LXR-mediated activation of macrophage stearoyl-CoA desaturase generates unsaturated fatty acids that destabilize ABCA1

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Abstract **Abnormal HDL metabolism among patients with diabetes and insulin resistance may contribute to their increased risk of atherosclerosis. ATP binding cassette transporter A1 (ABCA1) mediates the transport of cholesterol and phospholipids from cells to HDL apolipoproteins and thus modulates HDL levels and atherogenesis. Because fatty acids are increased in diabetes, we examined their effects on ABCA1 activity in cultured macrophages. cAMP analogs and ligands for the liver X receptor/retinoid X receptor (LXR/RXR) system can induce *Abca1* transcription in murine macrophages. When induced by cAMP, unsaturated but not saturated long-chain fatty acids inhibit apolipoprotein-mediated lipid efflux by destabilizing ABCA1 protein. Here, we show that the saturated fatty acids palmitate and stearate also destabilize ABCA1 when *Abca1* is induced by LXR/RXR ligands instead of cAMP. This was associated with increased palmitate and stearate desaturation by stearoyl-CoA desaturase (SCD), another gene product induced by LXR/RXR ligands. The SCD inhibitors conjugated linoleic acid and troglitazone nearly abolished ABCA1 destabilization by palmitate and stearate but not by linoleate. These results suggest that LXR/RXR ligands generate ABCA1-destabilizing monounsaturated fatty acids from their saturated precursors by activating SCD. Thus, with cholesterol-loaded macrophages exposed to saturated fatty acids, activated LXR/RXR may counteract the enhanced *ABCA1* transcription by reducing the ABCA1 protein content.**—Wang, Y., B. Kurdi-Haidar, and J. F. Oram. **LXR-mediated activation of macrophage stearoyl-CoA desaturase generates unsaturated fatty acids that destabilize ABCA1. *J. Lipid Res.* 2004.** 45: **972–980.**

Supplementary key words liver X receptor • ATP binding cassette transporter A1 • coenzyme A

Population studies have shown an inverse relationship between plasma HDL levels and risk for cardiovascular disease, implying that factors associated with HDL metabolism are atheroprotective. One of these factors is a cell membrane transporter called ATP binding cassette transporter A1 (ABCA1), which mediates the transport of excess cholesterol from cells to HDL apolipoproteins (1, 2). *ABCA1* mutations can cause Tangier disease (3, 4), a severe HDL deficiency syndrome characterized by cholesterol deposition in tissue macrophages and prevalent atherosclerosis (5–7). Genetic manipulations of ABCA1 expression in mice also affect plasma HDL levels and atherogenesis (8, 9).

ABCA1 expression is tightly regulated. Cholesterol loading of macrophages dramatically increases ABCA1 mRNA and protein levels (10, 11), consistent with a transporter that functions to rid cells of excess cholesterol. This regulation is mediated by nuclear liver X receptor (LXRα or LXRβ) and retinoid X receptor (RXR) (12, 13), which form heterodimers that are activated by oxysterols and retinoic acid, respectively (14). Analogs of cAMP also activate *Abca1* transcription in cultured murine macrophages by mechanisms distinct from the LXR/RXR system (15, 16).

Because of their robust transcriptional regulation of *ABCA1*, LXRs have become attractive new targets for drug development. One problem with current LXR agonists, however, is that they also induce enzymes of fatty acid production and desaturation, thus generating fatty livers and hypertriglyceridemia when administered to animals (17–19). The concept of targeting ABCA1 transcription to enhance cholesterol efflux from macrophages has also come under question. A study with mice revealed that there was a major discordance between ABCA1 mRNA and protein levels among tissues (20). This implicates posttranscriptional regulation of ABCA1 expression as playing a critical role in the overall activity of this pathway. This may also explain why linkage studies have suggested that the ABCA1 gene is a rare marker for people with low HDL lev-

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els and increased cardiovascular disease (21–23). It is possible that metabolic factors that reduce ABCA1 protein levels or impair activity contribute to the phenotype of this population.

In an attempt to identify metabolic factors that modulate ABCA1 expression or activity, we previously tested the effects of long-chain fatty acids on the ABCA1 pathway in cultured murine macrophages (24). Because of the possible confounding effects of LXR/RXR ligands on fatty acid metabolism, we induced ABCA1 in these cells with a cAMP analog. These studies showed that monounsaturated, diunsaturated, and polyunsaturated fatty acids reduced apolipoprotein-mediated cholesterol and phospholipid efflux from these cells by destabilizing ABCA1 protein. In contrast, the unsaturated fatty acids palmitate and stearate had no effect on ABCA1 protein levels or activity.

One of the transcriptional targets for LXR is stearoyl-CoA desaturase (SCD), which catalyzes the conversion of palmitoyl-CoA and stearoyl-CoA to palmitoleoyl-CoA and oleoyl-CoA, respectively (17, 25, 26). We reasoned that, when macrophages were treated with LXR/RXR ligands instead of a cAMP analog to induce *Abca1*, palmitate and stearate would become ABCA1 destabilizers after desaturation by activated SCD. This assumption was supported by a recent study showing that stearate as well as unsaturated fatty acids reduced ABCA1 protein levels in CaCo2 cells when *ABCA1* was induced by a LXR agonist (27). Here, we present evidence that LXR/RXR ligands activate macrophage SCD to generate ABCA1-destabilizing monounsaturated fatty acids from their saturated precursors.

EXPERIMENTAL PROCEDURES

Lipoproteins and apolipoprotein A-I

LDL and HDL were prepared by sequential ultracentrifugation in the density range 1.019–1.063 and 1.125–1.21 g/ml, respectively, and HDL was depleted of apolipoprotein E (apoE) and apoB by heparin-agarose chromatography (28). ApoA-I was purified from HDL and delipidated as described previously (28). LDL was acetylated by the method of Goldstein et al. (29).

Cell culture and lipid efflux

Murine J774 cells were maintained in DMEM (Invitrogen) containing 10% fetal bovine serum or incubated in serum-free DMEM plus 1 mg/ml fatty acid-free BSA (DMEM/BSA). To radiolabel cellular cholesterol, 1 μCi/ml [^{3}H]cholesterol (NEN Life Science Products) was added with 50 μg/ml acetylated LDL to DMEM/BSA during the 24 h immediately preceding treatments (15). Cells were then washed once with PBS containing 1 mg/ml BSA and incubated for 16–20 h with medium containing 5 mg/ml BSA and 0–0.25 mM fatty acids (molar ratios to BSA of 0–3.5) (24). To induce ABCA1, 0.5 mM 8-Br-cAMP, 10 μM 22(*R*)-hydroxycholesterol (Sigma), 10 μM 9-*cis*-retinoic acid (Sigma), or 2 μM T0901317 was added alone or in combination to this medium. To label phospholipids, 1 μCi/ml [methyl-^{3}H]choline chloride (NEN Life Science Products) was added to this medium followed by two washes of cells (24).

To measure lipid efflux, cells were incubated with DMEM/BSA with or without 10 μg/ml apoA-I for 2 h at 37°C and chilled on ice, and the medium was collected and centrifuged to remove detached cells. For cholesterol efflux, the medium was counted for ^{3}H and the cells were assayed for free and esterified [^{3}H]cholesterol after isolation by thin-layer chromatography (24). For phospholipid efflux, medium and cellular choline-labeled phospholipids were extracted in chloroform-methanol and assayed for ^{3}H radioactivity (24). ApoA-I-mediated lipid efflux is expressed as the fraction of ^{3}H-lipid released into the medium after subtraction of values obtained in the absence of apoA-I.

Metabolically labeled ABCA1 and immunoblot analysis

Macrophages were metabolically labeled by incubation for 15 min at 37°C with DMEM/BSA containing 100 μCi/ml [^{35}S]methionine (Amersham Pharmacia Biotech, Inc.). ABCA1 was isolated from 1% Triton X-100 digests of cells by immunoprecipitation and SDS-PAGE as described (10, 24). Each gel lane received immunoprecipitated protein corresponding to equal amounts of cells. [^{35}S]methionine-labeled ABCA1 was detected on gels by phosphorimaging (Cyclone; Packard Instrument Co.). For immunoblots of whole membrane ABCA1, microsomal membranes were isolated from homogenized cells by ultracentrifugation, membrane proteins were solubilized in SDS buffer and resolved by SDS-PAGE, and ABCA1 was identified by immunoblot analysis (15). Equal amounts of membrane protein were added per gel lane.

SCD activity

SCD activity was determined as described (30). J774 macrophages were incubated with 50 μM BSA-bound [1-^{14}C]palmitic acid (specific activity, 50 mCi/mmol) for 6 h. Lipids were extracted with chloroform-methanol. Fatty acid methyl esters were prepared by incubating cell extracts with 4% HCl/methanol at 60°C for 20 min, and fatty acyl groups were separated by thin-layer chromatography on silver nitrate-impregnated silica gel G plates using a mixture of hexane-diethyl ether (9:1). After visualization by dichlorofluorescein, corresponding spots were extracted with hexane and subjected to liqid scintillation counting.

Quantitation of ABCA1 mRNA by real-time RT-PCR

One-step real-time RT-PCR was conducted using the LightCycler (Roche) and 40 ng of total cellular RNA, prepared using the Genelute™ Mammalian Total RNA preparation kit (Sigma-Aldrich) according to the manufacturer's instructions. SYBR Green I-based real-time RT-PCR was carried out using 1 U of TaKaRa LA Taq™ polymerase (Panvera), 1× LA PCR™ Buffer II, 0.5 mM deoxynucleoside triphosphate, a 1:40,000 dilution of SYBR Green I dye (Roche), 0.5 μM each forward and reverse primers, and 2.5 U of the reverse transcriptase SuperScript™ II in a 10 μl reaction volume. The experimental LightCycler program started with a reverse transcription step of 15 min at 55°C followed by 2 min at 94°C and 33 cycles of amplification. Each cycle of amplification consisted of a denaturation step at 94°C for 15 s, an annealing step at 62°C for 20 s, and an extension step at 72°C for 40 s. The program was concluded with melting analysis to verify the specificity of amplification products. RT-PCR of ABCA1 was performed using the forward (5′-GCCTTGGCAGTGTCCAACATC-3′) and reverse (5′-TGTCTGAGAGACAGAGTAGTCTTC-3′) primers amplifying a 263 bp cDNA-specific fragment (ABCA1 amplicon). RT-PCR of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the control housekeeping gene, was performed using the forward (5′-GGTGCTGAGTATGTCGTGGAGTC-3′) and reverse (5′-CACAGTCTTCTGGGTGGCAGTG-3′) primers amplifying a 295 bp cDNA-specific fragment (GAPDH amplicon). The number of mRNA molecules per 40 ng of total cellular RNA in real-time RT-PCR was calculated against gene-specific standards consisting of dilutions containing fixed numbers of ei-

ther ABCA1 or GAPDH amplicon molecules. The quantification data obtained are presented as ABCA1 mRNA molecules normalized to the mRNA molecules of GAPDH.

ABCA1 degradation rate

J774 macrophages were cholesterol loaded with acetylated LDL for 24 h. After overnight incubation with DMEM/BSA containing 10 μM 22(*R*)-hydroxycholesterol and 10 μM 9-*cis*-retinoic acid, cells were washed and incubated for 2 h at 37°C in DMEM containing 5 mg/ml BSA alone or with 125 μM palmitate in the presence or absence of 20 μM troglitazone followed by incubations with the same medium containing 20 μg/ml cycloheximide. After 0, 1, 2, 3, and 4 h, ABCA1 was measured by immunoblot analysis (24).

RESULTS

Long-chain saturated and unsaturated fatty acids inhibit apoA-I-mediated lipid efflux in the presence of LXR/RXR ligands

Either cAMP analogs (15, 16) or LXR/RXR ligands induce ABCA1 expression in murine macrophages (24), thus markedly increasing apoA-I-mediated cholesterol and phospholipid efflux. We used real-time RT-PCR to measure the effects of these inducers on ABCA1 mRNA levels in cholesterol-loaded J774 macrophages (**Fig. 1A**). Consistent with previous results (15, 16), 8-Br-cAMP dramatically increased ABCA1 mRNA levels. The LXR ligand 22(*R*)-hydroxycholesterol increased ABCA1 mRNA by 30%, but this did not reach statistical significance compared with controls. The RXR ligand 9-*cis*-retinoic acid and the noncholesterol LXR agonist T0901317 each significantly increased ABCA mRNA levels. The combination of 9-*cis*-retinoic acid and 22(*R*)-hydroxycholesterol or T0901317 further increased ABCA1 mRNA levels. A similar pattern was observed when we measured the effects of LXR and RXR ligands on ABCA1 protein levels (Fig. 1B) and apoA-I mediated cholesterol efflux (Fig. 1C).

We showed previously that when ABCA1 was induced by 8-Br-cAMP, unsaturated but not saturated fatty acids inhibited apoA-I-mediated lipid efflux from J774 macrophages (24). To examine the effects of fatty acids when ABCA1 was induced by LXR/RXR ligands, we incubated cholesterol-loaded murine J774 macrophages with 22(*R*)-hydroxycholesterol and 9-*cis*-retinoic acid for 16 h in the absence or presence of different fatty acids and measured apoA-I-mediated [^{3}H]cholesterol and [^{3}H]phospholipid efflux. Similar to our previous results with 8-Br-cAMP-treated cells, the unsaturated fatty acids palmitoleate, oleate, linoleate, and arachidonate decreased apoA-I-mediated cholesterol (**Fig. 2A**) and phospholipid (Fig. 2B) efflux by 30–40% when ABCA1 was induced by 22(*R*)-hydroxycholesterol plus 9-*cis*-retinoic acid (Fig. 2A, B). In contrast to our previous findings, however, the saturated fatty acids palmitate and stearate also significantly decreased apoA-I-mediated cholesterol and phospholipid efflux. When we substituted the LXR agonist T0901317 for 22(*R*)-hydroxycholesterol, we observed the same inhibitory effects of long-chain fatty acids on cholesterol (Fig. 2C) and phospholipid efflux (Fig. 2D).

LXR/RXR agonists increase SCD activity in macrophages

One explanation for these findings is that, unlike cAMP analogs, LXR/RXR activators induce SCD in these cells, thus generating ABCA1-destabilizing palmitoleoyl-CoA and oleoyl-CoA from palmitoyl-CoA and stearoyl-CoA, respectively. To initially test this possibility, we examined the effects of LXR/RXR agonists on SCD activity by measuring the cellular content of radiolabeled palmitoleoyl and

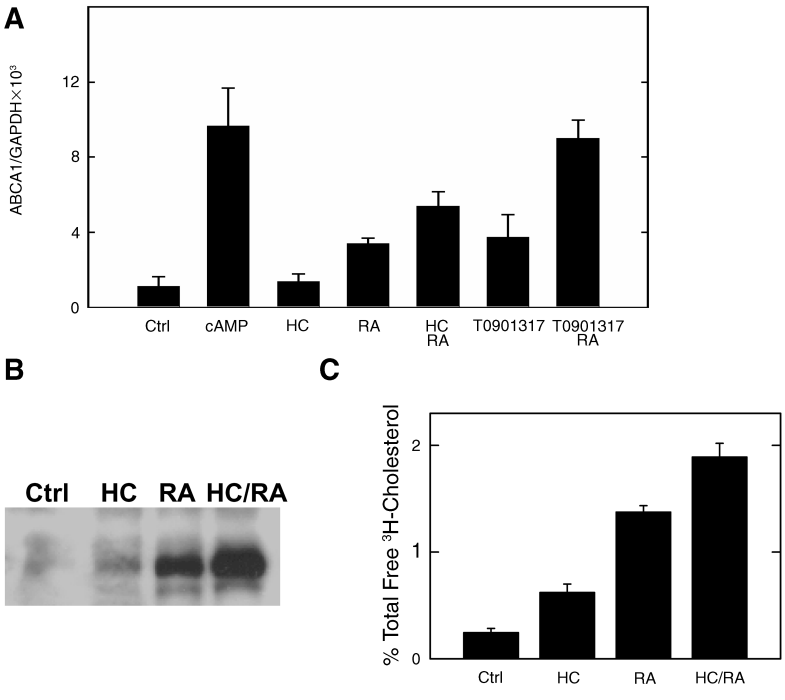

Fig. 1. A cAMP analog and liver X receptor/retinoid X receptor (LXR/RXR) ligands activate ATP binding cassette transporter A1 (ABCA1) in cholesterol-loaded J774 macrophages. Macrophages were incubated for 24 h with 50 μg/ml acetylated LDL and 1 mg/ml BSA with or without 0.5 mM 8-Br-cAMP (cAMP), 10 μM 22(*R*)-hydroxycholesterol (HC), 10 μM 9-*cis*-retinoic acid (RA), or 10 μM T0901317. Cells were then incubated with DMEM/BSA for 16 h. A: mRNA isolation and quantitation were conducted as described in Experimental Procedures. Each value is the mean ± SD of triplicate determinations. B: Membrane ABCA1 protein levels were assayed by immunoblot analysis. C: Apolipoprotein A-I (apoA-I)-mediated [^{3}H]cholesterol efflux was measured during subsequent 2 h incubations as described in Experimental Procedures. Each value is the mean ± SD of triplicate determinations. Results are representative of at least two similar experiments. Ctrl, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

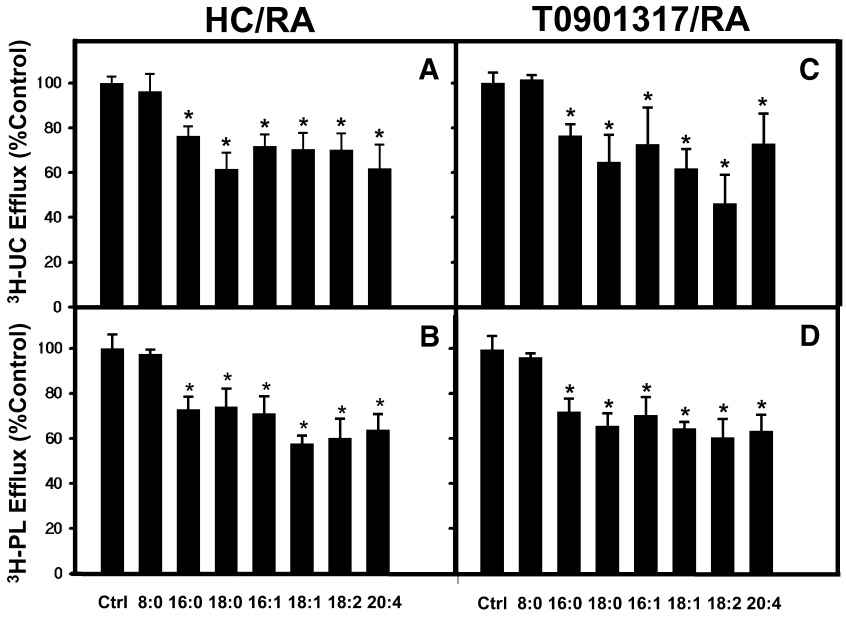

Fig. 2. Long-chain fatty acids inhibit lipid efflux. A and B: Cholesterol-loaded J774 macrophages were incubated for 16 h with 10 μM 22(*R*)-hydroxycholesterol plus 10 μM 9-*cis*-retinoic acid (HC/RA) with either 5 mg/ml BSA alone or BSA plus 125 μM of the indicated fatty acids (BSA molar ratio of 1.8). ApoA-I-mediated [^{3}H]cholesterol (UC; A) and [^{3}H]phospholipid (PL; B) efflux were measured during subsequent 2 h incubations. C and D: Cholesterol-loaded J774 macrophages were incubated for 16 h with 2 μM T0901317 and 10 μM 9-*cis*-retinoic acid with either 5 mg/ml BSA alone or BSA plus the indicated fatty acids (BSA molar ratio of 1.8). ApoA-I-mediated [^{3}H]cholesterol (UC; C) and [^{3}H]phospholipid (PL; D) efflux were measured during subsequent 2 h incubations. Each value is the mean ± SD of triplicate determinations. Asterisks indicate significant ($P < 0.03$) differences from controls (Ctrl).

oleoyl groups after incubating cells with [^{14}C]palmitate and [^{14}C]stearate, respectively. Treatment of cells with 8-Br-cAMP had no effect on the conversion of these fatty acids to their monounsaturated products (**Fig. 3**). In contrast, treatment with 22(*R*)-hydroxycholesterol plus 9-*cis*-retinoic acid increased this conversion greater than 2-fold. A similar increase occurred when the LXR agonist T0901317 was substituted for 22(*R*)-hydroxycholesterol. Because SCDs exclusively catalyze this conversion (30), these results indicate that LXR/RXR ligands stimulate SCD activity in these cells.

Conjugated linoleic acids and thiazolidinediones have been reported to suppress SCD expression or activity in cultured cells (30–36). We found that incubating cholesterol-loaded J774 macrophages with either *trans*-10,*cis*-12-conjugated linoleic acid (CLA) or the thiazolidinedione troglitazone (TRO) reversed the LXR/RXR ligand-induced increase in the conversion of palmitate and stearate to their monounsaturated forms (Fig. 3). Thus, both CLA and TRO inhibit SCD activity in these cells.

Palmitate and stearate reduce ABCA1 activity and protein levels in association with LXR/RXR-activated SCD

When we added either CLA or TRO to medium containing 22(*R*)-hydroxycholesterol plus 9-*cis*-retinoic acid, the inhibitory effects of palmitate and stearate on apoA-I-mediated cholesterol (**Fig. 4A**) and phospholipid (Fig. 4B) efflux were reversed. In contrast, the addition of CLA or TRO had no influence on the inhibitory effects of linoleate, consistent with a specific action on SCD.

Immunoblot analysis showed that incubating 22(*R*)-hydroxycholesterol/9-*cis*-retinoic acid-treated cells with palmitate, stearate, or linoleate reduced ABCA1 protein levels (Fig. 4C). The addition of CLA or TRO largely prevented the reduction caused by palmitate and stearate but not by linoleate. Thus, the inhibitory effects of palmitate and stearate on apoA-I-mediated lipid efflux and its reversal by SCD inhibitors were associated with changes in ABCA1 protein levels. These effects on ABCA1 activity and expression were observed over a physiologic molar ratio of palmitate to albumin (<2) (**Fig. 5**). These results support the concept that, in LXR/RXR-activated macrophages, saturated fatty acids are converted to monounsaturated fatty acids that in turn reduce ABCA1 protein levels.

Unsaturated fatty acids enhance ABCA1 degradation in LXR/RXR ligand-treated macrophages

We previously demonstrated that unsaturated fatty acids stimulate ABCA1 degradation in the presence of a cAMP analog (24). To identify the mechanism by which saturated fatty acids reduce ABCA1 protein levels in the presence of LXR/RXR ligands, we measured the effects of fatty acids on ABCA1 protein synthesis and degradation. To assay translation efficiency, we pretreated cells for 16 h with or without fatty acids, incubated cells for 15 min with [^{35}S]methionine, and measured the incorporation of radiolabel into immunoisolated ABCA1. Pretreating cells with

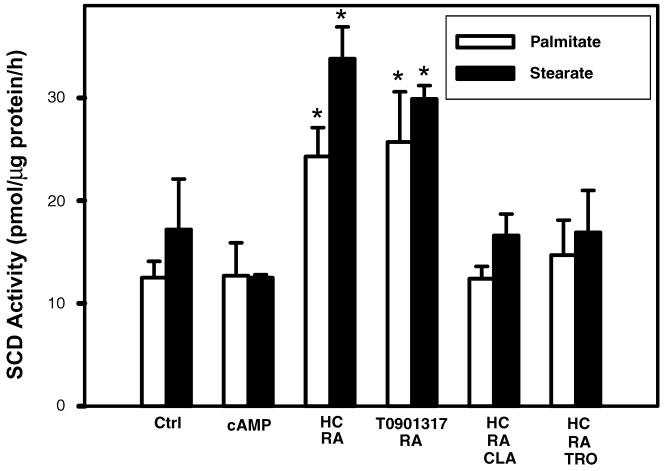

Fig. 3. LXR/RXR ligands activate stearoyl-CoA desaturase (SCD). Cholesterol-loaded macrophages were incubated for 16 h with 0.5 mM 8-Br-cAMP (cAMP), 10 μM 22(*R*)-hydroxycholesterol (HC) plus 10 μM 9-*cis*-retinoic acid (RA), or 2 μM T0901317 plus 10 μM 9-*cis*-retinoic acid (RA) with or without 50 μM conjugated linoleic acid (CLA) or 20 μM troglitazone (TRO) as indicated. A total of 50 μM BSA-bound [1-^{14}C]palmitate or [1-^{14}C]stearate (specific activity, 50 mCi/mmol) was then added to the same medium and incubated for 6 h. Assay for the ^{14}C incorporation into palmitoleate and oleate was conducted as described in Experimental Procedures. Each value is the mean ± SD of triplicate determinations. Ctrl, control.

palmitate, linoleate, or palmitate plus TRO did not significantly affect the incorporation of methionine into ABCA1 (**Fig. 6A**). Therefore, inhibition of transcription or translation efficiency cannot account for the fatty acid-induced decrease in ABCA1 protein levels.

To address the possibility that ABCA1 degradation rates are affected by fatty acids, we incubated 22(*R*)-hydroxycholesterol/9-*cis*-retinoic acid-treated cells with or without palmitate and TRO for 2 h, added cycloheximide to arrest protein synthesis, and monitored changes in ABCA1 levels during the cycloheximide treatment. After the 2 h preincubations with palmitate or palmitate plus TRO, there were no detectable differences in ABCA1 content between control and treated cells (Fig. 6B). After 1–3 h with cycloheximide, however, the palmitate-treated cells had lower ABCA1 compared with control cells and cells treated with palmitate plus TRO. After 4 h with cycloheximide, all detectable ABCA1 was degraded in control and treated cells, consistent with the rapid turnover of ABCA1 reported previously (24). These results show that palmitate enhances the rate of ABCA1 degradation in these LXR/RXR-activated macrophages by a TRO-inhibited process.

ApoA-I stabilizes ABCA1 protein by inhibiting calpain-mediated proteolysis (37, 38). To test the possible involvement of calpain in the fatty acid-enhanced degradation of ABCA1, we measured the effects of fatty acids on ABCA1 protein levels in the absence or presence of apoA-I or the calpain inhibitor calpeptin. Incubating cells with apoA-I alone increased the membrane ABCA1 content (Fig. 6C), consistent with a protein-stabilizing effect. The addition of either palmitate or linoleate decreased the ABCA1 content whether or not apoA-I was present. Treating cells with calpeptin dramatically increased ABCA1 levels, but the addition of linoleate still reduced these levels (Fig. 6D). These results indicate that fatty acids enhance ABCA1 degradation even when it is stabilized by apolipoproteins or calpeptin.

DISCUSSION

Our previous studies showed that, when ABCA1 is induced in cultured macrophages by a cAMP analog, monounsaturated and polyunsaturated long-chain fatty acids destabilize ABCA1 protein and impair the transport of excess cholesterol from cells to apoA-I (24). In contrast, the saturated long-chain fatty acids palmitate and stearate have no effect on ABCA1 activity or expression in these cells. Here, however, we show that palmitate and stearate

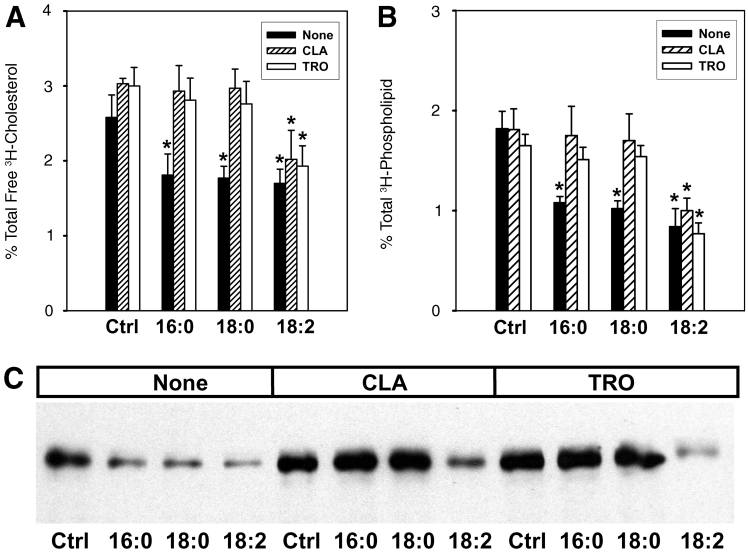

Fig. 4. SCD inhibitors attenuate the ABCA1 inhibitory effects of palmitate and stearate. Cholesterol-loaded J774 macrophages were incubated for 16 h with 10 μM 22(*R*)-hydroxycholesterol plus 10 μM 9-*cis*-retinoic acid with either 5 mg/ml BSA alone or BSA plus the indicated fatty acids (BSA molar ratio of 1.8) with or without 50 μM CLA or 20 μM TRO as indicated. ApoA-I-mediated [^{3}H]cholesterol (A) and [^{3}H]phospholipid (B) efflux were measured during subsequent 2 h incubations as described in Experimental Procedures. Each value is the mean ± SD of triplicate determinations. Membrane ABCA1 protein levels (C) were assayed by immunoblot analysis. Results are representative of two similar experiments. Ctrl, control.

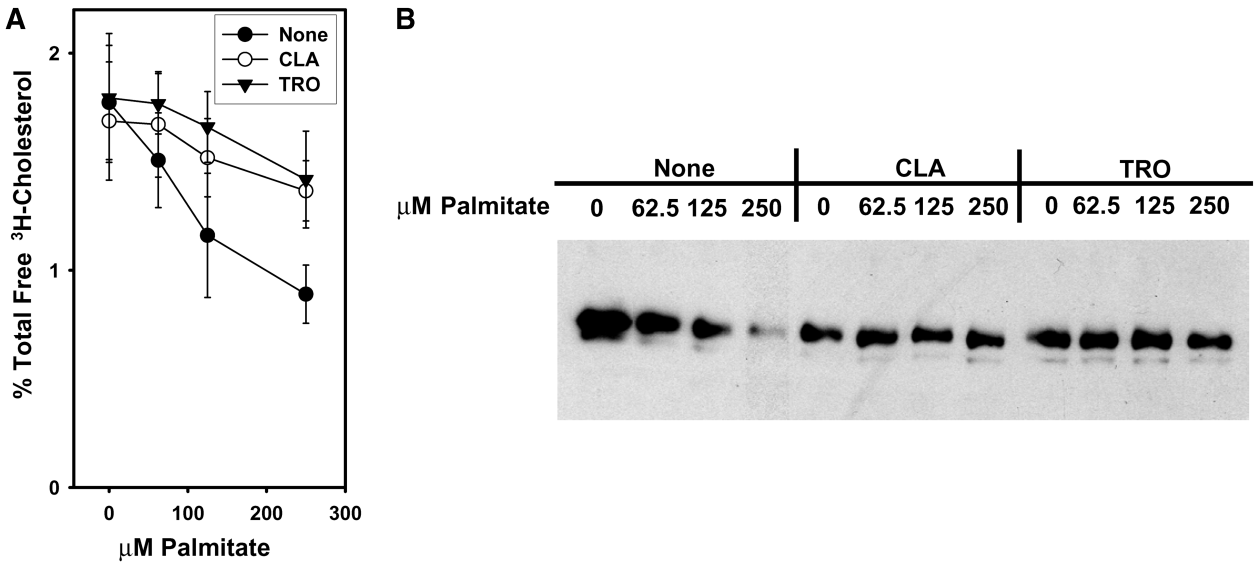

Fig. 5. Dose-dependent inhibitory effects of palmitate on ABCA1 activity and protein levels and their reduction by CLA and TRO. Cholesterol-loaded macrophages were incubated for 16 h with 10 μM 22(*R*)-hydroxycholesterol plus 10 μM 9-*cis*-retinoic acid and 5 mg/ml BSA plus 0–250 μM palmitate with or without 50 μM CLA or 20 μM TRO. ApoA-I-mediated [^{3}H]cholesterol efflux (A) was measured during subsequent 2 h incubations (mean ± SD, n = 3), and ABCA1 protein levels (B) were measured by immunoblot analysis. Results are representative of two similar experiments.

also destabilize ABCA1 when ABCA1 is induced by LXR/RXR ligands instead of a cAMP analog.

Desaturation of palmitate and stearate by SCD is likely to account for the destabilizing effects of these fatty acids on ABCA1 protein. With LXR/RXR ligand-treated macrophages, palmitate and stearate mimicked unsaturated fatty acids in inhibiting ABCA1-dependent cholesterol and phospholipid efflux and enhancing ABCA1 protein degradation. Treatment of cells with LXR/RXR ligands, but not with a cAMP analog, increased the conversion of palmitate and stearate to palmitoleate and oleate, respectively. This conversion occurs exclusively by an enzymatic process catalyzed by SCD (25). Palmitate and stearate desaturation and their effects on ABCA1 were blocked by CLA and TRO, two compounds shown previously to inhibit SCD in cultured cells (30–36). In contrast, CLA and TRO had no effect on the ability of linoleate to reduce ABCA1 activity or protein levels. Thus, the ability of saturated but not unsaturated fatty acids to destabilize ABCA1 was associated with SCD activity. These findings also imply that this destabilization requires the conversion of fatty acids to their acyl-CoA derivatives, as these are the substrates for SCD.

Murine macrophages express SCD1 and SCD2 isoforms, both of which are induced by LXR/RXR ligands (26). Although the relative specificity may differ, these isoforms appear to catalyze the desaturation of both palmitoyl-CoA and stearoyl-CoA to palmitoleoyl-CoA and oleoyl-CoA, respectively. CLA has been shown to suppress SCD expression and/or activity, depending on the cell type (30–34). Thiazolidinediones such as TRO have been shown to suppress *Scd1* transcription in murine adipocytes (35, 36), presumably through the activation of peroxisome proliferator-activated receptor γ (PPARγ). Although CLA and TRO may affect multiple cellular processes, it is likely that their inhibition of SCD is responsible for abolishing the ABCA1-destabilizing effects of palmitate and stearate. Both CLA and TRO appear to selectively inhibit SCD1 (31, 36), suggesting that this SCD isoform plays the major role in generating ABCA1-destabilizing fatty acids in these cells.

A previous study showed that cotransfection of cells with ABCA1 and either SCD1 or SCD2 perturbed plasma membrane lipid domains and inhibited ABCA1-dependent cholesterol efflux without affecting phospholipid efflux or ABCA1 protein levels (26). This study differed from ours in that the experiments were performed in the absence of exogenous fatty acids. The level of endogenous unsaturated fatty acids generated by SCD may have been too low to affect ABCA1 stability. Moreover, SCD-mediated alterations of plasma membrane phospholipids may be less dramatic in the presence of exogenous fatty acids. Nevertheless, these studies show that SCD has the potential to disrupt the ABCA1 pathway by several mechanisms.

Our studies have consistently shown that fatty acids reduce ABCA1 protein to a greater extent than they inhibit apoA-I-mediated lipid efflux. There are two experimental conditions that may contribute to this disparity. First, to avoid the complications of their interacting with apoA-I in solution, we performed the 2 h lipid efflux incubations in the absence of fatty acids. This would allow some recovery of ABCA1 protein. Second, we found that apoA-I-mediated lipid efflux tends to saturate with increasing expression of ABCA1. Thus, ABCA1 levels may need to decrease below a threshold before there is a large reduction in lipid efflux. For macrophages expressing moderate to high lev-

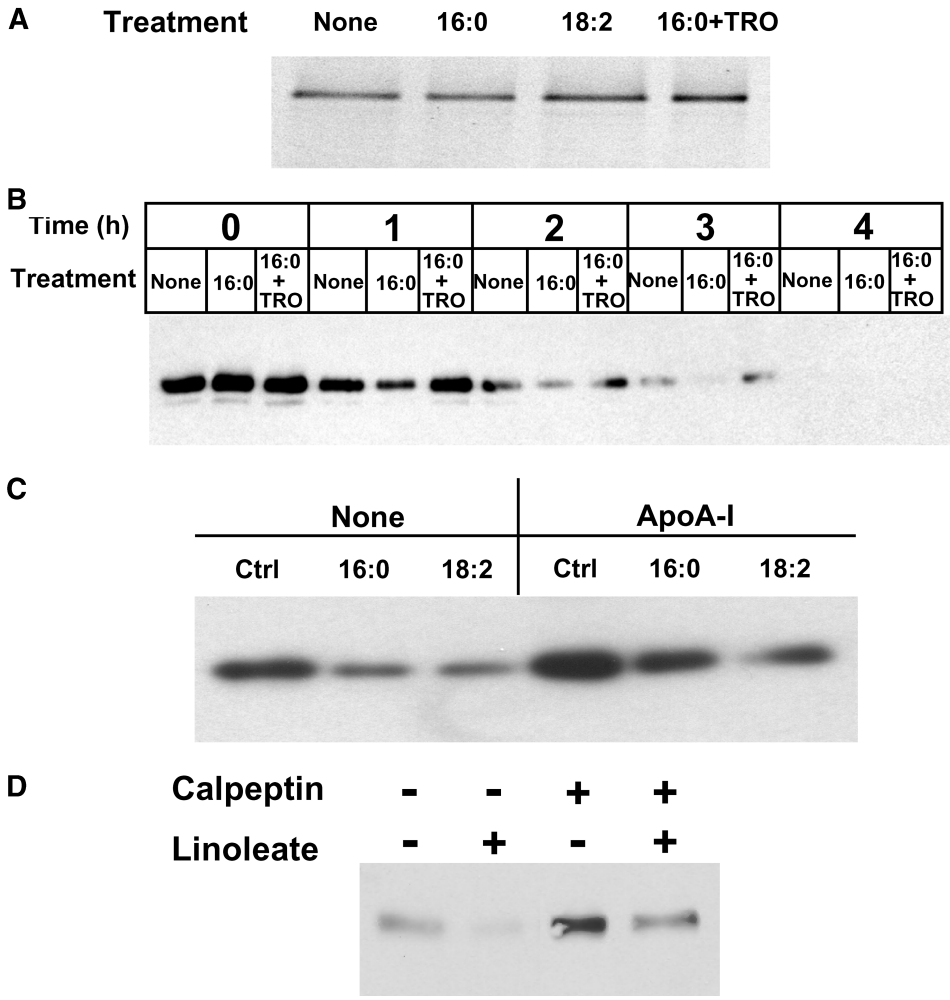

Fig. 6. Fatty acids enhance ABCA1 degradation in the absence or presence of apoA-I or a calpain inhibitor. A: Cholesterol-loaded macrophages were incubated for 16 h with 10 μM 22(*R*)-hydroxycholesterol plus 10 μM 9-*cis*-retinoic acid in the absence or presence of 125 μM palmitate, 125 μM linoleate, or palmitate plus 20 μM TRO. Cell proteins were radiolabeled with [^{35}S]methionine (100 μCi/ml) for 15 min, ABCA1 was isolated by immunoprecipitation and SDS-PAGE, and ^{35}S-labeled ABCA1 was detected by phosphorimaging. B: Cholesterol-loaded, 22(*R*)-hydroxycholesterol/9-*cis*-retinoic acid-treated macrophages were incubated for 2 h with 10 μM 22(*R*)-hydroxycholesterol plus 10 μM 9-*cis*-retinoic acid in the absence or presence of 125 μM palmitate with or without with 20 μM TRO. Cycloheximide (20 μg/ml) was added to the medium, and membrane ABCA1 levels were assayed by immunoblot analysis after an additional 0, 1, 2, 3, and 4 h. C: Macrophages were incubated for 16 h with 22(*R*)-hydroxycholesterol/9-*cis*-retinoic acid in the absence or presence of 125 μM palmitate, 125 μM linoleate, or 10 μg/ml apoA-I, and membrane ABCA1 levels were assayed by immunoblot analysis. Ctrl, control. D: Macrophages incubated for 18 h with 22(*R*)-hydroxycholesterol/9-*cis*-retinoic acid were then incubated for 6 h with the same medium with (+) or without (−) 125 μM linoleate and 40 μM calpeptin, and membrane ABCA1 levels were assayed by immunoblot analysis. Results are representative of at least two experiments.

els of ABCA1 in vivo, however, it is likely that continuous exposure to fatty acids will dramatically impair ABCA1-dependent lipid transport.

Although cAMP is also a potent *Abc1* inducer in murine macrophages, the more physiologic activators of *ABCA1* transcription are LXR/RXR ligands. Cholesterol loading of cells greatly increases ABCA1 expression by activating LXR/RXR (12, 13), presumably because cholesterol is metabolized to oxysterols that are ligands for LXR. These nuclear receptors, however, also activate the transcription of multiple genes involved in fatty acid production and metabolism, including *SCD* (17). Thus, with cholesterol-loaded macrophages exposed to saturated fatty acids, activated LXR/RXR may counteract the enhanced *ABCA1* transcription by reducing ABCA1 protein content.

There is emerging evidence that diverse proteolytic processes modulate ABCA1 protein levels. ABCA1 contains a cytosolic PEST sequence that, when phosphorylated, directs the calpain-mediated proteolysis of ABCA1 (38, 39). The interaction of apolipoproteins with cells prevents this PEST phosphorylation and thus stabilizes ABCA1 (39). Increasing the free cholesterol content of

macrophages to cytotoxic levels destabilizes ABCA1 by a process that at least partially involves proteosomes (40). We found that fatty acids decreased ABCA1 even when it was stabilized by apoA-I or a calpain inhibitor, suggesting a lack of involvement of calpain. We also found that fatty acid-mediated destabilization of ABCA1 was not prevented by lactacystin or ALLN (data not shown), implying that proteosomes are not involved. Thus, it is likely that fatty acids destabilize ABCA1 by a mechanism distinct from those described previously.

Fatty acids appear to have the ability to suppress ABCA1 transcription as well as enhance protein destabilization. Unsaturated fatty acids compete for oxysterol binding to LXR and thus antagonize the activation of LXR by oxysterols (41). Uehara et al. (42) reported that unsaturated fatty acids decreased ABCA1 mRNA in oxysterol-treated macrophages by a mechanism consistent with this antagonism. We found, however, that fatty acids had no effect on ABCA1 synthesis in LXR/RXR ligand-treated J774 macrophages. Similarly, Murthy et al. (27) showed that fatty acids reduced ABCA1 protein levels in oxysterol-treated CaCo2 cells without affecting mRNA levels. The different outcomes of these studies may reflect the relative concentrations of fatty acids and oxysterols available to compete for LXR interactions. These multiple inhibitory mechanisms suggest that suppressing ABCA1-mediated lipid secretion by unsaturated fatty acids plays a physiologic role in cellular lipid homeostasis, perhaps to retain excess cholesterol as a reservoir for membrane synthesis.

The ABCA1-destabilizing effects of fatty acids have important clinical implications. Type 2 diabetes and the metabolic syndrome are characterized by increased fatty acids, low plasma HDL levels, and prevalent cardiovascular disease (43, 44). The current and previous (24) studies show that both palmitate and oleate, the two most common fatty acids, destabilize ABCA1 over a fatty acid-to-albumin molar ratio within the range observed for subjects with these disorders. It is possible that impaired ABCA1-mediated cholesterol secretion from cells contributes to the low plasma HDL levels and enhanced atherosclerosis in these subjects.

These studies also have important implications for therapeutic approaches to treat cardiovascular disease. Based on their ability to stimulate *ABCA1* transcription, LXRs have become attractive pharmaceutical targets. The stimulatory effects of LXR agonists on fatty acid production and desaturation, however, would tend to destabilize ABCA1 protein and override their positive transcriptional effects. Our results provide additional support for the idea that SCD could be an effective therapeutic target. Mouse models with mutant or disrupted *Scd1* are deficient in plasma triglycerides and cholesteryl esters (45, 46). SCD inhibitors would not only produce a more favorable plasma lipid profile, they may also enhance cholesterol mobilization from tissues by protecting ABCA1. This is particularly relevant for thiazolidinediones, which are already prescribed for diabetes. It appears that therapeutic interventions designed to treat dyslipidemias, diabetes, and insulin resistance may have the additional cardioprotective benefit of enhancing the ABCA1-dependent cholesterol secretory pathway.

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