochondrial network fragmentation and decreased expression of mitofusin-1 (115). Mitochondrial dynamics in T2DM has been reviewed previously (152, 199). Furthermore, the critical roles of calcium transport and homeostasis in both skeletal muscle and cardiac mitochondria as well as its tight linkage with OXPHOS has also been examined (42, 65, 129). Exploring how calcium handling is changed in T2DM may provide greater insight into the OXPHOS alterations observed. More recently, they have provided convincing evidence to support a mitochondrial reticulum in skeletal muscle in which protonmotive force is generated primarily in complex IV-rich paravascular mitochondria and conducted down complex V-rich I-band mitochondria, where it is used for ATP generation (64). The theoretical basis of this electrochemical transmission, identifying Na^+ and/or K^+ as essential ions in the conduction from the cell periphery to the I-band segments, has been reported (131).

Ability of Exercise to Reverse Mitochondrial Dysfunction in T2DM

Exercise training has been shown not only to improve insulin sensitivity in patients with T2DM but also to help restore mitochondrial function (69, 102, 110, 145, 182, 189). In fact, in vivo mitochondrial function, which was decreased in T2DM patients, was normalized by exercise training in T2DM subjects up to control levels (110). In a separate study, it was shown that a behavioral weight loss program in T2DM patients, including ≥4 days of exercise at 60–70% maximal heart rate, increased skeletal muscle mitochondrial density, size, cardiolipin content, mtDNA content, citrate synthase activity, and NADH oxidase activity (182). Van Tienen et al. (189) showed that after 1 yr of exercise training twice/wk, longstanding T2DM patients had an increased PCr recovery rate constant and increased mitochondrial density and complex I activity of vastus lateralis muscle. Low-volume, high-intensity exercise was also shown to increase maximal citrate synthase activity as well as upregulate subunits of ETC complexes II and III in vastus lateralis muscle of T2DM patients (102). Ten weeks of aerobic training on a stationary bike two to three times/wk was enough to increase respiration with complex I-supported substrates, ETC_{max}, and mitochondrial lipid oxidation in vastus lateralis from T2DM patients (69). Others have found low-intensity exercise in the form of walking >150 min/wk over the span of 4 mo to increase the expression of metabolic enzymes UCP3 and PPARδ (59). The positive effects of exercise extend beyond T2DM patients, as aerobic exercise programs have been shown to improve insulin sensitivity and skeletal muscle mitochondrial function in both healthy subjects and nondiabetic obese participants (69, 110). The precise contribution that physical inactivity of T2DM subjects has on skeletal muscle mitochondrial dysfunction has not been quantified; however, it is plausible that increasingly sedentary lifestyles of T2DM patients may play a significant role for changes in skeletal muscle mitochondrial density and function. Evidence in support of this hypothesis comes from studies in skeletal muscle showing that mitochondrial respiration is similar in T2DM and nondiabetic subjects when physical activity is controlled for and that mitochondrial function in T2DM is restored to control levels following an exercise training program (145, 189). The dynamic nature of the mitochondrion, and of mitochondrial metabolism more specifically, makes it susceptible to environmental insults but also to the positive impact of exercise training and lifestyle modification.

Exercise training has been found to have systemic positive effects in patients with T2DM, as evidenced by decreased liver fat, improved β-cell function, bolstered endothelial function, enhanced cardiac structure and function, and attenuation of diabetic peripheral neuropathy development (13, 20, 30, 40, 91, 213). The precise mechanisms linking exercise training to the improved function of organs negatively impacted by T2DM have not been fully elucidated, but we suspect that, as in

skeletal muscle, improved mitochondrial function will be a significant contributor.

MITOCHONDRIAL DYSFUNCTION IN THE T2DM ENDOCRINE SYSTEM

Mitochondrial Dysfunction in T2DM Pancreatic β-Cells

Pancreatic β-cells serve as the body's thermostat in sensing glucose level and responding to insulin needs of the organism. When either of these abilities to sense or to respond is impaired, there are consequences to systemic metabolism. In a state of metabolic homeostasis, temporary fluctuations in blood glucose levels are easily corrected by small alterations in insulin secretion. However, in the setting of T2DM, chronic exposure to hyperglycemia and hyperlipidemia impairs β-cell function. Multiple groups have highlighted mitochondrial structural or functional abnormalities as key factors in this impairment (6, 51). Pancreatic β-cells from diabetic patients were shown to have increased expression of ETC complexes I and V though decreased ATP levels and ATP/ADP ratio (6). They explained this paradox by showing increased uncoupling protein 2 (UCP-2) expression in diabetic islet cells of which glucose-stimulated insulin production was decreased (6). Interestingly, it has recently been reported that high-intensity CrossFit training 3 days/wk for 6 wk can reverse β-cell dysfunction in T2DM patients (123). In a Goto Kakizaki T2DM rat model it was shown that pancreatic β-cells displayed mitochondrial network disintegration (51). Others have found increased β-cell apoptosis in Zucker diabetic fatty rats and high-fat diet-induced models of T2DM (165, 169). Increased ETC-derived reactive oxygen species (ROS) in high-glucose-treated MIN6 pancreatic β-cells was shown to contribute to decreased glucose-induced insulin secretion (156). The important role of mitochondria in optimal β-cell function is further evidenced by the age-related loss of mtDNA corresponding with declining insulin secretion (43, 124). Previously, it had been shown that mtDNA depletion in a mouse pancreatic β-cell line prevented adequate glucose-stimulated insulin secretion (168). Altogether, pancreatic β-cell mitochondria play a dynamic role in the process of insulin secretion. This begs the question of whether mitochondria-targeted therapeutics may be a viable treatment strategy to improve β-cell function in T2DM patients.

Mitochondrial Dysfunction in T2DM Adipose Tissue

Often overlooked as a passive organ, adipose tissue is insulin-sensitive, highly dynamic, and acutely responsive to various environmental factors. The strong linkage between obesity and T2DM provides ample motivation into exploring how adipocyte populations are altered in the diabetic setting. One such alteration widely found in adipose tissue from T2DM patients and animal models is that of mitochondrial dysfunction or decrement (32, 37, 47, 93, 151). It was reported that obese T2DM patients displayed increased mitochondrial ROS when compared with nondiabetic normal weight controls and concomitantly that mitochondrial antioxidant enzymes were downregulated in this group (32). It has also been shown that many ETC components have decreased expression in visceral adipose mitochondria of women with T2DM (47). This is further supported by work showing decreased expression of OXPHOS genes in adipose tissue of T2DM patients (125). However, others have provided data to support the notion that mitochondrial dysfunction is only present in obese T2DM patients (31). In animal models of T2DM, the decrease in mitochondrial number and protein content have been shown to be corrected by either daily voluntary wheel running or treatment with the insulin-sensitizing agent rosiglitazone (37, 93, 151). Interestingly, one study looked at five groups of obese mice with increasing hyperglycemia and found that three classes of proteins with marked change in expression were those of metabolism, signal transduction, and transcription factors (120). In the 3T3-L1 adipocyte cell line, treatment with high glucose and high glucose plus high free fatty acids resulted in increased ROS levels, loss of mitochondrial membrane potential, and downregulation of NRF1 and PGC-1, two important OXPHOS transcription factors (61). These findings suggest that changes in adipose tissue composition and secretion of inflammatory molecules may contribute to mitochondrial dysfunction in other organs of the T2DM patient.

MITOCHONDRIAL DYSFUNCTION IN THE T2DM CARDIOVASCULAR SYSTEM

Mitochondrial Dysfunction in the T2DM Myocardium

Mitochondrial dysfunction in the type 2 diabetic human myocardium has been observed by multiple groups, although the negatively impacted mitochondria-intrinsic processes leading to this impairment are varied (2, 4, 45, 115). It has been identified that increased oxidative stress and mitochondrial network fragmentation in right atrial tissue of diabetic patients may be contributors to the observed dysfunction (115). Others have echoed this reported increase of ROS in the human diabetic heart (2, 4). Some argue that this increase in metabolic stress may render the myocardium more susceptible to Ca^{2+}-induced mitochondrial permeability transition pore opening and further activation of the intrinsic apoptosis pathway (4). Indeed, this group found increased activity of caspase-9 in right atrial tissue from T2DM patients (4). Regarding the mitochondrial subpopulation most negatively impacted by the diabetic pathology, our laboratory has determined that cardiac SSM is most susceptible to T2DM insult (45). It was found that ETC complex I and IV activities and expression levels were decreased in SSM of T2DM patient right atrial tissue, whereas there was little to no difference in interfibrillar mitochondria (IFM) (45). The short- and long-term effects of insulin therapy or lifestyle modification on the levels of ROS and apoptosis activation in the diabetic human heart remain unexplored.

Murine models of T2DM have largely recapitulated the cardiac mitochondrial dysfunction described above (18, 23, 46, 84, 106, 109, 121, 181). These models may provide the researcher with more detailed insight into the mechanistic underpinnings of T2DM on cardiac mitochondria due to the ability to analyze the full heart and not atrial tissue alone. The in-depth understanding of mitochondrial-driven pathways such as energy substrate metabolism and ROS generation provided by T2DM animal models have proven invaluable. As an example, our laboratory observed downregulation of ATP6 in SSM of *db/db* hearts (164). Decreased expression of mtDNA-encoded genes in T2DM may also be a function of decreased

binding of mtTFA to the D-loop of mtDNA to initiate transcription (80). Previously, we have shown that the SSM subpopulation from cardiac tissue in the *db/db* model had decreased size, internal complexity, state 3 respiration, and ETC complex activities (46). Others have characterized mitochondrial energy substrate metabolism in the insulin-resistant *ob/ob* heart, finding precipitously decreased glucose metabolism with concomitant increased rates of palmitate oxidation (109). As with the human disease, increased ROS generation has been established as a hallmark of the T2DM cardiac phenotype in animal models. It has been found that there is increased H_2O_2 production when *db/db* cardiac mitochondria were provided with glutamate and malate in combination (84). Other groups have also reported increased mitochondrial-derived ROS as well as higher lipid and protein peroxidation products (23, 106). Another group showed that mitochondrial ROS were dramatically increased in cardiac mitochondria of Zucker diabetic fatty rats (18). Interestingly, some have documented the increased presence of myocardial lipid droplets in the diabetic heart, situated even closer to the mitochondria (23, 121). Boudina et al. (23) make a striking argument for fatty acid-induced mitochondrial uncoupling in the *db/db* heart to explain how increased FAO and ETC complex activities lead to increased mitochondrial-generated ROS, lower ATP synthase F1 α-subunit expression, and impaired OXPHOS capacity. Similar to the skeletal muscle, which is discussed above, the myocardium has also been found to possess a mitochondrial reticulum, albeit with greater segmentation (63). Whether cardiac mitochondrial networks are altered in T2DM or have a reduced capacity to undergo dynamic disconnection to preserve function remains unknown. Although the above mentioned diabetic animal models clearly share many cardiac mitochondrial similarities with T2DM patients, there remains an open opportunity to more closely mimic the insulin-treated T2DM patient in an animal model and determine whether combining novel mitochondria targeted therapeutics with traditional insulin therapy would provide improved cardiac function in T2DM preclinical models.

Mitochondrial Dysfunction in T2DM Vascular Endothelium

Endothelial dysfunction has been found to accompany T2DM in both the human pathology and animal model (36, 83, 162). This impairment in endothelial function can be explained in part by altered mitochondrial processes. The most prevalent observation in diabetic endothelial cells is the increased accumulation of mitochondrial-derived ROS (36, 83, 162). It has been shown that mitochondrial fission contributes to increased mitochondrial ROS production by knocking down mitochondrial fission proteins Fis1 or Drp1 and finding no increase in ROS production when stressed with high glucose (162). In a T2DM animal model generated by streptozotocin (STZ) injection followed by a high-fat diet, coronary endothelial cells were found to have higher mitochondrial ROS levels and lower superoxide dismutase 2 expression than nondiabetic controls (36). Thus, the increased mitochondrial ROS observed in the T2DM vasculature could be a function of both structural abnormalities and decreased antioxidant defense.

MITOCHONDRIAL DYSFUNCTION IN THE T2DM NERVOUS SYSTEM

Mitochondrial Dysfunction in the T2DM Central Nervous System

The connection between T2DM and dementia has been thoroughly studied. In the Honolulu-Asia Aging Study, it was shown that T2DM was associated with increased risk of total dementia, Alzheimer's disease, and vascular dementia (134). Risk of T2DM patients developing Alzheimer's disease was compounded by the co-occurrence of the APOE-ε4 allele (134). Some have pointed to the alterations in the cerebral glucose metabolic rate in the frontal, temporal-parietal, and cingulate brain regions of prediabetic or T2DM patients as an important factor in declining cognitive function (12). This phenomenon has been more extensively investigated in diabetic animal models. It has been reported that brain mitochondria from sucrose-treated T2DM mice have a decreased respiratory control ratio, membrane potential, and ATP/ADP ratio when compared with nondiabetic mice (29). This observed brain mitochondrial dysfunction in T2DM may be attributed to higher ROS production in combination with greater susceptibility to oxidative damage (143, 158). The influences of aging and amyloid-β exposure on brain mitochondria from diabetic Goto Kakizaki rats were examined, and it was reported that function was most compromised when T2DM, aging, and amyloid-β exposure were all present (118). Others utilizing the same diabetic model found that amyloid-β_{1-40} exposure of brain mitochondria induced increased H_2O_2 production, followed by decreased respiratory control ratio and ATP content; however, treatment with the antioxidant CoQ10 attenuated these negative effects (119). It is important to note that some have documented no change in brain mitochondrial function or biogenesis of young (6-mo-old) diabetic Goto Kakizaki rats (159). There is a need to identify the specific pathways implicated in other pathologies that synergize with the T2DM phenotype to produce mitochondrial dysfunction in the brain.

Mitochondrial Dysfunction in the T2DM Peripheral Nervous System

Diabetic peripheral neuropathy is a major complication for many T2DM patients, and the impairment of peripheral neurons in diabetics has been shown to be linked to structural or functional abnormalities of their mitochondria. One group reported enlarged mitochondria containing disrupted cristae in sciatic nerves of galactose-fed diabetic rats and in sural nerves of T2DM patients (78). In contrast, others have observed more numerous smaller, fragmented mitochondria in dorsal root ganglion neurons of *db/db* mice when compared with controls (54). Others have observed increased mitochondria in dorsal root axons of *db/db* mice (192). Regarding other peripheral nerve mitochondrial changes in T2DM, it has been shown that there is decreased expression of ETC complex proteins in dorsal root ganglia of *db/db* mice (153). It has also been reported that TCA cycle intermediates are lower in sural and sciatic nerves as well as dorsal root ganglia of *db/db* mice (70). This downregulation of TCA cycle flux was in concordance with an increased presence of protein and lipid oxidation found

in these tissues of diabetic mice (70). Determining the balance of mitochondrial fission and fusion in human T2DM peripheral nerves as well as how systemic antioxidant therapy may attenuate mitochondrial dysfunction in peripheral neurons remains unanswered for the field.

MITOCHONDRIAL DYSFUNCTION A UNIFYING THEME OF T2DM: THE ADAPTIVE THRESHOLD HYPOTHESIS

Mitochondrial dysfunction as a potentially unifying hypothesis accounting for both skeletal muscle insulin resistance and impaired pancreatic β-cell insulin secretion, two major events leading to T2DM, was first proposed by Lowell and Shulman (105) in a landmark viewpoint article in 2005. In the years since, organ-specific mitochondrial function in T2DM has been investigated much more thoroughly, as referenced in the above sections. Based on these findings, we suggest mitochondrial dysfunction to be a thread running deep in T2DM not only in organs that are primary drivers of the disease but also in those impacted secondarily. Current evidence supports a model where organs possess an adaptive threshold to genetic and environmental influences past the point where maladaptation occurs and the T2DM pathology progresses. Because certain organs play more prominent roles than others at different stages of T2DM pathogenesis, one would expect the adaptive thresholds of organs to be surpassed in a relatively predictive manner. However, because each organ functions in the context and environment of the organism, the adaptations and subsequent maladaptations of each organ in response to genetic and environmental insults leading to T2DM influence every other organ via the circulation. In this section, we present current evidence in support of this hypothesis.

Integration of T2DM Pathogenesis: Mitochondrial Dysfunction and Systemic Metabolism

Obesity-related T2DM is attributed partially to energy substrate imbalance both in the circulation and more importantly in the major effector organs of the diabetic condition: liver, skeletal muscle, and pancreatic β-islets. Energy substrate levels in the circulation are representative of external influences such as nutritional inputs and internal influences, including systemic energy substrate transport and metabolism in all bodily tissues. Excess nutrition and/or inactivity over an extended period in conjunction with genetic and epigenetic factors overwhelms the capacity of the organism to maintain strict control over circulating energy substrate levels.

In particular, increased hepatic lipid accumulation is a common observation in prediabetes and early-stage T2DM explained by higher circulating triacylglycerol levels (89). The culmination of lipid accumulation, aberrant lipid metabolism, and insulin resistance of the liver over time pushes the organ past its adaptive capability of increasing maximal mitochondrial respiration to respond to excess energy substrate accumulation, as in NAFLD to crossing its adaptive threshold characterized by decreased maximal respiration, mitochondrial uncoupling, and mitochondrial leakage, as in NASH (85). Interestingly, it was shown in the OLETF rat model of obesity that mitochondrial dysfunction may even precede NAFLD and hepatic insulin resistance (144). The impact of hepatic lipids on insulin resistance in T2DM has been definitively established and reviewed previously (136). Briefly, it has been demonstrated in vitro that palmitate treatment of hepatocyte cell lines and primary mouse hepatocytes reduces insulin receptor expression and activation of the insulin signaling cascade in a dose- and time-dependent fashion (154). Not only does the insulin-resistant fatty liver have a reduced capacity to efficiently convert the increased lipids being delivered to it by the circulation to energy via FAO and OXPHOS, but the lack of insulin signaling for the inhibition of gluconeogenesis also contributes to increased glucose production and secretion by the liver (111, 175). The downstream effects of increased circulating lipids, glucose, and inflammatory mediators following the development of insulin insensitivity in the liver help explain the tight inverse correlation found between intrahepatic triglyceride levels and whole body glucose disposal (74).

Increased circulating lipids following excess nutrition and chronically elevated circulating lipids and glucose following hepatic insulin resistance lead to the increased transport and altered metabolism of these substrates in skeletal muscle and pancreatic β-islets. Metabolic adaptations in skeletal muscle are first observed to accommodate the changing intracellular metabolite milieu; however, these adaptations, including increased shuttling of FFAs into cytotoxic lipid synthesis pathways and dysregulated energy substrate metabolism, lead to the adaptive threshold past the point at which there is an accumulation of inflammatory molecules, increased ROS, mitochondrial dysfunction, and insulin resistance (22, 90). The coexistence of hepatic and skeletal muscle insulin resistance contributes to hyperglycemia by both the increased production and decreased oxidation of glucose. As glucose production by the liver rises and its transport and catabolism by skeletal muscle falls, pancreatic β-cells must produce more and more insulin to achieve homeostatic levels. Long-term exposure to increasing concentrations of circulating glucose, FFAs, and inflammatory mediators contribute to β-cell,s reaching and surpassing their adaptive threshold from where lipotoxicity and glucotoxicity lead to increasing rates of ROS production and β-cell apoptosis (94, 165). The mechanisms of β-cell death in T2DM have been reviewed previously (52).

The progression along the adaptation/maladaptation continuum in each of the primary organs involved in T2DM pathogenesis, as described above, undoubtedly influences the function and thus progression along the adaptation/maladaptation continuum of organs secondarily impacted by prediabetic and diabetic conditions. Many of the negative effects observed in organs secondarily impacted by T2DM stem from the contribution of primary organs of T2DM pathogenesis to altered metabolite and inflammatory mediator profile of the systemic circulation. A case in point can be found in white adipose tissue (WAT). An adaptive response to declining glucose levels is the activation of WAT triglyceride lipase to mobilize FFAs to provide substrate for hepatic acetyl CoA (135). Insulin acts a negative regulator of this activation in WAT; however, in high-fat diet-fed mice and rats, WAT is not responsive to insulin's inhibitor effect, and consequently WAT lipolysis is enhanced, leading to even greater levels of circulating FFAs (135). Others have added that adipose tissue mitochondria of T2DM patients have heightened levels of both ROS and lipid peroxidation products (32). Alterations in lipid metabolism and the overproduction of mitochondrial ROS have been shown to contribute to cardiac dysfunction in both *db/db* mouse and Zucker diabetic fatty rat models of T2DM as well (18, 121).

The systemic metabolic effects of T2DM extend to peripheral nerves, where glycolytic intermediates are depleted and increased protein and lipid oxidation are observed (70). Because of the brain's high dependence on glucose as a fuel source, it is not surprising that long-term exposure of the cerebrum to the T2DM-altered circulating milieu induces cerebral insulin resistance, reduced cerebral glucose metabolic rate, and increased risk of dementia (12). Because of the mitochondrion's central role in glucose oxidation, FAO, OXPHOS, ROS generation, and apoptosis, the dysregulation of these pathways leading to increased circulating glucose and triglycerides, greater levels of intracellular and secreted cytotoxic lipid species and inflammatory mediators, and elevated mitochondria-derived ROS and apoptosis activation in key organs can all be linked to progression along the adaptation/maladaptation continuum of mitochondria across organs throughout T2DM progression.

mtDNA MUTATION AND VARIATION, NCRNA, EPIGENETICS, AND EPITRANSCRIPTOMICS: NEW PLAYERS IN MITOCHONDRIAL BIOLOGY

This section will cover novel experimental and technological innovations in mitochondrial biology. Athough we acknowledge that not much is known about the areas of ncRNA, epigenetics, and epitranscriptomics in the context of mitochondria, we present these topics as potential areas for future investigation in the setting of health and disease.

mtDNA Mutation and Variation in T2DM

The impact of mtDNA variants, whether homoplasmic or heteroplasmic, can be disastrous in the setting of metabolic disease. A litany of mtDNA mutations and single nucleotide polymorphisms have been found to be associated with T2DM (Table 1). Some groups have segregated their study populations into mitochondrial haplogroups based on mtDNA variants to determine whether a specific haplogroup is associated with increased or decreased risk of T2DM. One study showed that *haplogroup J* in a Finnish population was associated with maternally inherited T2DM (114). These results were further supported by a separate study that found *haplogroup J1* to be associated with T2DM (56). Others have reported that subjects of the mitochondrial *haplogroup B4* had an increased risk of T2DM, whereas those of *haplogroup D4* carried a decreased risk (100). Interestingly, a lower prevalence (0.1–0.2%) of the A3243G mtDNA variant in T2DM UK white Caucasian patients has been previously reported in T2DM Asian patient populations (157). The role of mitochondrial genetics in conferring increased or decreased risk of T2DM has been clearly established. One very relevant question that remains to be answered is whether more metabolically active tissues that generate more mitochondrial ROS have increased rates of mtDNA heteroplasmy in T2DM.

ncRNA in Mitochondria: miRNA, lncRNA, and circRNA

Recent work has demonstrated that ncRNA species such as microRNA (miRNA), long noncoding RNA (lncRNA), and circular RNA (circRNA) are found in the mitochondria (19, 48, 76, 126, 141, 173). Our laboratory has shown that STZ-treated diabetic mice have increased expression of miRNA-378 in cardiac IFM (76). MiRNA-378 was found to bind and inhibit the translation of the mtDNA-encoded ATP6 mRNA transcript, which contributed to decreased ejection fraction and fractional shortening in diabetic hearts (76). Others have also shown that a nuclear genome-encoded miRNA species can translocate to the mitochondria to regulate mtDNA-encoded genes, as they report miR-181c to bind and inhibit the translation of mt-COX1 mRNA (48). Mitochondrial lncRNA have been found to be both of nuclear and mitochondrial origin (19, 126, 141). Because of their versatility of functions, including binding of mRNA, miRNA, and epigenetic modifiers, the potential differential expression of mitochondrial lncRNA across organ systems in the setting of T2DM may prove important. The function of lncRNA as competitive endogenous RNA that sequesters the activity of complementary miRNA has been reviewed

Table 1. *mtDNA variations implicated in increased or decreased risk of T2DM*

mtDNA Alteration	Population	Variant Nature	Summary	Ref. No.
A3243G	Family	Heteroplasmic	Variant associated with familial DM and deafness	187
4399–14821 Deletion	Family	Heteroplasmic	Deletion associated with maternal inheritance of DM and deafness	14
A3243G	Japanese	Heteroplasmic	Variant associated with familial DM	77
C1310T, G1438A, A12026G	Japanese	Homoplasmic	Variants associated with T2DM	179
A3243G	Chinese	Heteroplasmic	Variant associated with T2DM	210
T14577C	Family	Heteroplasmic	Variant associated with T2DM	178
A5178C	Japanese	N/A	Variant associated with maternally-inherited T2DM	195
T16189C	UK Caucasians	Mostly Homoplasmic	Variant associated with T2DM	139
G3316A, T3394C	Chinese	N/A	Variants associated with T2DM	207
C8684T	Japanese	N/A	Variant associated with T2DM	67
T3394C, A14693G, T16189C	Chinese	A14693G Homoplasmic	Variants associated with T2DM	176
T4216C, A4917G	Caucasian-Brazilian	Homoplasmic	Variants associated with T2DM	44
G5231A, A12358G, G12372A	Asian	N/A	Variants associated with resistance to T2DM	60
T16189C	Taiwanese	N/A	Variant associated with T2DM	101
T16189C	Asian	N/A	Variant associated with T2DM	130
T3394C, G4491A, T16189C, T16519C; C5178A, A10398G	Chinese Han	N/A	Variants T3394C, G4491A, T16189C, and T16519C are positively correlated with T2DM; variants C5178A, A10398G negatively correlated with T2DM	96
16189–16193 Polycytosine Variant	Europid	N/A	Variant associated with T2DM	205

DM, diabetes mellitus; N/A, not available; T2DM, type 2 diabetes mellitus. The mtDNA base pair changes are indicated, followed by the population being studied, the nature of the mtDNA base pair variation, and a summary of the findings.

previously (180). Although the stoichiometry of complementary lncRNA and miRNA species may not near the 1:1 ratio required for full selective miRNA inhibition in the cytoplasm due to the large pool of miRNA, the action of lncRNA sponging in the much smaller pool of mitochondrial miRNA may be more pronounced. There are a variety of challenges and potential confounding factors when performing miRNA, lncRNA, or circRNA profiling of cells or tissue samples that have been outlined previously (140, 167, 174). It is important to take into consideration sample processing and RNA extraction methods to ensure minimal degradation of ncRNA in the tissue being studied (140). Understanding the strengths and weaknesses of qRT-PCR, hybridization-based methods, and high-throughput sequencing (RNA-seq) is also imperative when designing miRNA profiling experiments (140). The low abundance of lncRNA in most tissue types increases difficulty of quantification; however, Clark et al. (38) recently developed the technique of capture sequencing that was shown to be superior to qRT-PCR and RNA-seq for detection and quantification of low expressed transcripts such as lncRNA. The low expression of circRNA coupled with the absence of a poly(A) tail prevented the detection of this ncRNA species previously, but new methods of linearizing these transcripts and optimizing RNA-seq library preparation are improving their detection and quantification (174).

Accumulating evidence over the past decade has supported the observation of systemic intercellular miRNA transport via exosomes. It was first demonstrated that mouse mast cells secrete exosomes harboring functional mRNA and miRNA that can be received by both mouse and human mast cells (186). Others have shown Epstein-Barr virus-infected B cells secrete exosomes carrying EBV-miRNAs that are delivered to monocyte-derived dendritic cells where they act on their target transcripts (133). Along the lines of immune cell communication, exosomal miRNA from T cells have been shown to be transported to antigen-presenting cells (112). This mode of intercellular communication has also been found within the cardiovascular system (15, 68). A recent report showed that the interorgan exosomal transport of miRNA-15a from pancreatic β-cells to retinal tissue may contribute to diabetic retinopathy in T2DM (79). Determining the role of interorgan exosome-mediated ncRNA transport in states of health and disease will further illuminate the deep interconnectedness of organ systems.

Mitochondrial Epigenetic and Epitranscriptomic Regulation

Two of the most cutting edge topics of mitochondrial biology are mitochondrial epigenetics and epitranscriptomics. Shock et al. (166) were the first to establish the presence of DNA methyltransferase 1 (DNMT1) in the mitochondria along with describing the mitochondrial targeting sequence in this isoform. They further showed the presence of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) modifications of mtDNA (166). A later study reported 83 specific CpG sites found to be methylated in mtDNA (103). Another group has characterized the methylcytosine landscape of mtDNA from 39 separate human cell and tissue types (62). In support of mtDNA 5hmC modification, the presence of ten-eleven translocase, the enzyme responsible for 5mC hydroxylation to 5hmC, was observed in the mitochondria (53). The impact of T2DM on changes in mtDNA methylation and/or hydroxymethylation and how these changes effect mitochondrial function are wide-open questions for the field to investigate. There have been a variety of methods developed to detect mtDNA methylation, each with unique strengths and weaknesses (188). A couple potential challenges or confounding factors include incomplete mitochondrial purification or nuclear integration of mtDNA sequences (188).

It will also be of interest to look at cross-talk between the mitochondrion and nuclear epigenome in the setting of T2DM, and this dynamic in homeostasis and stress has been reviewed previously (108). Briefly, it has been shown that tissue-specific differentially methylated regions of the nuclear genome exist in a healthy state, accounting for the differences in function and thus gene expression in different tissues (104, 142). These tissue-specific methylation patterns represent the 70–80% of all CpGs that are methylated in most cell types and are the result of both the copying of methylation patterns following DNA replication primarily performed by DNMT1 and de novo methylation during development primarily attributed to DNMT3A and DNMT3B (127, 149, 212). DNA methylation has been shown to be dynamic, as CpG islands in the promoter regions of specific genetic loci may be more demethylated or heavily methylated to increase or decrease the expression of the specific gene in settings of disease such as that of T2DM. For instance, of the 41 genes reported to have differential expression between pancreatic β-cells of T2DM and nondiabetic subjects, 80% (34 genes) had an anticorrelation with their promoter methylation (193). Others found pancreatic duodenal homeobox 1 to be significantly downregulated in T2DM pancreatic β-cells, with 10 CpG sites in the distal promoter and enhancer regions of the gene shown to be much more heavily methylated in T2DM (202). Bisulfite sequencing of nuclear DNA isolated from cells or tissues of interest provides the average level of methylation for that population of cells at each desired site, which may then be correlated with expression of a specific gene. However, the average level of methylation at the exact distal and proximal gene regulatory elements and within the gene body itself required for a specific level of repression or de-repression of specific genes remains to be elucidated. Notably, recent advances in single-cell analysis have resulted in the ability to obtain methylome and transciptome data from the same cell (7, 39, 73). These illuminating single-cell multi-omics studies have further supported promoter methylation as a key influencer of gene repression (7, 73).

Methylation marks have also been observed on mtDNA-encoded mRNA transcripts (95, 155). The enzymes found in mitochondria to facilitate this mRNA N1-methyladenosine (m^1A) modification are TRMT10C and TRMT61B (95, 155). Because of the powerful translation activation or inhibition of the m1A modification, depending on its position on the mRNA transcript, T2DM-induced changes in the mitochondrial epitranscriptome may help to further explain mitochondrial dysfunction in this condition (Fig. 2) (95, 155).

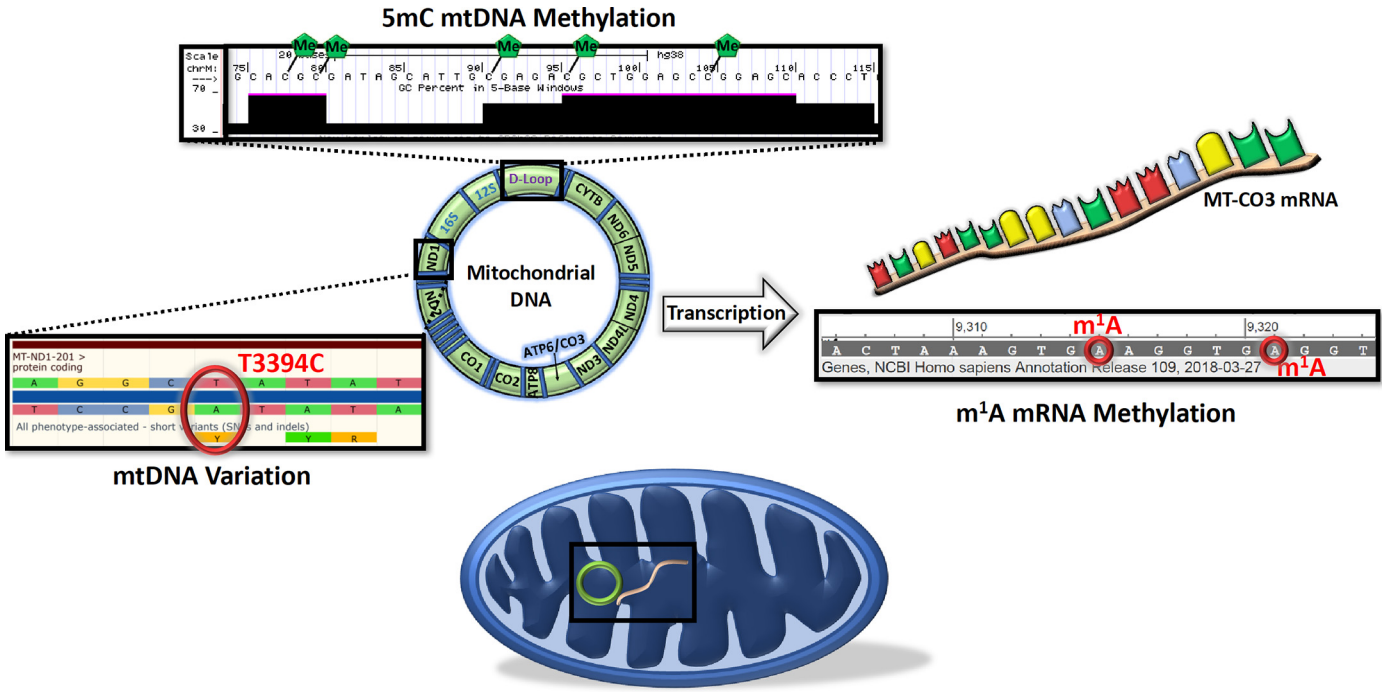

Fig. 2. mtDNA variation and epigenetic and epitranscriptomic regulation of the mitochondrion. An example of mtDNA variation in the ND1 gene that is associated with increased risk of type 2 diabetes mellitus is shown (Ensembl; *left*) (96, 176, 207). An example of specific mtDNA CpG sites in the D-Loop found to be methylated is shown (UCSC Genome Browser; *top*) (103). An example of specific N1-methyladenosine (m^1A) modifications of mtDNA-encoded MT-CO3 mRNA is shown (NCBI; *right*) (95). 5mC, 5-methylcytosine.

MITOCHONDRIA-TARGETED THERAPEUTICS FOR T2DM: RESTORING FUNCTION

Mitochondria-Targeted Antioxidant Therapy for T2DM

As described above, mitochondrial oxidative stress is a major contributor to mitochondrial dysfunction across organ systems in T2DM patients. This increase in ROS production in diabetic models is associated with alterations in both mitochondrial morphology and redox systems biology. In response to high-glucose treatment, it was found that the Clone 9 rat liver cell line and H9c2 rat myoblasts undergo dynamin-like GTPase DLP1/Drp1-mediated mitochondrial fragmentation, which is necessary for and precedes ROS overproduction (208). In a particularly illuminating study, Anderson et al. (3) found a strong link between high-fat diet with increased skeletal muscle mitochondria H_2O_2 emission, a more oxidized cellular redox state, and insulin resistance. They showed that mitochondrial H_2O_2 production was a major influencer of the intracellular redox environment by treating standard chow and high-fat diet-fed rats with the mitochondrial H_2O_2 scavenger SS31 and observing no change in oxidized glutathione or in reduced glutathione/oxidized glutathione ratio following acute glucose ingestion, whereas these measures were increased and decreased in both standard chow and high-fat diet-fed groups (3). The role of mitochondria in mediating insulin resistance in T2DM through changes in redox biology has been reviewed previously (58).

These observations have peaked the interest of many groups to determine the efficacy of mitochondria-targeted antioxidants, including mitoquinone (MitoQ), in diabetic models. MitoQ was found to decrease ROS, leukocyte-endothelium interactions, and TNFα level in leukocytes of T2DM patients (55). It was also shown to improve systemic insulin sensitivity and reduce pancreatic islet lipid peroxide levels in high-fat diet-fed obese mice (75). Further systemic benefit of MitoQ in T2DM is observed by improved renal function following administration to *db/db* mice (198, 201). Some attribute this positive effect to return of homeostasis in renal tubular cell mitophagy (201). Because of the presence of increased mitochondrial-derived ROS in T2DM musculoskeletal, cardiovascular, endocrine, and nervous systems, it is of high priority to determine whether MitoQ treatment helps to ameliorate the diabetes-induced mitochondrial dysfunction in these organ systems. Results indicating that catalase overexpression improves cardiomyocyte contractility in the agouti T2DM model hint at the potential benefit MitoQ may provide to the diabetic heart (204). Additional clinical and preclinical therapeutic approaches to repairing mitochondrial dysfunction in T2DM have been discussed previously (171).

Mitochondria-Targeted Metabolic Therapy for T2DM

Increased mitochondrial ROS in T2DM is intimately linked to changes in energy substrate metabolism and more inefficient OXPHOS. A common preventative or first-line T2DM therapeutic option is metformin, a member of the thiazolidinedione drug class. Metformin is a dynamic small molecule that has been reported to have multiple mechanisms of action in many different tissue types. These mechanisms of action share in common an alteration in energy substrate metabolism or OXPHOS. It was found that metformin activates AMP-activated protein kinase in hepatocytes and skeletal muscle with downstream effects that include decreased glucose production by the liver and increased glucose disposal into skeletal muscle (211). This observation of increased glucose uptake in skeletal

muscle following metformin treatment is further supported by others reporting increased glucose transporter 4 expression in soleus muscle of STZ-induced diabetic rats following metformin administration (34). Another group has observed that metformin treatment in STZ-induced diabetic mice attenuated atherosclerosis by decreasing endothelial mitochondrial fission and mitochondrial-derived superoxide generation (196). Yet others have identified inhibition of ETC complex I and thus decreased OXPHOS as a mechanism of action for metformin (5, 26, 27, 128). It is important to maintain a systems mindset when making sense of how metformin acts to improve the T2DM condition by inhibiting its multiple targets across organ systems.

Mitochondria-Targeted Gene Therapy for Restoring Mitochondrial Omics in T2DM

T2DM is a highly complex polygenic disease with substantial environmental influences. Although there are indeed genetic drivers in T2DM pathogenesis, significant alterations have been found at the mitochondrial proteome and transcriptome levels in the diabetic condition (1, 17, 28, 46, 76, 163). Our laboratory has reported the upregulation of the mitochondrial RNA import constituent polynucleotide phosphorylase and downregulation of mitochondrial protein import constituent mitochondrial heat shock protein 70 in the *db/db* myocardium to help explain these observations (163, 164). Following transgenic manipulation of these genes to their physiological levels, not only were miRNA transcript and protein expression more normalized to nondiabetic values, but mitochondrial function was also improved (163, 164). As described above, others have shown human antigen R and G-rich RNA sequence-binding factor 1 to facilitate the import of lncRNA into the mitochondria (126). Determining whether these and other important mediators of mitochondrial transport are dysregulated in one or more tissue types of T2DM patients will uncover important insights into mitochondrial regulation. This will provide further validation for the premise of using gene therapy to correct mitochondrial "omics" and help restore mitochondrial function in T2DM.

CONCLUSION

Mitochondrial dysfunction is the common thread across organ systems in T2DM patients. Although the specific pathophysiology observed in each T2DM tissue type is unique due to tissue-specific gene expression patterns influenced by the diabetic systemic milieu, many cellular processes that are gone awry connect to the mitochondrion. This unifying principle of the T2DM phenotype creates a paradigm shift in which considering novel therapies for T2DM comorbidities, including obesity, cardiovascular disease, peripheral neuropathy, and Alzheimer's disease, becomes increasingly focused. As new technologies such as epitranscriptomic analysis are being forged to uncover the amazing complexity of mitochondrial regulation, an expanding list of potential pharmacological targets is emerging. It will be the synergy between continuing to identify organism-wide driver pathways of T2DM-related mitochondrial dysfunction and advances in medicinal chemistry and mitochondrial drug delivery that may provide combination strategies to optimally treat this systemic condition.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.V.P., Q.A.H., and J.M.H. conceived and designed research; M.V.P. and Q.A.H. prepared figures; M.V.P., G.K.F., Q.A.H., A.J.D., A.K., and J.M.H. drafted manuscript; M.V.P., G.K.F., Q.A.H., A.J.D., A.K., and J.M.H. edited and revised manuscript; G.K.F., Q.A.H., A.J.D., A.K., and J.M.H. approved final version of manuscript.

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