Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 1271 – 1276

Journal of Nutritional Biochemistry

Unsaturated fatty acids repress expression of ATP binding cassette transporter A1 and G1 in RAW 264.7 macrophages☆

Chai Siah Ku[a], Youngki Park[a], Sara L. Coleman[b], Jiyoung Lee[a,*]

[a]Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA
[b]Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE 68583, USA

Received 5 February 2011; received in revised form 28 May 2011; accepted 13 July 2011

Abstract

Reverse cholesterol transport (RCT), a process to deliver excess cholesterol from the periphery to the liver for excretion from body, is a major atheroprotective property of high-density lipoproteins. As major transporters for cholesterol efflux in macrophages, ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1) are critical for RCT. We investigated mechanisms for the regulation of ABCA1 and ABCG1 expression by fatty acids (FA) in RAW264.7 macrophages. Cells were incubated with 100 μmol/L of palmitic, oleic, linoleic, linolenic or eicosapentaenoic acids in the absence or presence of T0901317, a liver X receptor (LXR) agonist. Unsaturated FA, but not saturated FA, significantly reduced ABCA1 and ABCG1 mRNA without the agonist. Trichostatin A (TSA), a histone deacetylase inhibitor, not only increased basal ABC transporter expression but abrogated the transcriptional repression by unsaturated FA. The increased basal ABCA1 and ABCG1 mRNA by TSA paralleled the increased peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ coactivator 1α expression, whereas LXRα and PGC-1β expression was significantly lowered. Although the repressive effect of ABCA1 and ABCG1 mRNA by unsaturated FA was abolished by T0901317, protein levels remained diminished. Chemical and genetic deficiency of protein kinase C δ did not abolish the repressive effect of linoleic acid on ABCA1 and ABCG1. In conclusion, unsaturated FA repressed ABCA1 and ABCG1 expression by two distinct mechanisms in RAW 264.7 macrophages: LXR-dependent transcriptional repression possibly by modulating histone acetylation state and LXR-independent posttranslational inhibition.

Keywords: ABCA1; ABCG1; RAW 264.7 macrophage; Fatty acids

1. Introduction

Since the hypothesis that high levels of plasma high-density lipoprotein (HDL) cholesterol are protective against coronary heart disease (CHD) was initially proposed in the early 1950s [1], epidemiological studies have consistently shown a strong inverse relationship between plasma HDL cholesterol levels and the incident of CHD [2–5]. Dyslipidemia, an underlying pathological condition for CHD and type 2 diabetes, is characterized by high plasma concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides as well as low plasma HDL cholesterol levels [6]. The antiatherogenic effect of HDL is due in part to the ability of HDL to promote cholesterol efflux from cells and to participate in reverse cholesterol transport (RCT) [7–9]. In the RCT, excess cholesterol is transported from the periphery to the liver for ultimate excretion from body, and HDL functions as the primary acceptor of cellular free cholesterol [10].

Removal of cholesterol from macrophages in arterial wall via the RCT is of significant importance in the prevention of atherosclerosis development. ATP binding cassette transporter A1 (ABCA1) and G1 (ABCG1) play a pivotal role in this process. ABCA1 facilitates the efflux of cellular cholesterol to extracellular acceptors, namely, lipid-free or lipid-poor apolipoprotein A-I [11–14]. In contrast, ABCG1 is highly expressed in macrophages and mediates the efflux of cholesterol to HDL_2 [15,16]. Deletion of macrophage *Abcg1* led to the deposition of free cholesterol and cholesteryl esters in various tissues, implicating its role in maintaining cellular cholesterol level [17,18]. Studies have shown that dysfunction of ABCA1 and ABCG1 in macrophages induces cholesterol accumulation and accelerates atherosclerosis, although some contradictory observations exist as to the role of ABCG1 in atherogenesis [19–24].

Transcription of ABCA1 and ABCG1 is primarily under the control of liver X receptors (LXRs) in response to cellular cholesterol levels

Abbreviations: ABCA1, ATP binding cassette transporter A1; ABCG1, ATP binding cassette transporter G1; CHD, coronary heart disease; HDAC, histone deacetylase; HDACi, HDAC inhibitor; HDL, high-density lipoprotein; LXR, liver X receptor; NCoR, nuclear receptor corepressors; SMRT, silencing mediators for retinoid and thyroid hormone receptors; PGC-1α, PPARγ coactivator 1α; PKCδ, protein kinase C δ; PLD2, phospholipase D_2; PPARγ, peroxisome proliferator-activated receptor γ; RCT, reverse cholesterol transport; siRNA, small interfering RNA; TSA, trichostatin A.

☆ Funding sources: This work was supported by National Science Foundation-EPSCoR grant EPS-0346476 and University of Nebraska Foundation Layman Award to J. Lee.

* Corresponding author. Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA. Tel.: +860 486 1827.
E-mail address: ji-young.lee@uconn.edu (J. Lee).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2011.07.007

[25]. Studies have shown that fatty acids can also alter ABCA1 expression at the transcriptional and posttranscriptional levels. Posttranscriptional repression of ABCA1 expression by unsaturated fatty acids in macrophages has been demonstrated by a series of studies conducted by Wang et al. [26] and Wang and Oram [27–29].In their studies, unsaturated fatty acids increase ABCA1 protein degradation in macrophages by activating phospholipase D_2 (PLD_2) and subsequently protein kinase C δ (PKCδ). This, in turn, phosphorylates ABCA1 serine residues for protein degradation. Other studies exist to demonstrate that fatty acids could alter transcription of ABCA1 and ABCG1. In human monocyte-derived macrophages, basal ABCA1 and ABCG1 mRNA levels were lowered by linoleic acid (18:2) compared with palmitic acid (16:0) [6]. Basal ABCA1 mRNA abundance in HepG2 and RAW 264.7 macrophages was reduced by eicosapentaenoic acid in a dose-dependent manner [30]. Unsaturated fatty acids, including oleic acid, arachidonic acid and eicosapentaenoic acid, also significantly lowered ABCA1 expression when an LXR agonist was present by decreasing ABCA1 promoter activity [30]. In RAW 264.7 macrophages, unsaturated fatty acids repressed ABCA1 and ABCG1 mRNA levels in the absence or presence of an LXR agonist, and mutations or deletion of direct repeat 4, a *cis*-acting element for LXR binding, in the promoters of ABCA1 and ABCG1 abolished the suppressive effects [31]. These studies suggest that unsaturated fatty acids repress the basal as well as LXR-activated ABC transporter expression.

Studies have demonstrated that unsaturated fatty acids, but not saturated fatty acids, reduce ABCA1 and ABCG1 expression. However, limited and contradictory observations exist as to the molecular mechanisms underlying the repressive effect of unsaturated fatty acids. This study shows that fatty acids commonly present in diet can alter gene expression via a modulation of histone deacetylation. In addition, an LXR-independent posttranscriptional inhibition of the ABC transporter expression exists in macrophages.

2. Methods and materials

2.1. Cell culture and fatty acid preparation

RAW 264.7 macrophages were purchased from ATCC (Manassas, VA, USA) and maintained in RPMI medium containing 10% fetal bovine serum (FBS), 100 kU/L penicillin,100 mg/L streptomycin, 1× vitamins and 2 mmol/L L-glutamine in a humidified incubator at 37°C with 5% CO_2. All cell culture supplies were purchased from Mediatech (Manassas, VA, USA).

Sodium salts of fatty acids (Nu-Chek, Elysian, MN, USA) were dissolved in the fatty-acid-poor and endotoxin-free bovine serum albumin (BSA) stock solution (2 mmol/L in PBS; EMD Chemicals, Gibbstown, NJ, USA) to a final concentration of 5mmol/L. The fatty acid and BSA mixture was purged with N_2 and sonicated in warm water bath (~40°C) until the solution became clear to form BSA/fatty acid complex (a molar ratio to BSA of 1:2.5). The complex was then filter sterilized and diluted with cell medium to reach a final concentration of 100 μmol/L of fatty acid and incubated in a water bath at 37°C for 1 h prior to addition to cells.

RAW264.7 macrophages were incubated without or with 10 μmol/L T0901317 (Sigma-Aldrich, St. Louis, MO, USA), an LXR agonist, in dimethyl sulfoxide (DMSO) for 18 h, after which cells were treated with BSA only (control) or 100 μmol/L of fatty acid for 12 h. Rottlerin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), a PKCδ inhibitor, at 5 or 10 μmol/L in DMSO was used to inhibit PKCδ. For this inhibitor experiment, cells were preincubated with the inhibitor for 2 h and subsequently with 100 μmol/L of fatty acid for 12 h. For experiments with trichostatin A (TSA; Sigma-Aldrich), a pan-histone deacetylase inhibitor (HDACi), RAW 264.7 macrophages were incubated with 100 μmol/L fatty acid in the absence or presence of 500 nmol/L of TSA for 24 h. Cells were cultured in complete medium devoid of FBS when they were treated with fatty acid to eliminate any potential contamination of fatty acids present in FBS. When inhibitors were dissolved in DMSO, control cells were incubated with the same amount of DMSO for a vehicle control.

2.2. Small interfering RNA (siRNA) transfection

RAW 264.7 macrophages were transfected with Silencer Negative Control scrambled siRNA (Ambion, Austin, TX, USA) or siGENOME PKCδ siRNA (Dharmacon, Lafayette, CO, USA) to knockdown PKCδ using DharmaFECT1 transfection reagent (Dharmacon) according to the manufacturer's protocol. Briefly, transfection reagent was diluted with a factor of 40 using cell medium void of antibiotics and FBS. Two micromoles per liter of siRNA solution was prepared in RNase-free sterile water and mixed with equal volume of cell medium void of antibiotics and FBS. Subsequently, the media containing transfection agent and siRNA were combined and incubated for 20 min at room temperature before being added to cells. Twenty-four hours later, the cells were washed twice with PBS and treated with BSA or 100 μmol/L of fatty acid for 24 h. In our preliminary experiments, knockdown of genes peaked at 24 h after transfection and was prolonged for up to 48 h.

Table 1
Quantitative real-time PCR primers

Gene	Forward primer	Reverse primer
ABCA1	5′-CGTTTCCGGGAAGTGTCCTA-3′	5′-GCTAGAGATGACAAGGAGGATGGA-3′
ABCG1	5′-AGGTCTCAGCCTTCTAAAGTTCCTC-3′	5′-TCTCTCGAAGTGAATGAAATTTATCG-3′
LXRα	5′-CGACAGAGCTTCGTCCACAA-3′	5′-ACAGCTCGTTCCCCAGCAT-3′
PPARγ	5′-GCCCACCAACTTCGGAATC-3′	5′-TGCGAGTGGTCTTCCATCAC-3′
PGC-1α	5′-AAGCTGAAGCCCTCTTGCAA-3′	5′-ACTGTACCGGGCCCTCTTG-3′
PGC-1β	5′-CGCTCCAGGAGACTGAATCCAGT-3′	5′-CTTGACTACTGTCTGTGAGGC-3′
GAPDH	5′-TGTGTCCGTCGTGGATCTGA-3′	5′-CCTGCTTCACCACCTTCTTGAT-3′

2.3. Total RNA isolation and quantitative real-time polymerase chain reaction (qPCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer's protocol. Reverse transcription for cDNA synthesis and qPCR analysis were performed as previously described [32,33]. Primers were designed according to GenBank database using the Primer Express software (Applied Biosystems, Austin, TX, USA). Lists of primer sequence are in Table 1.

2.4. Western blot analysis

Cell lysate was prepared and Western blot analysis was performed as previously described [32,33]. Rabbit ABCA1 antiserum was a generous gift from Dr. John Parks at Wake Forest University School of Medicine. ABCG1 and PKCδ antibodies were purchased from Novus Biologicals (Littleton, CO, USA) and Santa Cruz Biotechnology (Santa Cruz, CA USA), respectively. Monoclonal antibody against β-actin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as a loading control to normalize the data.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to identify statistically significant differences of treatments using GraphPad InStat 3 (GraphPad Software, Inc.). Two-way ANOVA was performed to analyze fatty acid effect and TSA effect and interaction on ABCA1 and ABCG1 mRNA expression. $P<.05$ was considered significant, and data are expressed as mean±S.E.M.

3. Results

3.1. Macrophage ABCA1 and ABCG1 expression was repressed by unsaturated fatty acids

RAW264.7 macrophages were treated with various fatty acids including palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1), 18:2, linolenic acid (18:3) and eicosapentaenoic acid (20:5) in the absence or presence of T091317, an LXR agonist. In the absence of the LXR agonist, both ABCA1 and ABCG1 mRNA levels were significantly repressed by all the unsaturated fatty acids tested compared with the control (Fig. 1A). However, when T0901317 was present, the repressive effect was completely abolished. Consistent with mRNA data in Fig. 1A, ABCA1 (~250 kDa) and ABCG1 (~60 kDa) protein levels were also markedly reduced by unsaturated fatty acids in the absence of T0901317 (Fig. 1B). Unsaturated fatty acids, however, were able to lower the ABC transporter protein levels when the cells were treated with the LXR agonist despite no changes in mRNA abundance observed.

3.2. Inhibition of histone deacetylase (HDAC) activity abolished the repressive effect of unsaturated fatty acids on ABCA1 and ABCG1 mRNA levels

We evaluated if modulations in histone acetylation status are involved in the unsaturated fatty acid-induced transcriptional

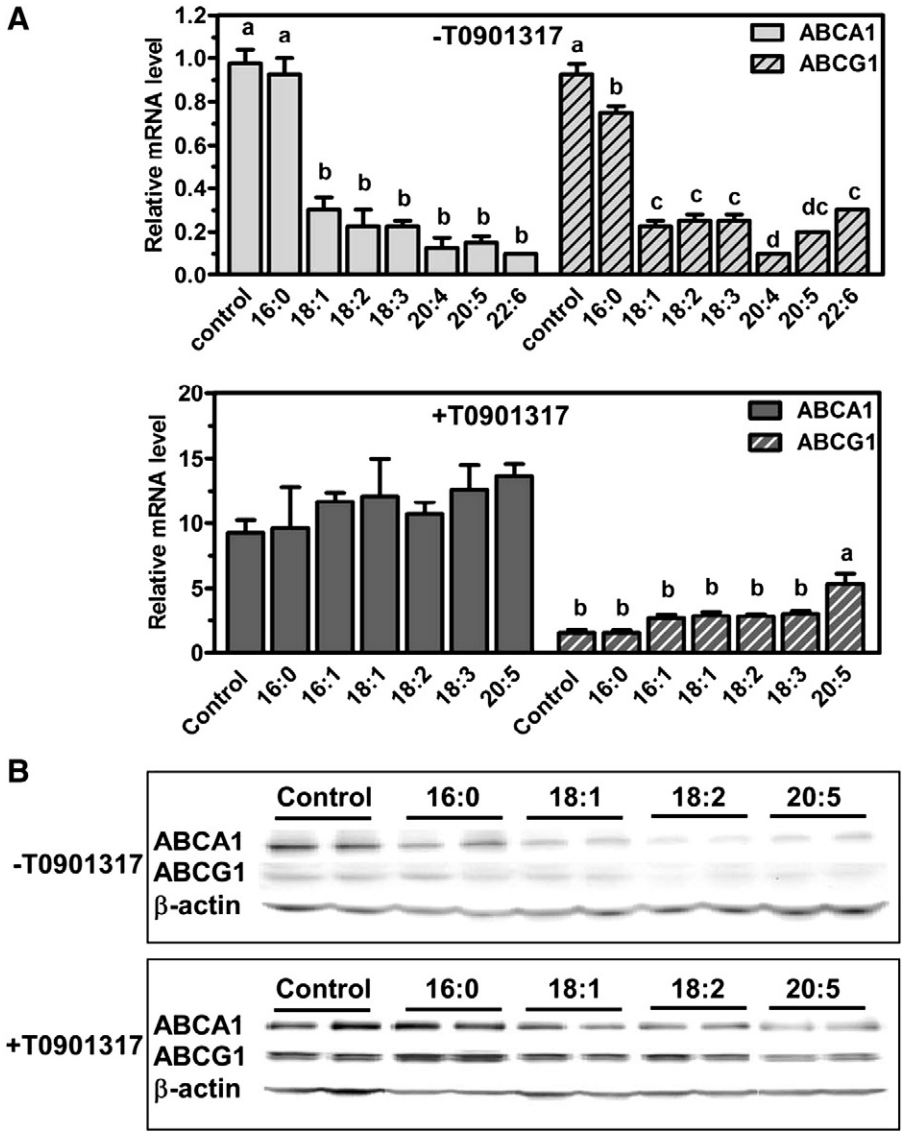

Fig. 1. Regulation of ABCA1 and ABCG1 expression by fatty acids in RAW 264.7 macrophages. Cells were incubated with or without LXR agonist T0901317 (10 μmol/L) for 18 h and subsequently with BSA only (control) or fatty acids (100 μmol/L) complexed with BSA for 12 h. (A) ABCA1 and ABCG1mRNA abundance by qPCR. Values are means±S.E.M, $n=6$. Bars without a common letter are significantly different, $P<.05$. (B) ABCA1 and ABCG1 protein by Western blot analysis with β-actin as a loading control. A representative blot of two or three separate experiments is shown.

repression of macrophage ABCA1 and ABCG1. RAW 264.7 macrophages were incubated with 16:0 and 18:2 in the presence of TSA. Consistent with the previous observation shown in Fig. 1, without TSA, both ABCA1 and ABCG1 mRNA levels were significantly repressed by 18:2 (Fig. 2). However, when the macrophages were treated with TSA, mRNA levels of ABCA1 and ABCG1 were significantly increased, and furthermore, the repression by 18:2 was completely abolished. It is of interest that, in the presence of TSA, 16:0-treated cells showed significantly lower ABCA1 and ABCG1 mRNA abundance than control and 18:2-treated cells.

TSA treatment altered the expression of other genes that have been linked to the regulation of ABCA1 and ABCG1 transcription. Whereas peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ coactivator 1α (PGC-1α) expression was significantly increased by TSA along with ABCA1 and ABCG1, LXRα and PGC-1β mRNA levels were significantly decreased by TSA in controls (Fig. 3).

3.3. Rottlerin did not abolish the unsaturated fatty acids-induced reduction in ABCA1 and ABCG1 proteins

Macrophage ABCA1 and ABCG1 protein levels were diminished by unsaturated fatty acids even with LXR activation in the condition which the repression of mRNA levels was abolished. Posttranscriptional regulation of ABCA1 by unsaturated fatty acids was suggested to result from destabilization of ABCA1 protein through its phosphorylation by PKCδ pathway [29]. To evaluate a role of PKCδ in the fatty acid regulation of ABCA1 as well as ABCG1 protein levels, we incubated RAW 264.7 macrophages with 16:0 or 18:2 in the presence of rottlerin, a commonly used PKCδ inhibitor, or after depletion of PKCδ using siRNA. Neither rottlerin nor PKCδ siRNA abolished the repressive effect of 18:2 on ABCA1 and ABCG1 proteins (Fig. 4A and B). Instead, the ABC transporter protein levels in 18:2-treated cells were shown least when cells were treated with rottlerin and PKCδ was deficient compared with no rottlerin control and scrambled control, respectively.

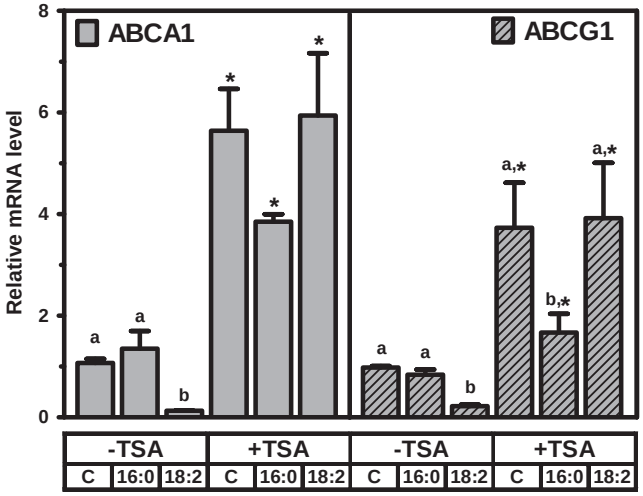

Fig. 2. Effect of TSA on ABCA1 and ABCG1 expression in RAW 264.7 macrophages. The cells were incubated with 500 nmol/L TSA for 20 h together with fatty acids (100 μmol/L) or BSA control (C) for qPCR. Means±S.E.M, n=6–13. Bars with a different letter within the same TSA treatment are significantly different (P<.05). *Within-group differences from −TSA to +TSA (P<.05).

4. Discussion

As major transporters for cholesterol efflux in macrophages, ABCA1 and ABCG1 play a pivotal role in maintaining cellular cholesterol homeostasis. Disturbance of the ABC transporter functions in macrophages, particularly in the arterial wall, could facilitate foam cell formation and consequently atherogenesis. The goal of this study was to understand molecular mechanisms for the regulation of macrophage ABCA1 and ABCG1 by fatty acids, whose plasma concentrations are commonly elevated in obesity-associated metabolic diseases, notably CHD [34–38]. We found that expression of ABCA1 and ABCG1 in macrophages is repressed by unsaturated fatty acids via two distinct mechanisms, i.e., LXR-dependent transcriptional inhibition and posttranscriptional regulation to facilitate protein degradation. In particular, the transcriptional repression by linoleate is likely mediated via modulations of histone deacetylation state. This study demonstrates that fatty acids commonly present in diet can regulate gene expression by altering HDAC in macrophages.

Several studies have reported that fatty acids modulate the expression of ABCA1 and ABCG1 [6,26–31,39,40]. The detailed regulatory mechanisms, however, remain largely enigmatic and contradictory. We observed that unsaturated fatty acids repressed

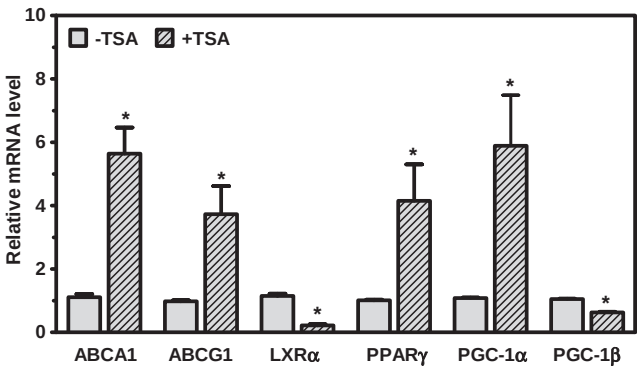

Fig. 3. Effect of TSA on the expression of genes related to transcriptional regulation of ABC transporters in RAW 264.7 macrophages. The cells incubated with TSA and fatty acids as described in Fig. 2 legend were analyzed by qPCR. Means±S.E.M., n=6–13, *P<.05 compared with −TSA control.

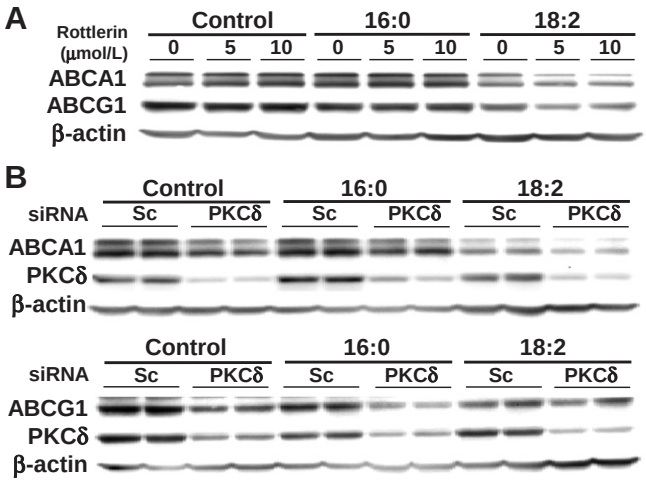

Fig. 4. Role of PKCδ in ABCA1 and ABCG1 protein levels in RAW 264.7 macrophages. (A) Cells were preincubated with rottlerin (5 and 10 μmol/L) for 2 h, after which they were treated with for 12 h as described in Fig. 1. ABCA1 and ABCG1 protein expression was measured by Western blot analysis using β-actin as a loading control. A representative blot of two separate experiments is shown. Densitometry analysis was conducted using β-actin for normalization, and relative ABCA1 and ABCG1 protein levels to BSA control without rottlerin are as follows from left to right: 1.00, 1.31, 1.26, 1.02, 1.12, 0.78, 0.57, 0.43 and 0.49 for ABCA1; 1.00, 1.13, 1.03, 0.77, 0.79, 0.65, 0.40, 0.32 and 0.44 for ABCG1. (B) Cells were transfected with 100 nmol/L of scrambled control (Sc) or PKCδ siRNA for 24 h and subsequently incubated with BSA only (control), 16:0 or 18:2 (100 μmol/L) for 24 h. A representative blot of two separate experiments is shown. Densitometry analysis of ABCA1 and ABCG1 protein relative to scrambled BSA control is as follows from left to right treatment as an average of two lanes: 1.00, 0.48, 0.77, 0.62, 0.47 and 0.24 for ABCA1; 1.00, 0.58, 0.81, 0.50, 0.58 and 0.38 for ABCG1.

mRNA abundance of ABCA1 and ABCG1 in RAW 264.7 macrophages and that the repression was abolished by an LXR agonist. Our results indicate that unsaturated fatty acids may inhibit the basal expression of the ABC transporters possibly in an LXR-dependent manner in macrophages. Involvement of LXR in the transcriptional repression of ABCA1 and ABCG1 in macrophages was also suggested by Uehara et al. [30,31]. Of importance is that unliganded LXR is suggested to repress the basal expression of the ABC transporters by recruiting co-repressors, such as nuclear receptor co-repressors (NCoR) and silencing mediators for retinoid and thyroid hormone receptors (SMRT), in macrophages but not in the liver [41]. As we observed the transcriptional repression of the basal ABCA1 expression by unsaturated fatty acids only in macrophages but not in HepG2 cells (manuscript in preparation), it can be presumed that unsaturated fatty acids could enhance recruitment of the co-repressor complex to unliganded LXR and/or its activity in the promoters of macrophage ABCA1 and ABCG1. Derepression of the ABC transporter expression by an LXR agonist in unsaturated fatty acid-treated cells further supports this speculation. However, we cannot rule out the possibility that the effect of TSA on ABCA1 and ABCG1 expression may be mediated through PPARγ pathway, as we observed a significant increase in PPARγ and PGC-1α by TSA, whereas LXRα mRNA levels were significantly reduced. Although LXR is the major transcription factor for the ABC transporter expression, LXR-independent but PPAR-dependent transcriptional regulation has been suggested [42]. Further study is necessary to identify major factors responsible for the fatty acid regulation of ABCA1 and ABCG1 expression.

Exchange of co-repressor with co-activator is widely used to switch function of transcription factors from active repression to transcriptional activation. Transcription factors that have been shown to interact with a co-repressor complex for transcriptional repression include activator protein 1 [43–45], nuclear factor κB [44–46], thyroid hormone receptor [47–49] and LXRα [41]. NCoR and SMRT are essential components of co-repressor complex and mediate active

transcriptional repression via activating HDAC in the complex [43,50,51]. Interestingly, we found that TSA, a pan-HDACi, markedly increased the basal ABC transporter mRNA and abolished the repressive effect of unsaturated fatty acids on the transporter expression in RAW 264.7 macrophages. The result led us to speculate that basal transcription of ABCA1 and ABCG1 may be repressed by an HDAC and that unsaturated fatty acid could further inhibit the transcription by inducing histone deacetylation in the promoters of the ABC transporters. TSA treatment also increased ABCA1 and ABCG1 mRNA levels in palmitate-treated macrophages, but the extent of the increase was markedly lower than control and linoleic acid-treated groups. Eighteen HDACs have been identified and divided into four classes based on their homology to yeast HDAC, subcellular localization and their enzyme activities [52]. HDACs are subject to posttranslational modifications, such as acetylation, methylation, phosphorylation, ubiquitination and sumoylation, that determine stability, localization, activity and protein–protein interaction of HDAC [53].We assume that fatty acids may modify HDAC posttranslationally, consequently altering its activity. Our data indicate that saturated and unsaturated fatty acids may have a differential effect on histone deacetylation in the promoters of ABCA1 and ABCG1 possibly by different modes of action or extent of the regulation. Several lines of evidence exist to support potential roles of fatty acids, such as short-chain fatty acid (e.g., butyrate) and 9-hydroxystearic acid, in modulating chromatin structure [54,55]. However, whether fatty acids commonly present in the diet could utilize this mode of transcriptional regulation is largely unknown. It should be further investigated whether long-chain fatty acids utilize HDAC to alter gene expression.

Turnover of ABCA1 protein is rapid, with a half-life of less than 1 h in macrophages [56,57], suggesting that posttranscriptional regulation could be an important determinant for its function. We found that unsaturated fatty acids reduced ABCA1 and ABCG1 protein levels in macrophages. Although T0901317 abolished the repression of the ABC transporter mRNA levels by unsaturated fatty acids, protein levels of both transporters remained to be diminished by unsaturated fatty acids compared with saturated fatty acids, suggesting that posttranscriptional repression of ABCA1 and ABCG1 is likely to exist in addition to the transcriptional inhibition. Reduced ABCA1 protein levels by 18:2 were at least partly due to faster protein degradation as demonstrated by the experiment using cycloheximide, a translation inhibitor (data not shown). It has been suggested that unsaturated fatty acids induce ABCA1 protein degradation by activating PLD_2 and PKCδ pathway that phosphorylates serine residues of ABCA1 for degradation [26–29]. We, however, found that PKCδ may function in an opposite direction, as its depletion by siRNA reduced ABCA1 and ABCG1 proteins and both genetic and chemical inhibition of PKCδ did not reverse the repressive effect of unsaturated fatty acids. The reason for the contradictory observations is not clear. However, we found similar results in HepG2 as well as FHs 74 Int cell lines (unpublished data), and further study is necessary to identify the role of PKCδ in the posttranscriptional regulation of the ABC transporters by fatty acids.

In summary, we found that unsaturated fatty acids repress the expression of ABCA1 and ABCG1 in macrophages at the transcription and posttranscription levels. In particular, our following observations support that unsaturated fatty acids lower ABCA1 and ABCG1 transcription in an LXR-dependent manner by altering HDAC in a corepressor complex: (1) repression of the ABC transporter mRNA levels by unsaturated fatty acids was completely repressed in the presence of an LXR agonist, (2) the ABC transporter mRNA levels were significantly increased by inhibiting HDAC activity using TSA, and (3) an inhibitory effect of linoleate on ABCA1 and ABCG1 mRNA was completely abolished by TSA. As macrophage ABCA1 and ABCG1 play a pivotal role in removal of cellular cholesterol, which can prevent foam cell formation and consequently atherogenesis, the inhibitory function of unsaturated fatty acids in the transporter expression could be regarded as unfavorable. Understanding of mechanisms by which fatty acids modulate the ABC transporters in macrophages is necessary to develop a therapeutic means to enhance beneficial effect of unsaturated fatty acids in the prevention of atherogenesis by potentiating their atheroprotective properties including reductions in triglycerides, anti-inflammation and antithrombosis [58–61].

Acknowledgments

C. S. Ku, Y. Park, and S. L. Coleman conducted experiments; J. Lee designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

References

[1] Barr DP, Russ EM, Eder HA. Protein–lipid relationships in human plasma. II. In atherosclerosis and related conditions. Am J Med 1951;11:480–93.
[2] Miller NE. The evidence for the antiatherogenicity of high density lipoprotein in man. Lipids 1978;13:914–9.
[3] Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977;62:707–14.
[4] Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989;79:8–15.
[5] Stein O, Stein Y. Atheroprotective mechanisms of HDL. Atherosclerosis 1999;144: 285–301.
[6] Mauerer R, Ebert S, Langmann T. High glucose, unsaturated and saturated fatty acids differentially regulate expression of ATP-binding cassette transporters ABCA1 and ABCG1 in human macrophages. Exp Mol Med 2009;41:126–32.
[7] Glomset JA. The plasma lecithins:cholesterol acyltransferase reaction. J Lipid Res 1968;9:155–67.
[8] Pieters MN, Schouten D, Van Berkel TJ. In vitro and in vivo evidence for the role of HDL in reverse cholesterol transport. Biochim Biophys Acta 1994;1225:125–34.
[9] Francone OL, Gong EL, Ng DS, Fielding CJ, Rubin EM. Expression of human lecithin-cholesterol acyltransferase in transgenic mice. Effect of human apolipoprotein AI and human apolipoprotein all on plasma lipoprotein cholesterol metabolism. J Clin Invest 1995;96:1440–8.
[10] Lacko AG, Pritchard PH. International symposium on reverse cholesterol transport. Report on a meeting. J Lipid Res 1990;31:2295–9.
[11] Kusuhara H, Sugiyama Y. ATP-binding cassette, subfamily G (ABCG family). Pflugers Arch 2007;453:735–44.
[12] Ohashi R, Mu H, Wang X, Yao Q, Chen C. Reverse cholesterol transport and cholesterol efflux in atherosclerosis. QJM 2005;98:845–56.
[13] Rogler G, Trumbach B, Klima B, Lackner KJ, Schmitz G. HDL-mediated efflux of intracellular cholesterol is impaired in fibroblasts from Tangier disease patients. Arterioscler Thromb Vasc Biol 1995;15:683–90.
[14] Francis GA, Knopp RH, Oram JF. Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier disease. J Clin Invest 1995;96: 78–87.
[15] Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci U S A 2004;101:9774–9.
[16] Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, et al. ABCG1 (ABC8), the human homolog of the *Drosophila* white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci U S A 2000;97:817–22.
[17] Out R, Hoekstra M, Hildebrand RB, Kruit JK, Meurs I, Li Z, et al. Macrophage ABCG1 deletion disrupts lipid homeostasis in alveolar macrophages and moderately influences atherosclerotic lesion development in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol 2006;26:2295–300.
[18] Baldan A, Pei L, Lee R, Tarr P, Tangirala RK, Weinstein MM, et al. Impaired development of atherosclerosis in hyperlipidemic Ldlr−/− and ApoE−/− mice transplanted with Abcg1−/− bone marrow. Arterioscler Thromb Vasc Biol 2006;26:2301–7.
[19] Francone OL, Royer L, Boucher G, Haghpassand M, Freeman A, Brees D, et al. Increased cholesterol deposition, expression of scavenger receptors, and response to chemotactic factors in Abca1-deficient macrophages. Arterioscler Thromb Vasc Biol 2005;25:1198–205.
[20] Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metabolism 2005;1:121–31.
[21] Out R, Jessup W, Le Goff W, Hoekstra M, Gelissen IC, Zhao Y, et al. Coexistence of foam cells and hypocholesterolemia in mice lacking the ABC transporters A1 and G1. Circ Res 2008;102:113–20.
[22] Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, et al. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. J Clin Invest 2007;117:3900–8.

[23] Haghpassand M, Bourassa PA, Francone OL, Aiello RJ. Monocyte/macrophage expression of ABCA1 has minimal contribution to plasma HDL levels. J Clin Invest 2001;108:1315–20.
[24] Tarling EJ, Bojanic DD, Tangirala RK, Wang X, Lovgren-Sandblom A, Lusis AJ, et al. Impaired development of atherosclerosis in Abcg1−/− Apoe−/− mice: identification of specific oxysterols that both accumulate in Abcg1−/− Apoe −/− tissues and induce apoptosis. Arterioscler Thromb Vasc Biol 30:1174–80.
[25] Naik SU, Wang X, Da Silva JS, Jaye M, Macphee CH, Reilly MP, et al. Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. Circulation 2006;113:90–7.
[26] Wang Y, Kurdi-Haidar B, Oram JF. LXR-mediated activation of macrophage stearoyl-CoA desaturase generates unsaturated fatty acids that destabilize ABCA1. J Lipid Res 2004;45:972–80.
[27] Wang Y, Oram JF. Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1. J Biol Chem 2002;277:5692–7.
[28] Wang Y, Oram JF. Unsaturated fatty acids phosphorylate and destabilize ABCA1 through a phospholipase D2 pathway. J Biol Chem 2005;280: 35896–903.
[29] Wang Y, Oram JF. Unsaturated fatty acids phosphorylate and destabilize ABCA1 through a protein kinase C delta pathway. J Lipid Res 2007;48:1062–8.
[30] Uehara Y, Engel T, Li Z, Goepfert C, Rust S, Zhou X, et al. Polyunsaturated fatty acids and acetoacetate downregulate the expression of the ATP-binding cassette transporter A1. Diabetes 2002;51:2922–8.
[31] Uehara Y, Miura Si, von Eckardstein A, Abe S, Fujii A, Matsuo Y, et al. Unsaturated fatty acids suppress the expression of the ATP-binding cassette transporter G1 (ABCG1) and ABCA1 genes via an LXR/RXR responsive element. Atherosclerosis 2007;191:11–21.
[32] Park YK, Rasmussen HE, Ehler SJ, Blobaum KR, Lu F, Schlegel VL, et al. Repression of proinflammatory gene expression by lipid extract of *Nostoc commune* var sphaeroides Kutzing, a blue–green alga, via inhibition of nuclear factor-kappa B in RAW 264.7 macrophages. Nutr Res 2008;28:83–92.
[33] Rasmussen HE, Blobaum KR, Park YK, Ehlers SJ, Lu F, Lee JY. Lipid extract of *Nostoc commune* var. sphaeroides Kutzing, a blue–green alga, inhibits the activation of sterol regulatory element binding proteins in HepG2 cells. J Nutr 2008;138: 476–81.
[34] Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 1994;93:2438–46.
[35] McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. Science 1992;258:766–70.
[36] Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. Diabetes 1988;37:1020–4.
[37] Perseghin G, Ghosh S, Gerow K, Shulman GI. Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. Diabetes 1997;46:1001–9.
[38] Shulman GI. Cellular mechanisms of insulin resistance. J Clin Invest 2000;106: 171–6.
[39] Ecker J, Langmann T, Moehle C, Schmitz G. Isomer specific effects of conjugated linoleic acid on macrophage ABCG1 transcription by a SREBP-1c dependent mechanism. Biochem Biophys Res Commun 2007;352:805–11.
[40] Murthy S, Born E, Mathur SN, Field FJ. Liver-X-receptor-mediated increase in ATP-binding cassette transporter A1 expression is attenuated by fatty acids in CaCo-2 cells: effect on cholesterol efflux to high-density lipoprotein. Biochem J 2004;377: 545–52.
[41] Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, et al. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. Mol Cell Biol 2003;23: 5780–9.
[42] Li AC, Binder CJ, Gutierrez A, Brown KK, Plotkin CR, Pattison JW, et al. Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARalpha, beta/delta, and gamma. J Clin Invest 2004;114:1564–76.
[43] Zhang J, Kalkum M, Chait BT, Roeder RG. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. Mol Cell 2002;9:611–23.
[44] Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. Cell 2004;116:511–26.
[45] Lee SK, Kim JH, Lee YC, Cheong J, Lee JW. Silencing mediator of retinoic acid and thyroid hormone receptors, as a novel transcriptional corepressor molecule of activating protein-1, nuclear factor-kappaB, and serum response factor. J Biol Chem 2000;275:12470–4.
[46] Baek SH, Ohgi KA, Rose DW, Koo EH, Glass CK, Rosenfeld MG. Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-kappaB and beta-amyloid precursor protein. Cell 2002;110:55–67.
[47] Rosenfeld MG, Glass CK. Coregulator codes of transcriptional regulation by nuclear receptors. J Biol Chem 2001;276:36865–8.
[48] Naar AM, Lemon BD, Tjian R. Transcriptional coactivator complexes. Annu Rev Biochem 2001;70:475–501.
[49] McKenna NJ, O'Malley BW. Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 2002;108:465–74.
[50] Li J, Wang J, Nawaz Z, Liu JM, Qin J, Wong J. Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. EMBO J 2000;19: 4342–50.
[51] Guenther MG, Lane WS, Fischle W, Verdin E, Lazar MA, Shiekhattar R. A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. Genes Dev 2000;14:1048–57.
[52] Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF. Histone deacetylases: unique players in shaping the epigenetic histone code. Ann N Y Acad Sci 2003;983:84–100.
[53] Brandl A, Heinzel T, Kramer OH. Histone deacetylases: salesmen and customers in the post-translational modification market. Biol Cell 2009;101:193–205.
[54] Jones DR, Divecha N. Linking lipids to chromatin. Curr Opin Genet Dev 2004;14: 196–202.
[55] Jansen MS, Nagel SC, Miranda PJ, Lobenhofer EK, Afshari CA, McDonnell DP. Short-chain fatty acids enhance nuclear receptor activity through mitogen-activated protein kinase activation and histone deacetylase inhibition. Proc Natl Acad Sci U S A 2004;101:7199–204.
[56] Arakawa R, Yokoyama S. Helical apolipoproteins stabilize ATP-binding cassette transporter A1 by protecting it from thiol protease-mediated degradation. J Biol Chem 2002;277:22426–9.
[57] Oram JF, Lawn RM, Garvin MR, Wade DP. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. J Biol Chem 2000;275:34508–11.
[58] Parks JS, Rudel LL. Effect of fish oil on atherosclerosis and lipoprotein metabolism. Atherosclerosis 1990;84:83–94.
[59] Demaison L, Moreau D. Dietary n-3 polyunsaturated fatty acids and coronary heart disease-related mortality: a possible mechanism of action. Cell Mol Life Sci 2002;59:463–77.
[60] Schaefer EJ. Effects of dietary fatty acids on lipoproteins and cardiovascular disease risk: summary. Am J Clin Nutr 1997;65:1655S–6S.
[61] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747–57.