Protection of mitochondria prevents high fat diet-induced glomerulopathy and proximal tubular injury

Hazel H. Szeto[1,2], Shaoyi Liu[1,2], Yi Soong[1,2], Nazia Alam[3,4], Glen T. Prusky[3,4] and Surya V. Seshan[5]

[1]Department of Pharmacology, Weill Cornell Medical College, New York, New York, USA; [2]Research Program in Mitochondrial Therapeutics, Weill Cornell Medical College, New York, New York, USA; [3]Department of Physiology, Weill Cornell Medical College, New York, New York, USA; [5]Department of Pathology, Weill Cornell Medical College, New York, New York, USA; and [4]Burke Medical Research Institute, White Plains, New York, USA

Obesity is a major risk factor for the development of chronic kidney disease, even independent of its association with hypertension, diabetes, and dyslipidemia. The primary pathologic finding of obesity-related kidney disease is glomerulopathy, with glomerular hypertrophy, mesangial matrix expansion, and focal segmental glomerulosclerosis. Proposed mechanisms leading to renal pathology include abnormal lipid metabolism, lipotoxicity, inhibition of AMP kinase, and endoplasmic reticulum stress. Here we report dramatic changes in mitochondrial structure in glomerular endothelial cells, podocytes, and proximal tubular epithelial cells after 28 weeks of a high-fat diet in C57BL/6 mice. Treatment with SS-31, a tetrapeptide that targets cardiolipin and protects mitochondrial cristae structure, during high-fat diet preserved normal mitochondrial structure in all kidney cells, restored renal AMP kinase activity, and prevented intracellular lipid accumulation, endoplasmic reticulum stress, and apoptosis. SS-31 had no effect on weight gain, insulin resistance or hyperglycemia. However, SS-31 prevented loss of glomerular endothelial cells and podocytes, mesangial expansion, glomerulosclerosis, macrophage infiltration, and upregulation of proinflammatory (TNF-α, MCP-1, NF-κB) and profibrotic (TGF-β) cytokines. Thus, mitochondria protection can overcome lipotoxicity in the kidney and represent a novel upstream target for therapeutic development.

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Correspondence: *Hazel H. Szeto, Department of Pharmacology, Weill Cornell Medical College, 1300 York Avenue, New York, New York, 10021, USA. E-mail: hhszeto@med.cornell.edu*

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Obesity is a major risk factor for the development of chronic kidney disease, even independently of its association with hypertension, diabetes, and dyslipidemia.[1–3] The primary pathologic finding of obesity-related kidney disease is glomerulopathy, with glomerular hypertrophy, mesangial matrix expansion, and focal segmental glomerulosclerosis.[3,4] Increasing evidence shows that podocyte stress is the primary driving force in the development of proteinuria and glomerulosclerosis. Patients with obesity-related glomerulopathy have reduced podocyte density, widened foot processes, and proteinuria.[4] Similar glomerular findings have been reported in mice after just 2 to 4 months on a high-fat diet (HFD), even before there is any significant increase in blood glucose.[5–9] Another prominent feature of obesity-related kidney disease is the accumulation of lipid vacuoles in podocytes, mesangial cells, and proximal tubular (PT) epithelial cells, suggesting that abnormal lipid metabolism and lipotoxicity may be the major cause of renal dysfunction.[3,10,11] Intracellular lipid accumulation causes oxidative stress, endoplasmic reticulum (ER) stress, cytoskeletal changes, and activation of proinflammatory processes.[10–12]

Mitochondrial fatty acid β-oxidation is the major source of adenosine triphosphate (ATP) for the kidney. Excess fatty acids that are not oxidized by mitochondria are esterified with glycerol and deposited as lipid droplets. Gene analysis of renal biopsy samples from diabetic nephropathy patients revealed significant upregulation of pathways involved in lipid uptake and decreased expression of enzymes supporting fatty acid β-oxidation.[10] Mice fed HFD also showed significant downregulation of key enzymes in β-oxidation and upregulation of enzymes involved in lipogenesis.[13] Importantly, there was a significant correlation between lipid metabolism gene expression and glomerular filtration rate in diabetic nephropathy.[10]

AMP kinase (AMPK) plays a major role in lipid metabolism. Activation of AMPK reduces fatty acid synthesis and increases uptake and oxidation of fatty acids by mitochondria. HFD decreases AMPK activity in multiple tissues, including the kidney.[13–15] Treatment with the AMPK activator (5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside) prevented glomerulopathy and tubulointerstitial fibrosis.[15,16]

Chronic 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside treatment also reduced lipid accumulation in tubular cells.[16]

There is evidence to suggest that obesity and HFD may inhibit mitochondrial fatty acid β-oxidation in the kidney. There are numerous reports of abnormal mitochondrial structures associated with obesity. PT mitochondria of *ob/ob* mice are small and rounded with loss of cristae membranes and matrix density.[17] Mitochondrial fragmentation, decreased ATP content, and increased H_2O_2 emission in kidney mitochondria have also been reported in mice after HFD, in *ob/ob* mice, and in a rat model of type 1 diabetes.[8,18,19]

Here we report structural changes indicative of lipid accumulation in PT cells and dramatic mitochondrial damage in tubular cells, podocytes, and glomerular endothelial cells after 28 weeks of HFD in C57BL/6 mice. Protection of mitochondrial structures by SS-31 (D-Arg-2′,6′-dimethylTyr-Lys-Phe-NH_2), a peptide that targets cardiolipin and protects mitochondrial cristae structure,[20–22] preserved normal mitochondrial structure in all kidney cells, restored AMPK activity, and prevented intracellular lipid accumulation and ER stress during HFD. SS-31 also prevented the loss of glomerular endothelial cells and podocytes, mesangial expansion, glomerulosclerosis, macrophage infiltration, and upregulation of proinflammatory and profibrotic cytokines. Interestingly, SS-31 had no effect on weight gain, insulin resistance, or hyperglycemia. These findings suggest that mitochondria protection can overcome lipotoxicity of the kidney and support the mitochondrion as a novel target for development of renal therapeutics.

RESULTS

HFD-induced increase in body weight and blood glucose are not affected by SS-31

C57BL/6 mice were maintained on a normal diet (ND) or HFD for 28 weeks. This specific diet has been reported to cause a significant increase in body weight, body fat, blood glucose, and insulin, after 16 weeks in C57BL/6 mice.[23] HFD mice received a 5-day regimen of low-dose streptozotocin (STZ) 4 weeks after the start of HFD to minimize hyperinsulinemia. HFD mice were then treated with either saline solution or SS-31 daily (1 mg/kg s.c.) for 8 to 28 weeks. The body weight of mice fed HFD was only slightly increased after 8 weeks of HFD relative to ND, but was dramatically increased after 28 weeks (Figure 1a). Nonfasting blood glucose was slightly increased after 8 weeks (Figure 1b), indicating that the dose of STZ did not cause a significant loss of pancreatic β cells. Oral glucose tolerance tests administered after 8 and 28 weeks of HFD revealed abnormal glucose clearance (Figure 1c and d), suggesting the development of insulin resistance before the onset of hyperglycemia. Plasma insulin has been reported to be significantly elevated in C57BL/6 mice after just 4 weeks of HFD.[13] Treatment with SS-31 had no effect on body weight, glucose clearance, or blood glucose after 8 and 28 weeks of HFD (Figure 1a–d).

SS-31 prevented HFD-induced glomerulopathy

Pathology in the early stages of obesity-related renal disease in humans is primarily related to glomerular changes.[24] Periodic acid–Schiff staining of mouse kidneys after 28 weeks of HFD revealed capillary collapse and mesangial expansion

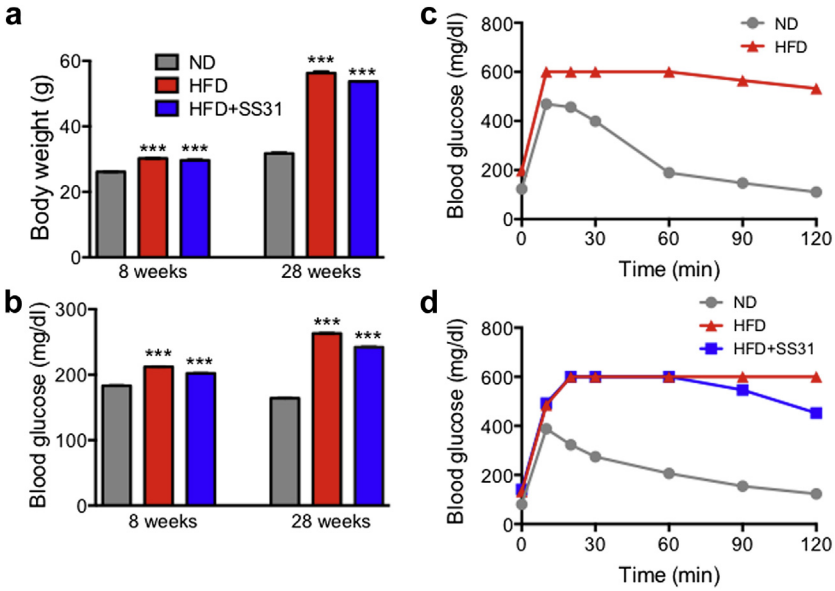

Figure 1 | SS-31 has no effect on body weight, blood glucose, and glucose tolerance test (GTT) caused by high-fat diet (HFD). (**a**) Body weight was significantly changed after 8 and 28 weeks of a high-fat diet ($n = 8$) compared with mice fed a normal diet (ND) ($n = 6$) (***$P <$ 0.001 compared with ND). Treatment with SS-31 had a very small, but statistically significant reduction in body weight in mice fed HFD ($n = 5$) ($P < 0.05$). (**b**) Nonfasting blood glucose was not changed after 8 weeks of HFD, suggesting that the low dose of streptozotocin had no significant effect on blood glucose. However, blood glucose was significantly increased after 28 weeks of HFD and was not affected by SS-31 treatment. (**c**,**d**) GTT revealed abnormal glucose clearance after both 8 and 28 weeks of HFD, indicating insulin resistance. This HFD-induced insulin resistance was not prevented by SS-31 treatment.