Stearoyl-CoA Desaturase Inhibits ATP-binding Cassette Transporter A1-mediated Cholesterol Efflux and Modulates Membrane Domain Structure*

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Liver X receptor/retinoid X receptor (LXR/RXR) transcription factors have been found to induce a number of genes involved in the regulation of cellular cholesterol efflux, including the ATP-binding cassette transporter A1 (ABCA1), which mediates the active efflux of cellular cholesterol and phospholipids to extracellular acceptors, such as apolipoprotein A-I (apoA-I). In a screen for macrophage LXR/RXR target genes, we identified stearoyl-CoA desaturases 1 and 2 (Scd1 and Scd2), and subsequently tested the hypothesis that SCD activity might modulate cellular cholesterol efflux. In HEK 293 cells co-transfection of ABCA1 with either SCD1 or SCD2 inhibited ABCA1-mediated cholesterol efflux but not phospholipid efflux. In Chinese hamster ovary (CHO) cells with moderate stable overexpression of SCD1, cholesterol efflux to apoA-I was inhibited by 73%, whereas phospholipid efflux and ABCA1 protein levels were unchanged. In contrast, cholesterol efflux to HDL2, which is not dependent on ABCA1, was increased 2-fold in CHO-SCD1 cells. The effect of SCD on cholesterol efflux to apoA-I was independent of acyl-CoA:cholesterol acyltransferase (ACAT) activity. SCD activity led to an increased content of plasma membrane monounsaturated fatty acids (18:1) at the expense of saturated fatty acids (18:0). As shown by confocal microscopy, SCD overexpression led to a decrease of Triton X-100-resistant domains in the plasma membrane, indicating a decrease in membrane-ordered regions. The data suggest that SCD changes membrane organization and depletes a specific pool of membrane cholesterol supporting ABCA1-mediated efflux, whereas increasing availability of cholesterol for passive efflux by HDL2, ABCA1-mediated cholesterol and phospholipid efflux may be uncoupled in pathological states associated with high SCD activity, as in hyperinsulinemic obese mice, or in animals treated with LXR agonists.

Cellular cholesterol efflux is central to the anti-atherogenic role of HDL1 and its apolipoproteins (1). Cholesterol efflux can involve several different pathways, including an active efflux to lipid-poor apolipoproteins mediated by ABCA1, and passive pathways mediated by diffusion through the aqueous medium or the binding of HDL to its receptor, scavenger receptor BI (SR-BI) (2, 3). Recent studies have identified ATP-binding cassette transporter A1 (ABCA1) as the mutant gene in patients with Tangier disease (4–6). The accumulation of cholesterol esters in the macrophages of Tangier disease patients indicates the key role of ABCA1 in mediating cellular cholesterol efflux. ABCA1 facilitates cholesterol and phospholipid efflux to extracellular lipid-poor apolipoproteins in liver, macrophages, and other tissues, initiating the formation of HDL (7, 8). Studies in Tangier disease patients and mouse models of ABCA1 overexpression or ablation suggest that ABCA1 has a protective role against atherosclerosis (9–13).

ABCA1 gene and protein expression are highly regulated on several different levels. Cholesterol efflux to apoA-I is increased in cells loaded with cholesterol (14). Cholesterol loading induces ABCA1 gene transcription, as a result of oxysterol activation of LXR (LXRα, LXRβ) that forms heterodimers with retinoid X receptor (RXR) on a direct repeat 4 element in the promoter of the ABCA1 gene (15). Emerging evidence suggests that ABCA1 is also regulated on a post-transcriptional level. Wang et al. (16) showed that polyunsaturated fatty acids reduced cellular ABCA1 protein content by enhancing ABCA1 degradation. In contrast, apoA-I and apoA-II stabilized ABCA1 protein without changing the mRNA, as a result of decreased degradation by thiols proteases (17).

The ability of ABCA1 to mediate cholesterol efflux is also modulated by trafficking and compartmentalization of cholesterol in cells. Overexpression of SR-BI in cells inhibits cholesterol efflux by the ABCA1 pathway, either by sequestering cholesterol in the membrane, or by promoting reuptake of newly effluxed cholesterol (18). In macrophages lipoprotein cholesterol deposited in late endosomes/lysosomes acts as a preferential source of cholesterol for ABCA1-mediated efflux (19). In these studies LXR/RXR activation increased cholesterol efflux to apoA-I, but the magnitude of the change was much less than the increase in ABCA1 protein levels. This finding raised the possibility that there might be other LXR/RXR target genes that could act to oppose ABCA1-mediated cholesterol efflux. In this study, we identified the LXR/RXR target gene, stearoyl-CoA desaturase; ACAT, acyl-CoA:cholesterol acyltransferase; SR-BI, scavenger receptor BI; LXR, liver X receptor; RXR, retinoid X receptor; SREBP, sterol regulatory element-binding protein; C6-NBD-SM, N-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoylphosphochole; Dil, dialkylindocarbocyanine; DMEM, Dulbecco's modified Eagle's medium; PBS, fetal bovine serum; BSA, bovine serum albumin.

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MATERIALS AND METHODS

Cell Culture—All cells were grown at 37 °C in a humidified 5% CO₂ incubator. Tissue culture reagents were from Invitrogen. All cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Transient and stable transfections were performed with LipofectAMINE 2000 (Invitrogen). The CHO-SCD1 cell line was established by transfecting CHO cells with pcDNA3.1/Hygro-mSCD1. Hygromycin-resistant clones were pooled for experiments. Total RNA with oligo(dT) primers were collected as described (19). 

Subtractive Hybridization—Selective subtractive hybridization was performed as described (20). Poly(A)(+) mRNA (2 µg) was made from treated or untreated thiglycolate-elicited mouse peritoneal macrophages. Selective subtractive hybridization was performed with a PCR-select cDNA subtraction kit (Clontech) according to the manufacturer’s recommendations with modifications (20). The subtracted cDNA libraries were generated by inserting the cDNA obtained after hybridization and PCR amplification into a cloning vector pCR-TOPO (Invitrogen). Individual clones were sequenced.

Northern Blot Analysis—Total RNA was extracted from macrophage using RNAzol (Tel-Test, Inc.). For Northern analysis, total RNA was separated on 1% agarose-formaldehyde gels (30 µg of RNA per lane), and transferred to Zeta-probe GT membranes (Bio-Rad). The membranes were hybridized with randomly primed 32P-labeled probes of cDNAs fragments overnight at 85 °C, and washed twice each for about 15 min at 65 °C with 0.2% SDS in 0.2× SSC. The membranes were then exposed to a phosphorimaging screen (Amersham Biosciences)

Quantitative Real Time PCR—RNA was treated with DNase I (DNA-free™, Ambion, Inc.). First strand cDNA was synthesized from 5 µg of DNAsfree-treated RNA with oligo(dT) primers using Superscriptase II (Invitrogen). cDNA from 6 individual samples was pooled for each condition for real time PCR analysis. Real time PCR was performed in triplicate and analyzed by using the MX4000 system (Stratagene).

Cytosol Cholesterol —To determine the level of cholesterol and its metabolites, erythrocyte membranes were isolated by differential centrifugation using an L8 ultracentrifuge (Beckman). Apolipoprotein B100 was isolated by differential centrifugation using Superspecies II (Invitrogen). Cholesterol was determined as described (18). The low density lipoprotein cholesterol was determined by enzymatic method.

Phospholipid Efflux—Cells were labeled with 1 µCi/ml [1,2-3H]cholesterol. Cells were then equilibrated in DMEM, 0.2% BSA, and then used for efflux experiments (2). The second method was with 10% FBS/DMEM. Cells were labeled with 1 µCi/ml [3H]-cholesterol in DMEM supplemented with 10% FBS for 24 h. The cells were then equilibrated in DMEM, 0.2% BSA for 30 min and then used for efflux experiments (2). For quantification, cells were treated as described above, with the exception of replacing 1% Triton X-100 with Medium 1 for control cells, and imaged on a Leica DMRB wide-field microscope (Leica Mikroskopie und Systeme GmbH, Germany) equipped with a cooled CCD camera (Frame Transfer MicroMax camera with a 512 × 512 back-thinned EEV chip, number 512BFP, Princeton Instruments) driven by Image-1/MetaMorph Imaging System software (Universal Imaging Corp., West Chester, PA). Images were acquired using a ×25 oil immersion objective (0.75 NA) to include a large number of cells in one field and to acquire fluorescence from the entire cell thickness. Dil C16 was imaged using a standard rhodamine filter set. Images were first background corrected (26), and then quantified by manually outlining each cell and taking the average fluorescence power associated with the cells. The ratio of average intensities obtained from the control and extracted cells of each cell line was determined as the percentage remaining cell-associated fluorescence. Statistical Analysis—Cholesterol and phospholipid efflux were expressed as the percentage of the radioactivity in the medium relative to the total radioactivity in cells and medium. Data shown are the average of at least three independent experiments with triplicates in each experiment unless specified otherwise.

Student’s t test.
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RESULTS

Stearoyl-CoA Desaturase Is Induced by LXR/RXR in Macrophages—In an attempt to identify genes that modulate the cholesterol efflux pathway, we carried out selective subtractive hybridization on mouse peritoneal macrophages treated with or without LXR/RXR activators, 22(R)-OH cholesterol (5 µM) and 9-cis-retinoic acid (10 µM). Among the genes induced by this treatment, we identified *scd2* (Fig. 1A). Scd1 and Scd2, expressed in an overlapping tissue distribution, catalyze the conversion of stearoyl-CoA to oleoyl-CoA and thereby regulate the ratio of monounsaturated to saturated fatty acids in cell membrane phospholipids (28). Quantitative real time PCR showed that both Scd1 and Scd2 are expressed in mouse peritoneal macrophages (11.7 and 35.4% of actin, respectively), and both were induced by treatment of macrophages with the synthetic LXR activator TO-901317 (1 nM) (Fig. 1B). Scd1 is 3-fold increase in SCD protein (the combined signal from Scd1 and Scd2) (Fig. 1D) and Scd2 protein, with no signal in control cells (Fig. 1D). In macrophages treated with LXR/RXR activators, there was a 5-fold induction of SCD protein (the combined signal from Scd1 and Scd2) (Fig. 1D). SCD activity is increased in the liver of ob/ob mice compared with wild type controls (Fig. 1E). SCD activity is increased in the liver of ob/ob mice compared with wild type controls (Fig. 1E). We next carried out experiments to evaluate SCD protein levels, we developed a specific SCD antibody using a peptide recognizing a common region in Scd1 and Scd2. As shown in 293 cells transfected with either Scd1 or Scd2, the antibody provided specific recognition of Scd1 and Scd2 protein, with no signal in control cells (Fig. 1C). In macrophages treated with LXR/RXR activators, there was a 5-fold induction of SCD protein (the combined signal from Scd1 and Scd2) (Fig. 1D), but no change in protein levels in mouse peritoneal macrophages (not shown).

SCD1 and SCD2 Specifically Inhibit ABCA1-mediated Cholesterol Efflux—We next carried out experiments to evaluate the effects of increased SCD levels on cellular cholesterol efflux. Transient co-expression of either Scd1 or Scd2 with ABCA1 in 293 cells resulted in a significant inhibition of cholesterol efflux to apoA-I (Fig. 2A). In contrast, phospholipid efflux to

Fig. 1. SCD mRNA and protein expression. A, peritoneal macrophages were isolated from C57BL/6 mice and plated in 6-well plates. Cells were treated with the indicated agents for 24 h. Total RNA was extracted and 30 µg was loaded in each lane. 22(R)-HC, 22(R)-hydroxycholesterol (5 µM); 9-cis-RA, 9-cis-retinoic acid (10 µM). B, macrophages treated with the synthetic LXR ligand TO-901317 (1 µM). cDNA from 6 individually treated wells was pooled for quantitative real time PCR. The values are the mean ± S.D. (from triplicates). C, total postnuclear lysates from HEK-293 cells transfected with the indicated plasmids were used to test SCD antibody. The membrane was also blotted with anti-FLAG M2 antibody (middle panel). D–F, SCD protein levels as detected by Western blot using a polyclonal antibody recognizing the C terminus of SCD. D, SCD expression in mouse peritoneal macrophages with or without LXR activation. Mouse peritoneal macrophages were treated with 5 µM 22(R)-hydroxycholesterol and 10 µM 9-cis-retinoic acid for 24 h. Postnuclear lysates were made and 75 µg of protein was loaded to each lane. E, SCD expression in wild type and ob/ob mouse liver postnuclear lysates. F, SCD expression in CHO and CHO-SCD1 cells.

Fig. 2. SCD 1 and SCD2 inhibit cholesterol efflux to apoA-I. A, HEK-293 cells were plated in 24-well plates and transiently transfected with the indicated plasmid constructs. Cells were labeled with [3H]cholesterol, 10% FBS for 24 h and equilibrated in DMEM, 0.2% BSA. Efflux was performed to 10 µg/ml apoA-I in DMEM, 0.2% for 20 h. *, p < 0.05 compared with cells transfected with ABCA1 alone. B, CHO and CHO-SCD1 were labeled with [3H]cholesterol, 10% FBS for 24 h and equilibrated in DMEM, 0.2% BSA. Efflux was performed to 10 µg/ml apoA-I in DMEM, 0.2% for 4 h. *, p < 0.05 compared with control CHO cells. Cholesterol efflux is [3H]cholesterol radioactivity in the medium divided by total radioactivity (cells plus medium) × 100. The values are the mean ± S.D.
apoA-I was slightly increased in cells expressing ABCA1 and either SCD1 or SCD2 (data not shown).

To further assess the role of SCD in modulating cellular cholesterol efflux, we developed CHO cell lines with stable expression of SCD1 (CHO-SCD1), and carried out cholesterol efflux experiments using a pool of clones. The CHO-SCD1 cells showed a moderate 4-fold increase in SCD protein expression (Fig. 1F), comparable with the relative SCD protein overexpression observed in macrophages treated with LXR/RXR activators, and in liver in ob/ob mice, compared with wild type mice (Fig. 1). Cholesterol efflux to apoA-I was decreased by 40% in CHO-SCD1 cells compared with control CHO cells (Fig. 2B).

In these experiments, cells were labeled by 24 h incubation with \[^{3}H\]cholesterol:PBS. In contrast, when cells were labeled by brief exposure to cyclodextrin, \[^{3}H\]cholesterol (30), there was a more pronounced 73% decrease in cholesterol efflux to apoA-I in CHO-SCD1 cells compared with control CHO cells (Fig. 3A). Time course experiments showed that the effect of SCD was observed throughout the 4-h efflux period (Fig. 3B). In contrast to cholesterol efflux, phospholipid efflux was slightly increased in CHO-SCD1 cells (Fig. 3C) and ABCA1 protein levels were identical in CHO and CHO-SCD1 cells (Fig. 3D).

In contrast to these findings, cholesterol efflux to HDL2, which is independent of ABCA1 (21), was increased 2-fold in CHO-SCD1 cells (Fig. 4A), and the effect was observed over a wide range of HDL concentrations (Fig. 4B). Cholesterol efflux to HDL can occur by passive diffusion (3) and can be facilitated by SR-BI (2). SR-BI protein levels were identical in control and SCD overexpressing cells (Fig. 4A), inset). Moreover, incubation of CHO cells with SR-BI neutralizing antibody showed only minor effects on cholesterol efflux, reflecting the low level of SR-BI protein in this cell type (2). Thus, SCD overexpression results in an increase in passive cholesterol efflux to HDL2, which is independent of ABCA1 and SR-BI.

**Mechanisms Underlying Inhibition of ABCA1-mediated Lipid Efflux by SCD**—Increased SCD activity is known to enhance cellular ACAT activity and apoA-I promotes cholesterol efflux from an ACAT-accessible pool (31, 32). Thus, we tested if an ACAT inhibitor would reverse the effect of SCD. However, at concentrations shown to be effective in inhibiting cellular cholesterol esterification (see “Materials and Methods”) the specific ACAT inhibitor, Dup128, did not alleviate the inhibitory effect of SCD on apoA-I-mediated cholesterol efflux (Fig. 5).

We next considered that in cells expressing SCD, ABCA1 might generate phospholipid-apoA-I complexes with a lower capacity to accept membrane cholesterol. To evaluate this possibility we conducted a media transfer experiment. Media con-
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containing complexes from control or SCD1 expressing CHO cells were transferred to a second set of cells (control or SCD1 expressing). These experiments showed that inhibition of cholesterol efflux was primarily a characteristic of cells expressing SCD1, and not of the media obtained from SCD1 expressing cells (Fig. 6).

The effects of SCD were less pronounced in cells subjected to a prolonged labeling procedure with FBS, [3H]cholesterol, compared with the rapid cyclodextrin labeling procedure (cf. Fig. 3 with Figs. 2 and 6). These results suggested that changes in plasma membrane composition or organization might be responsible for the effects of SCD. Cellular lipid measurements did not show any major differences in total cholesterol, free cholesterol, cholesterol ester, or phosphatidylcholine levels in control or SCD1 overexpressing cells (Table I). However, in SCD1 expressing cells there was a 71% increase in 18:1 to 18:0 ratio in plasma membrane phospholipid fatty acids (Table II). This -fold change in phospholipid fatty acids is comparable with that occurring in ob/ob liver compared with wild type liver (29).

Cholesterol-enriched membrane regions, known as liquid ordered domains or “rafts” (33), are formed from sphingolipids and glycolipids (34). Such regions are insoluble in Triton X-100. A new technique involving confocal microscopy of cells containing a fluorescent phospholipid marker (24) indicates that the major portion of plasma membrane is in the liquid ordered state, as shown by resistance to cold Triton X-100 extraction.

**TABLE I**

Lipid composition of CHO cells and CHO-SCD cells

| Lipid  | CHO      | CHO-SCD  |
|--------|----------|----------|
| TC (mg/mL) | 25.4 ± 1.3 | 23.7 ± 1.3 |
| FC (mg/mL) | 22.9 ± 1.5 | 20.0 ± 3.6 |
| CE (mg/mL) | 2.5 ± 1.6 | 2.8 ± 1.8 |
| PC (mg/mL) | 43.0 ± 3.3 | 41.4 ± 3.2 |

**Fig. 4.** SCD1 increases cholesterol efflux to HDL₅₀. Cells were labeled with [3H]cholesterol/cyclodextrin-cholesterol (8:1) complexes for 15 min. Efflux was performed to 15 μg/ml (A) or the indicated amounts (B) of HDL₅₀ in DMEM, 0.2% BSA for 4 h after equilibration in DMEM, 0.2% BSA. Cholesterol efflux is expressed as the percentage of total [3H]cholesterol radioactivity (cells plus medium) recovered in medium. The values are the mean ± S.D. *, p < 0.05 compared with CHO cells. The inset shows a Western blot of SR-BI.

**Fig. 5.** ACAT inhibitor does not reverse the effect of SCD1. Cells were labeled with [3H]cholesterol/cyclodextrin-cholesterol (8:1) complexes for 15 min. Dup128 (100 nM) or dimethyl sulfoxide were added to cells 24 h before efflux. Efflux was performed to 10 μg/ml apoA-I in DMEM, 0.2% BSA for 4 h after equilibration in DMEM, 0.2% BSA. Open bar, CHO cells; closed bar, CHO-SCD1 cells. *, p < 0.05 compared with CHO cells in same treatment.

**Fig. 6.** Inhibitory effect of SCD1 on cholesterol efflux is a feature of cells expressing SCD. Media transfer experiments were performed as described under “Materials and Methods.” The donor cells were depleted of cholesterol by incubating with 10 mM methyl-cyclo-dextrin for 30 min. Then the medium was changed to DMEM, 0.2% BSA containing 10 μg/ml apoA-I and was incubated for 4 h. This conditioned medium was transferred to [3H]cholesterol-labeled recipient cells for the efflux assay. The recipient cells were labeled with [3H]cholesterol, 10% FBS for 24 h and equilibrated in DMEM, 0.2% BSA. Efflux was performed for 4 h in conditioned medium, or to 10 μg/ml apoA-I in DMEM, 0.2% BSA as a control (first two bars). Open bar, CHO cell; closed bar, CHO-SCD1 cells. *, p < 0.05 compared with CHO cells in same treatment.
Methods.

Fatty acids were methyl esterified and determined by gas chromatography.

The portion of the plasma membrane is resistant to Triton X-100 (Fig. 7), resulting in the appearance of a small number of holes in the plasma membrane, indicating that in intact cells the major portion of the membrane is resistant to Triton X-100 (Fig. 7A), as reported (24). In CHO-SCD1 cells, there was a dramatic increase in the formation of Triton X-100-soluble regions, resulting in a “Swiss cheese” appearance (Fig. 7B). Thus, in SCD expressing cells liquid domains are increased, whereas liquid-ordered domains are decreased. Like the majority of plasma membrane proteins, ABCA1 has been reported to be present in Triton-soluble regions (24). Using antibodies against ABCA1-FLAG, we showed that the plasma membrane signal was abolished by treatment with cold Triton X-100, consistent with localization of ABCA1 in Triton-soluble regions (not shown) (35).

Scavenger receptor BI inhibits ABCA1-mediated cholesterol efflux (18), possibly as a result of direct binding of cholesterol to SR-BI or the re-uptake of effluxed cholesterol via SR-BI. SR-BI has been localized to Triton X-100-insoluble membrane regions (36). However, in CHO cells or CHO-SCD1 cells overexpressing SR-BI, there was no significant change in the formation of membrane liquid-ordered regions. SCD1 and SR-BI overexpression resulted in a comparable inhibition of cholesterol efflux, and in cells overexpressing both SCD1 and SR-BI the inhibition of cholesterol efflux was additive, indicating that SCD and SR-BI inhibit efflux by different mechanisms (Fig. 8).

TABLE II

Fatty acid composition of plasma membrane in CHO and CHO-SCD cells

| Fatty Acid | CHO | CHO-SCD |
|-----------|-----|---------|
| 8:0       | 10.6| 8.1     |
| 10:0      | N.D. | N.D.    |
| 12:0      | 5.2 | 3.5     |
| 14:0      | 18.5| 18.1    |
| 16:0      | 21.7| 24.6    |
| 16:1      | <1% | <1%     |
| 18:0      | 24.3| 19.0    |
| 18:1      | 20.0| 26.7    |

% of total detectable

CHO 10.6 N.D. 5.2 18.5 21.7 <1% 24.3 20.0
CHO-SCD 8.1 N.D. 3.5 18.1 24.6 <1% 19.0 26.7

* ND, nondetectable.

Discussion

In a screen for LXR target genes, we identified macrophage Scd1 and Scd2, similar to the earlier findings with hepatic Scd1 (37). To test the hypothesis that SCD might decrease cholesterol efflux mediated by ABCA1, we overexpressed SCD1 and SCD2 in 293 cells, and SCD1 in CHO cells, and showed a selective defective in ABCA1-mediated cholesterol efflux to apoA-I. The levels of overexpression of SCD1 protein were comparable with those induced by LXR/RXR activation, or by leptin deficiency in ob/ob mice. These findings suggest that SCD may be an important modulator of ABCA1-mediated cholesterol efflux in animals treated with LXR activators, or in different physiological states involving up-regulation of SCD. Mechanistic studies reveal that SCD alters plasma membrane phospholipid fatty acid composition and organization, decreasing the availability of cholesterol for efflux to apoA-I. In contrast, cholesterol efflux to HDL2 is increased by SCD1 overexpression, indicating a clear difference in availability of cholesterol efflux in the ABCA1 and passive diffusion pathways.

Cholesterol efflux to apoA-I and HDL are mediated by different pathways. Plasma HDL promotes cholesterol efflux through passive aqueous diffusion. This process may be SR-BI-dependent or -independent (3). This process is bidirectional and depends on a cholesterol concentration gradient between cell and lipoproteins (38). In this process, cholesterol must desorb from the lipid-water interface of plasma membrane before diffusing to and adsorbing into HDL, and the desorption step is rate-limiting. Lund-Katz et al. (39) showed that cholesterol desorbs more rapidly from unsaturated phosphatidylcholine bilayers than from saturated phosphatidylcholine bilayer because of greater Van der Waals attraction in the latter system. This may explain why cholesterol efflux to HDL2 is increased in CHO-SCD1 cells. Because SR-BI expression is unchanged by SCD, it is most likely the cholesterol desorption step that is enhanced in SCD1 expressing cells. The effects of SCD could
explain our previous finding that LXR/RXR activation increased cholesterol efflux to HDL₂ in mouse peritoneal macrophages (19), i.e. SCD expression was induced, resulting in increased efflux to HDL₂.

Our experiments add to the growing body of evidence that ABCA1-mediated phospholipid efflux can be dissociated from cholesterol efflux (21, 40, 41), and provide the first clear evidence showing that these processes may become uncoupled under physiological conditions. One interpretation of the dissociation of cholesterol from phospholipid efflux is that ABCA1 mediates lipid efflux in a two-step process, with initial formation of a phospholipid-apoA-I complex by ABCA1, followed by diffusion or insertion of cholesterol from a distinct cholesterol-enriched membrane microdomain. Our results could indicate decreased availability of cholesterol in membrane liquid-ordered domains and could be taken to support this model. However, the kinetics of phospholipid and cholesterol efflux are consistent with ABCA1 mediating coordinated efflux of cholesterol and phospholipid, possibly as a result of the formation of cholesterol-enriched microdomains contiguous with ABCA1 that are then solubilized by ABCA1-bound apolipoprotein (42). Moreover, ABCA1 is localized to membrane Triton X-100-soluble regions and mediates cholesterol efflux preferentially from these regions (Ref. 35, and this study). Thus, we favor an alternative interpretation that less cholesterol is available for efflux in the expanded Triton X-100-soluble domains containing ABCA1.

Because SCD activity is highly regulated, our findings provide the first evidence to suggest that ABCA1-mediated phospholipid and cholesterol efflux may sometimes be uncoupled in vivo. Under these circumstances formation of phospholipid-rich nascent HDL in hepatocytes could induce cholesterol efflux from other cells or tissues in a paracrine or endocrine fashion. Markedly increased SCD activity occurs in association with increased SREBP1c activity in the liver, as seen in SREBP1c transgenic mice (43), with fasting-refeeding (44) or in association with insulin resistance and hyperinsulinemia (29). Our data suggest that nascent HDL particles formed at the surface of hepatocytes by ABCA1 and apoA-I in insulin-resistant states (e.g. in ob/ob mice or in human metabolic syndrome) might be phospholipid-rich and cholesterol-poor. Such HDL particles would have an increased capacity to absorb cholesterol in the bloodstream, or in peripheral tissues from macrophage foam cells. Conversely, the decreased efflux of hepatic cholesterol via ABCA1 could lead to cholesterol accumulation in hepatocytes, possibly leading to increased cholesterol esterification or excretion into bile. Our observation that moderate changes in SCD substantially change membrane domain organization also raises the possibility that many different functions attributed to membrane liquid-ordered regions or rafts, such as nitric-oxide synthase activity (45), could be altered by changes in SCD expression.

In cells overexpressing SCD, ABCA1 protein is maintained and phospholipid efflux is slightly increased. This is different to the effects of the addition of exogenous fatty acids to cells (16). Thus, Wang and Oram (16) found that unsaturated fatty acids inhibited ABCA1-mediated phospholipid and cholesterol efflux by accelerating ABCA1 protein degradation. Our results also contrast with the inhibition of LXR-mediated gene transcription by exogenous polyunsaturated fatty acids (46). SCD overexpression led to increased incorporation of monounsaturated fatty acids into cellular phospholipids, whereas polyunsaturated fatty acids were unchanged (Table II). Endogenous and exogenous fatty acids function differently because of different cellular compartmentalization (31). SCD promotes formation of fatty acyl-CoAs that are readily incorporated into membrane phospholipids, whereas this may not be the case for exogously added unsaturated fatty acids.

LXR activators have emerged as therapeutic targets in the treatment of atherosclerosis (47). Our results indicate that the simultaneous up-regulation of ABCA1 and SCD by these treatments will have opposing effects on apoA-I-mediated cholesterol efflux. This may explain why such agents appear to have relatively modest effects on cholesterol efflux, despite marked up-regulation of ABCA1 (19). Because part of the increased expression of SCD likely results from an LXR-mediated increase in SREBP1c that in turn acts on the SCD promoter, LXR agonists that are selective for the ABCA1 promoter but not the SREBP1c promoter, would be predicted to have more favorable effects on macrophage cholesterol efflux via the ABCA1 apolipoprotein pathway, in addition to the benefit of not causing hypertriglyceridemia or fatty liver (29). Recently, mice deficient in SCD1 were shown to be resistant to dietary and genetic obesity (48, 49), suggesting that SCD inhibitors could be useful in the treatment of obesity. As noted above, inhibition of SCD activity in the liver is likely to have complex effects on cholesterol homeostasis and centripetal cholesterol transport. However, our results suggest that inhibition of SCD in the macrophage is likely to result in increased cholesterol efflux via the ABCA1 pathway and thus could be anti-atherogenic.

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