Inhibition of Stearoyl-Coenzyme A Desaturase 1 Dissociates Insulin Resistance and Obesity From Atherosclerosis

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Background—Stearoyl-coenzyme A desaturase 1 (SCD1) is a well-known enhancer of the metabolic syndrome. The purpose of the present study was to investigate the role of SCD1 in lipoprotein metabolism and atherosclerosis progression.

Methods and Results—Antisense oligonucleotides were used to inhibit SCD1 in a mouse model of hyperlipidemia and atherosclerosis (LDLr−/− Apob100/100). In agreement with previous reports, inhibition of SCD1 protected against diet-induced obesity, insulin resistance, and hepatic steatosis. Unexpectedly, however, SCD1 inhibition strongly promoted aortic atherosclerosis, which could not be reversed by dietary oleate. Further analyses revealed that SCD1 inhibition promoted accumulation of saturated fatty acids in plasma and tissues and reduced plasma triglyceride, yet had little impact on low-density lipoprotein cholesterol. Because dietary saturated fatty acids have been shown to promote inflammation through toll-like receptor 4, we examined macrophage toll-like receptor 4 function. Interestingly, SCD1 inhibition resulted in alterations in macrophage membrane lipid composition and marked hypersensitivity to toll-like receptor 4 agonists.

Conclusions—This study demonstrates that atherosclerosis can occur independently of obesity and insulin resistance and argues against SCD1 inhibition as a safe therapeutic target for the metabolic syndrome. (Circulation. 2008;118:1467-1475.)

Key Words: atherosclerosis ■ diabetes mellitus ■ fatty acids ■ inflammation ■ obesity
Experimental Design

Male apolipoprotein (Apo) B100-only, low density lipoprotein (LDL) receptor (LDLr)–deficient (LDLr<sup>−/−</sup>, Apob<sup>100/100</sup>) mice were used in this study. These mice were chosen on the basis of previous reports documenting their “human-like” lipoprotein profile,20 atherosclerosis susceptibility,20 and responsiveness to dietary fatty acids.21 All mice were on a mixed background (~75% C57BL/6 and ~25% 129Sv/Jae). At 6 weeks of age, the mice were switched from a diet of rodent chow to 1 of 2 synthetic diets containing 12% of energy as SFA-enriched fat (palm oil) or MUFA-enriched fat (oleate-enriched safflower oil) with 0.1% (wt/wt) cholesterol added. Table I of the online-only Data Supplement provides a complete analysis of the dietary fatty acid composition. In conjunction with diet, mice were injected biweekly with saline, 25 mg/kg of a nontargeting ASO (control ASO; 5′-TCCCAATTTACGAGAGCTTG-3′), or 25 mg/kg of an ASO targeting the knockdown of SCD1 (SCD1 ASO; 5′-GCCTTAACATCCAGAAGCT-3′). These phosphorothioate-modified ASO compounds were generously provided by Isis Pharmaceuticals, Inc (Carlsbad, Calif). Body weight was measured weekly, and food intake was measured at 4 and 8 weeks of diet/ASO treatment. All experimental animals were killed after 20 weeks of parallel dietary and ASO treatment. All mice were maintained in a pathogen-free animal facility, and experimental protocols were approved by the institutional animal care and use committee at the Wake Forest University School of Medicine.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

SCD1 ASO Treatment Inhibits Hepatic and Adipose SCD1 Function

After 20 weeks of ASO treatment, liver and adipose SCD1 mRNA levels were reduced by 99% and 83% to 94%, respectively (Figure 1A). Furthermore, SCD1 protein expression was undetectable by Western blot in both liver and adipose tissue (Figure 1B). Hepatic SCD1 activity was reduced by >95% after 20 weeks of treatment (Figure 1C). After only 4 weeks of ASO treatment, hepatic SCD1 protein and activity levels were reduced by >90% (data not shown). Importantly, SCD1 protein expression in skeletal muscle and skin was not altered by 20 weeks of SCD1 ASO treatment (data not shown). This tissue-specific pattern of knockdown is likely due to the intrinsic pharmacokinetic properties of ASOs and has previously been described with other ASO compounds of similar chemistry in mice.22,23

SCD1 Inhibition Prevents Diet-Induced Obesity and Insulin Resistance in Hyperlipidemic Mice

In agreement with previous reports,7–11 SCD1 inhibition prevented diet-induced obesity in LDLr<sup>−/−</sup>, Apob<sup>100/100</sup> mice. (Figure 2A through 2C). Epididymal fat pad mass was reduced by ~85% on the saturated diet and ~80% on the MUFA-rich diet compared with control ASO–treated mice (Figure 2C), which could not be explained by reductions in food intake (Figure 2D) and may result from the previously described role of SCD1 in energy expenditure.7,8,24 After only 4 weeks of treatment, fasting insulin levels were significantly lower in SCD1 ASO–treated mice (Figure 2E). In parallel, SCD1 inhibition significantly improved glucose tolerance (Figure 2F) and insulin tolerance (Figure 2G). Importantly, the effects of SCD1 inhibition on adiposity and insulin resistance could not be reversed by dietary MUFA supplementation (Figure 2). Collectively, these data support the notion that ASO-mediated inhibition of SCD1 is efficacious in the prevention of diet-induced obesity and insulin resistance.10,17

SCD1 Inhibition Promotes Atherosclerosis

En face morphometric analysis showed that SCD1 ASO–treated mice had 2.7-fold (SFA diet) and 2.6-fold (MUFA diet) increases in total aortic lesion area (Figure 3B) compared with control ASO–treated mice. Interestingly, this SCD1 ASO–driven augmentation of atherosclerotic lesion...
area seemed to be regional in nature (Figure 3A and 3C). When the control and SCD1 ASO groups were compared, no significant differences were found in en face lesion area in the aortic arch (Figure 3C). However, modest increases were found in the thoracic aorta lesion area and highly significant increases in the abdominal aorta lesion area when SCD1 was inhibited (Figure 3C). In fact, SCD1 inhibition caused a striking 5-fold (MUFA diet) to 7-fold (SFA diet) increase in abdominal aortic lesion area, where >70% of the abdominal aorta was covered with lesion in SCD1-inhibited mice (Figure 3A and 3C). Biochemical analysis of the complete set (n=8 to 15 per group) of whole aortas from this study revealed that SCD1 inhibition resulted in significant increases in both free and esterified cholesterol compared with either saline- or control ASO–treated mice (Figure 3D and 3E). Furthermore, SCD1 inhibition resulted in enrichment of SFA and depletion of MUFA in aortic CE and TG (Figure 3F and 3G). Although less dramatic than the effects seen in CE (Figure 3F) and TG (Figure 3G), aortic phospholipid was likewise significantly depleted of MUFA (Figure 3H), and desaturation indexes (16:1/16:0 and 18:1/18:0) were significantly reduced with SCD1 inhibition (data not shown). Importantly, dietary MUFA did not prevent SCD1 ASO–mediated promotion of aortic atherosclerosis (Figure 3). In agreement with en face (Figure 3A through 3C) and biochemical (Figure 3D and 3E) analyses, histological evaluation of cross sections from the proximal aorta revealed that SCD1 inhibition promoted the accumulation of cholesterol clefts and necrotic core formation (online-only Data Supplement Figure I). Similar histological lesion characteristics were seen.

Figure 2. SCD1 inhibition prevents diet-induced obesity and insulin resistance in LDLr−/−/Apob100/100 mice. Starting at 6 weeks of age, mice were fed diets enriched in 0.1% (wt/wt) cholesterol and either SFA or MUFA for a period up to 20 weeks, in conjunction with biweekly injections (25 mg/kg) of saline (◼ in bar graphs, green ▲ in line graphs), a nontargeting control ASO (◼ in bar graphs, ❏ in line graphs), or SCD1 ASO (◼ in bar graphs, red ▲ in line graphs). Photographs (A), body weights (B), and epididymal fat pad mass (C) of mice after 20 weeks of diet and ASO treatment. Data in B and C represent the mean±SEM from 8 to 15 mice per group; values not sharing a common superscript differ significantly (P<0.05). D, Food intake was measured after 8 weeks of diet and ASO treatment. No significant differences were detected. E, Fasting plasma insulin levels were measured after 4 weeks of diet and ASO treatment. Data represent the mean±SEM from 5 mice per group; values not sharing a common superscript differ significantly (P<0.05). Glucose tolerance tests (F) and insulin tolerance tests (G) were performed after 16 weeks of diet and ASO treatment. Data shown in F and G represent the mean±SEM from 5 mice per group. *Significantly different from the control ASO group within each diet group (P<0.05).
in thoracic and abdominal aortic sections (data not shown). Collectively, these data provide evidence that SCD1 inhibition promotes SFA- and cholesterol-rich atherosclerotic lesion formation in LDLr<sup>−/−</sup> Apob<sup>100/100</sup> mice.

**SCD1 Inhibition Promotes SFA Enrichment of Plasma Lipoproteins**

In agreement with previous reports,<sup>1−3</sup> our results showed that SCD1 inhibition prevented diet-induced hypertriglyceridemia (Figure 4A). In contrast, total plasma cholesterol was only modestly (186 mg/dL in control ASO group versus 124 mg/dL in SCD1 ASO group) reduced after 20 weeks of the SFA diet but was not significantly altered under any other conditions (Figure 4B). When lipoprotein cholesterol distribution was analyzed, we discovered that SCD1 inhibition decreased very low-density lipoprotein (VLDL) cholesterol, had no effect on LDL cholesterol levels, and significantly reduced HDL cholesterol (Figure 4C and 4D). These SCD1 ASO–driven reductions in VLDL and HDL cholesterol levels were accompanied by reductions in plasma ApoE and ApoAI, whereas plasma ApoB and lecithin:cholesterol acyltransferase were not altered by SCD1 inhibition (Figure 4G). Furthermore, VLDL particles were significantly smaller in SCD1 ASO–treated mice (Figure 4F), possibly because of depletion of TG-rich core (Figure 4A). However, LDL and HDL particle sizes were not altered by SCD1 ASO treatment (Figure 4F). Finally, SCD1 inhibition resulted in reductions of MUFAs with highly significant enrichments of SFAs in LDL-CE and similar but less impressive fatty acid shifts in HDL-CE (Figure 4E). Collectively, SCD1 inhibition resulted in dramatic alterations in plasma lipoprotein metabolism, including diminished plasma TG, VLDL cholesterol, HDL cholesterol, VLDL size, and ApoE and ApoAI levels and striking enrichment of plasma lipoproteins with SFAs. Im-
portantly, none of the SCD1 ASO–driven alterations in lipoprotein metabolism were prevented by dietary MUFAs (Figure 4A, 4B, and 4E and data not shown).

SCD1 Inhibition Prevents Diet-Induced Steatosis

It has been well documented that mice lacking SCD1 are protected against hepatic steatosis under a variety of conditions.1,4–7 In addition to confirming these reports, we set out to characterize hepatic cholesterol metabolism in SCD1 ASO–treated mice because cholesterol-rich atherogenic ApoB-containing lipoproteins are believed to originate from the liver.25 As expected, SCD1 inhibition resulted in striking reductions in hepatic steatosis (online-only Data Supplement Figure IIA), manifested as a 93% reduction in hepatic TG concentration and an 81% reduction in hepatic CE concentration (online-only Data Supplement Figure IIB) compared with control ASO–treated mice. This reduction in hepatic triglycerides mass may be partially a result of a reduction in SREBP1c protein expression (online-only Data Supplement Figure IIF), with parallel downregulation of SREBP1c target genes (online-only Data Supplement Figure IIE), including fatty acid synthase, acetyl-coenzyme A carboxylase, and mitochondrial glycerol-3-phosphate acyltransferase. Quite unexpectedly, hepatic free cholesterol concentration was significantly increased by 1.5-fold (SFA diet) or 2.4-fold (MUFA diet; data not shown) when SCD1 was inhibited (online-only Data Supplement Figure IIB). The hepatic free cholesterol increase seen with SCD1 ASO treatment was accompanied by compensatory downregulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, upregulation of Cyp7A1, but normal ATP-binding cassette protein G5 (ABCG5) mRNA expression (online-only Data Supplement Figure IIE). Protein expression of ABCG5 also was not altered by SCD1 ASO treatment (online-only Data Supplement Figure IIF). Interestingly, ACAT2 protein expression was slightly increased with SCD1 ASO treatment (online-only Data Supplement Figure IIF) without corresponding changes in mRNA expression (data not shown), indicating posttranscriptional regulation. During isolated liver perfusion, it was found that SCD1 inhibition did not significantly alter the secretion rate of TG, CE, free cholesterol, or phospholipids (online-only Data Supplement Figure IIC). A trend was found toward a decrease in TG secretion rate with SCD1 ASO treatment, but it was not statistically significant.
A closer look at the fatty acid composition of hepatic CE, TG, and phospholipids revealed that SCD1 inhibition promoted the enrichment of SFA into hepatic CE and TG but not phospholipids (online-only Data Supplement Figure IIC). None of the SCD1 ASO–driven alterations in hepatic lipid metabolism were prevented by dietary MUFAs (data not shown). Collectively, these data support previous observations\(^7,8,10,12,14,17\) that SCD1 inhibition is efficacious in the prevention of hepatic steatosis, but surprisingly, augmented free cholesterol concentrations also were present.

**SCD1 Inhibition Exacerbates Toll-Like Receptor 4–Driven Proinflammatory Response in Macrophages**

Macrophages can play a pivotal role in the pathogenesis of atherosclerosis through multiple mechanisms, including their well-known role in producing atherogenic cytokine signals.\(^26\)

It has been demonstrated that SFAs promote inflammatory cytokine secretion in macrophages through a toll-like receptor 4 (TLR4)–dependent mechanism.\(^27–30\) Because SCD1 inhibition resulted in striking SFA enrichment of LDL-CE (Figure 4E), we hypothesized that macrophage lipids may likewise become enriched in SFAs, thereby enhancing TLR4-dependent proinflammatory cytokine secretion. We found that in vivo SCD1 ASO treatment for 6 weeks reduced SCD1 mRNA and protein expression by \(90\%\) and \(50\%\), respectively, in isolated macrophages (Figure 5A). In addition, macrophages isolated from SCD1 ASO–treated mice had a significantly decreased 16:1/16:0 ratio and an increased proportion of linoleic acid (18:2; \(n=6\)) in isolated phospholipids (Figure 5B). Similar effects were observed in neutral lipids (Figure 5C). The increased proportion of linoleic acid is consistent with the reported effect of SCD1 inhibition on FFA composition.\(^12\)

SCD1 inhibition exacerbated the proinflammatory response to Kdo2-lipid A (Figure 5D and 5E). This was accompanied by increased expression of proinflammatory cytokines (Figure 5F). Western blot analysis of TLR4, MyD88, and CD14 protein expression in freshly isolated macrophages (2-hour culture) demonstrated the proinflammatory effect of SCD1 inhibition.
lipids, with SCD1 inhibition resulting in significant decreases in 16:1/16:0 and 18:1/18:0 ratios (data not shown). Interestingly, when macrophages isolated from SCD1 ASO–treated mice were challenged with a TLR4 agonist (10 ng/mL Kdo2-lipid A), marked hypersensitivity was apparent (Figure 5C and 5D). In support of this, SCD1 inhibition resulted in augmented TLR4-driven proinflammatory gene expression (Figure 5C) and cytokine secretion (Figure 5D) in isolated macrophages, although basal expression (ie, the absence of TLR4 ligand) of inflammatory cytokines was not significantly different between macrophages isolated from control- and SCD1 ASO–treated mice (data not shown). When we examined the expression of key proteins involved in TLR4-dependent signal transduction,31,32 we found a slight reduction in TLR4 expression, no change in MyD88, and a slight reduction in CD14 in SCD1-inhibited macrophages (Figure 5F). Furthermore, canonical TLR4–MyD88–dependent activation of mitogen-activated protein kinases, phosphorylation of inhibitor α-kinase α/β, and downstream degradation of inhibitory κB-kinase α was not different between control- and SCD1 ASO–treated macrophages (Figure 5E). However, the TLR4–MyD88–independent driven tyrosine phosphorylation of STAT1, a well-known interferon-β–dependent event,33 was markedly elevated in SCD1-inhibited macrophages (Figure 5E).

Discussion

Although the presence of the metabolic syndrome seems to be associated with atherosclerotic CVD outcomes in humans,1–3 dissociation of the two has previously been reported.34 Results from this study provide evidence that the metabolic syndrome can be completely dissociated from atherosclerosis in mice. That is, in the extreme case of leanness and insulin sensitivity induced by SCD1 ASO treatment, atherosclerosis progressed independently. Results from this study support the notion7–18 that SCD1 inhibitors may be efficacious in preventing many aspects of the metabolic syndrome (diet-induced obesity, hepatic steatosis, insulin resistance, hypertriglyceridemia), but perhaps at the expense of the artery wall. To help explain this unexpected finding, we propose a working model in which SCD1 inhibitors promote atherosclerosis (Figure 6). Briefly, we believe that inhibition of SCD1 in the liver results in secretion of VLDL particles that are highly enriched in SFA-rich CE, giving rise to SFA–CE–rich LDL particles. These SFA–CE–rich LDL particles deliver abundant SFA to macrophages, which also have diminished SCD1 expression, resulting in alterations in membrane lipid composition, enhanced TLR4-driven tyrosine phosphorylation of STAT1, and ultimately enhanced inflammatory cytokine secretion. This proinflammatory phenotype ultimately promotes atherosclerosis.

In support of this model, a role for SCD1 in protecting against another inflammation-driven disease (dextran sulfate sodium–induced colitis) was recently reported.35 In this report, Chen et al35 elegantly demonstrated that mice lacking SCD1 had elevated dextran sulfate sodium– and bacteria-driven inflammatory gene expression and exaggerated colitis, findings analogous to our results. This study, as well as ours, supports the long-standing notion that SFAs are potent proinflammatory molecules.27–30 Hence, one of the key roles of SCD1 may be to suppress inflammation by preventing excessive accumulation of SFAs themselves and downstream metabolites such as stearoyl-lyso phosphatidylcholine35 and ceramide.36 Importantly, this study now joins several recent reports that have demonstrated unexpected harmful consequences of inhibiting SCD1.18,35,37

The molecular mechanism(s) by which SCD1 inhibition promotes atherosclerosis (Figure 3), inflammatory colitis,35 frank diabetes,18 and cholestasis37 have not been clearly elucidated. More work is needed to address these unexpected outcomes if SCD1 inhibitors are to be pursued as CVD therapeutics in humans. In addition to SCD1 inhibitors, TLR4 antagonists have been suggested as potential CVD therapeutics, but whether TLR4 plays a role in atherosclerosis in humans has been a matter of intense debate.38–40 Indeed, more work is needed to investigate whether TLR4 is necessary for SFA-dependent induction of diseases such as atherosclerosis and the other diverse pathologies associated with SCD1 inhibition18,35,37 (Figure 3). Performing SCD1 inhibition studies in TLR4-deficient mice will no doubt provide useful insight into the necessity of TLR4 in promoting both
endogenous and dietary SFA-driven atherosclerosis and other inflammatory diseases.

One potential unifying mechanism driving the multiple pathologies seen under conditions of SCD1 deficiency may involve the previously documented function of SCD1 in modulating the formation of cholesterol- and SFA-rich membrane microdomains, better known as “lipid rafts.” It has previously been shown that overexpression of SCD1 in macrophages results in decreased abundance of lipid-ordered domains or lipid rafts. This finding correlates well with the recent report that mice lacking SCD1 in a leptin-deficient background have massive accumulation of free cholesterol and SFA in pancreatic β cells. Our study further supports this idea, given that SCD1 inhibition resulted in accumulation of free cholesterol and SFA in the liver (online-only Data Supplement Figure IIB and IID), aorta (Figure 3E through 3H), and isolated macrophages (data not shown). Collectively, these data suggest that SCD1 may play a crucial role in limiting accumulation of lipids (cholesterol and SFAs) known to segregate into membrane liquid-ordered domains, which could potentially alter membrane-associated signal transduction.

Conclusions

The present study demonstrates that inhibition of SCD1 protects against development of the metabolic syndrome but may promote atherosclerosis. These results do not support the idea that obesity and insulin resistance are causatively linked to atherosclerosis and argue against SCD1 inhibition as a safe therapeutic target for treatment of CVD.

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Disclosures

None.

References


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The metabolic syndrome has become a leading health concern in developed countries. Importantly, the presence of the metabolic syndrome has been shown to be a predictor of atherosclerotic cardiovascular disease extent in humans. Simply by catalyzing the conversion of long-chain saturated fatty acids to monounsaturated fatty acids, stearoyl-coenzyme A desaturase 1 (SCD1) has been shown to promote multiple aspects of the metabolic syndrome. Therefore, inhibition of SCD1 is currently regarded as a promising therapeutic strategy, yet little information exists on whether SCD1 inhibition should be approached with caution and that SCD1 inhibition may not necessarily be a treatment for atherosclerosis and its complications.
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