

# Lipotoxicity in Diabetic Nephropathy: The Potential Role of Fatty Acid Oxidation

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Cellular toxicity mediated by lipids (lipotoxicity) has been implicated in the pathophysiology of metabolic syndrome and diabetes mellitus. Genetic analyses now implicate lipotoxicity in susceptibility to type 2 diabetes mellitus-associated nephropathy (T2DN), a pathway that had previously been unexplored. A genome-wide association study in Japanese patients identified a single nucleotide polymorphism in the acetyl-CoA carboxylase  $\beta$  (*ACACB*) gene associated with T2DN. Replication analyses suggest that this same polymorphism may be a diabetic nephropathy risk allele in other ethnic groups. The *ACACB* gene (also called *ACC2* or acetyl-CoA carboxylase 2) plays a critical role in intracellular fatty acid (FA) oxidation. This manuscript reviews the physiology of FA metabolism and adverse cellular effects that can result from dysregulation of this process. It is hypothesized that glomerular and tubular dysfunction can be induced by increases in intracellular FA concentrations, a process that may be enabled by genetic risk variants. This novel glucolipotoxicity hypothesis in T2DN warrants further investigation.

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Over the last decade, dysregulated fatty acid (FA) oxidation has been implicated as an effector pathway in the pathophysiology of metabolic syndrome, atherosclerosis, cardiomyopathy, and diabetes mellitus (1). An imbalance between circulating and cytosolic FA levels resulting in excessive intracellular accumulation of FAs and their derivatives (diacylglycerol, ceramides) underlies the spectrum of insulin-deficient states, including insulin resistance in metabolic syndrome, insulin resistance and relative insulin deficiency in type 2 diabetes (T2D), and absolute insulin deficiency in type 1 diabetes (T1D) (2).

Diabetic nephropathy (DN) is a serious complication that develops in a significant yet limited proportion of patients with T1D and T2D. Glycemic control and genetic predisposition have been explored as major pathogenic determinants. To determine how DN develops, the long-held theory that glomerular and tubular toxicity result from hyperglycemia (glucotoxicity) has been evaluated extensively at the molecular level for contributing factors, including generation of advanced glycation end products; activation of protein kinase C, TGF $\beta$ -Smad, and mitogen-activated protein kinase pathways; enhanced production of reactive oxygen species; increased extracellular matrix deposition, and increased tubulointerstitial fibrosis (3,4). Despite these investigations, the renal pathology in animal models of glucotoxicity does not reliably replicate what occurs

in human DN, and specific factors that cause, propagate, or predict DN remain elusive.

DN is believed to be a complex polygenic trait; that is, the clinical and histopathologic phenotype depends on alleles in multiple genes. On the basis of this hypothesis, scientists have taken full advantage of unbiased genetic studies as human genome-wide association studies have been used in large diabetic cohorts. Although several candidate genes have been detected, individual alleles account for only a small proportion of disease risk, risk alleles have been only partially replicated in other ethnic groups, and cellular gene expression or downstream function has not been explored.

However, Maeda *et al.* recently performed a large-scale genotype analysis of 754 Japanese patients with type 2 diabetes mellitus-associated nephropathy (T2DN) and 558 T2D controls lacking nephropathy. A polymorphism in a noncoding region of the acetyl-CoA carboxylase  $\beta$  gene (*ACACB*; also called *ACC2*) exhibited the strongest association with proteinuria (rs2268388, intron 18,  $P = 1.4 \times 10^{-6}$ , odds ratio [OR] = 1.61, 95% confidence interval [CI] 1.33 to 1.96, additive model). Replication followed in nine independent cohorts encompassing nearly 2000 T2DN patients and 2000 T2D non-nephropathy controls of various non-African ethnicities; this confirmed that the same landmark single nucleotide polymorphism (SNP) in *ACACB* (rs2268388) had similar strong associations with proteinuria, with comparable ORs and  $P$  values in additive models ( $P = 5.35 \times 10^{-8}$ , OR = 1.61, 95% CI: 1.35 to 1.91 by meta-analysis of all nine cohorts) (5). The landmark SNP was found with a frequency of approximately 25% in DN patients and 17% in controls, but its frequency was not significantly different in patients with ESRD in these populations. However, these find-

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ings were later extended to T2D-associated ESRD in Chinese and European Americans; these groups again revealed the strongest disease association with the rs2268388 SNP (OR = 2.39, 95% CI 1.20 to 4.75 in Chinese; and OR = 1.61, 95% CI 1.22 to 2.13 in European Americans, both recessive) (6). Of note, a statistically significant association was not detected in patients with T1D-associated nephropathy or in African Americans (Barry I. Freedman, personal communication).

The possibility that dysregulated FA metabolism could play an important role in the genesis and propagation of DN integrates with the similar role of lipids in atherogenesis, T2D, metabolic syndrome, and obesity—all clinical conditions associated with varying degrees of albuminuria (7). This new hypothesis sheds a different light on the link between albuminuria and cardiovascular events. The tenet that glomerular cell glucotoxicity leads to progressive albuminuria and a subsequent parallel graded risk of cardiovascular events (atherosclerosis) may in fact be the representation of a common pathophysiologic denominator: multisystem cellular FA toxicity. Moreover, clinical interventions aimed solely at intensive glucose control with insulin and oral hypoglycemic agents (Action to Control Cardiovascular Risk in Diabetes trial and Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation trial) did not significantly reduce the negative cardiovascular outcomes (8,9). Conversely, multifactorial interventions incorporating lipid-lowering agents significantly reduced the risk of cardiovascular complication, microvascular disease, and death (Steno-2 studies and the Fenofibrate Intervention and Event Lowering in Diabetes study) (10–13).

The suggestion that lipids may play a role in DN is not entirely novel, but it has not yet been explored to its fullest potential. Previous genetic studies implicated polymorphisms in two other genes that affect important components of lipid metabolism: the apoE and apoB genes (14,15). Numerous models of metabolic syndrome and diabetes (mice fed high-fat diets, leptin impaired db/db and ob/ob mice, streptozotocin-treated rats) display upregulated lipogenic genes and develop glomerular and tubular lipid deposits (16–19). Genetic or pharmacologic alteration of lipogenic genes (sterol regulatory element binding protein-1 [SREBP-1] and peroxisome proliferator-activated receptors [PPAR $\alpha$ , PPAR $\gamma$ ]) in diabetic animals mitigates diabetic kidney injury (20–23). In the kidneys of diabetic humans, intraglomerular lipid deposits were described first in 1936 by Kimmelstiel and Wilson and subsequently observed by other researchers (24,25). Patients with DN present an altered serum lipid profile characterized by elevated triacylglycerol (TAG; also called triglyceride)-rich lipoproteins beginning in the earliest stages of microalbuminuria; in contrast, subjects with diabetes and normoalbuminuria do not have elevated plasma TAG levels. Significant direct relationships between the stage and progression of DN with total cholesterol and TAG levels have been reported. Pharmacologic interventions with lipid-lowering medications (statins, fibrates) have been shown to improve progression of albuminuria and GFR decline in clinical intervention groups and to attenuate kidney inflammatory reactions in experimental intervention models (26,27).

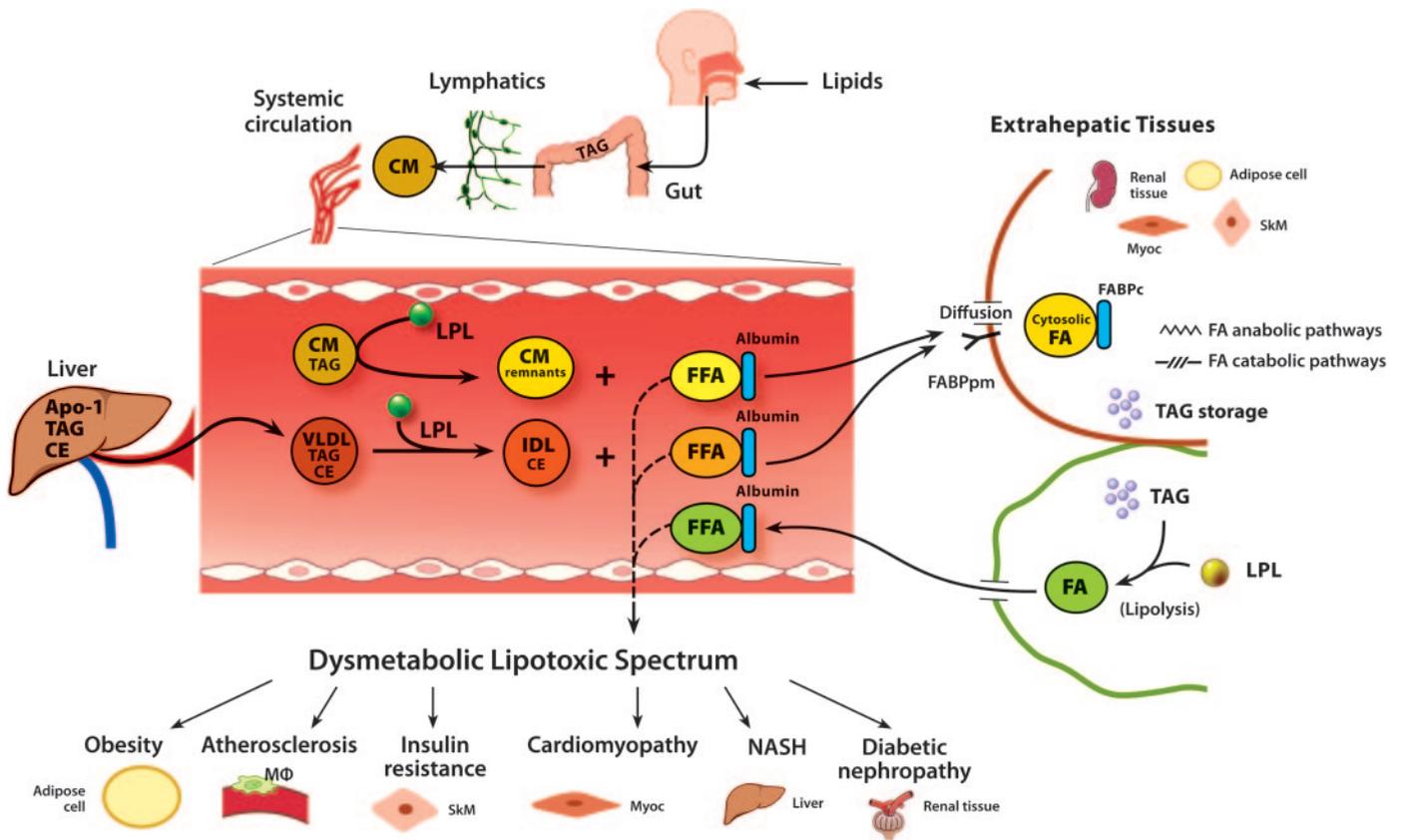
## FA Metabolism and the ACC Enzyme

Normal circulating free fatty acid (FFA) concentrations are under neurohormonal adrenergic control and range between 0.2 and 0.6 mM; high levels have been reported in metabolic syndrome, diabetes, obesity, hepatic steatosis, cardiomyopathy, myocardial ischemia, and atherosclerosis (28–30). Circulating FFAs arise from exogenous or endogenous sources (Figure 1), and intracellular homeostasis reflects a balance between the processes that generate and utilize FFAs (31) (Table 1 and Figure 2).

The ACC enzyme catalyzes intramitochondrial conversion of acetyl-CoA to malonyl-CoA and through the latter product is pivotal in controlling the rate of FA intramitochondrial  $\beta$ -oxidation (Figure 2). The ACC enzyme exists in two isoforms—ACACA (or ACC1) and ACACB (or ACC2)—that have differential tissue expression and functions. Lipogenic tissues (liver and adipose) express mainly isoform A, and ACACA-derived malonyl-CoA enters the pathway of long-chain FA biosynthesis, which can further be incorporated into TAGs and phospholipids. In contrast, isoform B is predominantly expressed in the heart and skeletal muscle, and ACACB-derived malonyl-CoA is a potent inhibitor of FA oxidation in these tissues through allosteric binding of carnitine palmitoyltransferase (CPT1) (Figure 2). ACC enzyme activity is controlled at the transcriptional level by several transcription factors (PPARs, SREBPs, and carbohydrate responsive element binding protein [ChREBP]) and at the posttranslational level by molecular modulators (AMP-activated protein kinase [AMPK] and stearoyl CoA desaturase through an indirect effect on AMPK) (Figure 2).

In the kidney, expression of the ACC isoforms, malonyl-CoA, and CPT1 has not been thoroughly evaluated. However, evidence suggests that in wild-type adult mice, ACACB mRNA is expressed in glomerular and tubular epithelial cells, albeit at lower levels than in adipose tissue, heart, and skeletal muscle. *In vitro* functional analysis on cultured human renal proximal tubular epithelial cells with plasmid transfection of DN-risk SNP rs2268388 demonstrated significant enhancer activity, with more than a 10-fold increased expression for the minor allele indexed to the promoter alone ( $P = 0.0005$ ) and a nearly 2-fold increased expression for the minor allele indexed to the major allele ( $P = 0.045$ ) (5). Glomerular ACACB expression was also noted to be increased in diabetic db/db mice compared with control mice, and adenovirus-induced ACACB overexpression in human renal proximal tubular epithelial cells resulted in marked upregulation (>20-fold) of proinflammatory genes through the p38-mitogen-activated protein kinase pathway (32).

Molecules known to regulate ACC function (PPAR $\alpha$ , PPAR $\gamma$ , AMPK, SREBP-1, and ChREBP) have been studied in the experimental diabetic kidney, albeit in a context not specifically inclusive of effects on FA oxidation and ACC regulation (33–35). For example, evidence for the role of AMPK on kidney function is developing. AMPK is predominantly expressed in podocytes under basal conditions. In the adiponectin-null mouse, AMPK is inactivated in podocytes and associated with altered morphology, foot process effacement, and proteinuria, and activation of AMPK has been shown to restore podocyte morphology *in vitro* and normalize albuminuria (36).

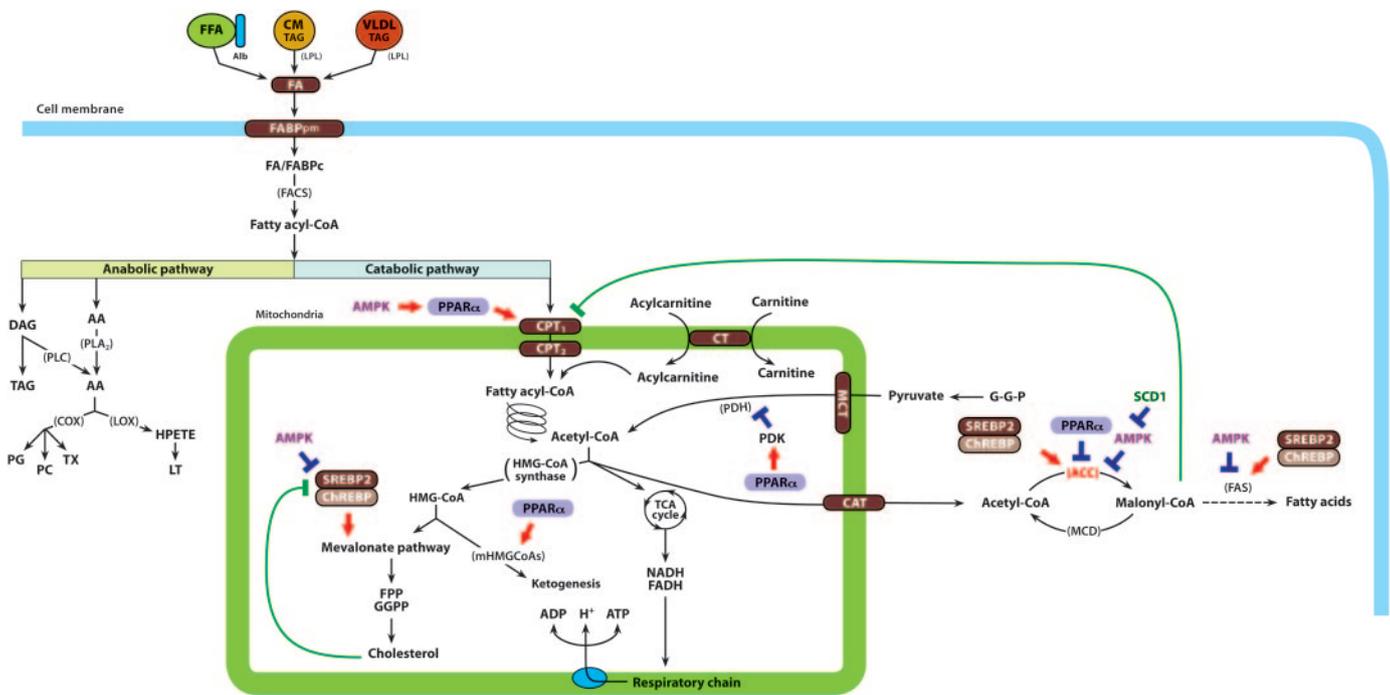


**Figure 1.** Circulating FFAs: origins and lipotoxic effects. Systemic FFAs are present in three forms: (1) FFAs released from the cells and bound to albumin, (2) exogenous FFAs complexed as TAGs in chylomicrons (CMs), and (3) endogenous FFAs complexed in TAGs in VLDL. Ingested TAGs are hydrolyzed to FFAs and monoacylglycerol by pancreatic enzymes, and these hydrolysis products are solubilized by bile acids or absorption by intestinal enterocytes. The absorbed FFAs and monoacylglycerol are re-esterified to TAGs, which are assembled into CMs that are secreted into the lymphatic system and ultimately drain into the systemic circulation via the thoracic duct. Within the liver, synthesized apo (*i.e.*, apoB and apoE), cholesterol esters (CEs), and TAGs are assembled into VLDL particles that are secreted into the plasma. Lipoprotein lipase (LPL), an enzyme present on the endothelial cell surface, hydrolyzes the TAG component of CMs and VLDL, releasing FFAs that bind albumin or are taken up by cells (*i.e.*, muscle and adipose tissue) and resulting in CM remnants and intermediate-density lipoprotein (IDL), respectively. Circulating FFAs gain entrance into tissues via diffusion or specialized plasma membrane fatty acid binding proteins (FABPpm). Once in the cytosol, FFAs bind to cellular fatty acid binding proteins (FABPc) and engage in anabolic/synthetic pathways (phospholipid, DAG, and ceramide synthesis), catabolic/oxidation pathways, or are stored as TAGs. Intracellular lipolysis releases FFAs from TAGs, which can efflux to the extracellular space via diffusion and bind to serum albumin. Elevated levels of circulating FFAs of any origin are associated with various tissue toxic effects (lipotoxicity), among which renal disease expands the spectrum of cardiometabolic disorders. *M $\phi$* , macrophage; *SkM*, skeletal muscle; *Myoc*, myocardium; NASH, nonalcoholic steatohepatitis.

**Table 1.** Summary of cellular FFA functions

Function	Mechanism
Cell membrane structural integrity	Lipid bilayer synthesis (PL and cholesterol synthesis) Lipid rafts and caveolae
Energy (ATP) production	Mitochondrial FA $\beta$ oxidation
Posttranslational protein modification	Prenylation (lipidation)
Intracellular signaling and transcriptional regulation	FA pathway intermediates or FA ligation of nuclear receptors
Intracellular lipid storage	MAG, DAG, and TAG synthesis

PL, phospholipid; MAG, monoacylglycerol; DAG, diacylglycerol.



**Figure 2.** FA cellular pathways and regulatory control. FFAs are imported into the cellular compartment via diffusion or FABPpm. Cytosolic FFAs bind to FABPc and are converted into fatty acyl-CoA by the action of fatty acyl-CoA synthase (FACS). Fatty acyl-CoA may engage in anabolic pathways and be converted into DAG or PLs with roles in the maintenance of cell membrane fluidity and proper function. FFAs may be esterified to DAG, forming TAGs for cytosolic neutral lipid storage. AA release results from lipolysis of DAG by phospholipase C (PLC) and of PLs by phospholipase A2 (PLA2); subsequent AA stereospecific oxygenation yields prostaglandins (PGs), prostacyclin (PC), or thromboxanes (TX) via cyclooxygenase (COX) or 5-HPETE (5-hydroperoxyeicosatetraenoic acid) and leukotrienes (LT) via lipoxygenase (LOX). Cytosolic fatty acyl-CoA is transported into the mitochondria via the carnitine shuttle system. CPT1 is located on the outer mitochondrial membrane, whereas CPT2 is located on the inner mitochondrial membrane. In parallel, carnitine translocase (CT) recirculates carnitine between the mitochondrial and cytoplasmic compartments. Acetyl-CoA, a product of FA oxidation, can also be formed by glucose oxidation. Glucose enters the cell via GLUT transporters, is converted into glucose-6-phosphate, and then into pyruvate, the latter entering the mitochondria via monocarboxylate transporters (MCTs). Pyruvate is then converted into acetyl-CoA via pyruvate dehydrogenase complex (PDH). This represents the point of interaction between FA and glucose metabolism, or the Randle cycle (glucose-FA cycle). Acetyl-CoA can meet one of four fates, not mutually exclusive: (1) engage in the citric acid cycle (TCA cycle), producing NADH and FADH<sub>2</sub> that act as electron donors in the electron transport chain (or respiratory chain) to produce ATP; (2) convert to hydroxy-methyl-glutaryl CoA (HMG-CoA) by HMG-CoA synthase and enter the mevalonate pathway (with intermediates farnesyl-pyrophosphate [FPP], geranyl-geranyl-pyrophosphate [GGPP], and end product cholesterol); (3) enter the ketone synthesis pathway under action of mitochondrial hydroxy-methyl-glutaryl CoA (mHMG-CoAS); or (4) export into the cytosol (through transient conversion into acetyl-carnitine by the carnitine acyltransferase enzyme system [CAT]), where it is then converted to malonyl-CoA in a reaction catalyzed by ACC. Cytosolic homeostasis of malonyl-CoA is maintained by the degrading enzyme malonyl-CoA decarboxylase (MCD). Lipid and glucose metabolism intermediates and enzymes are under the modulatory control of other enzymes (AMPK, stearoyl CoA desaturase [SCD1], and pyruvate dehydrogenase kinase [PDK]), transcription factors (PPAR $\alpha$ ; SREBP, ChREBP), or their own feedback control.

## Potential Mechanisms of Dysregulated FA Oxidation-Induced DN

Adipocytes have the unique ability to store excess FFAs in lipid droplets, a plasticity not seen in other cells. In nonadipose tissues, excess cytosolic FFAs lead to cell dysfunction and death by promoting endoplasmic reticulum stress and excess production of reactive oxygen species, processes collectively designated as “lipotoxicity.” This mechanism is the foundation of a growing spectrum of diseases currently known to encompass obesity, T2D, cardiomyopathy, atherosclerosis, and nonalcoholic steatohepatitis (Figure 1). On the basis of the evidence

described above, it is conceivable that analogous FA toxicity extends to T2D-associated renal injury in genetically predisposed individuals, implicating T2DN in the spectrum of lipotoxic diseases (Figures 1 and 3).

The mechanism of lipotoxic renal injury could be divided into generic cellular stress (akin to responses observed in other tissues) and renal-cell-specific stress (Figure 3). The kidneys, similar to the heart, oxidize FFAs as the preferred energy source, with ketoacid oxidation providing for the remaining energy need (37). Experimentally, downregulation of FA oxidation leads to insufficient ATP generation in the kidney and excessive

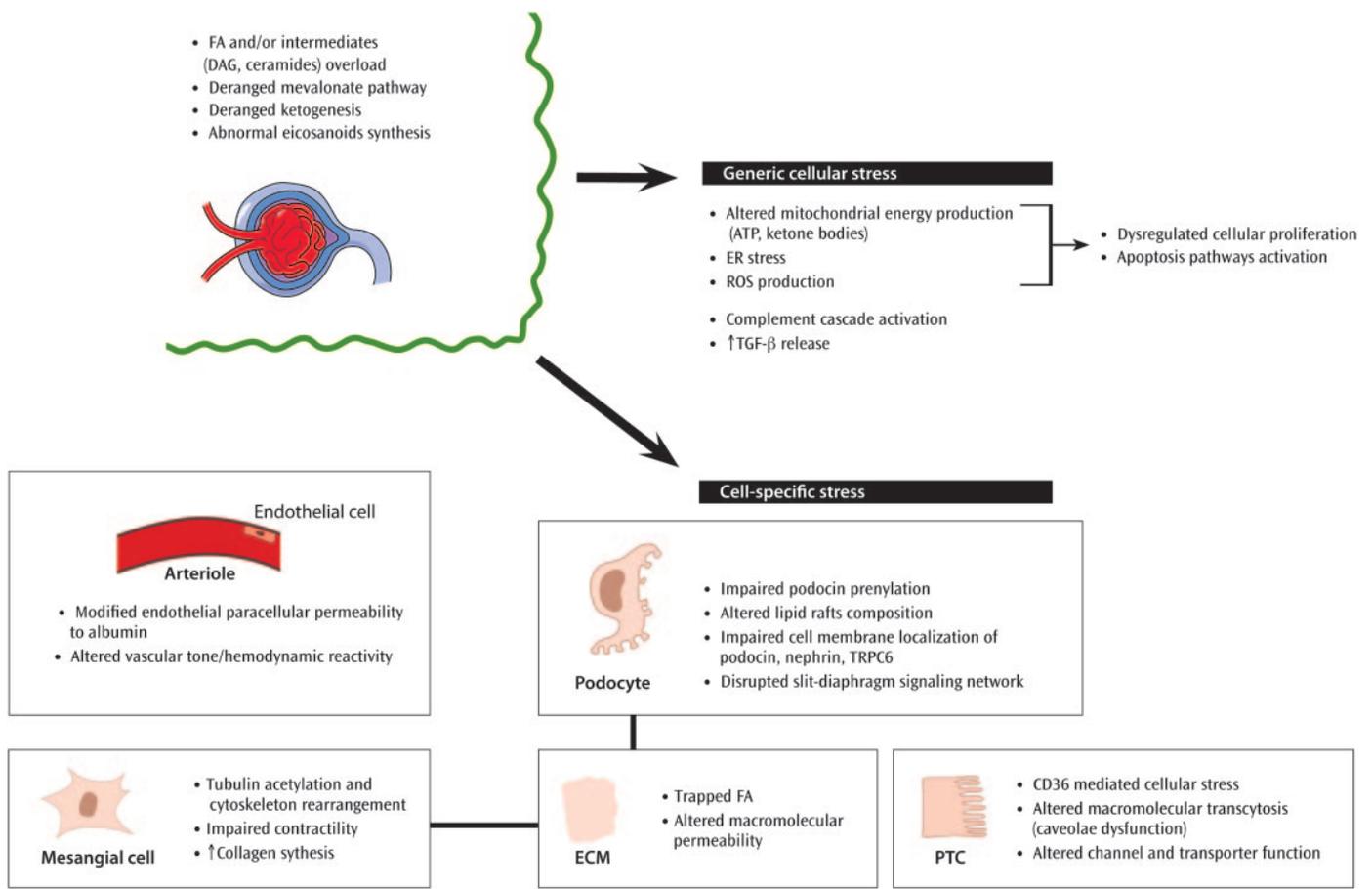


Figure 3. Mechanism of lipid-induced cellular toxicity. FA overload or products derived from misdirected intracellular FA pathways (e.g., enhanced eicosanoids, DAG, or ceramide synthesis) may lead to cellular dysfunction via generic stress pathways or cell-specific pathways. ER, endoplasmic reticulum; ROS, reactive oxygen species; ECM, extracellular matrix.

albumin excretion (38). In T2D characterized by insulin resistance, proximal tubular cells (PTCs) reabsorb glucose from glomerular filtrate via the insulin-independent sodium-glucose cotransporter; this glucose cellular import is not afforded by glomerular cells harboring insulin-dependent glucose transporters. It can be postulated that tubular cells may have additional options for short-term energy generation by substituting fuels, an untenable mechanism in glomerular cells, which offers a potential mechanistic explanation for earlier development of glomerular functional and histologic changes seen in DN versus delayed tubulointerstitial changes.

A lipotoxic-podocyte-centric view can also be hypothesized at several dimensions. First, studies using immunoelectron microscopy revealed that podocin and nephrin must be spatially inserted within the cholesterol-rich segments (lipid rafts) of the podocyte slit diaphragm to maintain the proper podocin-nephrin and podocin-transient receptor potential cation channel 6 [TRPC-6] interactions and podocyte functions (39–43). Second, posttranslational podocin modification via palmitoylation (covalent attachment of FA) enables the accurate routing to lipid rafts (44). Therefore, dysregulated FA oxidation can affect podocin palmitoylation and thus proper membrane insertion; FA overload can cause altered lipid composition of the lipid rafts and therefore interfere with

network signaling of the podocin-nephrin-TRPC-6 actin cytoskeleton, triggering a cascade of well described downstream pathologic effects. Similar derangement can be envisioned in mesangial cells, which contain caveolae (lipid rafts in the form of plasma membrane invaginations).

AA pathway products are known to control renal microvascular tone, and evaluation of their fractions in the context of altered FA metabolism merits investigation to improve our understanding of the glomerular hyperfiltration that is often present in incipient DN stages.

At the tubular level, filtered albumin has attached to FFAs; evidence suggests that it is the FA component in albuminuric states that exerts proinflammatory and profibrotic effects because delipidated albumin does not induce these effects (45). CD36, proposed to be a PTC receptor for FFA bound to albumin, was found to be overexpressed in human DN; *in vitro*, this receptor mediated PTC apoptosis and proinflammatory and profibrotic responses (46,47). In this context, it is conceivable that increased CD36 expression may represent an adaptive response to high levels of circulating FFAs, which in turn might be caused by improper FA tissue oxidation and mobilization from adipose tissue.

Beyond the essential role that intracellular FAs appear to

play in the pathophysiology of DN, lipid accumulation in the kidney has been observed in other models of renal disease, including the remnant kidney model. In a recent study by Kim and colleagues, experimental 5/6 nephrectomy lead to upregulation of ACC with marked lipid accumulation in the remnant kidney along with other important changes in the molecular architecture of cellular lipid homeostasis, including intense upregulation of the proximal tubule megalin-cubulin complex, which facilitates uptake of protein-bound lipids; marked upregulation of scavenger receptor class A and class E lectin-like oxidized LDL receptor 1, which mediate uptake of oxidized lipids and lipoproteins; downregulation of hydroxy-methylglutaryl CoA reductase; downregulation of SREBP-1 and PPAR $\alpha$ ; and upregulation of ChREBP (48). Megalin has been identified as a key molecule in the pathogenesis of tubulointerstitial injury, providing a molecular link between PTC lipid overload and renal failure (49).

In conclusion, lipotoxicity undergirds numerous pathophysiologic states and is caused by intracellular accumulation of saturated FAs and/or FA downstream metabolic pathway derivatives. A single variant of the enzyme controlling FFA oxidation (the *ACACB* gene) has recently been shown to be robustly associated with T2DN in numerous cohorts of individuals from different genetic backgrounds (5,6). The *ACACB* minor allele likely mediates this risk through the canonical toxic cytosolic FFA accumulation as a consequence of enhanced *ACACB*-mediated inhibition of CPT1 and reduced FA oxidation. Perhaps a dual glucolipotoxic metabolic insult (genetic risk inheritance of altered FA oxidation coupled with a glucotoxic environment) orchestrates the multicellular dysfunction seen in DN. This genetic association will undoubtedly catalyze several studies attempting to delineate ACC isoform expression in the kidney, genotype-based *ACACB* control of FA oxidation in kidney cells under normal and hyperglycemic conditions, and its role in DN pathophysiology. Targeting key molecules involved in lipid influx and FA oxidation with the goal of restoring cellular FA concentration and FA-derived pathways is a putative intervention that could benefit patients with DN if this fundamental hypothesis proves to be correct in well designed basic and translational studies.

## Disclosures

None.

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