Stearoyl-CoA Desaturase Deficiency, Hypercholesterolemia, Cholestasis, and Diabetes

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Previous studies showed that mice deficient in Scd1 *had a reduced level of liver triglyceride and an improvement in insulin sensitivity. We studied* Scd1$^{-/-}$ *mice on a very low-fat, high-carbohydrate lipogenic diet. The animals were almost entirely devoid of high-density lipoprotein (HDL). Nonetheless, they were hypercholesterolemic and had cholestasis. These changes were reversible with oil containing both mono- and polyunsaturated fat, but not entirely reversible with just triolein, suggesting that* Scd1 *deficiency increased the requirement for polyunsaturated fat. We also found that the* Scd1$^{-/-}$ *mice on a normal chow diet had dramatically improved insulin sensitivity. However,* leptin$^{ob/ob}$ Scd1$^{-/-}$ *mice had worse diabetes than* leptin$^{ob/ob}$ Scd1$^{wt/wt}$ *mice.*

Key words: diabetes, hypercholesterolemia, leptin deficiency stearoyl-CoA desaturase (Scd1) deficiency

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INTRODUCTION

Stearoyl-CoA desaturase (Scd1) catalyzes the introduction of a double bond at the Δ_9 position of palmitoyl-CoA (16:0) and stearoyl-CoA (18:0) to produce the monounsaturated fatty acids (MUFAs) palmitoleoyl-CoA (16:1) and oleoyl-CoA (18:1), respectively.[1] The dietary requirement for MUFA is difficult to ascertain because of the capacity for MUFA synthesis conferred by Scd1.

Scd1 is widely expressed, particularly in lipogenic tissues such as liver and adipose tissue. Like other lipogenic genes, it is positively regulated by insulin and leptin and by several known lipogenic transcription factors, sterol-responsive element-binding protein-1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP). In addition, it is positively regulated by the liver-X-receptor (LXR).

High-carbohydrate diets increase the expression of lipogenic enzymes, including Scd1, and the rate of triglyceride synthesis in the liver. This induction is attenuated in mice deficient in *Scd1*.[2,3] *Scd1* deficiency was reported to reduce serum triglycerides.[4] However, we have found that in some mouse strains, *Scd1* deficiency, although it still reduces hepatic triglyceride levels, does not reduce serum triglycerides. These studies have all been conducted in animals receiving an ample supply of polyunsaturated fat. Long-term feeding of *Scd1*-deficient animals with lipogenic diets containing a minimum amount of polyunsaturated fat had not been tested.

In addition to its effects on lipid metabolism, *Scd1* deficiency has dramatic effects on adipogenesis[5,6] and insulin sensitivity.[7] We have previously speculated that hepatic lipogenesis, through competition for some gluconeogenic substrates, might decrease the rate of gluconeogenesis.[8] Thus, the effect of *Scd1* deficiency on obesity-induced diabetes could have both an anti-diabetic effect (increased insulin sensitivity) and a pro-diabetic effect (increased gluconeogenesis.

We studied obesity-induced diabetes by studying two mouse strains that, when made obese with the *leptin^{ob}* mutation, differ in diabetes susceptibility. As originally shown in the classic studies of Coleman,[9] we have found that the C57BL/6 strain is transiently and mildly hyperglycemic when it carries the *leptin^{ob}* mutation. In contrast, the BTBR *leptin$^{ob/ob}$* mice are severely diabetic.[10] Gene expression studies carried out in these

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two strains revealed that the level of lipogenic gene expression, including *Scd1*, was higher in the C57BL/6 *leptin*$^{ob/ob}$ mice than in the BTBR *leptin*$^{ob/ob}$ mice.[11] Similar studies in lipodystrophic mice showed a positive correlation between lipogenic gene expression, hepatic triglyceride content, and resistance to diabetes.[12] Another angle on strain differences emphasized triglyceride clearance rather than triglyceride production and secretion. Studies comparing leptin-deficient obese or lipodystrophic C57BL/6 and FVB strains found that increased triglyceride clearance in the C57BL/6 strain effectively diverted triglyceride away from muscle.[12] These studies concluded that this change in triglyceride partitioning would tend to improve whole-body insulin sensitivity, even though it would worsen hepatic insulin sensitivity.

RESULTS AND DISCUSSION

Very Low-Fat/High-Carbohydrate Diet and *Scd1* Deficiency

Scd1$^{-/-}$ C57BL/6 mice were obtained by backcrossing 129/sv mice carrying the null mutation into the C57BL/6 background for at least five generations. The mice were fed a standard chow diet (PMI 5008. Formulab), a very low-fat diet (VLF, TD03045), VLF + coconut (TD03138), or VLF + canola (Harlan Teklad). When maintained on the VLF diet, the *Scd1*$^{-/-}$ mice developed hypercholesterolemia. Their cholesterol levels were elevated 250%, whereas cholesterol only increased by 20% in the wild-type animals on the same diet.

Despite the increase in total cholesterol, the *Scd1*$^{-/-}$ mice had a nearly complete loss of HDL. The hypercholesterolemia was due to a large increase in VLDL and LDL. In addition, by agarose gel electrophoresis, we were able to detect lipoprotein-X. To better understand the basis for the increase in LDL, we measured the rate of clearance of a ^{125}I-LDL tracer from the bloodstream of the mice. The fractional clearance rate of the LDL tracer was reduced by 50% in the VLF *Scd1*$^{-/-}$ mice. The magnitude of the decrease was approximately commensurate with the increase in LDL, indicating that reduced clearance was the major mechanism underlying the increase in LDL. We also estimated triglyceride secretion by measuring the increment in serum triglyceride following the injection of poloxamer 407 to inhibit lipoprotein lipase.[13] Although liver triglyceride levels were reduced by 82% in the VLF *Scd1*$^{-/-}$ mice, there was no difference in triglyceride secretion between these mice and the wild-type mice on the same diet. In addition to the lipid disorders, the VLF *Scd1*$^{-/-}$ mice had a 50-fold elevation in serum bile acids and a 6-fold increase in bilirubin, mostly conjugated, all consistent with cholestasis. In addition, the presence of lipoprotein X is symptomatic of cholestasis.[14] Despite cholestasis, there was no defect in bile flow or bile salt secretion. However, biliary phospholipids were decreased substantially (by 44%).

Both the lipoprotein and the cholestasis phenotypes were reversible by supplementing the VLF diet with canola oil, but not with coconut oil, suggesting that mono- and/or polyunsaturated fat deficiency was an underlying problem in the *Scd1*$^{-/-}$ mice on the VLF diet. To better understand which lipid source was critical for this correction, we supplemented VLF mice with triolein in order to provide a pure source of MUFA. The triolein supplementation was able to abolish cholestasis, improve hepatic function, and restore HDL cholesterol to near normal levels. However, triolein did not reduce the very high levels of LDL in the VLF *Scd1*$^{-/-}$ mice. The latter defect could only be reversed by polyunsaturated fat.

The increased cholesterol in the bloodstream was primarily in free cholesterol, which is consistent with the accumulation of lipoprotein-X. This is also the case in animals with a deficiency of lecithin cholesterol acyltransferase (LCAT). The *Scd1*$^{-/-}$ mice on the VLF diet had a 43% reduction in LCAT activity. Although this helps to account for an increase in free cholesterol, it does not explain the complete loss of HDL, because animals heterozygous for a null allele at *Lcat* have half the normal level of HDL.[15]

We carried out microarray analysis of gene expression in the liver to learn what genes are dysregulated under the conditions of *Scd1* deficiency and the VLF diet. As expected, there were many genes whose expression changed; unexpectedly, the expression of many of the genes involved in lipid transport across the canalicular membrane was not significantly changed. The bile salt export pump (ABCB11/BSEP) mRNA abundance was reduced by one-half in the *Scd1*$^{-/-}$ mice on the VLF diet. It is possible that the balance of mono- and polyunsaturated fats has more of an effect on the activity of lipid transporters than it does on their level of expression.

Scd1 and Diabetes Susceptibility in Leptin-Deficient Obese Mice

We carried out hyperinsulinemic-euglycemic clamp studies to quantitate the level of insulin sensitivity in lean *Scd1*$^{-/-}$ mice. In this procedure, insulin levels are raised and glucose is infused to maintain euglycemia. The glucose infusion rate required to maintain euglycemia is a measure of insulin sensitivity. The *Scd1*$^{-/-}$ mice had a striking 3-fold increase in the glucose infusion rate, indicating a large increase in whole-body insulin sensitivity. By measuring the accumulation of ^{14}C-deoxyglucose, we were able to quantitate glucose uptake into muscle and adipose tissue. Heart and soleus muscle had an increase of about 2.5-fold in glucose uptake, but

overall, the uptake of glucose into the heart became quite dominant over all other insulin-sensitive tissues. The liver also showed an increased level of insulin sensitivity, as evidenced by a virtual total suppression of hepatic glucose output under the hyperinsulinemic conditions (Figure 1A).

We introgressed the Scd^{null} allele into BTBR $leptin^{ob/ob}$ mice, which normally develop diabetes between 6 and 10 weeks of age. The effect of the *Scd1* mutation was to increase the severity of the hyperglycemia. At 6 weeks of age, glucose levels in obese male mice was 474 mg/dL in the *Scd1*-deficient mice compared with 272 mg/dL in the $Scd1^{wt/wt}$ mice ($P < 0.0001$). In females, the corresponding values were 344 and 214 mg/dL ($P < 0.002$), respectively. Insulin levels dropped by about 35% in both sexes. Although a reduction in fasting insulin can be a sign of increased insulin sensitivity, in this case, because of the rise in glucose, it appeared to be more likely an indication of reduced insulin secretion. Indeed, when challenged with an intraperitoneal glucose bolus, the mice secreted less than one-half as much insulin as did BTBR $leptin^{ob/ob}$ $Scd1^{wt/wt}$ mice. Insufficient insulin secretion can be a consequence of an intrinsic defect in the process by which β-cells sense glucose and secrete the appropriate amount of insulin. Alternatively, it can be caused by a reduction in β-cell mass. When we isolated islets from the mice, we noted that about 50% of the islets in the BTBR $leptin^{ob/ob}$ $Scd1^{-/-}$ mice were small and had an opaque circle in the center, giving them a "fried egg" appearance. These islets had a more than 80% reduction in insulin content. Thus, the insulinopenia of the BTBR $leptin^{ob/ob}$ $Scd1^{-/-}$ mice is due to the presence of the abnormal islets and their loss of insulin.

Monounsaturated fatty acyl-CoAs are the preferred substrates for the incorporation of fatty acids into the *sn*-2 position of glycerolipids and cholesterol esters. Thus, the production of monounsaturated fatty acids can be limiting for the amount and type of these lipids. In addition, to the extent that palmitate might affect the production of ceramide and sphingolipids, desaturation

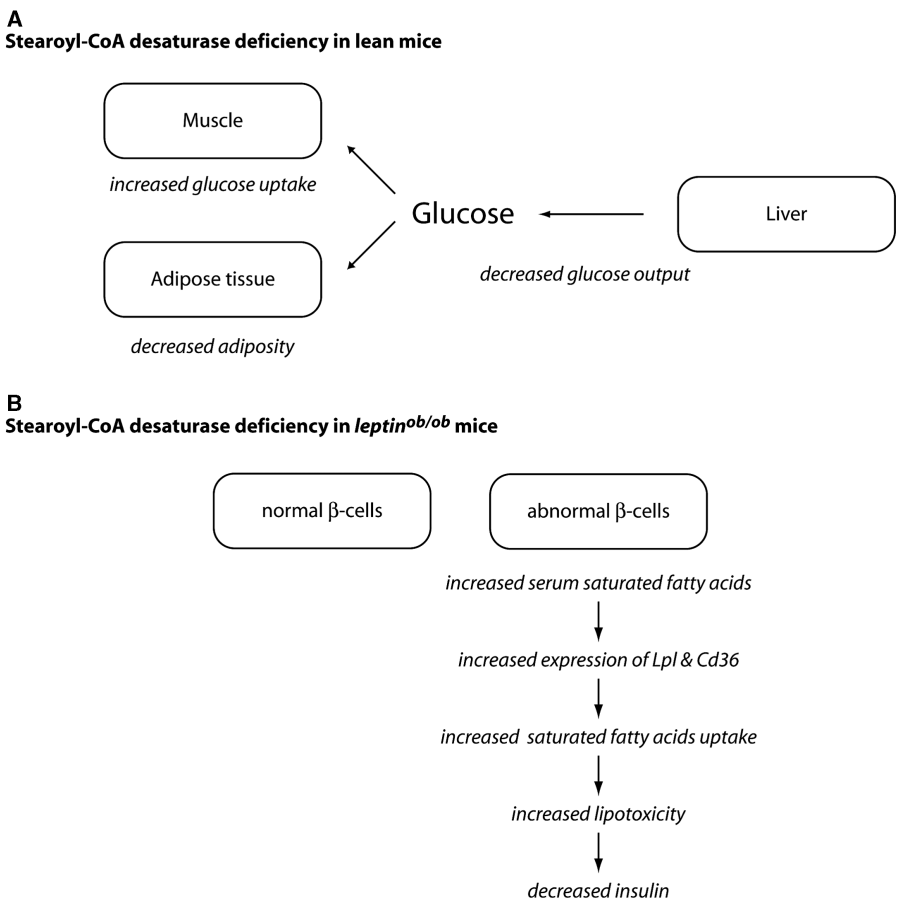

Figure 1. A, Consequences of *Scd1* deficiency in lean mice. The loss of *Scd1* leads to increased insulin sensitivity. This in turn causes increased muscle glucose uptake and decreased hepatic glucose output. In addition, the mice have a profound loss of adipose tissue mass. B, Consequences of *Scd1* deficiency in *leptinob/ob* mice. A large fraction of the pancreatic islets in these mice have an abnormal morphology. These islets have a dramatic induction in *Lpl* and *Cd36*. Thus, we speculate that increased uptake of saturated fatty acids leads to lipotoxicity of the β-cells.

of palmitate can play a role in the flux through the sphingolipid pathway.

As expected when an animal has a reduction in MUFA synthesis, tissues and serum showed an increase in palmitate and stearate relative to palmitoleate and oleate. However, the abnormal islets in the BTBR *leptin*$^{ob/ob}$ *Scd1*$^{-/-}$ mice had 3-fold more palmitate and stearate as free fatty acid and 4-fold more palmitate in triglyceride than the normal-looking islets.

We surveyed gene expression by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). The most striking changes in gene expression in the abnormal versus the normal-looking islets were in *Lpl* and *Cd36*. *Lpl* was up-regulated about 10-fold and *Cd36* about 167-fold in the abnormal islets. Both of these changes would be expected to increase the lipid load on the β-cells, through enhanced lipolysis of lipoprotein triglyceride and through enhanced fatty acid transport into the cells (Figure 1B). In vitro, chronic exposure of β-cells to fatty acids alters secretory function and induces apoptosis.[16-21] Thus, the combination of increased saturated fatty acid availability and increased fatty acid transport can explain a potential lipotoxic effect of *Scd1* deficiency on the islets.

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FINANCIAL DISCLOSURE

Dr. Attie reported owning stock in Xenon Pharmaceuticals, Inc., a company developing inhibitors of stearoyl-CoA desaturase.

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