Shaping Up Mitochondria in Diabetic Nephropathy

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Abstract
Mitochondrial medicine has experienced significant progress in recent years and is expected to grow significantly in the near future, yielding many opportunities to translate novel bench discoveries into clinical medicine. Multiple lines of evidence have linked mitochondrial dysfunction to a variety of metabolic diseases, including diabetic nephropathy (DN). Mitochondrial dysfunction presumably precedes the emergence of key histologic and biochemical features of DN, which provides the rationale to explore mitochondrial fitness as a novel therapeutic target in patients with DN. Ultimately, the success of mitochondrial medicine is dependent on a better understanding of the underlying biology of mitochondrial fitness and function. To this end, recent advances in mitochondrial biology have led to new understandings of the potential effect of mitochondrial dysfunction in a myriad of human pathologies. We have proposed that molecular mechanisms that modulate mitochondrial dynamics contribute to the alterations of mitochondrial fitness and progression of DN. In this comprehensive review, we highlight the possible effects of mitochondrial dysfunction in DN, with the hope that targeting specific mitochondrial signaling pathways may lead to the development of new drugs that mitigate DN progression. We will outline potential tools to improve mitochondrial fitness in DN as a novel therapeutic strategy. These emerging views suggest that the modulation of mitochondrial fitness could serve as a key target in ameliorating progression of kidney disease in patients with diabetes.


Introduction
Diabetic nephropathy (DN) is the most common microvascular complication of diabetes and the leading cause of ESKD in the United States (1,2). Despite improvements in current therapies (3,4), there is still significant risk of DN progression and, thus, widespread innovative is urgently needed to mitigate this issue.

Mitochondria are organelles responsible for producing most of the ATP, the energy currency of the cell. Each mitochondrion is enveloped in a double-membrane structure, an outer and an inner mitochondrial membrane. The inner mitochondrial membrane circumscribes and delineates the mitochondrial matrix that contains hundreds of proteins and houses the mtDNA. As the power houses of the cell, the principal function of mitochondria is to generate energy in the form of ATP. Using a series of redox reactions via the electron transport chain (ETC) system. This process is also known as aerobic respiration or oxidative phosphorylation (OXPHOS) (5) (Figure 1). The efficiency in which these electrons are transported to generate ATP is one means by which mitochondrial fitness is defined. In addition to energy production, mitochondria are also involved in a wide range of biologic activities, such as reactive oxygen species (ROS) generation, calcium signaling, metabolic signaling, proliferation, cancer, and apoptosis (6).

The number of mitochondria present in a cell depends on the metabolic requirements of that cell. Because the kidney is one of the most metabolically active organs in the body, it is not surprising to find that kidneys are very rich in mitochondrial content (7–9). Importantly, the mitochondria of the different cell types in the kidney are heterogeneous in quantity, form, and function. For example, proximal and distal tubules are rich in mitochondrial density, whereas tubular cells of the collecting duct and loop of Henle have far fewer mitochondria (10,11). Furthermore, whereas mitochondria in proximal tubules are highly dependent on mitochondria and OXPHOS to meet their energy needs, podocytes have an intermediate density of mitochondria, and exhibit a flexible phenotype using both OXPHOS and glycolysis to balance their energy needs (12–14).

Recent evidence suggests that mitochondria are critically involved in the pathogenesis and progression of DN (15–20). This review summarizes recent updates on the pathobiologic significance of improving mitochondrial fitness in DN.

OXPHOS and Progression of DN
OXPHOS can be separated into two interconnected parts: the ETC and the chemiosmosis to generate ATP. The ETC is composed of four macromolecular complexes found in the inner mitochondrial membrane of the mitochondria, including complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-quinone oxidoreductase), complex III (ubiquinol-cytochrome b6f oxidoreductase), and complex IV (cytochrome c oxidase). Electrons are passed from one complex to another in a series of redox reactions through the iron-sulfur cores of these complexes. Energy released from these reactions...
is then converted to a proton gradient by proton extrusion at complexes I, III, and IV. The energy stored by the proton gradient is used by complex V (ATP synthase) to produce ATP, a process called chemiosmosis. Thus, the ETC and chemiosmosis together compose the process of OXPHOS in the mitochondria (Figure 2). The proper function and activity of ETC is critical for mitochondrial fitness. Previous reports linking progression of DN with ETC identified

Figure 1. | Overview of mitochondrial fitness. Mitochondria are involved in a number of key biologic processes in the cell, including energy production, redox signaling, calcium homeostasis, inflammation, senescence, innate immune response, and mitophagy.

Figure 2. | Oxidative phosphorylation pathway and mitochondrial reprogramming in diabetic nephropathy. When electron transport chain and mitochondrial fitness are impaired, mitochondrial dysfunction ensues, leading to increased production of reactive oxygen species (ROS) and mitochondrial fragmentation, decrease in cristae formation and mitochondrial membrane potential, and reduced mitochondrial biogenesis. CI, complex I; CII, complex II; CIII, complex III; CIV, complex IV; CoQ, coenzyme Q; CV, complex V; Cyt C, cytochrome c; DN, diabetic nephropathy; e⁻, electron; GPX, glutathione peroxidase; H⁺, hydrogen ion; H₂O, water; H₂O₂, hydrogen peroxide; O₂⁻, superoxide; PRX, peroxiredoxin; SOD, superoxide dismutase.
diabetes is shown in Figure 3 and Tables 1 on mitochondrial bioenergetic changes in different phases of elucidated (16,17,26,43,44). Our interpretation of current data between OCR and progression of DN still remains to be fully these seemingly different observations, the correlation be-

hand, OCR in glomeruli and podocytes were decreased both declined with progression of albuminuria (41). On the other 
cortex and proximal tubular cells (37 after induction of diabetes), OCR was increased in the renal 
progresses (23 that complex I, III, and IV activities are reduced as DN 

dysregulated complex I, III, and/or IV activity in mitochondria from either whole diabetic kidney or cortex in a number 
of established animal models of DN (21–27). Mutations leading to compromised complex I function have been demonstrated to contribute to kidney damage in animal models and human pathologies (28–32). Whereas complex I activity seemed to be increased in the early phase of diabetes in some studies (21,22), a large body of evidence has ultimately shown that complex I, III, and IV activities are reduced as DN progresses (23–27). Likewise, complex I activity in glomeruli and podocytes was shown to be significantly diminished in DN (33,34). Consistent with these observations, at least one study has demonstrated that ATP levels in mitochondria of the kidney cortex were decreased with progression of DN (16), whereas other published studies indicated that ATP production was unchanged (35,36). Notably, when mitochondrial fitness was assessed in these experiments by monitoring oxygen consumption rate (OCR), it was observed that, during very early phases of diabetes in animal models (1–4 weeks after induction of diabetes), OCR was increased in the renal cortex and proximal tubular cells (37–40), and then slowly declined with progression of albuminuria (41). On the other hand, OCR in glomeruli and podocytes were decreased both in the early and late phases of diabetes (17,42,43). Because of these seemingly different observations, the correlation between OCR and progression of DN still remains to be fully elucidated (16,17,26,43,44). Our interpretation of current data on mitochondrial bioenergetic changes in different phases of diabetes is shown in Figure 3 and Tables 1–3. The increased OXPHOS during the early phases of DN, we believe, could reflect the response to an excess of metabolic flux under diabetic conditions (7). In support of a central role for dysregulated ETC in DN, marked changes in the formation of ETC complexes and supercomplexes have also been identified in patients with diabetes (45,46). For instance, complex IV staining score was shown to be significantly lower in the kidneys of patients with diabetes compared with that in normal kidney tissues (47), and the protein levels of complex IV subunit, MTCO2 (mitochondrially encoded cytochrome C oxidase II), in human postmortem glomeruli were significantly decreased in patients with type 2 diabetes who had lower eGFR than in those with higher eGFR or control subjects without diabetes (48).

It has long been known that increased mitochondrial ROS induces mtDNA damage, protein modifications, and lipid peroxidation, resulting in mitochondrial dysfunction, such as decreased complex activities, compromised supercomplex formation, and inefficient electron transport (7,17,49). The free radical theory of diabetic microvascular complication, also known as “the unifying hypothesis,” proposed by Brownlee and colleagues (50) in early 2000, linked mitochondrial ROS production to mitochondrial dysfunction and end-organ damage in diabetic complications, including DN. This hypothesis suggests that excess mitochondrial ROS causes mitochondrial and cellular damage, conveying progression of DN. Although many important questions need to be carefully addressed regarding the value and the limitations of this hypothesis, several important studies have clearly shown enhanced mitochondrial ROS in kidneys of diabetic mice leading to podocyte depletion and apoptosis (15). We have recently used a mitochondrial matrix-localized, redox sensitive, green fluorescent protein biosensor transgenic mouse to assess the effect of mitochondrial ROS in a type 2 model of diabetic mouse (34). The results revealed increased mitochondrial ROS in the kidneys of diabetic mice. Importantly, the increase in ROS was mitigated by treating the mice with the mitochondria-targeted antioxidant, mitoTEMPO, or genetic bypass of complex I, preventing mitochondrial ROS generation (34). These findings are in contrast with another study evaluating mitochondrial ROS in the kidneys of diabetic mice using dihydroethidium (24). This study indicated that markers of oxidative stress were present in the diabetic kidney, but that mitochondrial ROS was decreased, implicating, in part, NADPH oxidases as a source of enhanced total ROS (24). It is unclear why the two methods used in live animals appear to be in contrast, but these may be the results of differences in the biophysical properties of the reporter, reporter localization, or sensitivity to crosstalk between mitochondrial sources of ROS and sources such as NADPH oxidase 4 (24,34,51).

Another recent study focusing on podocyte-specific deletion of ATP-binding cassette A1 also convincingly showed that progression of DN was prevented by elamipretide (20). The authors found that podocyte-specific deletion of ATP-binding cassette A1 resulted in the progression of DN,

Figure 3. | Progressive changes in mitochondrial respiratory function in diabetic nephropathy. Changes associated with electron transport chain, complex activities, and ATP in diabetic nephropathy. OCR, oxygen consumption rate.
increased cardiolipin, and peroxidation of cardiolipin. Prevention of cardiolipin peroxidation was protective in their model, and suggestive that mitochondrial ROS could potentially be both a driving and responding factor to DN (20,49). Taken together, these observations emphasize the difficulties in evaluating mitochondrial ROS as a cause of kidney injury, but provide strong evidence on the importance of mitochondrial ROS and ETC as key determinants of mitochondrial fitness and progression of DN.

Mitochondrial Dynamics
Mitochondria are highly motile organelles, the shape and size of which are determined through a coordinated action of the mitochondrial fission and fusion processes, collectively known as mitochondrial dynamics. Cells maintain a characteristic mitochondrial morphology, ranging from fragmented to fused elongated networks, maintaining their fitness and optimal ATP production under different metabolic conditions (52) (Figure 4). Mitochondrial fission divides this double-membrane organelle into daughter mitochondria, whereas mitochondrial fusion joins independent and separate mitochondria into one mitochondrion. The process of mitochondrial dynamics is highly regulated. A family of dynamin-related GTPase proteins is best characterized in regulating mitochondrial fission. The dynamin-related protein 1 (Drp1) is the best characterized mechanoenzyme of mitochondrial fission. During mitochondrial fission, Drp1 is recruited and oligomerized around the mitochondrial fission furrow. Its recruitment and stabilization on the mitochondrial outer membrane is regulated by post-translational modifications and involves interactions with its known mitochondrial receptors, including mitochondrial fission factor 1, mitochondrial fission factor, and mitochondrial dynamics.

Table 1. Mitochondrial respiratory complexes activity

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Animals’ Age (or wk after induction of diabetes)</th>
<th>Diabetic Animal Model</th>
<th>Complex Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole kidney</td>
<td>4 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>CI activity ↑</td>
<td>(21)</td>
</tr>
<tr>
<td>Whole kidney</td>
<td>6 wk</td>
<td>Mice (C57BL/6) treated with STZ and unilateral nephrectomy db/db mice (C57BLKS/J)</td>
<td>Rotenone sensitive NADH oxidation ↑</td>
<td>(22)</td>
</tr>
<tr>
<td>Whole kidney</td>
<td>13 wk of age</td>
<td>Mice (C57BL/6j) treated with STZ</td>
<td>CI activity ↓</td>
<td>(23)</td>
</tr>
<tr>
<td>Whole kidney</td>
<td>24 wk</td>
<td>Mice (C57BL/6j) treated with STZ</td>
<td>CI activity ↓</td>
<td>(24)</td>
</tr>
<tr>
<td>Whole kidney</td>
<td>25–27 wk</td>
<td>Mice (TALLYHO/Jng [type 2 diabetes + obesity model]) db/db mice (C57BLKS/J)</td>
<td>CI + CIII activity ↓</td>
<td>(25)</td>
</tr>
<tr>
<td>Renal cortex</td>
<td>24 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>CIV activity ↓</td>
<td>(26)</td>
</tr>
<tr>
<td>Renal cortex</td>
<td>12 mo</td>
<td>Lewis rats treated with STZ and insulin</td>
<td>CIV activity ↓</td>
<td>(27)</td>
</tr>
<tr>
<td>Glomeruli</td>
<td>16 and 32 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>CI activity ↓</td>
<td>(33)</td>
</tr>
<tr>
<td>Podocytes</td>
<td>16 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>CI activity ↓</td>
<td>(34)</td>
</tr>
</tbody>
</table>

STZ, streptozotocin; CI, complex I; CIV, complex IV; CIII, complex III; CII, complex II.

Table 2. Mitochondrial ATP production

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Animals’ Age (or wk after induction of diabetes)</th>
<th>Diabetic Animal Model</th>
<th>ATP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole kidney</td>
<td>12 wk of age</td>
<td>Akita mice (C57BL/6j)</td>
<td>CI-driven ATP →</td>
<td>(35)</td>
</tr>
<tr>
<td>Renal cortex</td>
<td>4, 8, and 16 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>CI-driven ATP →</td>
<td>(36)</td>
</tr>
<tr>
<td>Renal cortex</td>
<td>18–20 wk of age</td>
<td>db/db mice (C57BLKS/J, female)</td>
<td>Total ATP ↑ (4 and 8 wk)</td>
<td>(16)</td>
</tr>
<tr>
<td>Renal cortex</td>
<td>32 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>Total ATP ↑ (16 wk)</td>
<td>(16)</td>
</tr>
<tr>
<td>Podocytes</td>
<td>24 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>CII-driven ATP ↓</td>
<td>(43)</td>
</tr>
<tr>
<td>Podocytes</td>
<td>24 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>Total ATP ↓</td>
<td>(17)</td>
</tr>
</tbody>
</table>

CI, complex I; CII, complex II; STZ, streptozotocin.
proteins of 49 and 51 kDa (53) (Figure 4, left panel). Additional stabilization and activation of Drp1 come from both interactions with actin and the mitochondria-specific phospholipid, cardiolipin (54,55). Although the exact sequence of translocation and activation is still under investigation, it is known that once Drp1 is activated on the mitochondrial outer membrane, it drives mitochondrial fission (Figure 4).

The opposing process of mitochondrial fusion involves several dynamin-related GTPase proteins. The outer membrane fusion is mediated by mitofusin 1 and 2, which can homo- and hetero-typically interact to join mitochondria (56). Inner membrane fusion is dependent on the activation of dynamin-related GTPase, optic atrophy 1. Optic atrophy 1 also plays a key role in stabilizing mitochondrial cristae (57).

We and others have shown that progression of DN shifts the mitochondrial equilibrium toward mitochondrial fission, resulting in mitochondrial fragmentation and decreased mitochondrial fitness (15,19,43). We have elucidated a high glucose–mediated, Drp1-induced mitochondrial fragmentation that leads to a significant decrease in mitochondrial fitness. Specifically, we have outlined a mechanism in which hyperglycemia-driven activation of rho-associated, coiled-coil-containing protein kinase 1 phosphorylates Drp1 at serine 637/656 (human/rat) in podocytes and endothelial cells of the kidney, triggering mitochondrial ROS and a kidney phenotype similar to DN (15,19).

We have also shown that enhanced mitochondrial fission is characteristic of podocytes in mouse models of DN (43). Indeed, podocyte-specific deletion of Drp1 in db/db diabetic mice protected against DN, improving both biochemical and histologic key features of DN. Depletion of Drp1 reduced albuminuria and mesangial expansion, while inhibiting podocyte loss and foot effacement. These improvements were shown to correlate with improved mitochondrial fitness in podocytes from these mice. Cultured podocytes from these animals had significantly improved mitochondrial bioenergetics accompanied by the restoration mitochondrial morphology.

More recently, to demonstrate the importance of Drp1 serine 600 phosphorylation in vivo, a diabetic knockin mouse model was established in our laboratory (19). This model has a mutated serine to alanine at the amino acid position 600, such that the mutant Drp1 could no longer be phosphorylated at this position. Similar to the Drp1 knockout mice, these mice were protected against DN, with reduced albuminuria, mesangial expansion, and glomerular basement membrane thickness. The notion of dysregulated mitochondrial dynamics as an underlying molecular mechanism in diabetic complications is supported by several studies using biopsy sample–proven human data in patients with diabetes in different tissues (58–60). Indeed, fragmented mitochondria were more dominant in podocytes and proximal tubular cells of patients with biopsy sample–proven DN than in those cells of control subjects without diabetes (61,62). We have also demonstrated that Drp1 phosphorylation is apparent in biopsy specimens from patients with DN (19). In addition, Drp1 and mitochondrial fission factor 1 staining were increased, whereas mitofusin 2 staining was decreased, in the tubules of patients with DN compared with in the same lesions of healthy controls (62), which is all consistent with a critical role of mitochondrial dynamics machinery being involved in DN progression.

### Table 3. Mitochondrial oxygen consumption rate

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Animals’ Age (or wk after induction of diabetes)</th>
<th>Diabetic Animal Model</th>
<th>OCR</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole kidney</td>
<td>12 wk of age</td>
<td>Akita mice (C57BL/6j)</td>
<td>CI-driven state II, III, IV OCR → (35)</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>2 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>CI-driven state II, III, IV OCR → (40)</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>4, 8, and 16 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>State IV OCR ↑ (16)</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>23 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>CI-driven state III OCR → (4 and 8 wk)</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>24 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>CI-driven state III OCR ↑ (16)</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>32 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>CI-driven state III OCR ↑ (16)</td>
<td></td>
</tr>
<tr>
<td>Renal cortex</td>
<td>12 mo</td>
<td>Lewis rats treated with STZ and insulin</td>
<td>Ci-driven state III OCR ↓ (27)</td>
<td></td>
</tr>
<tr>
<td>Cortical and medullary cells</td>
<td>4 wk</td>
<td>Wister rats treated with STZ</td>
<td>Cortical cells: total OCR ↑ (38)</td>
<td></td>
</tr>
<tr>
<td>Cortical and medullary cells</td>
<td>4 wk</td>
<td>Sprague-Dawley rats treated with STZ</td>
<td>Medullary cells: total OCR ↑ (39)</td>
<td></td>
</tr>
<tr>
<td>Cortical tubular cells</td>
<td>8–9 wk</td>
<td>Wister rats treated with STZ and insulin</td>
<td>CI-driven state III OCR ↑ (27)</td>
<td></td>
</tr>
<tr>
<td>Proximal tubular cells</td>
<td>2 wk</td>
<td>Wister rats treated with STZ</td>
<td>CI-driven state III OCR ↑ (37)</td>
<td></td>
</tr>
<tr>
<td>Glomeruli</td>
<td>1 wk and 3 wk</td>
<td>Mice (DBA/2J) treated with STZ</td>
<td>Reverse respiratory capacity ↓ (42)</td>
<td></td>
</tr>
<tr>
<td>Podocytes</td>
<td>24 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>Basal and maximal OCR ↓ (43)</td>
<td></td>
</tr>
<tr>
<td>Podocytes</td>
<td>24 wk of age</td>
<td>db/db mice (C57BLKS/J)</td>
<td>Basal and maximal OCR ↓ (17)</td>
<td></td>
</tr>
</tbody>
</table>

OCR, oxygen consumption rate; CI, complex I; CII, complex II; STZ, streptozotocin.

*Glutamate/malate-driven state III OCR was unchanged, whereas pyruvate- and malate-driven state III OCR was significantly decreased.
Mitophagy and Mitochondrial Fitness

Mitophagy is a selective physiologic process in which damaged mitochondria are engulfed by autophagosomes, and subsequently degraded by lysosomes (63). Removal of damaged mitochondria is promoted by translocation of autophagy machinery to the impaired mitochondria, and is classified as either a ubiquitin-dependent or -independent pathway. The ubiquitin-dependent pathway is regulated by the phosphatase and tensin homolog–induced putative kinase 1 (PINK1) and Parkin (63,64). In healthy cells under a steady state, PINK1 is transported into mitochondria and degraded (63,65). However, once mitochondria are damaged and depolarized, PINK1 and Parkin accumulate on the mitochondrial outer membrane, resulting in the autophagic degradation of mitochondria (Figure 4) (63,65). PINK1 recruits and phosphorylates both the E3-ligase Parkin and ubiquitin to create the poly-ubiquitin chains to serve as the recognition signal for autophagic proteins and mitochondrial destruction. The ubiquitin-independent pathway is less well understood and involves autophagic receptors, which can localize to mitochondria and interact with microtubule-associated protein 1A/1B light chain 3 to trigger mitochondrial destruction and involves an ever-increasing list of candidates (63,64).

It has been shown that dysregulated mitophagy results in cellular damage and apoptosis. Basal levels of mitophagy are relatively high in podocytes and appear necessary for mitochondrial homeostasis in these cells. In contrast, basal mitophagy is low in proximal tubular cells but readily induced when these cells are metabolically stressed (66,67). This dichotomy suggests that mitophagy has distinct physiologic functions in these two cell types. PINK1/Parkin-mediated mitophagy levels are reported to be significantly decreased in DN in animal models and in patients with DN (68,69). The defect has been linked to decreased expression of both PINK1 and Parkin, which mitigates clearance of defective mitochondria in both podocytes and tubular cells (68,70). Overexpression of forkhead box class O1 in podocytes of db/db mice has been shown to restore expression of PINK1 and PINK1/Parkin-mediated mitophagy, protecting from progression of DN (68). In addition, an excess accumulation of damaged mitochondria due to reduced clearance of autophagosome in kidney cells was also reported in DN, leading to marked increase in mitochondrial ROS and inflammation (63,69,71). Similarly, injection of the mitochondrially targeted antioxidant, MitoQ, was reported to restore mitophagy in tubular cells, in part by increasing PINK1 expression (70).

Further evidence of a central role for mitophagy in DN came from some recent genetic studies. Importantly, podocyte-specific knockout of autophagy related 5 in
strepptozotocin-treated or high-fat diet–treated diabetic mice accelerated diabetes-induced podocytopathy and glomerulosclerosis (72,73). In addition, deletion of autophagy related 5 in proximal tubules in a diet-induced model of obesity displayed exacerbated proteinuria via mammalian target of rapamycin–mediated inhibition of mitophagy (74). Finally, kidney biopsy specimens of patients with DN have revealed increased staining in glomeruli for the mitophagy insufficiency marker p62 (73). This protein is a specific target for mitophagy degradation, and its accumulation is considered a marker for disrupted mitophagy (69,71,73). Taken together, these studies suggest that impairment of the mitophagy process may contribute to the development and progression of DN.

Mitochondrial Biogenesis

Mitochondrial biogenesis is the biologic process by which cells increase mitochondrial mass to cope with cellular demands (75,76). The regulation of mitochondrial biogenesis is largely achieved through an interconnected set of transcription factors that link environmental cues to cellular energy status in the cell (51,77). Among these transcriptional factors, the peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) family of transcriptional coactivators is considered to serve as the master regulator of mitochondrial biogenesis (78). It is highly expressed in tissues with high energy demands, such as the brown fat, heart, and kidneys (78). PGC1α dimerizes with partner transcription factors to regulate target gene transcription at their respective DNA response elements. The heterodimerization of these factors is referred to as coactivation and results in modulation of many genes involved in mitochondrial function. Transcription factors, such as nuclear respiratory factor 1, nuclear respiratory factor 2, and the estrogen-related receptors, are among multiple key transcription factors coactivated by PGC1α (79,80). The interaction between PGC1α and these DNA-binding transcriptional factors allows for the concerted control of multiple mitochondrial genes, leading to regulation of mitochondrial biogenesis, respiration, fatty acid β-oxidation, tricarboxylic acid cycle, and OXPHOS. However, the result and relative contribution of these binding partners are highly tissue specific and explain, at least in part, the different metabolic programs elicited in different cell and tissue types (12,81).

The reduced efficiency of mitochondrial biogenesis and downregulation of PGC1α have been frequently reported in DN (17,24,82). Indeed, a growing body of evidence supports that progression of DN is closely related to the PGC1α signaling pathway (24,76,83). Whereas upregulation of PGC1α has been demonstrated in the early phase of diabetes (84), PGC1α has been shown to be significantly downregulated in later phases of diabetes and human kidney tissues of patients with DN (17,24,47,82). We have recently shown a novel layer of PGC1α regulation by taurine upregulated gene 1 (Tug1), a long noncoding RNA (17). We found that Tug1 is significantly decreased in podocytes of diabetic mice and in glomeruli of patients with DN and, notably, overexpression of Tug1 protected the mice from DN. Furthermore, our data suggest that a key target of Tug1 in the diabetic milieu was PGC1α. Importantly, we established that overexpressed Tug1 contributed not only to increases expression of PGC1α but also to the increased complex I activity, OCR, and ATP production, and less ROS and mitochondrial fission, suggesting a critical role of Tug1 on mitochondrial fitness.

Mitochondria as Biomarkers of DN Progression

A great deal of effort has recently been directed toward identifying key blood or urinary biomarkers for DN. The ideal biomarker would offer improved sensitivity and specificity for kidney damage, and be consistently and reliably detectable in either blood or urine. The search for these biomarkers is driven by the notion that early detection and treatment of DN is key to delaying outcomes such as dialysis. Additionally, early diagnosis is key to interventions that may be able to arrest and prevent further progression.

As highlighted above, mitochondrial dysfunction is a major determinant of metabolic homeostasis. Sharma et al. (47) used gas chromatography–mass spectrophotometry to examine urine for biomarkers of DN. They identified 13 urine metabolites, 12 of which were consistent with depressed mitochondrial respiration. This same study found elevated mtDNA in the urine, consistent with another study that identified increased mtDNA in the PBMCs of patients with DN (85). These reports give some promise for examining mtDNA in both blood and urine. However, a more recent study with a 2-year clinical follow-up was unable to correlate mtDNA in the urine with any clinically relevant measures of DN (86). An inherent problem with mtDNA in the urine or plasma as biomarkers is that its source is unclear and it is unlikely to be a tissue-specific biomarker. Although there is not a definitive conclusion on the utility of mtDNA as a biomarker at this time, metabolomic and proteomic approaches to identify suitable biomarkers still hold promise (87–89).

Pharmacologic Targeting of Mitochondria

Several therapeutics aimed at improving mitochondrial fitness and ameliorating DN progression have attempted to target the energy-sensing pathways of the cell in the mitochondria. The energy sensor of the cell, 5′ AMP-activated protein kinase (AMPK), has recently gained interest as a target due to its broad role in maintaining mitochondrial fitness (24). This enzyme can be activated by several agonists, including metformin, cinacalcet, anthocyanin, and 5-aminomidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) (90). Importantly, a growing body of evidence suggests that AMPK is markedly decreased in kidneys of diabetic mice and humans with diabetes (91–93). Administration of AICAR, an agonist of AMPK, has been shown to prevent DN in diabetic models, at least in part, through increased mitochondrial biogenesis and increased ETC activity (24,94,95). A similar approach has been aimed at a second cellular nutrient sensor, Sirtuin 1, an NAD+-dependent protein deactylase, that promotes mitochondrial biogenesis. A selective agonist of Sirtuin 1, BF175, reduced albuminuria, podocyte loss, and reduced oxidative stress in OVE26 diabetic mice in part by restoring PGC1α levels and mitochondrial fitness (96).

Successful stabilization of cardiolipin, a mitochondrial phospholipid, has also been shown to improve several key features DN (97,98). Cardiolipin plays a central role
in the structural formation of cristae and organization of ETC. Elamipretide is a small mitochondrially targeted tetrapeptide that appears to preferentially bind to cardiolipin, stabilizing it and promoting OXPHOS, as well as protecting against mitochondrial ROS. An AKI model in rats demonstrated protection of mitochondrial cristae morphology in proximal tubules using elamipretide (99). Taken together, these preclinical studies suggest that targeting fundamental mitochondrial mechanisms could potentially benefit DN progression.

Future Perspective

In summary, recent advances in mitochondrial medicine have given us more understanding of the mechanism underlying mitochondrial dysfunction in DN. Because mitochondrial fitness plays a central role in cell viability and kidney function, improving mitochondrial fitness in the kidney can be a promising therapeutic target to prevent DN progression. The emerging view of targeting key players implicated in mitochondrial reprogramming, however, is that they are cell and context specific. Understanding how mitochondrial fitness supports cell adaptation to changes in the environment requires a more refined view of how mitochondrial fitness evolves in the kidney cells as DN progresses.

Disclosures

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Author Contributions

F. Danesh provided supervision and was responsible for project administration and resources; F. Danesh and D. Galvan were responsible for validation; F. Danesh, D. Galvan, and K. Mise conceptualized the study, wrote the original draft, and reviewed and edited the manuscript; F. Danesh and K. Mise were responsible for validation; F. Danesh, D. Galvan, and K. Mise contributed to funding acquisition; D. Galvan and K. Mise were responsible for project administration; F. Danesh and K. Mise conceptualized the study, wrote the original draft, and reviewed and edited the manuscript; F. Danesh, D. Galvan, and K. Mise were responsible for methodology; K. Mise was responsible for data curation and formal analysis.

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