

# Nutrient Sensing, Autophagy, and Diabetic Nephropathy

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**T**he prevalence of diabetic nephropathy, a serious complication of diabetes, has been increasing worldwide. Therefore, there is an urgent need to identify a new therapeutic target to prevent diabetic nephropathy. “Nutrient-sensing” pathways are generally well conserved among eukaryotes. Accumulating evidence indicates that alteration of nutrient-sensing pathways and subsequent impairment of cell function in insulin-sensitive organs of mammals are involved in the pathogenesis of type 2 diabetes. According to recent reports, nutrient-sensing in the kidney also seems to be altered under diabetic conditions. In this review, we discuss the possibility that nutrient-sensing pathways can be a therapeutic target for diabetic nephropathy and suggest future directions for research.

## NUTRIENT SENSING, AUTOPHAGY, AND DIABETIC NEPHROPATHY

Each cell has the ability to recognize and specifically respond to nutrient fuel substrates, such as glucose, lipids, and amino acids, to ensure their efficient use. These nutrient-sensing pathways appear critical for cellular homeostasis, for coping with starvation, and making the most of nutrient abundance. These pathways also represent important regulators of cell growth and proliferation, motility, mitochondrial function, autophagy, and survival (1–4).

Nutrient sensing is highly conserved across eukaryotic species. These pervasive regulatory pathways use posttranslational modifications of target proteins to link substrate availability to cellular homeostasis and stress responses (1–4). The best known of these pathways include the mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), and the sirtuins (SIRT). Under low-energy conditions, AMPK and SIRT are activated by increases in intracellular AMP and NAD<sup>+</sup> levels, respectively (1,2). In excessive nutrient conditions, mTOR is activated (3,4).

Each of these nutrient-sensing pathways has been implicated in the pathogenesis of obesity and diabetes, including actions on  $\beta$ -cell, adipocyte, hepatic and skeletal muscle metabolism, and the central regulation of nutrition (1–4). However, the same pathways may also be directly relevant to the development and progression of diabetes complications (Fig. 1). In particular, the cells of the kidney

appear especially vulnerable to the effects of over-nutrition, conveyed via the aforementioned energy-sensing pathways (5–7).

Autophagy, a lysosomal degradation pathway, plays a crucial role in removing protein aggregates as well as damaged organelles to maintain intracellular homeostasis during various stress conditions (8,9) that are involved in the pathogenesis of diabetic nephropathy. Interestingly, autophagy is regulated by the above-mentioned nutrient-sensing pathways (9–11). Thus, alteration of these nutrient-sensing pathways under diabetic conditions may impair the autophagic response stimulated by intracellular stress, which may lead to exacerbation of organelle dysfunction and subsequently result in diabetic nephropathy.

These findings led us to hypothesize that these signaling pathways are involved in pathogenesis of diabetic nephropathy and may be a potential therapeutic target for the prevention of diabetic nephropathy. Here, we provide a review and perspective regarding the potential actions of nutrient-sensing pathways and autophagy in diabetic nephropathy.

## mTOR IN DIABETIC NEPHROPATHY

Of the many nutrient-sensing pathways, mTOR has received the most attention for its potential actions in diabetic nephropathy. mTOR (also known as FK506 binding protein 12-rapamycin associated protein 1 or FRAP1) is a serine/threonine protein kinase whose activity is associated with cellular nutrient levels and redox status (12,13). An excess of nutrient components increases the activity of mTOR, either by direct interactions (i.e., in response to glucose or amino acids) or indirectly via metabolic signaling pathways, including insulin and other growth factors (4,12,13).

mTOR provides the enzymatic activity for two distinct protein complexes. The best characterized is mTOR complex 1 (mTORC1), which is composed of mTOR, regulatory-associated protein of mTOR (raptor), mammalian LST8/G-protein  $\beta$ -subunit-like protein (mLST8/G $\beta$ L), PRAS40, and DEP domain protein that interacts with mTOR (DEPTOR) (4,12). mTORC1 receives nutrient input via small GTPases, such as Rag and Rheb (14,15). An increase in amino acid levels and growth factors activate mTORC1 via Rag and Rheb activation, respectively. Activation of mTORC1 results in phosphorylation of ribosomal S6 kinase (S6K) and eukaryotic translation-initiation factor 4E-binding protein on several sites (4,12). This stimulates ribosome biogenesis and protein synthesis, subsequently leading to cell growth. mTORC1 activity is inhibited by the bacterial toxin rapamycin (marketed as sirolimus). Indeed, the anticell growth effect of sirolimus is thought to be largely mediated by its ability to prevent translation initiation.

Diabetic nephropathy is characterized by dysregulated cell growth and cell-cycle participation, with uncontrolled hypertrophy, exaggerated proliferative responses, and increased apoptosis in mesangial and proximal tubular cells (16). In the diabetic kidney, there is significant early activation of mTOR-dependent pathways in both animal models and humans with diabetes (17–24). Moreover, podocyte-specific

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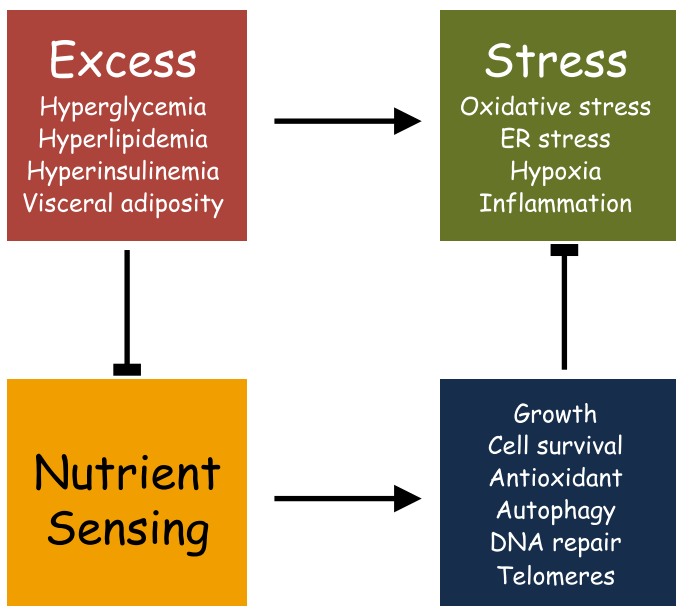


FIG. 1. The potential role of nutrient-sensing pathways in the pathogenesis of diabetes complications.

activation mTORC1 recapitulates many features of diabetic nephropathy, including mesangial expansion and proteinuria (25). The mTOR inhibitor rapamycin has also been extensively studied in experimental diabetic nephropathy. Beneficial actions on tubular and glomerular hypertrophy, mesangial expansion, glomerular basement-membrane thickening, tubular epithelial-to-mesenchymal transition, macrophage recruitment, and reduction in proteinuria have been reported (17–24) (Table 1). To what extent these findings reflect its direct renal actions, or indirect effects to the systemic immune system or glucose-lipid metabolism, continues to be debated. However, recent

studies using podocyte-specific repression of raptor, a component of mTORC1, to attenuate mTOR signaling have demonstrated benefits with respect to proteinuria, glomerulosclerosis, and mesangial matrix expansion in murine models of diabetes (25,26).

Other studies have reported an increase in proteinuria and glomerulosclerosis following treatment with the mTOR inhibitor rapamycin in some patients and selected animal models (27,28). Certainly, mTORC1 activity modulates the expression of podocyte slit diaphragm proteins and cytoskeleton structure in vitro (29). In addition, outside diabetes, podocyte-specific deletion of mTORC1 signaling also results in progressive glomerulosclerosis (25,26), with a phenotype similar to that observed in podocyte-specific insulin receptor-deficient mice (it should be remembered that insulin stimulates mTOR signaling) (30). Taken together, these data suggest that achieving a level of mTOR signaling consistent with energy and cellular stress may be the best way to achieve renal health, and that functionally too much or little mTOR may have its own physiological consequences.

In addition, mTOR is the catalytic unit for a second protein complex, the mTOR complex 2 (mTORC2), which also contains the rapamycin-insensitive companion of mTOR (riCTOR), mLST8/GβL, mammalian stress-activated protein kinase interacting protein 1 (mSIN1), and Protor-1 or Protor-2 (31). Although, mTORC2 is also activated by growth factors, unlike the mTORC1, its actions are rapamycin insensitive, at least in the short term (31). Consequently, the overall effects of mTOR in the diabetic kidney cannot simply be inferred from studies with rapamycin. The exact role and regulatory mechanisms of mTORC2 are less well understood, and few studies have investigated the role of mTORC2 in the kidney. Unlike mTORC1 knockout, mTORC2 knockout, specifically in the podocytes of non-diabetic mice, shows no phenotype, which would suggest that the mTORC2 function in podocytes is not essential (26). However, mTORC2 in podocytes plays a protective role against stress-induced damage to the filtration barrier (26). Consistent with this finding, the phenotypes in double

TABLE 1  
Studies demonstrating the potential renal actions of mTORC1-dependent signaling in experimental diabetic nephropathy

Experimental type	Renal outcome/phenotype	Mechanism	Reference
S6 kinase 1 <sup>-/-</sup> mice	Renal hypertrophy↓	Inhibition of p70S6 kinase	Chen et al. (17)
Rapamycin ( <i>db/db</i> mice)	Renal and glomerular hypertrophy↓	eEF2 kinase phosphorylation and laminin β1 expression	Sataranatarajan et al. (22)
Rapamycin (STZ-diabetic mice)	Renal hypertrophy↓	Inhibition of p70S6 kinase	Sakaguchi et al. (21)
Sirolimus (STZ-diabetic rats)	Glomerular hypertrophy↓, podocyte loss↓	Decreases of TGF-β and VEGF expression	Wittmann et al. (23)
Rapamycin ( <i>db/db</i> mice)	Albuminuria↓, glomerular lesion↓	Inhibition of p70S6 kinase	Mori et al. (19)
Rapamycin (STZ-diabetic rats)	Albuminuria↓, glomerular lesion↓, inflammation↓	Decreases of TGF-β, VEGF and MCP-1 expression	Yang et al. (24)
Rapamycin (STZ-diabetic rats)	Albuminuria↓, glomerular lesion↓	Decreases of TGF-β, CTGF and α-SMA expression	Lloberas et al. (18)
Gas6 <sup>-/-</sup> mice	Glomerular hypertrophy↓, mesangial expansion↓	Inhibition of Akt and p70S6 kinase	Nagai et al. (20)
Podocyte-specific TSC1 knockout mice	Albuminuria↑, glomerular lesion↑	Activation of mTORC1	Inoki et al. (25)
Podocyte-specific raptor knockout mice	Albuminuria↓, glomerular lesion↓	Inactivation of mTORC1	Gödel et al. (26)

CTGF, connective tissue growth factor; MCP-1, monocyte chemo-attractant protein-1; PARP, poly(ADP-ribose) polymerases; PTECs, proximal tubular epithelial cells; α-SMA, α-smooth muscle actin; STZ, streptozotocin; TGF-β, transforming growth factor β; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor.

knockout of mTORCs in podocyte are more severe than in single mTORC1 knockout; this supports the notion that mTORC2 exerts its protective role against podocyte injury induced by mTORC1 depletion (26). mTORC2 also phosphorylates serum- and glucocorticoid-induced protein kinase 1, the serine/threonine protein kinase Akt/protein kinase B, and protein kinase C (PKC) (31), all of which are known to be crucial causal factors for the development and progression of diabetic nephropathy; this suggests that mTORC2 may be involved in the pathogenesis of diabetic nephropathy. However, further investigations need to be conducted to clarify this issue.

In the diabetic kidney, the pathogenic role of mTOR in the proximal tubules is still poorly understood. Cellular hypertrophy and apoptosis in the proximal tubules are the main characteristics of diabetic nephropathy (21,32). High glucose-induced mTOR activation is associated with both phenotypes in the proximal tubular cells (21,32). mTOR could be involved in the pathogenesis of tubular lesions in diabetic nephropathy, although further studies using proximal tubule-specific mTOR knockout mice are necessary to confirm this.

### SIRT1 IN DIABETIC KIDNEY DISEASE

Sirtuins (silent information regulator 2 [Sir2]) are protein deacetylases that respond to changing cellular  $\text{NAD}^+$  levels associated with metabolic and redox stresses by deacetylating proteins that contribute to cellular regulation, adaptation, and survival (1). Sirtuins are best known for their acknowledged link with longevity associated with calorie restriction as well as being the putative target of the plant-derived polyphenol resveratrol (1). There are seven mammalian sirtuins (SIRT1 to SIRT7), which share a conserved  $\text{NAD}^+$  binding and catalytic core domain, but which differ in acylprotein substrate specificity, binding partners, and intracellular localization (1). The most studied mammalian sirtuin is SIRT1, which has been broadly implicated in a range of metabolic processes, including lipid and glucose metabolism and weight regulation/adiposity (1). Under conditions of calorie restriction, increased intracellular  $\text{NAD}^+$  levels promote the activity of SIRT1, leading to changes in energy metabolism and the stimulation of stress-resistance pathways, including antioxidant release, DNA repair, telomere maintenance, and autophagy (1,11). The overexpression of SIRT1 in transgenic mice results in lower body weight, greater metabolic activity, and reduced glucose and lipid levels—phenotypic features similar to those observed in calorie-restricted mice (33). By contrast, high-fat diet-induced insulin resistance and hyperglycemia are all associated with decreased expression and activity of SIRT1 (1).

Although most research emphasis has been placed on the actions of SIRT1 in cancer, metabolism, and aging, the dysregulation of growth and stress responsiveness associated with diabetic kidney disease are also potentially linked to SIRT1 expression. In the kidney, SIRT1 is preferentially expressed in the inner medulla and renal interstitium, where it is thought to protect against oxidative stress, partly through induction of cyclooxygenase-2 (34). SIRT1 deficiency accentuates renal fibrosis following unilateral ureteral obstruction (UUO), whereas treatment with SIRT1 activators decreases renal apoptosis and fibrosis after UUO injury (34). Consistent with these “antioxidant” actions, overexpression of SIRT1 in proximal tubular epithelial cells increases the expression of the intracellular

antioxidant catalase, which confers protection against cisplatin-induced injury (35). Antiapoptotic effects of SIRT1 overexpression have also been reported in cultured mesangial cells (36,37).

Like the UUO model, diabetic nephropathy is characterized by progressive and cumulative atrophy and apoptosis of tubular epithelial cells, which precedes manifestations including tubular dilatation, peritubular fibrosis, and subsequent nephron dropout. In the diabetic kidney, up to 51% of glomeruli may be attached to atrophic tubules, and up to 17% of glomeruli may be “atubular” (38). Although a number of metabolic and hemodynamic changes associated with diabetes can modulate the expression of apoptosis-regulatory genes, among the most important effectors appears to be oxidative stress, since tubular apoptosis in diabetes can be partly prevented by antioxidants. Given the known tubuloprotective effects of SIRT1 in other models, recent researchers have also explored its potential actions in the diabetic kidney.

In experimental models of both type 1 and type 2 diabetes, the expression and activity of SIRT1 have been reported to be reduced in the kidney (39,40). Nonetheless, studies using SIRT1 activators in models of both type 1 and type 2 diabetes have reported renoprotective benefits (41,42). For example, we have recently shown that resveratrol treatment in *db/db* mice, a model of type 2 diabetes, results in improved renal functional and histological abnormalities, such as albuminuria, mesangial expansion, glomerular and interstitial fibronectin accumulation, and interstitial macrophage infiltration (42). However, in this study, resveratrol also reduced intracellular reactive oxygen species in SIRT1-knocked-down cells, suggesting that its actions to exert antioxidative effects, at least in proximal cells, were independent of the SIRT1 signaling (42). Indeed, recent studies have suggested that resveratrol and the other small molecules (SRT1720, SRT2183, SRT1460) may not be direct activators of SIRT1, but mediate their physiological effects via off-target activities (43). Consequently, the true role of SRT in the diabetic kidney or potential benefits from increasing SIRT1 activity remains to be established. Nonetheless, overexpression of SIRT1 in transgenic mice is able to reduce circulating levels of proinflammatory cytokines, adipokines, and other prooxidant molecules associated with chronic exposure to a high-fat diet (44). Insofar that these mediators also contribute to renal damage in type 2 diabetes, it is likely that at the very least the development of legitimate SIRT1 agonist may prove beneficial in the diabetic kidney, albeit indirectly.

There is also emerging evidence that SIRT1 participates in the regulation of sodium balance and blood pressure control. Diabetes is associated with an increase in fractional and absolute sodium reabsorption in the proximal tubule. This is partly determined by renal hypertrophy, which increases proximal salt reabsorption simply by mass action. Indeed, inhibitors of tubular growth reduce salt reabsorption and hyperfiltration in direct proportion to their effect on kidney size (45). However, an important additional stimulus for salt reabsorption may be excess energy levels, mediated via nutrient sensors such as SIRT1. That there is a fundamental link between active (energy intensive) reabsorption of salt and energy supplies is hardly surprising, given that the oxygen consumption/tissue weight by the kidney is exceeded only by that of the beating heart. Salt reabsorption is significantly down-regulated following calorie restriction, partly mediating its antihypertensive

effect. SIRT1 also represses the expression of apical sodium channel, ENaC (46). For the same reasons, it is possible to speculate that energy excess associated with diabetes and obesity may contribute to avid sodium retention, hyperfiltration, and hypertension.

#### AMPK IN DIABETIC KIDNEY DISEASE

AMPK is a ubiquitously expressed heterotrimeric kinase that plays a key role in cellular energy homeostasis (2). AMPK consists of an  $\alpha$ , a  $\beta$ , and a  $\gamma$  subunit and is activated by the upstream kinases such as calcium calmodulin-dependent protein kinase kinase (CaMKK) and serine/threonine kinase 11 (LKB1) via phosphorylation of threonine residue 172 (47). Activation of AMPK by CaMKK and LKB1 is dependent, respectively, on intracellular calcium and the AMP:ATP ratio (47). During energy-depleted conditions, intracellular concentrations of AMP rise while ATP levels fall, leading to increased activation of AMPK and phosphorylation of its multiple substrates to enhance catabolism and suppress anabolic energy consumption. In excess energy states, reduced AMPK activation stimulates protein synthesis, cell growth, and storage. AMPK activity is also independently regulated by circulating hormones and cytokines, including bradykinin and the adipokines leptin and adiponectin (2,47). AMPK is also induced by the antidiabetic agent metformin, thiazolidinediones, and the peroxisome proliferator-activated receptor  $\alpha$  agonist fenofibrate, which may partly contribute to these renoprotective actions in diabetic nephropathy (2,47–50).

The metabolic effects of AMPK activation have been extensively characterized and include effects on glucose and lipid metabolism, mitochondrial function, and exercise-induced glucose utilization. However, the actions of AMPK in the kidney are less well understood. AMPK is widely expressed in all renal cell types, including podocytes, mesangial cells, glomerular endothelial cells, and tubular cells, especially the mitochondrial rich cells of the proximal tubule and thick ascending limb of the loop of Henle. The activity of AMPK in the kidney appears to be reduced in experimental models of diabetes (42).

One of the earliest structural changes in the diabetic kidney is hypertrophy and hyperplasia of the tubuli of the cortex and the outer medulla. Early studies have demonstrated that diabetic rats exhibit a 15% increase in whole kidney weight within 72 h of induction of diabetes with streptozotocin (51). Most of this increase may be accounted for by proliferation, hypertrophy, and elongation of proximal tubular cells. These changes appear to be driven by tubular glucose reabsorption because they are attenuated by phlorizin, an inhibitor of  $\text{Na}^+$ /glucose cotransport (52). However, the mechanism(s) by which increased tubular glucose flux is sensed and subsequently stimulates the increased production of growth factors and suppresses anti-proliferative mediators is a matter of ongoing research. One possible candidate for the role of “metabolic master-switch” is AMPK (5). Exposure of tubular cells to hyperglycemia *in vitro* results in reductions in AMPK phosphorylation and cellular hypertrophy that can be inhibited by metformin and AICAR, while expression of kinase-inactive AMPK augments glucose-induced protein synthesis (53). Similarly, in experimental models of type 1 diabetes, pharmacological activation of AMPK has been shown to attenuate renal hypertrophy (53). However, the renal actions of metformin, like those of resveratrol, are probably more complicated, with a range of effects that appear to be independent of

AMPK, since the effects are also observed in AMPK-deficient tubular cells and not reproduced by the AMPK agonist, AICAR (53).

The accumulation of intracellular glycogen granules (the so-called Armanni-Ebstein lesion) is perhaps the best-known tubular change associated with diabetes. Its pathological significance remains to be established, although it is widely considered to be injurious. Certainly, the Fanconi-Bickel syndrome, a disorder associated with tubular glycogen accumulation due to a mutation of the GLUT-2 transporter, is also associated with progressive diabetic-like changes in the glomeruli (54). Large glycogen accumulations are thought to alter the cellular architecture with loss of basal infoldings and apical microvilli and, ultimately, caspase-mediated apoptosis. One of the metabolic signals stimulating tubular glycogen synthesis in the diabetic kidney appears to be reduced tubular AMPK activity since AMPK directly phosphorylates and inactivates glycogen synthase (55). AMPK deficiency may also contribute to triglyceride accumulation through suppressed lipolysis and enhanced renal lipogenesis, in part mediated by reduced AMPK-mediated phosphorylation of the lipogenic enzyme acetyl-CoA carboxylase (2,47).

AMPK may also partly mediate the renal effects of the abundant circulating adipokine adiponectin. Serum adiponectin concentrations are increased in individuals with type 1 diabetes, especially those with chronic kidney disease (56). Moreover, elevated adiponectin levels are associated with increased risk of progressive nephropathy in adults with type 1 diabetes (56). It has been suggested that this elevation may be a response to renal injury, rather than its cause. Certainly, adiponectin is able to activate AMPK in podocytes, mesangial cells, and glomerular endothelial cells, which leads to cytoprotective responses, including reduced oxidative stress (57). Furthermore, adiponectin knockout mice exhibit increased albuminuria and effacement of podocyte foot processes (58). Similarly, administration of adiponectin to cultured podocytes leads to increased AMPK activity and reduced permeability to albumin. By contrast to type 1 diabetes, adiponectin levels are reduced in patients with type 2 diabetes, reflecting its link with visceral adiposity. Because diabetic nephropathy may therefore occur with both low and high adiponectin levels, any direct role for adiponectin in diabetic kidney disease appears less likely.

Finally, AMPK is also implicated in the regulation of tubular sodium reabsorption, the most energy-intensive process in the kidney (5). In order to ensure efficient tubular functions, it is thought that AMPK maintains tight coupling between energy metabolism and tubular transport. AMPK activation in low-energy states leads to the inactivation of key ion transporters, including the cystic fibrosis transmembrane conductance regulator, the epithelial sodium channel, the  $\text{Na}^+$ - $\text{K}^+$ - $2\text{Cl}^-$  cotransporter, and the vacuolar  $\text{H}^+$ -ATPase (5). By contrast, reduced AMPK activity, as is observed in diabetes, is associated with increased tubular sodium reabsorption. This may be important for the pathogenesis of hyperfiltration and, ultimately, salt-dependent hypertension.

#### AUTOPHAGY IN DIABETIC RENAL DISEASE

Autophagy is the catabolic process by which intracellular components are degraded through the lysosomal machinery (8,9). Autophagy plays a critical role in removing components that have become damaged or dysfunctional as a result

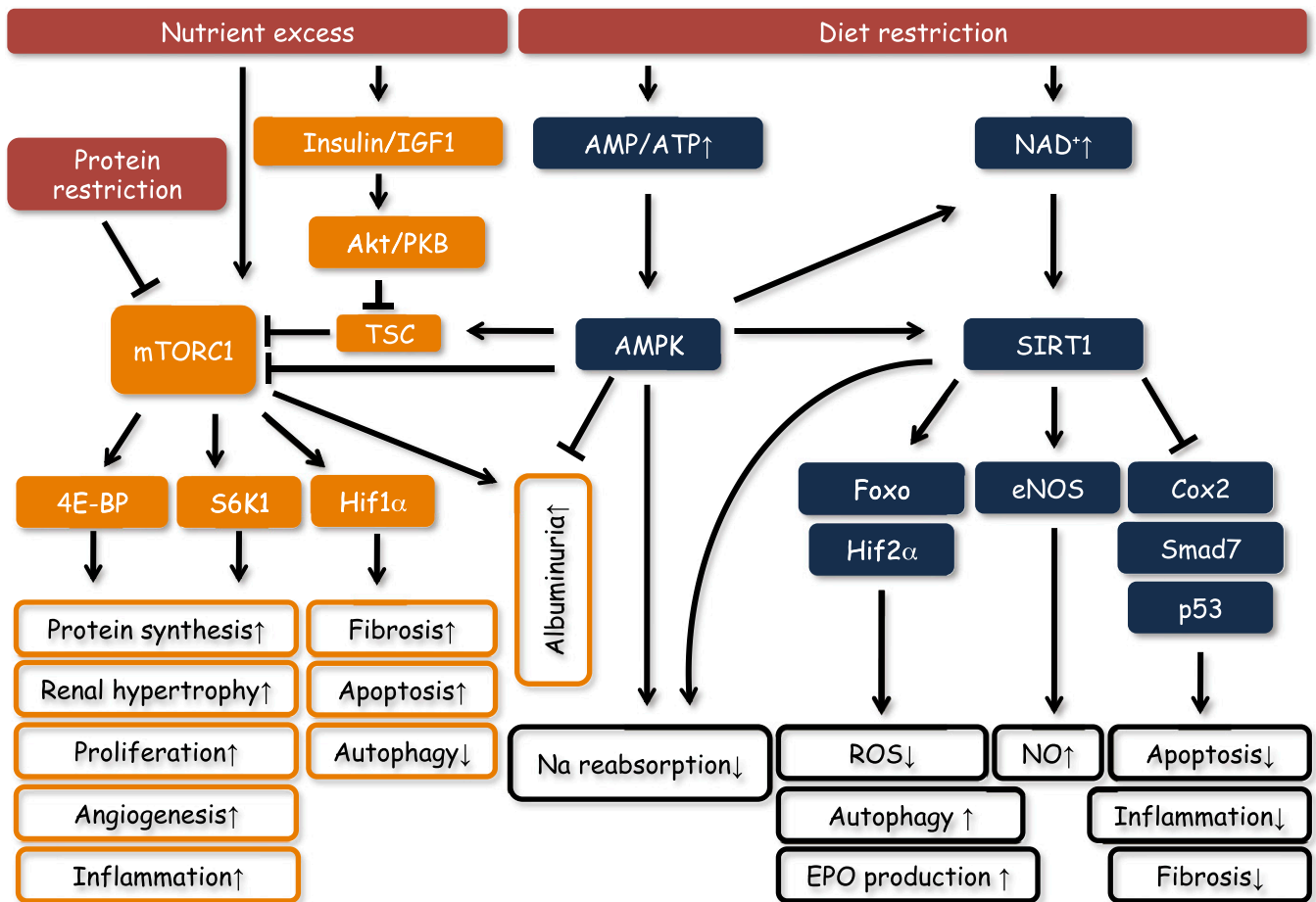


FIG. 2. The three nutrient-sensing pathways, mTOR, AMPK, and SIRT1, may be independently and coordinately involved in the pathogenesis of diabetic nephropathy. 4E-BP, 4E-binding protein; COX2, cyclooxygenase-2; eNOS, endothelial nitric oxide synthase; EPO, erythropoietin; FoxO, forkhead box class O; Hif2 $\alpha$ , hypoxia-inducible factor 2 $\alpha$ ; IGF1, insulin-like growth factor 1; NO, nitric oxide; PKB, protein kinase B; ROS, reactive oxygen species; TSC, tuberous sclerosis complex.

of exposure to cellular stressors (8,9). However, its actions in health and disease are far more complicated than simply replacement and renewal. Autophagy is tightly regulated to ensure an optimal balance between the synthesis and degradation, use, and storage and recycling of cellular products. Even healthy components may be jettisoned for the greater good of cellular homeostasis. One of the chief regulators of autophagy is cellular energy levels that determine which components are essential via the above-mentioned nutrient-sensing pathways (7,9–11). If energy levels become depleted, autophagy is activated to pare down energy expenditure and provide substrate resources for cells. For example, inhibition of mTOR or activation of AMPK and SIRT1, which occurs during nutrient starvation, activates nonselective autophagy (10,11).

When renal cells are exposed to conditions that lead to stress and injury, including hypoxic, genotoxic, oxidative, and endoplasmic reticulum (ER) stress, autophagy is upregulated to maintain cellular homeostasis via the degradation/turnover of cytoplasmic components, such as damaged proteins and organelles (3,9). For example, ER dysfunction results in the generation of misfolded proteins, which accumulate if their rate of production exceeds the readiness and capacity for autophagy. Equally, exposure of podocytes to angiotensin II increases autophagy through increased oxidative stress (59). In these settings, autophagy is an important

survival factor, without which stress-induced apoptosis would be more likely. Autophagy is especially important for maintenance of postmitotic cells, such as podocytes, which have only limited capacity for regeneration. Indeed, podocyte-specific deletion of autophagy-related 5 leads to proteinuria and glomerulopathy in aging mice (60).

In states of energy excess, autophagy is downregulated to make the most of nutrient abundance. Although in the short term this is beneficial, the failure of autophagy may ultimately contribute to the accumulation of cell damage and aging (7,60). Autophagy deficiencies may also contribute to increased chronic renal injury associated with hypoxia (7), ischemia-reperfusion (61), and cisplatin-induced damage (62), partly by promoting apoptosis. In the hypertrophic diabetic kidney, not only is the capacity for autophagy reduced, but because there is high exposure to cellular stresses, the need for cytoprotective autophagy is significantly increased. Diabetic kidney disease is associated with the intracellular accumulation of periodic acid-Schiff–positive lysosomal dense bodies, chiefly in the straight proximal tubules (S2 and S3). These lysosomal bodies, containing multilamellar inclusions, are classically considered to be accumulated phospholipid membranes indicative of membrane damage and lipid peroxidation. However, more than simply as markers of injury, some of these granules may be independently involved in the

generation of intracellular oxidative stress and cellular dysfunction (63).

Although autophagy has beneficial actions, it is also suggested to be one means of programmed cell death (known as type II cell death). Whether autophagy is causally related to cell death or represents a cell's ultimate effort for survival is unclear. Certainly when apoptosis is inhibited, autophagic cell death can be induced, possibly via oxidative stress. The role or existence of autophagic cell death in the diabetic kidney has not been established.

#### DIABETES: A RENAL PERSPECTIVE

From a kidney's point of view, diabetes is a bonanza state of nutrient excess. Even when glucose and lipids are under control, signals from the brain, fat, liver, and other metabolic sites remind the kidney of the surfeit and the need to "make hay while the sun shines." This occurs despite increased cellular stresses, which should promote autophagy, regeneration, and biogenesis and would normally serve to slow down metabolism. It is possible to speculate that these mixed messages, and ultimately their disconnection, contribute to renal dysfunction in diabetes (Fig. 1).

How can we protect the kidney against diabetes? Multi-target modulation of nutrient sensors may be one possible target to enhance autophagy and promote the tissue repair required to attenuate renal damage in diabetes (Fig. 2). As a potential proof of concept, intermittent fasting in a streptozotocin-induced and Wistar fatty rats is associated with increased renal SIRT1 activity and renoprotection (40,64). Although such nutritional interventions in clinical diabetes are impractical, multitarget interventions directed against nutrient-sensing pathways to simulate starvation represent an emerging new therapeutic approach for the prevention and management of diabetic nephropathy. However, these pathways play a critical role in cellular differentiation as well as anabolic processes that are required for the maintenance of functional organisms. Therefore, further investigations, especially ones focusing on adequate levels and tissue/cell-specificity of manipulation of these pathways, are needed.

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