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MICROVASCULAR COMPLICATIONS—NEPHROPATHY (M AFKARIAN AND B ROSHANRAVAN, SECTION EDITORS)

Crosstalk Between Lipids and Mitochondria in Diabetic Kidney Disease

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Abstract

Purpose of Review The goal of this review is to review the role that renal parenchymal lipid accumulation plays in contributing to diabetic kidney disease (DKD), specifically contributing to the mitochondrial dysfunction observed in glomerular renal cells in the context of DKD development and progression.

Recent Findings Mitochondrial dysfunction has been observed in experimental and clinical DKD. Recently, Ayanga et al. demonstrate that podocyte-specific deletion of a protein involved in mitochondrial dynamics protects from DKD progression. Furthermore, our group has recently shown that ATP-binding cassette A1 (a protein involved in cholesterol and phospholipid efflux) is significantly reduced in clinical and experimental DKD and that genetic or pharmacological induction of ABCA1 is sufficient to protect from DKD. ABCA1 deficiency in podocytes leads to mitochondrial dysfunction observed with alterations of mitochondrial lipids, in particular, cardiolipin (a mitochondrial-specific phospholipid). However, through pharmacological reduction of cardiolipin peroxidation DKD progression is reverted.

Summary Lipid metabolism is significantly altered in the diabetic kidney and renders cellular components, such as the podocyte, susceptible to injury leading to worsened DKD progression. Dysfunction of the lipid metabolism pathway can also lead to mitochondrial dysfunction and mitochondrial lipid alteration. Future research aimed at targeting mitochondrial lipids content and function could prove to be beneficial for the treatment of DKD.

Keywords Diabetic kidney disease · Podocyte · Mitochondria · Cardiolipin · ABCA1 · Lipid metabolism

Introduction

Diabetic kidney disease (DKD) is the most common cause of end-stage kidney disease (ESKD) [1]. Podocyte loss (podocytopenia) is a clinical feature of DKD and an independent predictor of DKD progression in patients with type 1 (T1D) and type 2 (T2D) diabetes [2–5]. However, the cause of podocytopenia in DKD remains to be established. In both clinical and experimental DKD, glomerular hypertrophy and increased glomerular filtration rate in the early stage of DKD is a risk factor for the development and progression to ESKD [6–9].

Among several contributors to clinical and experimental DKD, it was recently demonstrated that renal accumulation of lipids correlates with the development of glomerulosclerosis [6, 8, 10]. Cholesterol accumulates in glomerular cells in DKD even when the cholesterol synthesis pathway which can be targeted by statins, is not affected [8, 10]. In experimental DKD, therapeutic strategies targeting intracellular lipid metabolism reduce proteinuria and mesangial

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expansion [8, 11], strongly suggesting that altered lipid metabolism in the kidney may be at least partially responsible for disease development and progression in DKD.

Moreover, mitochondrial dysfunction has been described as a key event in the development of diabetic complications and occurs in addition to other mitochondrial defects such as in mitochondrial biogenesis, number, morphology, and dynamics, all of which have been described in hyperglycemic states [12]. In fact, a reduction in oxygen consumption rates (OCR), increased mitochondrial DNA damage, and reduced mitochondrial oxidative phosphorylation have been described in DKD [13–17].

Lipid accumulation may contribute to the mitochondrial dysfunction observed in DKD. Here, we aim to review the process by which lipid accumulation may render mitochondria dysfunctional in the diabetic kidney, highlighting mechanistic insights observed in other model systems.

Lipids in DKD

Lipid metabolism dysregulation has been described in diabetes. This dysregulation occurs systemically, contributing to hyperlipidemia, which is prevalent in diabetic patients, but also at the intracellular level leading to specific target organ dysfunction such as the heart, liver, and kidney [6, 8, 18–20]. Lipid accumulation in the kidney cortex of distinct DKD mouse models has been described by us and several others [6, 8, 10]. Cell lipid homeostasis is constrained by adjusting of lipid uptake, synthesis, usage, and storage. The significant kidney lipid classes are phospholipids, triglycerides, and non-esterified (free) fatty acids. Modulation of gene expression of several lipid-related genes involved primarily in cholesterol uptake and efflux has also been described in association with lipid accumulation in DKD, specifically, in patients with DKD, [8, 21••]. Cholesterol uptake gene expression is significantly increased, while genes regulating cholesterol efflux are significantly decreased. Additionally, genes involved in fatty acid oxidation were found to be significantly reduced, contributing to chronic kidney disease progression [6]. Similarly, accumulation of cholesterol and triglycerides was reported in the *Akita* mouse model of T1D [22, 23]. Together this data support the notion that intracellular accumulation of cholesterol and triglycerides may add to the development and progression of DKD. Although expression of sterol regulatory element-binding protein 1 (SREBP1), an important potential mediator of kidney fibrosis, has been shown to be significantly increased in DKD experimental models, SREBP1 inhibition did not result in improved DKD outcome in T1D mice [22, 24]. This suggests that inhibiting cholesterol synthesis with statin treatment is not an effective method to improve DKD patient outcomes. However, recent data suggest that cholesterol efflux plays an important role in DKD progression and may present novel therapeutic options.

Podocytes, highly specialized epithelial cells of the glomerular filtration barrier, express all genes and proteins participating in cellular cholesterol homeostasis. In patients with early DKD, glomerular expression of ATP-binding cassette transporter 1 is decreased [8], while no changes in low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-CoA reductase were reported. In mice with experimental Alport syndrome, we recently reported that cholesterol efflux and trafficking-related genes were differentially affected in kidney cortex and isolated glomeruli [25], suggesting that cholesterol homeostasis plays a very important role in the progression of kidney disease of both metabolic and non-metabolic origin.

The Role of ATP-Binding Cassette Transporters in DKD

The ATP-binding cassette (or ABC) family of transporters contains a large number of transmembrane proteins expressed in almost all organisms and consists of 49 members divided into A to G subfamilies. Many of ABC family genes were linked to inherited diseases, such as Tangier disease (ABCA1), cystic fibrosis (ABCC7), Dubin-Johnson syndrome (ABCC2), or hyperinsulinemic hypoglycemia of infancy (ABCC8) (reviewed in [26]). In the kidney, ABC family transporters were found to play a critical role in the function of renal proximal tubules [27–29], to cause resistance against steroid treatment of nephrotic syndrome in children [30] or to regulate cholesterol homeostasis [31, 32].

Increased cellular cholesterol can result from impaired cholesterol efflux due to downregulation of ATP-binding cassette transporter A1 (ABCA1) expression [10, 33], which mediates the efflux of cholesterol and phospholipids to apolipoproteins. In the non-obese diabetic mouse model of T1D, decreased ABCA1 expression in the kidney and in circulating macrophages is observed [33]. We reported decreased ABCA1 expression in glomerular transcripts from 70 patients with T2D and early DKD. Similar results were obtained in a separate cohort of patients with T2D and were associated with the presence of podocyte lipid droplets by electron microscopy analysis of kidney biopsies [8]. Podocytes treated with sera obtained from patients with both T1D and T2D resulted in significant reduction of ABCA1 expression as well as lipid droplet accumulation [21••, 34]. This observation was also confirmed in DKD mouse models as shown in the *ob/ob* and *db/db* mice. Interestingly, ABCA1 expression has been shown to correlate with markers of DKD progression both clinically and experimentally [21••].

Loss of ABCA1 function due to mutations in ABCA1 can be observed in patients with Tangier disease. These patients have foamy podocytes on kidney biopsies [35] but develop only minimal proteinuria, suggesting that ABCA1 deficiency may be sufficient to cause lipid accumulation but not sufficient to cause glomerular cell injury. In fact, we demonstrated that neither ABCA1 siRNA in podocytes in vitro nor

podocyte-specific deletion of ABCA1 in vivo is sufficient to cause glomerular injury [34]. However, we have recently described loss of ABCA1 expression and function to be a susceptibility factor in DKD rendering podocytes susceptible to injury and contributing to worsened DKD progression, as demonstrated both in vitro and in vivo [21••]. Furthermore, both pharmacological induction of cholesterol efflux with cyclodextrin [8] and genetic overexpression of ABCA1 [34] are sufficient to prevent the progression of DKD or DKD-like glomerulosclerosis, suggesting ABCA1's role in driving disease progression. This does not rule out other key genes involvement in disease progression as other ABC transporters have also been shown to be downregulated in DKD [36]. Loss of ABCA1 function due to mutations in ABCA1 can be observed in patients with Tangier disease. These patients have foamy podocytes on kidney biopsies [35] but develop only minimal proteinuria, suggesting that ABCA1 deficiency may be sufficient to cause lipid accumulation but not sufficient to cause glomerular cell injury. In fact, we demonstrated that neither ABCA1 siRNA in podocytes in vitro nor podocyte-specific deletion of ABCA1 in vivo is sufficient to cause glomerular injury [34]. However, we have recently described loss of ABCA1 expression and function to be a susceptibility factor in DKD rendering podocytes susceptible to injury and contributing to worsened DKD progression, as demonstrated both in vitro and in vivo [21••]. Furthermore, both pharmacological induction of cholesterol efflux with cyclodextrin [8] and genetic overexpression of ABCA1 [34] are sufficient to prevent the progression of DKD or DKD-like glomerulosclerosis, suggesting ABCA1's role in driving disease progression. This does not rule out other key genes involvement in disease progression as other ABC transporters have also been shown to be downregulated in DKD [36].

The Role of Cholesterol in DKD

In a retrospective study on patients with T2D, low HDL cholesterol and high triglyceride levels, a sign of diabetic dyslipidemia, were shown to be independent risk factor for development of DKD over 3 years [37]. Other prospective studies suggest that hypercholesterolemia is another predictor of DKD [38, 39]. In animal models of diabetes and murine podocytes in vitro, statin administration showed decreased lipid peroxidation, increased antioxidant levels, reduced accumulation of advanced glycosylation end products, and reduced podocyte injury [40–43]. However, treatment with lipid-lowering agents has not been clearly shown to be beneficial in slowing clinical DKD progression, suggesting that tissue-specific rather than systemic dyslipidemia may play a role in the pathogenesis of DKD.

Toxic lipids such as free cholesterol, esterified cholesterol, ceramide, and lysophosphatidylcholine are all found to accumulate in tissues including kidneys under conditions of insulin resistance, suggesting a crosstalk of hyperglycemia and altered peripheral lipid metabolism (reviewed in Ref. [44]). Toxic lipids such as free cholesterol, esterified cholesterol, ceramide, and lysophosphatidylcholine are all found to accumulate in tissues including kidneys under conditions of insulin resistance, suggesting a crosstalk of hyperglycemia and altered peripheral lipid metabolism (reviewed in Ref. [44]). In the kidney glomerulus, altered lipid metabolism causes podocyte apoptosis, enhances excessive extracellular matrix production and macrophages infiltration via increasing lipogenesis or reducing efflux and oxidation (reviewed in Ref. [45]). Our group and other researchers reported that cholesterol accumulates in the kidney cortex from mouse models of DKD as well as in glomeruli of affected patients [8, 46]. We also demonstrated an important link between local glomerular inflammation and impaired cholesterol efflux. In particular, glomerular production of TNF stimulates nuclear factor of activated T cells (NFAT) which in turn can suppress ABCA1 expression. In fact, inflammation was demonstrated as another hit to increased accumulation of lipids in podocytes of *db/db* mice through increased expression levels of low density lipids, cleavage-activating protein, and sterol regulatory element-binding protein 2 (SREBP2) [47, 48]. Recently, elevated levels of angiotensin II, a risk factor of initiation and progression of chronic kidney disease, have been shown to correlate with podocyte injury via increased expression of cholesterol-uptake-related molecules such as LDL receptor, SREBP1, SREBP2, HMGCR, and decreased expression of ABCA1 [49]. Another study has demonstrated that diabetes-induced cholesterol accumulation in podocytes is controlled by small GTPase Arf6 [50], which is related to the recycling disorder of ABCA1. Our studies determined the relative contribution of free and esterified cholesterol to podocyte injury via manipulation of ABCA1 and SOAT1 [21••, 34]. In a parallel study, administration of the cholesterol sequestrant cyclodextrin to *BTBR ob/ob* mice restored renal parenchymal cholesterol content and normalized proteinuria despite increasing circulating cholesterol. These findings suggest that cholesterol accumulation in glomerular cells is unrelated to the amount of systemic cholesterol and that dyslipidemia and local lipid metabolism may play a different role in the progression of DKD and its related cardiovascular complications.

The Role of Fatty Acids and Triglycerides in DKD

Other than cholesterol and phospholipids, lipid droplets are also characterized by triglycerides. Interestingly, intracellular and circulating triglycerides are also increased in clinical and experimental models of DKD [10, 21••]. Intracellular triglycerides are composed of free fatty acids and glycerols. Briefly, long-chain fatty acid uptake into the cell occurs via the cluster of differentiation 36 (CD36) receptor. Once intracellular, the

fatty acids can form part of the triglyceride and phospholipid components of the lipid droplets [51, 52].

CD36, a protein involved in free fatty acids (FFA) uptake, cholesterol absorption, and the activation of inflammatory pathways [53–55], has been observed to be upregulated in DKD experimental models [56–59]. Specifically, renal tubular epithelial cells (HK-2) cultured in high-glucose (30 mM) media have significantly increased CD36 expression [58] due to an increase in peroxisome proliferator-activated receptor γ (PPARγ) and significant accumulation of lipid droplets [59]. We reported that cholesterol efflux dysregulation is a key mediator of podocyte injury in several glomerular disorders [59]. While we reported that cholesterol efflux dysregulation is a key mediator of podocyte injury in the experimental Alport syndrome mouse model [25], others demonstrated a role of cholesterol influx [60], as well as of CD36-induced FFA in podocyte injury and in the NFAT mouse model [34]. Clinical and experimental studies have suggested that dysregulation of FFA metabolism in podocytes plays a pivotal role in obesity-related glomerulopathy and DKD pathogenesis [52, 54]. CD36 has been also shown to promote podocyte apoptosis via pyrin domain-containing 3 (NLRP3) inflammasome in nephrotic syndrome, others demonstrated a role of cholesterol influx in Alport renal tubules injury [60], as well as of CD36-induced FFA-mediated podocyte apoptosis in conditionally immortalized mouse podocyte cell line MPC5 [56]. Clinical and experimental studies have suggested that dysregulation of FFA metabolism in podocytes plays a pivotal role in obesity-related glomerulopathy and DKD pathogenesis (reviewed in Ref. (52)). CD36 has been also shown to promote apoptosis in mouse podocyte cell line MPC5 via pyrin domain-containing 3 (NLRP3) inflammasome under treatment with LDL and IL-1β [61].

Furthermore, high glucose-mediated increase in reactive oxygen species was prevented by CD36 knockdown in renal tubular epithelial HK-2 cells, suggesting that fatty acid uptake contributes to pathological mechanisms of DKD [57]. Similarly, several genes involved in fatty acid synthesis, including sterol regulatory binding protein 1 (SREBP1) and fatty acid synthase (FAS), were observed to be significantly increased in Akita and OVE26 mouse models of T1D with progressive kidney disease [10]. Increased fatty acid synthesis was also met with a decrease in gene expression of genes important in fatty acid oxidation (FAO). Several groups have reported reduced FAO gene expression in distinct DKD experimental mouse models, such as Akita and OVE26 [10] and BTBR *ob/ob* [21••]. Specifically, we recently demonstrated that human podocytes cultured in the presence of sera obtained from T2D patients with DKD have significantly reduced expression of genes important in FAO. This was also observed in BTBR *ob/ob* mouse model and in the model of ABCA1 deficiency [21••] in BTBR *ob/ob* mice [21••].

Collectively, these data suggest an increase in fatty acid uptake and accumulation of triglycerides in lipid droplets with a reduction in FAO, which further suggests that lipid accumulation does not permit for proper intracellular fuel utilization. Moreover, targeting and increasing FAO and lipid utilization pharmacologically or genetically proved to be beneficial in improving kidney disease progression [62].

Sphingolipids in DKD

Sphingolipids have been shown to play a significant role in normal cell and tissue homeostasis as well as in the development and progression of numerous diseases. They also interfere with many signaling pathways [63], including glomerular disorders (reviewed in Ref. (64)) [64]. Biologically active sphingolipids such as ceramide, ceramide-1-phosphate (C1P) and sphingosine-1-phosphate (S1P) attracted the most attention due to its roles in cell differentiation, membrane fluidity, protein anchoring, immune activation, insulin sensitivity, autophagy, and cell death.

S1P may have a potential as a therapeutic target in neurodegenerative disorders [65–67], rheumatoid arthritis [68], cancer [69–72], renal oncology [73], acute kidney injury [74, 75], and nephrotic syndrome [76]. Notably, it has been shown that in obese *ob/ob* mice plasma S1P levels are increased [77, 78], as well as in another rodent models of T1D [79]. In cultured C2C12 myoblasts S1P can stimulate basal glucose uptake [80], while in rat primary adipocytes S1P stimulates lipolysis [81]. Overexpression of sphingosine kinase 1 in mice on high-fat diet was associated with improved insulin sensitivity [82] and ceramide reduction in muscles [83]. In hepatocytes, S1P has been shown to contribute to cell survival probably via protein-kinase B (AKT) [84, 85] and to ameliorate glucose intolerance and insulin resistance [86]. The role of active sphingolipids in the kidney functioning is not very well studied yet. It has been demonstrated that the renal levels of S1P were increased in the streptozotocin mouse model [87]. Another study in humans demonstrated that mutations in *SGPL1* gene, encoding S1P lyase 1, is associated with development of nephrotic syndrome [88, 89]. In mice, *Sgpl1* gene knockout causes progression of foot processes effacement and severe proteinuria [88]. Using of FTY720 compound, an unselective S1P receptor agonist, in streptozotocin-induced diabetic nephropathy in rats demonstrated renoprotective effect [90].

Despite what might be expected, pro- or anti-inflammatory role of C1P is still a matter for discussion [91–94]. According to one of the current points of view, in contrast to S1P, C1P is most likely released by damaged cells [95]. In renal mesangial cells, knockout of ceramide kinase, an enzyme that generates C1P from ceramide, resulted in propagation of prostaglandin E2 and might be sufficient for treatment glomerular diseases [96]. Interestingly, in hepatocytes ceramide C16:0 was identified as a negative regulator of fatty acid oxidation in obesity [97]. We previously demonstrated elevated expression of sphingomyelin phosphodiesterase acid-like 3b (SMPDL3b),

a lipid-raft associated protein [98] that negatively regulates plasma membrane fluidity when its expression is reduced [99], in glomeruli from patients with DKD and in glomeruli of *db/db* mice [100]. Interestingly, SMPDL3b overexpression was also observed in normal human podocytes exposed to the sera of patients with DKD [100]. This was also associated with inability of DKD sera-treated podocytes to phosphorylate AKT in response to insulin suggesting insulin resistance [8]. In our recent study, we demonstrated that SMPDL3b overexpression negatively affects the availability of C1P and that human podocytes in state of SMPDL3b overexpression are not able to phosphorylate AKT in response to insulin stimulation, which could be eliminated with exogenous administration of C1P [101•]. In the same study, in vivo experiments showed that podocyte-specific *Smpdl3b* deficiency in diabetic mice protect them from the development of DKD. Podocyte-specific *Smpdl3b* deficiency was associated with the restoration of the C1P content in kidney cortexes of mice, and most importantly with the restoration of podocyte-specific AKT phosphorylation [101•]. Thus, our data reveals that SMPDL3b is a master modulator of insulin signaling in human podocytes.

Mitochondrial Defects and Dysfunction in DKD

Recently, it has been reported that mitochondrial DNAs and RNAs are altered in blood cells from patients with DKD and in renal cells cultured in high-glucose conditions [16]. In addition, hyperglycemia-induced mitochondrial reactive oxygen species (ROS) production occurs in podocytes [102, 103], and we have recently demonstrated that human podocytes cultured in the presence of sera obtained from patients with DKD have a significant increase ROS [21••]. Furthermore, ABCA1 knockdown podocytes have a significant increase in superoxide dismutase 2 (SOD2), a mitochondrial ROS scavenger. This suggests that ROS production is increased in ABCA1 deficiency; however, the compensatory elevation of SOD2 could explain why ABCA1 deficiency alone is not detrimental to podocytes, and we have recently demonstrated that human podocytes cultured in the presence of sera obtained from patients with DKD have a significant increase ROS [21••]. Furthermore, ABCA1 knockdown podocytes have a significant increase in superoxide dismutase 2 (SOD2), a mitochondrial ROS scavenger [21••]. This suggests that ROS production is increased in ABCA1 deficiency with compensatory elevation of SOD2 indicating ABCA1 deficient alone is not detrimental to podocytes, but when challenged in a diabetic state (with reduced SOD2) it results in worsened podocyte injury [21••]. Furthermore, in DKD, enzymatic ROS generation mostly involves NADPH oxidase (Nox) pathways while non-enzymatic pathways include the mitochondrial electron transport chain (mETC), advanced glycation products (AGEs), glucose autoxidation, and other pathways. It has become clear that hyperglycemia-induced generation of superoxide plays an important role in the development of DKD [104–106]. In experimental models of DKD and H_2O_2 treated tubular cells, the mitochondrial ATP content and production are decreased [107]. In hepatocytes and myoblasts, high-glucose conditions increase ROS in association with morphological changes in the mitochondrial shape. While it is not clear if increased fission or decreased fusion contribute to these morphological changes, inhibition of fission or promotion of fusion is sufficient to prevent high glucose-induced increases in ROS [108]. Furthermore, in DKD, enzymatic ROS generation mostly involves NADPH oxidase (Nox) pathways while non-enzymatic pathways include the mitochondrial electron transport chain (mETC), advanced glycation products (AGEs), glucose autoxidation and other pathways. It has become clear that hyperglycemia-induced generation of superoxide plays an important role in the development of DKD [104–106]. In experimental models of DKD and H_2O_2-treated tubular cells, the mitochondrial ATP content and production are decreased [107]. In hepatocytes and myoblasts, high*glucose conditions increase ROS in association with morphological changes in the mitochondrial shape. While it is not clear if increased fission or decreased fusion contribute to these morphological changes, inhibition of fission or promotion of fusion is sufficient to prevent high glucose induced increases in ROS [108].

Mitochondrial Fission and Fusion

Mitochondrial fission and fusion are necessary dynamics that allow for the proper mitochondrial network maintenance. During fission mitochondria are split into two sister organelles, whereas during fusion two mitochondria will merge to produce one larger organelle, a process originally described in yeast [109]. Briefly, fission is primarily mediated by dynamin related protein 1 (Drp1) and its receptors which allow for it to be properly recruited to the outer mitochondrial membrane to carry out fission, such as mitochondrial fission 1 (FIS1). Furthermore, fusion is primarily regulated by the interactions between mitofusin 1 and 2 (MFN1/2) [110].

Several experimental and clinical studies using DKD models have reported mitochondrial fragmentation in the kidney of these models [111]. Mitochondrial fragmentation is associated with increased fission dynamics. Interestingly, when Drp1 is knocked out in the podocyte of *db/db* mice, they are protected from the development of DKD, suggesting mitochondrial fission to play an important role in the progression of DKD [112]. Furthermore, cultured mouse podocytes treated with palmitic acid experience an increase in mitochondrial fragmentation and Drp1, as well as an increase in podocyte injury. However, when these mouse podocytes are co-treated

with palmitic acid and berberine (inhibits palmitic acid-mediated upregulation of Drp1), then mitochondrial fragmentation and podocyte injury is diminished [113]. Similarly, human renal proximal tubular epithelial cells treated with high glucose experience a significant downregulation of MFN1 met with an increase in DRP1 [114]. Interestingly, we demonstrated that diabetic mice with a podocyte-specific deletion of ABCA1 experience a significant reduction in gene expression of genes important in fusion, and were observed to have mitochondrial morphological alterations as compared to their diabetic controls [21••].

Mitochondria and the Oxidative Phosphorylation (OXPHOS) System

Mitochondria are highly dynamic organelles which form a tubular network that constantly changes by fission and fusion. They fulfill pleiotropic functions as they are the main source of ATP for fundamental cell functions, they are involved in fatty acid and amino acid metabolism and they play a role in reactive oxygen species (ROS) production [115]. The inner membrane of mitochondria forms invaginations (cristae) accommodates five OXPHOS complexes (Complex CI–CV). Cristae morphology varies depending on the energy demand of the tissue, i.e., they are closely stacked in tissues with high energy demand but are less closely stacked in tissues, for example liver, which has low energy demand. CI, CIII, and CIV are oxidoreductases and often found associated in supercomplexes. CII is a dehydrogenase, CV is an ATP synthase. These complexes form the OXPHOS system [116] of mitochondria. CI and CIII are the main sites of superoxide production in renal mitochondria [117]. While CI-generated superoxide is released to the mitochondrial matrix, CIII-generated superoxide is released to both, the matrix and the intermembrane space [118, 119]. Renal mitochondria of rats with STZ-induced diabetes show a significant reduction in CIII activity associated with increased ROS production and decreased respiratory activity suggesting that selective alterations in CIII may play a central role in diabetes-related mitochondrial dysfunction [120].

Mitochondrial Lipids and the OXPHOS System

Although, mitochondria are considered to be sterol poor organelles, several other lipids play a pivotal role in maintaining mitochondrial function. Specifically, mitochondrial membranes are primarily composed of phospholipids, including but not limited to phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), cardiolipin (CL), and phosphatidic acid (PA) [121, 122]. Interestingly, several lipids are found to be important in stabilizing and contributing to proper OXPHOS system function within the inner mitochondrial membrane (IMM) [123].

PE-deficient cells demonstrate a reduction in complex IV activity, met with impaired mitochondrial membrane potential, suggesting an important role of PE in cytochrome c oxidase activity [124]. Furthermore, purification of the individual complexes has demonstrated that CL, PI, PE, and PA are found to be integral members of complex III formation [122]. CL is a mitochondrial-specific phospholipid, making up about 10–15% of total mitochondrial lipids. PE-deficient cells demonstrate a reduction in complex IV activity, met with impaired mitochondrial membrane potential, suggesting an important role of PE in cytochrome c oxidase activity [124]. Furthermore, purification of the individual complexes has demonstrated that CL, PI, PE, and PA are found to be integral members of complex III formation [122]. CL is a mitochondrial-specific phospholipid, making up about 10–15% of total mitochondrial lipids [121] and is at the center of mitochondrial physiology [125]. Interestingly, several studies have shown CL to be intricately involved in membrane stability of OXPHOS supercomplexes (SC), and individual complex III, as well as in respiration, ATP production and cytochrome c activity, and is at the center of mitochondrial physiology [125]. Interestingly, several studies have shown CL to be intricately involved in membrane stability of OXPHOS supercomplexes (SC), and individual complex III, as well as in respiration, ATP production and cytochrome c activity [124, 126–128].

However, the role of lipid accumulation and alterations on renal mitochondrial complex formation and mitochondrial dysfunction has not been thoroughly investigated until recently. As previously mentioned ABCA1 expression is significantly reduced in DKD models. Interestingly, podocytes with siRNA knockdown of ABCA1 have significant alterations in the OXPHOS complexes, including an increase in supercomplex formation, met with a redistribution of intermediate complexes $CIII_2$ and CIII-CIV. This remodeling was also met with a significant reduction in oxygen consumption and CIII activity [21••]. These data suggest that alterations in lipid metabolism significantly alter mitochondrial functions in the context of DKD (Fig. 1); however, further studies need to be conducted in order to understand if the mitochondrial redistribution observed is a compensatory mechanism or if it is indeed pathological. Furthermore, CL content is significantly increased in ABCA1 knockdown podocytes associated with the previously mentioned mitochondrial dysfunction. This confirms the finding that fibroblasts obtained from patients affected by Tangier disease have a significant accumulation in CL content [129–131]. CL is an integral part of the inner mitochondrial membrane and vital for maintaining proper mitochondrial function and we recently demonstrated in the state of ABCA1 deficiency and DKD, alterations in CL content play a crucial role in disease and will be further discussed next.

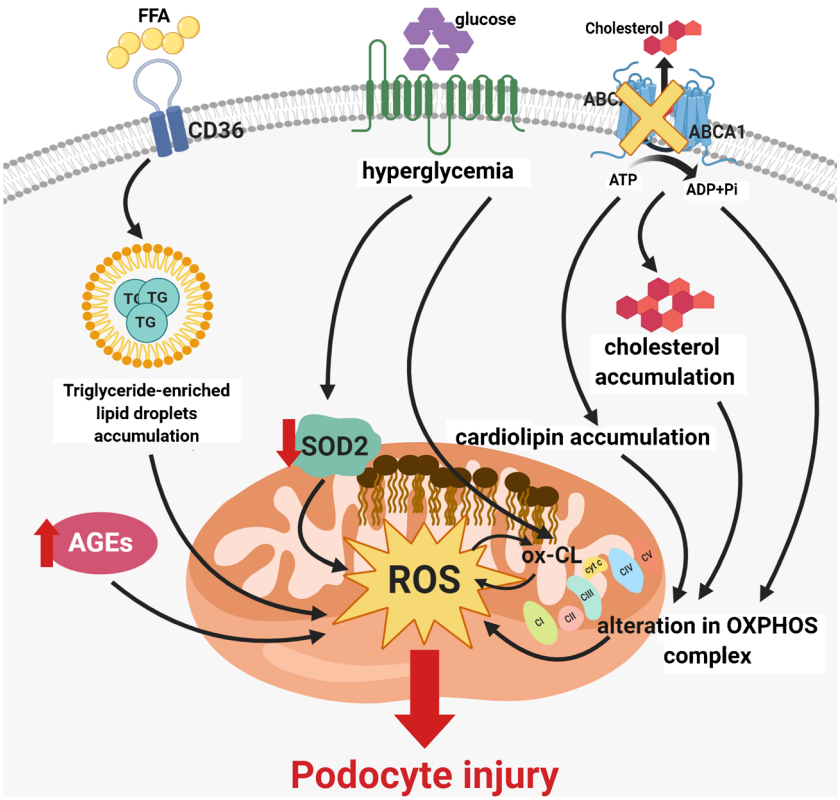

Fig. 1 Role of mitochondria in podocyte injury associated with DKD. Decreased expression of ATP-binding cassette transporter 1 (ABCA1) leads to increased cholesterol and cardiolipin accumulation and alteration in OXPHOS complex; however, in a hyperglycemic state, superoxide dismutase 2 (SOD2) is reduced and together this contributes to elevated reactive oxygen species (ROS) production and oxidized cardiolipin (ox-CL). Alteration of free fatty acids (FFA) uptake through platelet glycoprotein 4 (CD36) causes triglyceride (TG)-enriched lipid droplets accumulation and increases ROS production. Hyperglycemia and increased production of advanced glycosylation end products (AGEs) lead to ROS production and podocyte injury in DKD

The Role of Cholesterol, Cardiolipin, and Peroxidized Cardiolipin in Mitochondria Membrane Fluidity and Permeability

CL plays critical roles in mitochondrial biogenesis, morphology, fusion and fission, respiration, and protein import [132]. CL is mostly synthesized from phosphatidic acid (PA) produced at the ER. Thus, accumulation of CL is dependent on the transfer of PA from the ER to the inner mitochondrial membrane (IMM) [133, 134]. High CL levels and phosphatidylethanolamine (PE) in the mitochondria membrane are required to maintain important mitochondrial functions, including cristae formation and stabilization of the respiratory complexes [124]. High CL levels and phosphatidylethanolamine (PE) in the mitochondria membrane are required to maintain important mitochondrial functions, including cristae formation and stabilization of the respiratory complexes [124].

Mitochondria regulate various forms of cell death including apoptosis and necrosis. Apoptosis can be initiated using two distinct signaling pathways, either intrinsic (where pro-apoptotic proteins are activated within the cell) or extrinsic (where pro-apoptotic ligands bind to receptors on the cell surface). When the ligand binds the death receptors, this stimulates caspase 8 translocation from the cytosol to the mitochondria, a process which requires CL [135]. Once in the mitochondria, caspase 8 increases mitochondrial permeability, through oligomerization of Bax and Bcl-2, which allows for the release of cytochrome c from the outer mitochondrial membrane (OMM). Interestingly, CL must be present at the OMM in order for all these processes to occur. Furthermore, CL regulates cytochrome c binding to the IMM and any change in CL through remodeling, oxidation, or content will release cytochrome c to initiate cell death. CL oxidation has been shown to weaken the affinity of CL to cytochrome c, thereby contributing to increased apoptosis [135–139].

The lipid composition of the OMM and IMM differs significantly [140, 141]. While the IMM is enriched with CL, the OMM has only low concentrations of CL. As mentioned previously mitochondria are cholesterol poor organelles, however, in pathological conditions, cholesterol accumulation in the MM has been described where it is associated with changes in the IMM permeability and function of the resident proteins [142]. Interestingly, MM fluidity is enhanced with increasing CL concentration [143]. CL binds to CI, CIII, CIV, and CV of the OXPHOS system and plays an important role in the assembly of the supercomplexes [144–146]. CL is particularly vulnerable to ROS-induced damage which in turn contributes to mitochondrial dysfunction [147, 148]. Recent literature has demonstrated that treatment with elamipretide, a selective cardiolipin peroxidase inhibitor improved podocyte content and kidney injury in high-fat diet-fed mice [149].

Our group has recently demonstrated that alterations of the fatty acid side chain composition of the CL found in the kidney cortex of *db/db* mice were associated with increases in CL oxidation, as well as DKD progression, including mesangial expansion and reduced podocyte content. However, when *db/*

db mice were treated with a pharmacological inducer of ABCA1 the CL fatty acid side chain remodeling was conserved, resulting in reduced CL oxidation and DKD progression. Furthermore, DKD experimental models of ABCA1 deficiency were treated with elamipretide and also observed to have improved podocyte content and slower progression of DKD [21••].

Conclusions

Altered renal cell lipid metabolism contributes to clinical and experimental DKD. Detailed knowledge of specific lipids, lipid modifying enzymes, and the receptors implicated in their actions, is essential to move from a novel molecular mechanism of glomerular cell damage in DKD to new therapeutic options. Here, we summarized the clinical and experimental evidence supporting a role of lipids in the development of DKD, with particular attention to these lipids causing mitochondria dysfunction. It is clear that alterations in lipid trafficking, lipid storage, and membrane lipids all affect mitochondrial function and can contribute not only to DKD progression but also to other diseases where altered lipid metabolism is observed in conjunction with mitochondrial dysfunction and cellular injury. As the knowledge that podocyte mitochondria function and lipid metabolism are connected, novel therapeutic strategies will become available. Notwithstanding, our current understanding of these signaling cascades is incomplete and future work in this area is needed.

Authors' Contributions GMD and AM prepared a draft of the manuscript. AM focused primarily on the "Lipids in DKD" section and GMD focused primarily on the "Mitochondrial Defects and Dysfunction in DKD" section. AF reviewed and improved the entire manuscript.

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Compliance with Ethical Standards

Conflict of Interest Alessia Fornoni is an investor on pending or issued patents (US 10,183,038 and US 10,052,345) aimed at diagnosing or treating proteinuric kidney diseases. She stands to gain royalties from the future commercialization of these patents. She is Chief Scientific Officer of L&F Health LLC and is a consultant for Variant Pharmaceuticals. Variant Pharmaceuticals has licensed worldwide rights from L&F Research to develop and commercialize hydroxypropyl-beta-cyclodextrin for the treatment of kidney disease. She is the founder of LipoNexT LLC. She is also supported by Roche and Boehringer Ingelheim.

Michelle Ducasa and Alla Mitrofanova declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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