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Acyl-CoA synthetase 1 is required for oleate and linoleate mediated inhibition of cholesterol efflux through ATP-binding cassette transporter A1 in macrophages

Jenny E. Kanter¹, Chongren Tang², John F. Oram², and Karin E. Bornfeldt¹

¹Department of Pathology, Diabetes and Obesity Center of Excellence, University of Washington School of Medicine, Seattle, WA 98109

²Department of Medicine, Diabetes and Obesity Center of Excellence, University of Washington School of Medicine, Seattle, WA 98109

Abstract

Diabetes and insulin resistance increase the risk of cardiovascular disease caused by atherosclerosis through mechanisms that are poorly understood. Lipid-loaded macrophages are key contributors to all stages of atherosclerosis. We have recently shown that diabetes associated with increased plasma lipids reduces cholesterol efflux and levels of the reverse cholesterol exporter ABCA1 (ATP-binding cassette transporter A1) in mouse macrophages, which likely contributes to macrophage lipid accumulation in diabetes. Furthermore, we and others have shown that unsaturated fatty acids reduce ABCA1-mediated cholesterol efflux, and that this effect is mediated by the acyl-CoA derivatives of the fatty acids. We therefore investigated whether acyl-CoA synthetase 1 (ACSL1), a key enzyme mediating acyl-CoA synthesis in macrophages, could directly influence ABCA1 levels and cholesterol efflux in these cells. Mouse macrophages deficient in ACSL1 exhibited reduced sensitivity to oleate- and linoleate-mediated ABCA1 degradation, which resulted in increased ABCA1 levels and increased apolipoprotein A-I-dependent cholesterol efflux in the presence of these fatty acids, as compared with wildtype mouse macrophages. Conversely, overexpression of ACSL1 resulted in reduced ABCA1 levels and reduced cholesterol efflux in the presence of unsaturated fatty acids. Thus, the reduced ABCA1 and cholesterol efflux in macrophages subjected to conditions of diabetes and elevated fatty load may, at least in part, be mediated by ACSL1. These observations raise the possibility that ABCA1 levels could be increased by inhibition of acyl-CoA synthetase activity *in vivo*.

Keywords

acyl-CoA synthetase; ATP-binding cassette transporter A1; cholesterol efflux; high-density lipoprotein; lipid metabolism; macrophage

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Send correspondence to: K.E. Bornfeldt, Department of Pathology, Diabetes and Obesity Center of Excellence, 815 Mercer Street, University of Washington, Seattle, WA 98109-8055. Tel: (206) 543-1681; Fax: (206) 543-3567; bornf@u.washington.edu.

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1. Introduction

Cardiovascular complications, due to atherosclerosis, are one of the main causes of mortality and morbidity in people with diabetes. However, the underlying mechanism is unknown [1–2]. Macrophages play a key role in lesions of atherosclerosis by accumulating cholesteryl esters and releasing a plethora of mediators of inflammation and plaque disruption [3]. We have previously reported that macrophages from animal models of diabetes in combination with elevated plasma lipids have reduced cholesterol efflux to apoA-I, reduced levels of the reverse cholesterol transporter ATP-binding cassette transporter A1 (ABCA1), and increased intracellular levels of cholesterol [4–5]. ABCA1 effluxes free cholesterol and phospholipids to apolipoprotein A-I (apoA-I), the first step in reverse cholesterol transport. Defects in ABCA1-mediated reverse cholesterol transport result in increased intracellular cholesterol accumulation, and ABCA1 plays a critical role in protecting against atherosclerosis in both humans and animals [6]. We and others have demonstrated that ABCA1 levels in macrophages are reduced by factors associated with diabetes and insulin resistant states, such as an increased fatty acid load [7–9]. The unsaturated fatty acids oleate and linoleate are most effective in degrading ABCA1, whereas saturated fatty acids first need to be unsaturated by stearoyl-CoA desaturase [9].

Long-chain acyl-CoA synthetases (ACSLs) are enzymes required for thioesterification of long-chain fatty acids into their acyl-CoA derivatives, which is the first committed step in fatty acid metabolism [10]. Of the five ACSL isoforms expressed in mammals, ACSL1 is abundantly expressed in macrophages [11]. ACSL1 acts on both unsaturated and saturated fatty acids in intact cells [12–13]. We therefore investigated whether ACSL1 could directly influence ABCA1 and cholesterol efflux from macrophages exposed to elevated levels of the unsaturated fatty acids oleate and linoleate (18:1 and 18:2, respectively). Our results demonstrate that ACSL1 plays an important role in mediating fatty acid-induced degradation of ABCA1 and the subsequently reduced cholesterol efflux to apoA-I in macrophages.

2. Materials and Methods

2.1. Generation and isolation of ACSL1-deficient macrophages

Mice with myeloid-specific deletion of ACSL1 (ACSL1^{M-/-} mice) on the C57BL/6 background were generated using the LysM-Cre-loxP system, as previously described [14–15]. Wildtype (WT) controls consisted of Acs11^{wt/wt};Cre^{+/+} littermates. Adult wild type and ACSL1^{M-/-} mice were injected with 2 ml of thioglycollate (40 mg/ml) intraperitoneally. Five days later, the ascites was collected by sterile lavage and the macrophages were adherence purified, as previously described [11].

2.2. Isolation of macrophages from fat-fed mice with elevated free fatty acid levels

Male LDL receptor (LDLR)-deficient mice, 12 weeks of age, were fed a diabetogenic diet with 0.5% added cholesterol (DDC) for 12 weeks to induce obesity and to elevate plasma non-esterified fatty acids [16]. Littermate *Ldlr*^{-/-} controls were fed a regular chow diet. Mice were monitored weekly for body weight changes. Five days prior to euthanasia, thioglycollate was injected to allow for harvest of elicited macrophages, as described above. At the end of the 12 weeks, macrophages and plasma were harvested. Non-esterified fatty acids were measured in EDTA-collected plasma using a colorimetric assay from Wako Chemicals (Richmond, VA).

2.3. Expression of wild type and mutant ACSL1 in *E. coli* and in J774.A1 macrophages

Residues in the ATP/AMP-binding sites of the *E. coli* Acs1 ortholog FadD are required for ACSL enzymatic activity [17]. Two enzymatically inactive murine ACSL1 mutants were

generated using the QuickChange XL Site-directed Mutagenesis Kit (Stratagene, La Jolla, CA). A phenylalanine at position 276 was mutated into an alanine (F276A) in the first ATP/AMP binding site and a glutamate was mutated into an alanine (E463A) at the second site. For expression in *E. coli*, WT or mutated ACSL1 was sub-cloned into pGEX-4T-3 generating GST-fusion proteins (Promega, Madison, WI). Bacterial cultures were grown overnight in the presence of antibiotics followed by a 1:20 dilution in fresh LB media. Cultures were grown to O.D.₆₀₀ of 0.4–0.6 prior to induction of protein expression. Protein expression was induced using 1 mmol/l isopropyl β -D-1-thiogalactopyranoside (IPTG) and cultures were allowed to grow for 3–4 h (O.D.₆₀₀ < 6.0). Total bacterial lysate or purified protein (MagneGST™ Protein Purification system, Promega) was used for subsequent analysis.

For expression in mammalian cells, WT or mutated ACSL1 was sub-cloned into the pBM retroviral vector, followed by transfection of ecotropic Phoenix cells (Orbigen, San Diego, CA), as previously described [12]. To accomplish a higher viral titer, transfected Phoenix cells were puromycin-selected (2 μ g/ml). J774.A1 macrophages (American Type Culture Collection, Manassas, VA) were transduced for 24 h, and subsequently selected using puromycin (10 μ g/ml). Selected J774 macrophages were maintained in DMEM with 10% FBS, 25 mmol/l D-glucose, 1% penicillin-streptomycin, and 1% non-essential amino acids.

2.4. Analysis of mRNA and protein levels and ACSL enzymatic activity

Levels of *Acs11* and *Abca1* mRNA were determined using real-time PCR. Total RNA was isolated using Qiagen RNeasy® Mini Kits. To remove trace genomic DNA, all samples were DNase treated. Total RNA was quantitated on the Mx4000® Multiplex QPCR System using the RiboGreen® RNA Quantitation Kit (Molecular Probes, Eugene, OR). Quantitative PCR was performed on an Mx4000® Multiplex QPCR System (Stratagene, La Jolla, CA) with samples loaded in triplicate using approximately 30 ng of total RNA. Total RNA from pooled samples was used for standard curves at 1:2 serial dilutions. For detection of *Acs11*, the primers GCGGAGGAGAATTCTGCATAGAGAA (forward) and ATATCAGCACATCATCTGTGGAAG (reverse) were used, and for *Abca1* the primers GGACATGCACAAGGTCCTGA (forward) and CAGAAAATCCTGGAGCTTCAAA (reverse) with the probe 6FAM-AATGTTACGGCAGATCAAGCATCC-BHQ1 were used. The *Acs11* and *Abca1* mRNA levels were normalized to that of *Rn18s*, detected with the primers CATTAAATCAGTTATGGTTCCTTTGG (forward) and CCCGTCGGCATGTATTAGCT (reverse) and the probe HEX-TTACCACAGTTATCCAAGTAGGAGAGGAGCGAG-BHQ1. Quantitative PCR was run in a 20 μ l reaction using Stratagene Brilliant® Single-Step QRT-PCR Kit (2 μ l 10 $\times$ core RT-PCR buffer, 5.0 mmol/l MgCl₂, 400 nmol/l each primer, 200 nmol/l probe, 0.2 mmol/l each dNTP, 75 nmol/l passive reference dye, 0.8 μ l RT/Block, 1.0 unit of SureStart TaqDNA-polymerase) with PCR cycling conditions of 45°C for 30 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 30 seconds, 60°C for 1 minute. Primers and fluorescent labeled TaqMan® probes were from Integrated DNA Technologies (Skokie, IL) or Invitrogen.

For protein analysis, total cell lysates (10–50 μ g) were loaded onto SDS-PAGE gels, separated and transferred onto nitrocellulose membranes. Detection was accomplished by using anti-ABCA1 antibodies (rabbit polyclonal; 1:1000-fold dilution; Novus Biologicals, Littleton, CO) and an anti-Myc antibody (mouse monoclonal clone 9B11; 1:1000-fold dilution; Cell Signaling Technology, Danvers, MA), and horseradish peroxidase-conjugated secondary antibodies. Total ACSL activity was measured as the rate of conversion of [³H]-18:1 into [³H]-18:1-CoA, as described previously [11–12].

2.5. Measurements of cholesterol efflux and cellular triacylglyceride levels

To investigate the role of ACSL1 in apoA-I-mediated cholesterol efflux, macrophages were loaded with 50 $\mu\text{g/ml}$ acetylated LDL (acLDL) and [^3H]-cholesterol overnight. ABCA1 expression was then induced using 22-hydroxycholesterol and 9-cis retinoic acid (10 $\mu\text{mol/l}$) alone or together with oleic acid (18:1) or linoleic acid (18:2) overnight. Stock solutions of the sodium salts of the fatty acids (Nu-check Prep Inc, Elysian, MN) were prepared in sterile, endotoxin free water. At the day of the experiment fresh fatty acids were added to media containing 0.5% fatty acid free, low endotoxin BSA (Sigma-Aldrich, St. Louis, MO). Cholesterol efflux was measured during a subsequent 2 h period with or without 10 $\mu\text{g/ml}$ of apoA-I. Cholesterol efflux was calculated, as described previously [8]. Cellular triacylglyceride levels were measured by a colorimetric kit from Sigma (St. Louis, MO).

2.6. Statistical analysis

Statistical analysis was performed using two-tailed unpaired Student's *t*-test when comparing two groups. One or two-way ANOVA was used to compare more than two parameters with Bonferroni post hoc tests. Probabilities of less than 0.05 were considered statistically significant.

3. Results

3.1. ACSL1-deficient macrophages are protected against oleate- and linoleate-mediated inhibition of cholesterol efflux to apoA-I and degradation of ABCA1

The generated ACSL1^{M-/-} mice exhibited dramatically reduced levels of *Acs1* mRNA in macrophages (Fig. 1A) and an approximate 40% reduction in total ACSL activity *in vitro* (Fig. 1B). Mono- and di-unsaturated fatty acids, such as oleate and linoleate, have previously been demonstrated to inhibit apoA-I-mediated cholesterol efflux from cells [7–8]. Accordingly, in WT macrophages, 225 $\mu\text{mol/l}$ oleic acid (18:1) or linoleic acid (18:2) reduced cholesterol efflux to apoA-I (Fig. 1C). Strikingly, ACSL1-deficient macrophages were protected against fatty acid-induced inhibition of cholesterol efflux (Fig. 1C). The protection of ABCA1 protein levels in 18:1-stimulated ACSL1-deficient macrophages was not mediated by an increased ABCA1 transcription or mRNA stability, since no significant differences in *Abca1* mRNA levels were observed between WT and ACSL1-deficient macrophages under basal or 18:1-stimulated conditions (Fig.1D).

Instead, the reduced cholesterol efflux to apoA-I in the presence of 18:1 or 18:2 was accompanied by a reduction of ABCA1 protein in WT macrophages (Fig. 1E). Consistent with the protective effects of ACSL1 on cholesterol efflux in cells challenged with fatty acids, ACSL1-deficiency protected the cells against fatty acid-mediated reduction of ABCA1 (Fig. 1E). On the other hand, ACSL1-deficiency had no effect on ABCA1 protein levels in the absence of these fatty acids (Fig. 1E).

Together, the results demonstrate that ACSL1-deficiency protects macrophages against the reduced cholesterol efflux due to ABCA1 degradation in cells exposed to an increased 18:1 or 18:2 load.

3.2. Elevated fatty acids do not regulate macrophage ACSL1

ACSL1 has been shown to be up-regulated in the stomavascular fraction of adipose tissue enriched in fatty acids in mice fed a high fat diet [18]. Additionally, it has been shown that ACSL1 can be induced by peroxisome proliferator-activated receptors (PPARs) [19], and 18:1 has been suggested to stimulate PPAR γ activity [20]. Therefore, we investigated if 18:1 could induce ACSL1 expression in macrophages as a potential mechanism behind its ability to induce ABCA1 degradation.

Thioglycollate-elicited macrophages were stimulated with 225 $\mu\text{mol/l}$ 18:1 for 24 h. Interestingly, *Acs11* mRNA was not induced by 18:1 (Fig. 2A) at a concentration that induced both *Cd36* and carnitine palmitoyltransferase 1 α (*Cpt1a*), known PPAR γ and PPAR α target genes (data not shown). To investigate whether ACSL1 was induced in macrophages exposed to elevated plasma fatty acid levels *in vivo*, we fed male LDLR-deficient mice a diabetogenic diet with 0.5% added cholesterol (DDC) to induce weight gain (Fig. 2B) and elevated plasma non-esterified fatty acid levels (Fig. 2C). Similar to what we observed *in vitro*, *Acs11* mRNA was not induced in macrophages harvested from mice fed the DDC (Fig. 2D) strongly suggesting that macrophage ACSL1 is not regulated by elevated fatty acids *in vitro* or *in vivo*. Thus, the ability of unsaturated fatty acids to promote ABCA1 degradation is unlikely to be due to upregulation of ACSL1 expression.

3.3. Overexpression of ACSL1 exacerbates fatty acid-mediated loss of ABCA1 and cholesterol efflux

To ascertain that the above described effects of ACSL1 were indeed due loss of ACSL1 activity, we generated retroviral vectors to overexpress WT ACSL1. In addition, two enzymatically inactive ACSL1 mutants were generated. Two ATP/AMP consensus sites have been described and mutated in the *E. coli* ortholog FadD [17]. A phenylalanine to alanine substitution at site 276 in the first ATP/AMP (F276A) and a glutamate to alanine substitution at site 463 (E463A) in the second ATP/AMP site were generated. These mutations correspond to Y213A and E361A mutations in FadD lacking acyl-CoA activity described by Weimar and colleagues [17]. Enzymatic activity or lack of activity was verified by expressing ACSL1 or the two mutants in *E. coli* (Fig. 3A). Similar to what was reported for FadD [20], mutations at these sites resulted in an almost complete loss of ACSL1 enzymatic activity (Fig. 3B). Furthermore, induced ACSL activity could be inhibited by triacsin C, but not by rosiglitazone (Fig. 3C), consistent with the known susceptibility of recombinant rat ACSL1 to these pharmacological inhibitors [21].

Next, mouse J774.A1 macrophages were transduced with WT ACSL1 (either myc-tagged or untagged ACSL1), the ACSL1 mutants F276A, E463A, or the empty pBM vector. Overexpression of WT ACSL1 led to increases in both total ACSL enzymatic activity (data not shown) and immunoprecipitable ACSL1-specific activity (Fig. 3D). The two ACSL1 mutants did not demonstrate increased ACSL1 activity over empty vector control, despite expression levels similar to that of WT ACSL1 (Fig. 3D, lower panel). Increased ACSL1 activity has been shown to increase fatty acid-driven triacylglyceride accumulation [22–23]. Indeed, macrophages overexpressing ACSL1 exhibited increased triacylglyceride accumulation after stimulation with 18:1, whereas the ACSL1 mutants had no effect (Fig. 3E). Together, these results demonstrate that overexpression of ACSL1 results in biologically active ACSL1.

We next investigated whether overexpression of ACSL1 would affect ABCA1 and cholesterol efflux in a manner opposite that of ACSL1-deficiency. As shown in Figures 4A–C, this was indeed the case, as overexpression of ACSL1 increased the cells' sensitivity to 18:1-mediated reduction in ABCA1 protein levels (Figs. 4A–B). This reduction in ABCA1 protein levels correlated with an attenuation of apoA-I-mediated cholesterol efflux in macrophages overexpressing ACSL1 (Fig. 4C).

These results strongly suggest that the increased cholesterol efflux and increased ABCA1 levels in ACSL1-deficient macrophages subjected to unsaturated fatty acid stimulation are a result of loss of ACSL1 enzymatic activity.

4. Discussion

Mono- and di-unsaturated fatty acids have previously been demonstrated to increase degradation of ABCA1, leading to reduced apoA-I-dependent cholesterol efflux from macrophages [8]. Saturated fatty acids can also induce degradation of ABCA1 if they are first converted to unsaturated fatty acids by stearoyl-CoA desaturase [9]. Fatty acid-induced degradation of ABCA1 is inhibited by the non-specific ACSL inhibitor triacsin C [24], which inhibits several of the ACSL isoforms, including ACSL1 [21,25]. Here we present evidence that fatty acid-mediated degradation of ABCA1 is indeed dependent on thioesterification of the fatty acids into acyl-CoAs, and that this is mediated by ACSL1 in primary mouse macrophages. Because total ACSL activity was reduced by only approximately 40% in ACSL1-deficient macrophages, reflecting the remaining ACSL activity of other ACSL isoforms in these cells, these results suggest that ACSL1 plays a key role in esterifying fatty acids destined to mediate ABCA1 degradation. This indicates that other ACSL isoforms, and other enzymes with capacity to thioesterify fatty acids, cannot compensate for the loss of ACSL1, suggesting that ACSL isoforms may have distinct functions within the cell. The concept of specific functions of different ACSL isoforms in different tissues is strengthened by recent data. In heart and liver, ACSL1-deficiency blunts β -oxidation [13–14]. ACSL5-deficiency, on the other hand, increases aspects of β -oxidation in hepatocytes [26]. It is possible that the different functions of ACSL isoforms are due to differences in subcellular localization. Thus, ACSL1 is associated with the plasma membrane in some cells [27], where it might be perfectly situated to regulate the function of ABCA1 in response to increased fatty acid load. Alternatively, different biological effects of ACSL isoforms might be due to differences in fatty acid substrate preference. For example, ACSL1 has a preference for oleic acid when overexpressed in human smooth muscle cells, whereas ACSL4 has a much stronger preference for arachidonic acid [12].

In the present study, we did not observe a significant effect of oleate on *Abca1* mRNA levels, suggesting that the ability of oleate to reduce ABCA1 protein levels are primarily due to reduced ABCA1 stability, consistent with previous studies [8]. However, linoleate reduces both ABCA1 stability and *Abca1* mRNA levels in macrophages under some conditions [28], and this effect is most likely mediated by reduced *Abca1* promoter activity [7]. No significant effect of ACSL1-deficiency on *Abca1* mRNA levels was observed in the present study under basal conditions or oleate-stimulated conditions, suggesting that the increased ABCA1 levels in ACSL1-deficient macrophages are due primarily to increased ABCA1 protein stability. The mechanism whereby ACSL1 regulates ABCA1 protein stability warrants further studies. ABCA1 degradation has been shown to be enhanced by phospholipase D₂ activity [24], protein kinase C δ phosphorylation [29], calpain [30] and ubiquitination [31]. It is possible that ACSL1 promotes one or several of these pathways, or mediates ABCA1 degradation by an independent pathway.

Importantly, the effect of ACSL1 on ABCA1 and cholesterol efflux is clearly observed in macrophages exposed to elevated levels of 18:1 or 18:2, whereas basal levels of ABCA1 and cholesterol efflux are unaffected by ACSL1-deficiency. Thus, ACSL1 does not regulate ABCA1 or cholesterol efflux unless the cell is subjected to elevated fatty acid load, such as is likely to occur in the setting of diabetes in combination with increased triglyceride and fatty acid levels. Consistently, diabetic mice with elevated levels of plasma triglycerides exhibit reduced ABCA1 protein levels in both macrophages and kidneys [4,7], concomitant with increased neutral lipid accumulation in macrophages [4]. On the other hand, diabetic mice without elevated plasma triglycerides and fatty acids do not show increased neutral lipid accumulation in macrophages (our unpublished observations), suggesting that macrophage cholesterol efflux is unaltered. The liver appears to be less susceptible, and diabetes does not consistently downregulate ABCA1 in the liver [4,7]. A more dramatic

hepatic lipid accumulation and steatosis has recently been shown to be associated with reduced ABCA1 levels [32].

In summary, we have demonstrated a novel and direct link between acyl-CoA synthesis by ACSL1 and degradation of ABCA1 with subsequently reduced cholesterol efflux to apoA-I in macrophages exposed to increased fatty acid load (Fig. 4D). These findings raise the possibility that ACSL1 might be targeted to modulate levels of ABCA1 and cholesterol efflux *in vivo*.

Research Highlights

- Acyl-CoA synthetase 1 (ACSL1) mediates fatty acid-induced degradation of ATP-binding cassette transporter A1 (ABCA1) in macrophages
- ACSL1-mediated loss of ABCA1 causes reduced cholesterol efflux to apolipoprotein A-I
- Reduced levels of ABCA1 in macrophages in the setting of diabetes and insulin resistance might be caused, in part, by ACSL1

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Figure 1. Macrophage ACSL1-deficiency protects against oleate- and linoleate-mediated degradation of ABCA1

Thioglycollate-elicited macrophages were harvested 5 days after thioglycollate injection by sterile lavage. A. Total mRNA was reverse transcribed, and specific primers were used to detect *Acs1* mRNA using real-time PCR. B. Total ACSL activity was measured as the rate of formation of [³H]-18:1-CoA from [³H]-18:1 acid. C. Macrophages were stimulated with acLDL for 24 h followed by an additional 24 h induction of ABCA1 in the absence or presence of 225 μmol/l oleic acid (18:1) or linoleic acid (18:2). Following fatty acid challenge, [³H]-cholesterol efflux was measured in the absence or presence of 10 μg/ml of apoA-I. D. Macrophage *Abca1* mRNA levels were analyzed using real-time PCR after a 24

h incubation in the absence (control) or presence of 225 $\mu\text{mol/l}$ 18:1. E. Macrophages were treated similarly as in C, but lyzed and analyzed for ABCA1 protein content by Western blot. The results were normalized to GAPDH and expressed as means $\pm$ SEM. All experiments were performed at least 3 times in independent experiments. NS, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by two-tailed unpaired Student's t -test.

Figure 2. Elevated fatty acid levels do not induce ACSL1 in macrophages

Thioglycollate-elicited macrophages were stimulated in the absence (control) or presence of 225 μ mol/l 18:1 for 24 h, and total RNA was then extracted and subjected to reverse transcription followed by real-time PCR using specific primers to *Acs11* (A) (n=6). Male LDLR-deficient mice were fed a diabetogenic diet with 0.5% added cholesterol (DDC) or regular chow for 12 weeks. Body weight changes were monitored weekly (B). Plasma levels of non-esterified fatty acids (NEFA) were measured in plasma collected in EDTA using a colorimetric assay from Wako (C). Levels of *Acs11* mRNA in thioglycollate-elicited macrophages harvested from mice fed chow or DDC for 12 weeks (D). The results are expressed as means $\pm$ SEM, n=4–5 (unless otherwise specified), *** $p < 0.001$ using a 2-way ANOVA comparing the curves in B; and by Student's *t*-test in C. There were no significant differences between cells under basal conditions versus oleate-stimulated conditions in C. NS, non-significant ($p > 0.05$)

Figure 3. Generation of wildtype ACSL1 and ACSL1 mutants lacking enzymatic activity
 Wildtype murine ACSL1 and the ACSL1 mutants F276A and E463A were expressed in *E. coli* (A–C) or J774 macrophages (D–E). A. Expression of wildtype ACSL1 and mutants in *E. coli* following induction by IPTG. B. ACSL activity in bacterial lysates was measured as in figure 1. C. ACSL activity in bacterial lysates was measured in the presence or absence of the ACSL4 inhibitor rosiglitazone or the non-specific ACSL inhibitor triacsin C. D. Myc-tagged ACSL1 was overexpressed in J774.A1 macrophages using a retroviral approach. Specific ACSL1 activity was determined in the macrophages overexpressing ACSL1, or enzymatically inactive mutants using only immunoprecipitated (IP) myc-tagged ACSL1 (pBM empty vector). E. Accumulation of triacylglycerides (TAG) in J774 macrophages

expressing wildtype ACSL1 or the inactive mutants was measured following stimulation with oleate by a kit from Sigma. The results are expressed as means $\pm$ SEM. All experiments were performed at least 3 times in independent experiments. ** $p < 0.01$ by one-way ANOVA.

Figure 4. Macrophage overexpression of ACSL1 leads to increased sensitivity to oleic acid-stimulated degradation of ABCA1

Myc-tagged ACSL1 was overexpressed in J774.A1 macrophages using a retroviral approach. A. Macrophage ABCA1 protein after stimulation with oleic acid (18:1) at the indicated concentrations was analyzed by Western blot and quantified in B. C. Cholesterol efflux to apoA-I was measured as described in Materials and Methods. The results are expressed as means $\pm$ SEM. All experiments were performed at least 3 times in independent experiments. ** $p < 0.01$, *** $p < 0.001$ by Student's t-test in B and by comparing the curves by two-way ANOVA in C. D. Schematic depiction of the role of ACSL1 in mediating the degradation of ABCA1 under elevated fatty acid load.