Free fatty acids and their metabolism affect function and survival of podocytes

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Podocyte injury and loss critically contribute to the pathogenesis of proteinuric kidney diseases including diabetic nephropathy. Deregulated lipid metabolism with disturbed free fatty acid (FFA) metabolism is a characteristic of metabolically unhealthy obesity and type 2 diabetes and likely contributes to end-stage kidney disease irrespective of the underlying kidney disease. In the current review, we summarize recent findings related to FFAs and altered renal FFA metabolism with a special focus on podocytes. We will outline the opposing effects of saturated and monounsaturated FFAs and a particular emphasis will be given to the underlying molecular mechanisms involving insulin resistance and endoplasmic reticulum homeostasis. Finally, recent data suggesting a critical role of renal FFA metabolism to adapt to an altered lipid environment will be discussed.

Keywords: podocyte, diabetic nephropathy, saturated and monounsaturated free fatty acids, lipid metabolism, lipotoxicity, endoplasmic reticulum stress, β-oxidation

INTRODUCTION

Diabetic nephropathy (DN) is the major cause of end-stage renal disease, and most affected patients have type 2 diabetes (1, 2). Injury and loss of the glomerular epithelial cells or podocytes are critical in the pathogenesis of DN (3–6). Importantly, the epidemic of DN and type 2 diabetes parallels the obesity epidemic (7, 8), which also drives other causes of chronic kidney disease (CKD) including obesity-related glomerulopathy and secondary focal segmental glomerulosclerosis [reviewed in Ref. (9)]. Obesity-related glomerulopathy has been found to be more associated with

serum triglyceride (TG) levels and ectopic lipid accumulation in the kidney than with obesity *per se* (10). Lipid excess and deregulated lipid metabolism in the kidney are increasingly recognized as pathogenic factors not only in the development and progression of obesity-related renal disease and DN but they may also contribute to CKD irrespective of the underlying pathology (9). Excessive lipid droplets can be found in different renal cell types including podocytes (9, 11). Accumulation of lipids in non-adipose tissues can contribute to cellular dysfunction and cell death, a phenomenon that is called lipotoxicity. Elevated plasma free fatty acids (FFAs) and disturbed FFA metabolism critically contribute to lipotoxicity. However, to which extent and how the various FFAs and their metabolites such as diacylglycerols (DAGs) and TGs are pathogenic or are even part of a protective, adaptive process is under debate. Recent data also indicate that cholesterol accumulation in podocytes plays a critical pathogenic role in DN and contributes to lipotoxicity (12).

This review will highlight the potential consequences of altered FFA levels and disturbed FFA metabolism with a special focus on podocytes. If adaptation fails, lipotoxicity with insulin resistance and endoplasmic reticulum (ER) stress may ultimately result in podocyte death. Recent advances in the underlying cellular processes will be summarized and may help to foster further research to find and translate novel therapeutic strategies. In

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; ATF6, activating transcription factor 6; Bcl-2, B-cell lymphoma 2; BiP, heavy chain binding protein; CHOP, C/EBP homologous protein; chREBP, carbohydrate responsive element-binding protein; CKD, chronic kidney disease; CPT, carnitine palmitoyltransferase; DAG, diacylglycerol; DGAT, acyl-CoA:diacylglycerolacyltransferase; DN, diabetic nephropathy; DR5, death receptor 5; ER, endoplasmic reticulum; ERO1, ER oxidoreductase 1; FFA, free fatty acid; Gadd34, growth arrest and DNA damage-inducible protein 34; IRE1, inositol-requiring enzyme 1; IRS, insulin receptor substrate; JNK, c-Jun NH$_2$-terminal kinase; mTORC1, mammalian target of rapamycin complex 1; MUFA, monounsaturated fatty acid; PERK, PKR(protein kinase RNA)-like ER kinase; PKC, protein kinase C; ROS, reactive oxygen species; SCD, stearoyl-CoA desaturase; SFA, saturated fatty acid; SREBP, sterol regulatory element-binding protein; TG, triglyceride; TRAIL, tumor necrosis factor related apoptosis inducing ligand; UPR, unfolded protein response; Xbp1, X-box binding protein.

addition, a particular emphasis will be laid on discussing cellular adaptive responses, which might be interesting targets in supporting podocytes dealing with an altered lipid environment.

PLASMA FREE FATTY ACIDS

A main source of lipids is plasma FFAs, which are hydrolyzed and relieved from adipocyte TG stores and carried by plasma albumin to provide energy for tissues during fasting (13). In addition, fatty acids hydrolyzed from liver-derived low density lipoprotein TG by lipoprotein lipases are also contributing to the FFA pool in tissues (14). FFA levels are underlying diurnal fluctuations with low postprandial levels where most FFAs are taken up by the adipose tissue and increased levels during states of fasting (15). In contrast to the general belief, obesity is not generally associated with increased fasting FFA levels, and this association only exists in certain groups of obese and type 2 diabetic patients (16). Elevated FFA levels are associated with and may result from insulin resistance and increased lipolysis (15, 17–19). *Vice versa*, insulin resistance can be the result of elevated FFAs (20). In addition, adipocytes of obese individuals can become defective in FFA uptake, which contributes to elevated FFA levels and promotes ectopic fat deposition (21). This might be of interest as plasma FFA composition partially reflects dietary fatty acid composition (22) and saturated FFAs (SFAs), and monounsaturated FFAs (MUFAs) have distinct effects on cell metabolism and function. In this context, a recent intervention trial is of interest, which demonstrated that a Mediterranean diet enriched with extra-virgin olive oil is effective in the primary prevention of cardiovascular diseases and diabetes (23, 24), and it has been suggested that this beneficial effect at least in part results from the high content of MUFAs in olive oil (24). Further interventional studies are warranted to test whether dietary shifting of the FFA balance toward unsaturated FFAs can prevent and delay the progression of obesity-related renal diseases and DN.

OPPOSING EFFECTS OF SFAs AND MUFAs

The SFAs, palmitic and stearic acid, together with the MUFA oleic acid account for 70–80% of plasma FFAs (25, 26). Interestingly, in most cell types including podocytes mainly SFAs are inducing lipotoxicity such as insulin resistance and cell death (27, 28). By contrast, MUFAs can prevent SFA induced lipotoxicity (**Table 1**), i.e., an equimolar combination of palmitic and oleic acid does not lead to podocytes death (28). Most of the current understanding of the opposing effects has been derived from studies with hepatocytes, muscle cells, and pancreatic β-cells linking the detrimental actions of SFAs to SFA-derived metabolites such as DAGs and ceramide (29). DAG-mediated activation of protein kinase C (PKC) δ and increased levels of ceramide are associated with the intrinsic mitochondrial apoptotic pathway, e.g., increased mitochondrial membrane permeability and cytochrome *c* release (30–32). Cytochrome *c* release is also observed in palmitic acid treated podocytes (preliminary data). Of note, some studies show partially conflicting findings in the light of the effects of increased ceramide synthesis, TG accumulation, and β-oxidation with its associated reactive oxygen species (ROS) (33–36). In human podocytes, ceramide accumulation has been linked to palmitic acid-induced insulin resistance (27); however, the ceramide synthase inhibitor fumonisin B1 is not ameliorating survival of murine podocytes
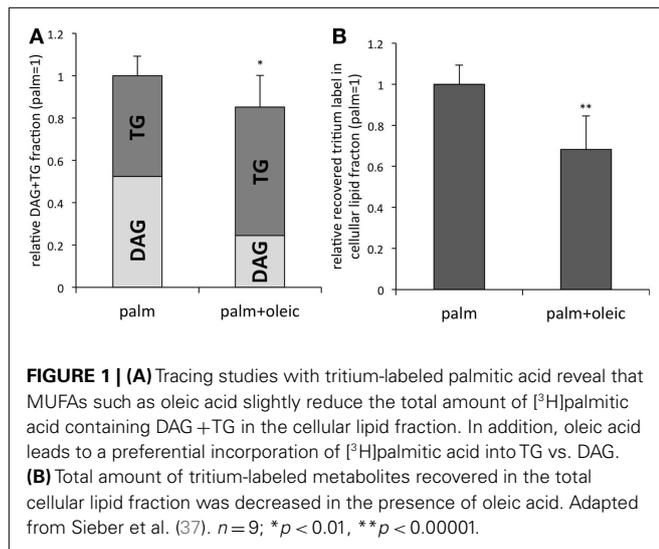
Table 1 | Beneficial effects of MUFAs on podocytes treated with palmitic acid.

	Action of MUFAs (e.g., oleic acid) in podocytes treated with palmitic acid	Reference
Cell viability	Prevention of podocyte death	(28)
ER stress/ UPR	Induction of the adaptive UPR (e.g., BiP)	(28)
	Prevention of CHOP induction	(28)
Insulin resistance	Improved insulin sensitivity	Unpublished observation
Lipid metabolism	Increased fatty acid β-oxidation	(37)
	Reduced accumulation of palmitic acid in DAG	**Figure 1A** (37)
	Preferential incorporation of palmitic acid into TG	**Figure 1A** (37)
	Increased DGAT gene expression	Unpublished observation
	Reduced accumulation of palmitic acid derived metabolites in the total lipid fraction	**Figure 1B** (37)

exposed to palmitic acid (37). Of note, tracing studies with tritium-labeled palmitic acid could show that MUFAs, such as oleic acid, slightly but significantly reduce the total amount of intracellular [³H]palmitic acid containing DAG and TG (**Figure 1A**). More importantly, oleic acid leads to preferential incorporation of [³H]palmitic acid derived metabolites into TGs, which is accompanied by a reduction of the [H³]palmitic acid containing DAG fraction [**Figure 1A**, adapted from Sieber et al. (37)]. Furthermore, oleic acid stimulates β-oxidation of palmitic acid (37) and may be beneficial simply by reducing the levels of palmitic acid and its toxic metabolites (38), which might be reflected by the overall decreased recovery of tritium derived from labeled palmitic acid in the total cellular lipid fraction (**Figure 1B**). In summary, although the beneficial effects of MUFAs are not completely understood, recent work including studies in podocytes points toward facilitated incorporation of palmitic and its metabolites into TGs and increased palmitic acid β-oxidation, which have been postulated to prevent from accumulation of toxic SFA metabolites (29, 35, 37).

INSULIN RESISTANCE

In human (27) and murine (preliminary data) podocytes, palmitic acid induces insulin resistance. Studies in hepatocytes and skeletal muscle cells linked palmitic acid-induced insulin resistance to alternate serine/threonine phosphorylation of insulin receptor substrate (IRS) 1, 2, and AKT by either ROS-mediated JNK (c-Jun NH₂-terminal kinase) activation or by DAG activation of PKC (39–41). In podocytes, insulin signaling could be ameliorated by JNK inhibition (preliminary data). However, this was not sufficient to ameliorate survival of podocytes chronically exposed to palmitic acid (preliminary data). Of note, JNK activation is also downstream of disturbed ER homeostasis referred to as ER stress, which has been causatively linked to palmitic acid-induced podocyte death (28) (see below). Reduced insulin sensitivity is observed in glomeruli of obese and diabetic rats (42) and normal

FIGURE 1 | (A) Tracing studies with tritium-labeled palmitic acid reveal that MUFAs such as oleic acid slightly reduce the total amount of [^{3}H]palmitic acid containing DAG +TG in the cellular lipid fraction. In addition, oleic acid leads to a preferential incorporation of [^{3}H]palmitic acid into TG vs. DAG. **(B)** Total amount of tritium-labeled metabolites recovered in the total cellular lipid fraction was decreased in the presence of oleic acid. Adapted from Sieber et al. (37). $n = 9$; *$p < 0.01$, **$p < 0.00001$.

insulin signaling seems critical for podocyte function and survival as podocyte-specific insulin receptor knockout mice develop albuminuria and glomerulosclerosis (43). In summary, these findings point toward a potential FFA-mediated role of insulin resistance in the development and progression of obesity-related renal disease and DN.

ER STRESS

FFA mediated ER stress has been associated with the pathogenesis of obesity and type 2 diabetes where it is extensively studied in the light of pancreatic β-cell failure and the onset of type 2 diabetes (44, 45). ER stress is also observed in the tubulointerstitial and glomerular compartment of renal biopsies obtained from patients with DN (28, 46, 47). Importantly, ameliorating ER stress has been shown to attenuate DN in a type 1 diabetes mouse model (48, 49).

Disturbed ER homeostasis decreases the ER folding capacity and thereby leads to accumulation of unfolded and misfolded proteins, which in turn initiates the unfolded protein response (UPR). The UPR is primarily an adaptive response to maintain proper ER function (50, 51) and involves three signaling branches that are mediated by the ER transmembrane receptors PERK (PKR-like ER kinase), ATF6 (activating transcription factor 6), and IRE-1 (inositol-requiring enzyme 1), which ultimately lead to translational attenuation, ER-associated protein degradation, increased ER chaperone expression, and ER membrane synthesis. If ER stress persists, cells initiate apoptosis, which has been linked to the proapoptotic transcription factor C/EBP homologous protein (CHOP) (52–55). In podocytes, palmitic acid-induced ER stress results in the induction of several UPR markers/effectors, including the ER chaperone heavy chain binding protein (BiP), Gadd34 (Growth arrest and DNA damage-inducible protein), as well as alternate splicing of X-box binding protein 1 (Xbp1), and upregulation of CHOP [(28) and unpublished results]. Contrariwise, the monounsaturated palmitoleic and oleic acids alone upregulated BiP but not CHOP (28). As BiP is known to protect from palmitic acid-induced apoptosis (56), the upregulation of the ER chaperone

BiP by MUFAs likely contributes to their beneficial effect. Furthermore, MUFAs attenuate palmitic acid-induced upregulation of CHOP in podocytes, and gene silencing of CHOP protects against palmitic acid-induced podocyte death, which points to a causative role for CHOP (28). A recent study suggests that the detrimental effects of SFAs are linked to activation of mTORC1 (mammalian target of rapamycin complex 1) and subsequent CHOP upregulation (57). Similarly, CHOP deficient mice are protected from DN as well as age-related albuminuria (58). Surprisingly, however, CHOP levels were either unchanged or significantly downregulated in the tubulointerstitial (46) and glomerular (28) compartment of renal biopsies obtained from patients with DN, which could indicate that CHOP positive cells may die and be removed from the tissue. An alternative explanation might be that during the progression of DN, there is a selection of podocytes adapted to the altered environment. The apoptotic actions of CHOP are not completely understood (59); however, proapoptotic targets include GADD34 (60), DR5 (TRAIL Receptor-2) (61), and ERO1α (ER oxidoreductase-1α) (62). In addition, CHOP has been associated with downregulation of anti-apoptotic Bcl-2 (63).

Saturated FFA-mediated ER stress and subsequent UPR have been associated with altered ER membrane composition and disrupted ER integrity (64). In addition of being activated by unfolded proteins, the ER stress sensor IRE-1 has been shown to be sensitive to alterations in ER membrane lipid composition (65). This is as in β-cells, palmitic acid-induced ER stress does not correlate with unfolded proteins (66). Interestingly, in preliminary experiments, specific IRE-1 inhibition with the small molecule compound 4μ8C attenuates palmitic acid-induced podocyte death, which is in accordance with the crucial role of IRE-1 in determining cell fate (67, 68).

CELLULAR ADAPTIVE RESPONSES: REGULATION OF β-OXIDATION

SFA lipotoxicity has been linked to lipid accumulation including TGs (69, 70) and increased ROS derived from enhanced β-oxidation (39). However, recent findings indicate that increased FFA β-oxidation as well as TG synthesis may not have to be harmful in any case, but may reflect a protective adaptive response helping podocytes to deal with elevated FFA levels.

Specifically, increasing fatty acid oxidation reduces the susceptibility of podocytes to palmitic acid (38). Fatty acid β-oxidation can be enhanced by AICAR (5-Aminoimidazole-4-carboxamide ribonucleotide), an agonist of the energy-sensor AMPK (AMP-activated protein kinase), which inactivates the acetyl-CoA carboxylase (ACC) and thereby reduces levels of the natural carnitine palmitoyltransferase 1 (CPT1) inhibitor malonyl-CoA. The protective effect of Aicar on palmitic acid could be reversed by inhibiting CPT1, the rate-limiting enzyme of fatty acid β-oxidation. Similarly, ACC-silenced podocytes were less susceptible to palmitic acid (38). Importantly, several recent genome-wide association studies in type 2 diabetic patients found a single-nucleotide polymorphism in a non-coding region of ACC2 to be strongly associated with proteinuria (71–73). The polymorphism is associated with increased ACC2 expression (71), which tends to increased levels of the CPT1 inhibitor malonyl-CoA and diminished fatty acid β-oxidation capacity.

Of interest, the adipocyte-derived hormone adiponectin, a physiological activator of AMPK signaling, slightly improves survival of podocytes treated with palmitic acid (38). However, the protective effect could only be seen in the presence of high glucose, which is known to reduce AMPK signaling (74) and thereby allowed to uncover the effect of adiponectin (38). This observation is in line with and gives a further explanation for the known renoprotective effect of adiponectin (74).

A recent study found an altered gene expression profile of key enzymes of fatty acid metabolism in glomeruli of patients with DN (37). Of note, an upregulation of all three CPT1 isoforms and a downregulation of ACC2 were found (37), which both suggest disposition for increased fatty oxidation. An increase in fatty acid oxidation likely contributes to a protective, adaptive response by decreasing the load of toxic SFAs (38). A second study, however, found a decreased expression of CPT1 in DN (11). However, these results were obtained from whole kidneys (11) and not from glomerular extracts (37), which may explain these discrepancies.

CELLULAR ADAPTIVE RESPONSES: SCD1 EXPRESSION AND REGULATION OF TG SYNTHESIS

Both aforementioned gene expression analyses found an increased expression of stearoyl-CoA desaturases (SCD) 1 in diabetic kidneys (11, 37) and by immunohistochemistry the glomerular upregulation of SCD1 could be mainly localized to podocytes (37). SCDs desaturate SFAs to MUFAs and thereby provide acyl-CoA:diacylglycerolacyltransferases (DGATs), which catalyze the final step in TG synthesis, with their preferred substrates (75, 76). In glomeruli, the expression of DGAT1 was also found to be increased (37), and together these results indicate not only facilitated conversion of toxic SFAs to MUFAs but also stimulation of TG synthesis.

In obesity, accumulation of TG has been linked to peripheral lipotoxicity (69, 70); however, TG storage might not be pathogenic *per se* as endurance-trained athletes show higher TG levels as well as higher insulin sensitivity in skeletal muscle, a phenomenon known as the "athletes' paradox" (77, 78). Also, transgenic mice overexpressing the TG-synthesizing enzyme DGAT1 in the heart have an increased TG content, but improved cardiac function (79). Moreover, mice overexpressing chREBP (carbohydrate response element-binding protein) fed a high-fat diet have increased hepatic levels of SCD1 and DGAT1 and show elevated insulin sensitivity despite increased hepatic steatosis (80). On the other hand, at some point, renal lipid accumulation may become harmful and a reduction in lipid overload by farnesoid X receptor agonists, which reduce the lipid synthesis regulator SREBP-1 (sterol
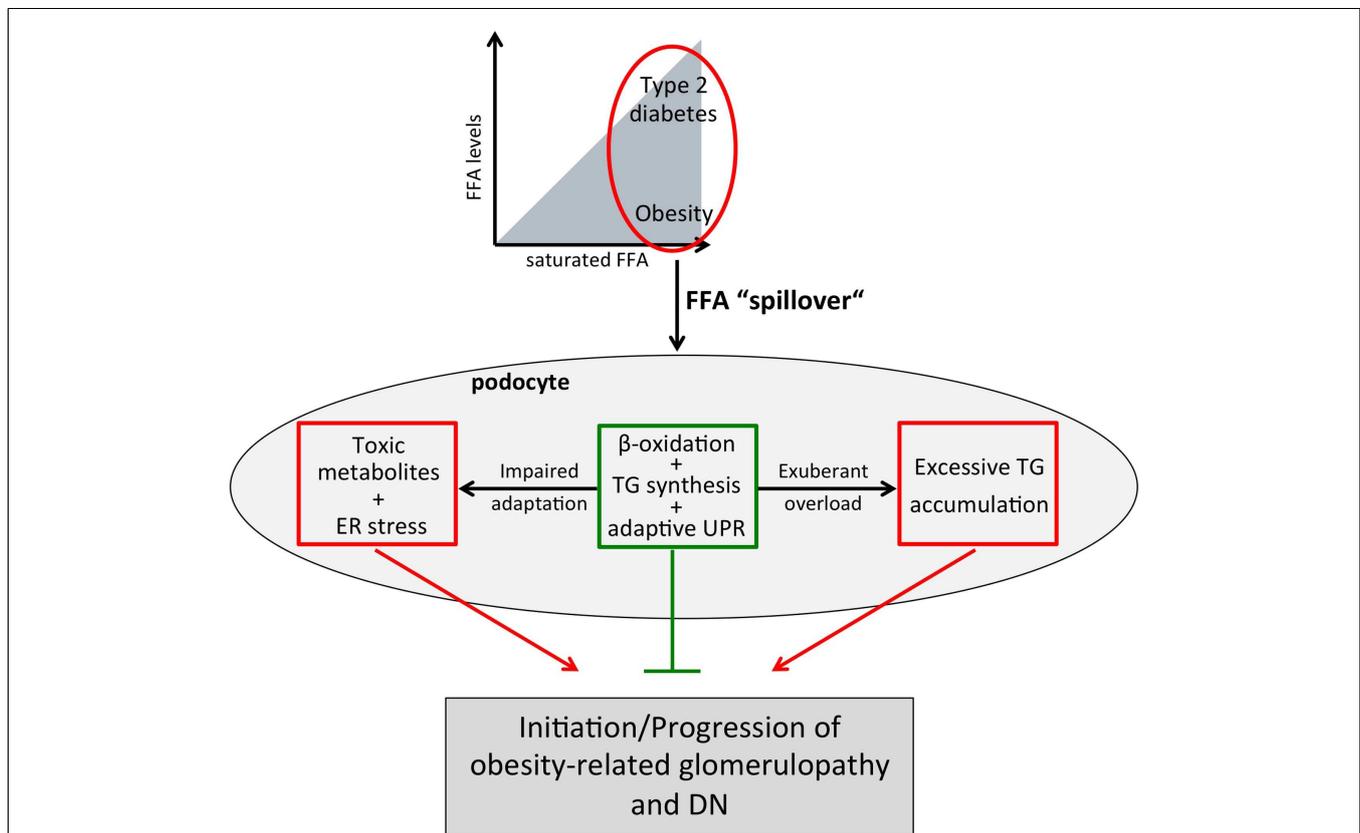
FIGURE 2 | Working model for increased plasma FFA levels and/or a shift toward SFAs on podocytes. In obesity and type 2 diabetes, increased adipose tissue lipolysis and/or a FFA uptake defect of adipocytes together with increased dietary FFA intake results in elevated plasma FFAs and a "spillover" of FFAs to non-adipose tissues including the kidney and podocytes. Podocytes may adapt to the altered lipid environment by upregulating fatty acid β-oxidation, TG synthesis, and the adaptive branch of the UPR. However, impaired adaptive capacity (e.g., genetic) or chronic "overload" leading to accumulation of toxic FFA metabolites and/or excessive TG storage may lead to diminished podocyte function and ultimately podocyte death resulting in obesity-related glomerulopathy and DN.

regulatory element-binding protein), has been shown to slow the progression of DN (81) and obesity-induced nephropathy (82). It can be hypothesized that the extent, location, and context of TG deposition determine lipotoxicity, and specifically, if it prevents accumulation of even more toxic SFA metabolites such as ceramide and DAGs, the beneficial effects may outweigh [**Figure 2** (35)].

Pharmacological stimulation of SCDs by liver X receptor agonists protects podocytes from palmitic acid-induced cell death and this protective effect is lost in podocytes deficient of SCD1 and 2. Also, SCD1 and 2 double deficient podocytes are more susceptible to palmitic acid, but genetic overexpression of SCD1 is protective. These data suggest that the abovementioned increased expression of SCD1 in podocytes likely is part of a protective, adaptive response, which helps podocytes dealing with FFAs.

SUMMARY AND CONCLUSION

Disordered lipid metabolism and renal lipid accumulation are not only associated with obesity-related renal disease and DN but there is also growing insight that they contribute to the disease process. Recent human and experimental studies suggest that disturbed FFA metabolism plays a critical role in disordered lipid metabolism. As podocytes are highly susceptible to the saturated palmitic acid, but protected by MUFAs as well as increased expression of SCDs, the upregulation of SCD1 in podocytes of diabetic kidneys likely is part of a protective mechanism against SFAs and their toxic metabolites. The toxicity of SFAs in podocytes is partially explained by induction of ER stress and insulin resistance. Several mechanisms can explain the protective effect of MUFAs in podocytes including attenuation of the palmitic acid-induced CHOP upregulation and stimulation of fatty acid β-oxidation. The potentially crucial importance of β-oxidation is supported by genome-wide association studies in type 2 diabetic patients, which found that a single-nucleotide polymorphism in ACC2 favoring impairment of β-oxidation is associated with proteinuria. The observation that the protective effect of MUFAs is associated with a shift of palmitic acid from DAG to TG suggests that "limited" accumulation of TGs in podocytes does not have to be deleterious, but may prevent accumulation of even more toxic FFA metabolites. In conclusion, recent data not only suggest that podocytes are highly susceptible to FFAs, but they have also the potential to adapt to a certain extent to an altered lipid environment (**Figure 2**). In light of these findings, obese or type 2 diabetic patients with reduced ability for an adaptive response to a disturbed lipid metabolism are likely more prone to develop proteinuria and CKD (**Figure 2**). Therefore, novel strategies supporting podocytes in their adaptive responses may help to prevent and delay progression of CKD.

ACKNOWLEDGMENTS

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