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

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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

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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 released 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 post-prandial 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 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 acid 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

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<thead>
<tr>
<th>Table 1</th>
<th>Beneficial effects of MUFAs on podocytes treated with palmitic acid</th>
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<tr>
<td>Action of MUFAs (e.g., oleic acid) in podocytes treated with palmitic acid</td>
<td>Reference</td>
</tr>
<tr>
<td>Cell viability</td>
<td>Prevention of podocyte death</td>
</tr>
<tr>
<td>ER stress/UPR</td>
<td>Induction of the adaptive UPR (e.g., BiP)</td>
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<tr>
<td>Insulin resistance</td>
<td>Improved insulin sensitivity</td>
</tr>
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<td>Lipid metabolism</td>
<td>Increased fatty acid β-oxidation</td>
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<tr>
<td></td>
<td>Reduced accumulation of palmitic acid in DAG</td>
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<td></td>
<td>Preferential incorporation of palmitic acid into TG</td>
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<td></td>
<td>Increased DGAT gene expression</td>
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<td>Reduced accumulation of palmitic acid derived metabolites in the total lipid fraction</td>
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BIP but not CHOP (28). As BIP is known to protect from palmitic acid-induced proteolysis of CHOP (28) and unpublished results. Contrariwise, the proapoptotic transcription factor C/EBP homologous protein, 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 FFAs may protect against ER stress (61). It should be noted that the beneficial 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).

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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...
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 genetic overexpression of SCD1 is only associated with obesity-related renal disease and DN but there is still the potential to adapt to a certain extent to an altered lipid environment ([Figure 2](#)).

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.

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