Role of Bile Acid–Regulated Nuclear Receptor FXR and G Protein–Coupled Receptor TGR5 in Regulation of Cardiorenal Syndrome (Cardiovascular Disease and Chronic Kidney Disease)

Moshe Levi

It is well known that chronic kidney disease (CKD) is associated with increased risk of cardiovascular disease. In fact, studies have shown that cardiovascular event rates and all-cause mortality increase as a function of CKD as determined by decrease in estimated glomerular filtration rate and presence of microalbuminuria.1,2

In recent years, the term cardiorenal syndrome (CRS) has been proposed to define the relationship between cardiac disease and renal disease. Ronco et al have defined CRS as a pathophysiologic disorder of the heart and kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other.3–6 CRS Type 1: acute CRS is defined as sudden worsening of cardiac function (acute cardiogenic shock or acutely decompensated heart failure), leading to acute kidney injury. CRS Type 2: chronic CRS is defined as chronic abnormalities in cardiac function (chronic congestive heart failure) causing progressive and potentially permanent CKD. CRS Type 3: acute renovascular syndrome is defined as sudden worsening of kidney function (acute kidney injury or acute glomerulonephritis) causing acute cardiac disorder (heart failure, arrhythmia, or ischemia). CRS Type 4: chronic renovascular syndrome is defined as CKD contributing to decreased cardiac function, cardiac hypertrophy, and increased risk of adverse cardiovascular events. CRS Type 5: secondary CRS is defined as systemic condition (obesity, diabetes mellitus, sepsis) causing simultaneous cardiac and renal dysfunction.

In this review, I will be discussing mainly the type 5 CRS associated with obesity and diabetes mellitus and models of atherosclerosis and vascular calcification. In addition to discussing renal disease, I will also be discussing vascular disease because vascular dysfunction is a well-characterized complication of renal disease, and vascular dysfunction is also a leading cause of cardiac disease.

Role for Altered Renal Lipid Metabolism in the Pathogenesis of Kidney Disease in Obesity and Diabetes Mellitus

Our work early on determined an important role for altered renal lipid metabolism in the pathogenesis of kidney disease in obesity and diabetes mellitus. In a series of studies using rodent models of type I diabetes mellitus,7–9 high-fat diet–induced obesity and insulin resistance,10,11 type 2 diabetes mellitus,12,13 and aging,14,15 we determined that abnormal lipid metabolism mediated by increased expression and activity of the sterol regulatory element–binding proteins (SREBPs) and carbohydrate regulatory element–binding protein (ChREBP) and decreased activity of the peroxisome proliferator–activated receptor alpha (PPARα) and liver X receptor (LXR) played an important role in kidney disease (Figures 1 and 2).

In streptozotocin-induced diabetes mellitus in the rat, we found marked increases in SREBP-1 and fatty acid synthase expression, resulting in increased triglyceride accumulation. Treatment of diabetic rats with insulin prevented the increased renal expression of SREBP-1 and the accumulation of triglyceride. The role of hyperglycemia in the upregulation of SREBP-1 was confirmed in renal cells cultured in a high glucose media. High glucose induced increased expression of SREBP-1a and -1c mRNA, SREBP-1 protein, and fatty acid synthase, resulting in increased triglyceride content. To determine a direct role for SREBP in mediating the increase in renal lipids and glomerulosclerosis, we studied SREBP-1a transgenic mice with increased renal expression of SREBP-1. The increase in SREBP-1 was associated with increased expression of fatty acid synthase and acetyl CoA carboxylase, resulting in increased triglyceride content, increased expression of transforming growth factor-β1 and vascular endothelial growth factor, mesangial expansion, glomerulosclerosis, and proteinuria. Our study therefore indicated that renal SREBP-1 expression is increased in diabetes mellitus and that SREBP-1 plays an important role in the increased lipid synthesis, triglyceride accumulation, mesangial expansion, glomerulosclerosis, and proteinuria by increasing the expression of transforming growth factor-β and vascular endothelial growth factor.7

Additional studies were performed in Akita and OVE26 mice, which are 2 genetic models of type 1 diabetes mellitus. Diabetic nephropathy was characterized by mesangial expansion and loss of podocytes, resulting in glomerulosclerosis and proteinuria, and is associated with increased expression of profibrotic growth factors, proinflammatory cytokines, and increased oxidative stress. We have also found significant

From the Division of Renal Diseases and Hypertension, Department of Medicine, University of Colorado AMC, Aurora.
Correspondence to Moshe Levi, Division of Renal Diseases and Hypertension, University of Colorado AMC, 12700 E 19th Ave, Aurora, CO 80045.
E-mail Moshe.Levi@ucdenver.edu
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increases in renal triglyceride and cholesterol content. The increase in renal triglyceride and cholesterol content was associated with (1) increased expression of SREBP-1c and ChREBP, which collectively results in increased fatty acid synthesis, (2) decreased expression of peroxisome proliferator–activated receptor PPARα and PPARδ, which results in decreased fatty acid oxidation, and (3) decreased expression of farnesoid X receptor (FXR) and small heterodimer partner. The increase in cholesterol content was associated with (1) increased expression of SREBP-2 and 3-hydroxy-3-methylglutaryl-CoA reductase, which results in increased cholesterol synthesis and (2) decreased expression of LXR-α, LXR-β, and ATP-binding cassette transporter-1, which results in decreased cholesterol efflux. Our results had further indicated that in type 1 diabetes mellitus, there is altered renal lipid metabolism favoring net accumulation of triglycerides and cholesterol, which are driven by increases in SREBP-1, ChREBP, and SREBP-2 and decreases in FXR, LXR-α, and LXR-β, which may also play a role in the increased expression of profibrotic growth hormones, proinflammatory cytokines, and oxidative stress.9

In addition to diabetes mellitus, obesity and metabolic syndrome are associated with glomerulosclerosis and proteinuria. Using a model of diet-induced obesity and insulin resistance, C57BL/6J mice that were fed a high-fat, 60 kcal % saturated (lard) fat diet developed obesity, hyperglycemia, and hyperinsulinemia compared with those that were fed a low-fat, 10 kcal % fat diet. In contrast, A/J mice were resistant when fed the same diet. C57BL/6J mice with high-fat diet exhibited significantly higher levels of renal SREBP-1 and SREBP-2 expression than those mice with low-fat diet, whereas in A/J mice, there were no changes with the same treatment. The increases in SREBP-1 and SREBP-2 expression in C57BL/6J mice resulted in renal accumulation of triglyceride and cholesterol. There were also significant increases in the renal expression of plasminogen activator inhibitor-1, vascular endothelial growth factor, type IV collagen, and fibronectin, resulting in glomerulosclerosis and proteinuria. To determine a role for SREBPs per se in modulating renal lipid metabolism and glomerulosclerosis, we performed studies in SREBP-1c (−/−) mice. In contrast to control mice, in the SREBP-1c (−/−) mice with high-fat diet, the accumulation of triglyceride was prevented, as well as the increases in plasminogen activator inhibitor-1, vascular endothelial growth factor, type IV collagen, and fibronectin expression. Our results therefore indicated that diet-induced obesity causes increased renal lipid accumulation and glomerulosclerosis in C57BL/6J mice via an SREBP-1c-dependent pathway.10

db/db mice on the friend virus B (FVB) genetic background with loss-of-function mutation of the leptin receptor (FVB-Lepr(db) mice or FVB db/db) develop severe diabetic nephropathy, including glomerulosclerosis, tubulointerstitial fibrosis, increased expression of type IV collagen and fibronectin, and proteinuria, which is associated with increased renal mRNA abundance of transforming growth factor-β, plasminogen activator inhibitor-1, and vascular endothelial growth factor. Electron microscopy demonstrated increases in glomerular basement membrane thickness and foot process (podocyte) length. We found that there is a marked
increase in neutral lipid deposits in glomeruli and tubules by oil red O staining and biochemical analysis for cholesterol and triglycerides. We also detected a significant increase in the renal expression of adipocyte differentiation–related protein (adipophilin), a marker of cytoplasmic lipid droplets. We examined the expression of SREBP-1 and -2, transcriptional factors that play an important role in the regulation of fatty acid, triglyceride, and cholesterol synthesis. We found significant increases in SREBP-1 and -2 protein levels in nuclear extracts from the kidneys of FVB db/db mice, with increases in the mRNA abundance of acetyl-CoA carboxylase, fatty acid synthase, and 3-hydroxy-3-methylglutaryl-CoA reductase, which mediates the increase in renal triglyceride and cholesterol content. Our results indicate that in FVB db/db mice, renal triglyceride and cholesterol accumulation is mediated by increased activity of SREBP-1 and -2. Of note, when the above mice originally generated in Dr Streamson Chu’s laboratory at Columbia University were rederived at Jackson Laboratories, the resulting phenotype was different. This may have been because of different FVB strains, diets, and barrier (pathogen-free conditions, ie, microbiome). We therefore also performed studies with db/db mice obtained from Jackson Laboratories in the BKS genetic background. Our results essentially confirmed our findings with the FVB db/db mice.

Parallel studies in aging mice also indicated a significant role for altered lipid metabolism in modulating renal disease.

Role for Bile Acid–Activated Receptors in the Pathogenesis of Kidney Disease in Obesity and Diabetes Mellitus

The above findings of increased SREBP and ChREBP activity and decreased PPARα activity in obesity and diabetes mellitus led to the investigation of the role of the bile acid–activated nuclear hormone receptor FXR in modulation of lipid metabolism and renal disease. There was indeed emerging evidence in the liver that FXR is an important negative regulator of SREBP-1 and ChREBP and a positive regulator of PPARα activity. Accordingly, INT-777 also induced renal expression of master regulators of mitochondrial biogenesis, inhibitors of oxidative stress, and inducers of fatty acid β-oxidation, including sirtuin 1, sirtuin 3, and Nrf-1. Increased activity of sirtuin 3 was evidenced by normalization of the increased acetylation of mitochondrial superoxide dismutase 2 and isocitrate dehydrogenase 2 observed in untreated db/db mice. Accordingly, INT-777 decreased mitochondrial H2O2 generation and increased the activity of superoxide dismutase 2, which associated with decreased urinary levels of H2O2 and thioribarbituric acid reactive substances. Furthermore, INT-777 decreased renal lipid accumulation. INT-777 also prevented kidney disease in mice with diet-induced obesity. In human podocytes cultured with high glucose, INT-777 induced mitochondrial biogenesis, decreased oxidative stress, and increased fatty acid β-oxidation. Compared with normal kidney biopsy specimens, kidney specimens from patients with established ORG or DN expressed significantly less TGR5 mRNA, and the levels correlated with disease progression. Our results indicate that TGR5 activation induces mitochondrial biogenesis and prevents renal oxidative stress and lipid accumulation, establishing a role for TGR5 in inhibiting kidney disease in obesity and diabetes mellitus (Figures 3 and 4).

In view of important roles for FXR and TGR5 in modulating kidney disease in obesity and in diabetes mellitus in collaboration with the Intercept scientists, we have also characterized a dual FXR-TGR5 agonist INT-767 (6α-ethyl-23(S)-methyl-3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) decreased proteinuria, podocyte injury, mesangial expansion, fibrosis, and CD68 macrophage infiltration in the kidney. INT-777 also induced renal expression of master regulators of mitochondrial biogenesis, inhibitors of oxidative stress, and inducers of fatty acid β-oxidation, including sirtuin 1, sirtuin 3, and Nrf-1. Increased activity of sirtuin 3 was evidenced by normalization of the increased acetylation of mitochondrial superoxide dismutase 2 and isocitrate dehydrogenase 2 observed in untreated db/db mice. Accordingly, INT-777 decreased mitochondrial H2O2 generation and increased the activity of superoxide dismutase 2, which associated with decreased urinary levels of H2O2 and thioribarbituric acid reactive substances. Furthermore, INT-777 decreased renal lipid accumulation. INT-777 also prevented kidney disease in mice with diet-induced obesity. In human podocytes cultured with high glucose, INT-777 induced mitochondrial biogenesis, decreased oxidative stress, and increased fatty acid β-oxidation. Compared with normal kidney biopsy specimens, kidney specimens from patients with established ORG or DN expressed significantly less TGR5 mRNA, and the levels correlated with disease progression. Our results indicate that TGR5 activation induces mitochondrial biogenesis and prevents renal oxidative stress and lipid accumulation, establishing a role for TGR5 in inhibiting kidney disease in obesity and diabetes mellitus (Figures 3 and 4).

In view of important roles for FXR and TGR5 in modulating kidney disease in obesity and in diabetes mellitus, we have also characterized a dual FXR-TGR5 agonist INT-767 (6α-ethyl-3α,7α,23-trihydroxy-24-nor-5β-cholan-23-sulfate sodium salt). We found that INT-767 is a potent agonist for both FXR (mean EC(50), 30 nmol/L by PerkinElmer AlphaScreen assay) and TGR5 (mean EC(50), 630 nmol/L by time resolved-fluorescence resonance energy transfer),
the first compound described to date to potently and selectively activate both BA receptors.

In preliminary studies in streptozotocin diabetic mice and db/db mice, we have found that treatment of mice INT-767 has significant effects to prevent development of albuminuria and glomerular disease.

**Role for Bile Acid–Activated Receptors in the Pathogenesis of Atherosclerosis and Vascular Calcification in Apolipoprotein E KO and Low-Density Lipoprotein Receptor KO Mice With CKD**

We found that FXR also play an important role on vascular calcification on apolipoprotein E (ApoE) knockout mice (KO) with 5/6 nephrectomy-induced CKD.29 We found that FXR was highly induced during osteogenic differentiation of bovine calcifying vascular cells (CVCs) and in the aorta of ApoE KO mice with CKD, which are common tissue culture and mouse model, respectively, for aortic calcification. FXR activation by a synthetic FXR agonist, 6alpha-ethyl chenodeoxycholic acid (INT-747), inhibited phosphate-induced mineralization and triglyceride accumulation in CVCs. FXR-dominant negative expression augmented mineralization of CVCs and blocked the anticalcific effect of INT-747, whereas VP16FXR that is a constitutively active form reduced mineralization of CVCs. INT-747 treatment also increased phosphorylated c-Jun N-terminal kinase. SP600125 (specific c-Jun N-terminal kinase inhibitor) significantly induced mineralization of CVCs and alkaline phosphatase expression, suggesting that the anticalcific effect of INT-747 is attributable to c-Jun N-terminal kinase activation. We also found that INT-747 ameliorates CKD-induced vascular calcification in ApoE KO mice with 5/6 nephrectomy, without affecting the development of atherosclerosis.29

In additional studies, we have treated ApoE KO mice and low-density lipoprotein receptor KO mice with the FXR-TGR5 dual agonist INT-767. INT-767 treatment drastically reduced serum cholesterol levels and significantly reduced atherosclerotic plaque formation in both ApoE KO and low-density lipoprotein receptor KO mice.30 INT-767 decreased the expression of proinflammatory cytokines and chemokines in the aortas of ApoE KO mice through the inactivation of nuclear factor kappa-B. In addition, J774 macrophages treated with INT-767 had significantly lower levels of active nuclear factor kappa-B, resulting in cytokine production in response to LPS through a PKA-dependent mechanism. This study demonstrates that concurrent activation of FXR and TGR5 attenuates atherosclerosis by reducing both circulating lipids and inflammation.

**Summary**

In summary, our studies indicate an important role for altered lipid metabolism mediated by significant alterations in nuclear hormone receptors, transcription factors, and G protein–coupled receptors in modulating kidney disease in diabetes mellitus, obesity and aging, and atherosclerosis and vascular calcification in low-density lipoprotein receptor KO and ApoE KO mice with 5/6 nephrectomy-induced CKD. Studies discussed in this review indicate that in these conditions, FXR and TGR5 agonists play an important role in preventing progression of kidney disease, atherosclerosis, and vascular calcification. Future studies will also need to determine whether FXR and TGR5 agonists have similar beneficial effects in preventing cardiac dysfunction in CKD.

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