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Adipocyte Modulation of High-Density Lipoprotein Cholesterol

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Abstract

Background—Adipose harbors a large depot of free cholesterol. However, a role for adipose in cholesterol lipidation of HDL *in vivo* is not established. We present the first evidence that adipocytes support transfer of cholesterol to HDL *in vivo* as well as *in vitro* and implicate ABCA1 and SR-BI, but not ABCG1, cholesterol transporters in this process.

Methods and Results—Cholesterol efflux from wild-type (WT), ABCA1−/−, SR-BI−/− and ABCG1−/− adipocytes to apoA-I and HDL3 were measured *in vitro*. 3T3L1-adipocytes, labeled with ^{3}H-cholesterol, were injected intraperitoneally (IP) into WT, apoA-I transgenic and apoA-I−/− mice and tracer movement onto plasma HDL monitored. Identical studies were performed with labeled WT, ABCA1−/− or SR-BI−/− mouse-embryonic-fibroblast (MEF) adipocytes. The effect of TNFα on transporter expression and cholesterol efflux was monitored during adipocyte differentiation. Cholesterol efflux to apoA-I and HDL3 was impaired in ABCA1−/− and SR-BI−/− adipocytes respectively, with no effect observed in ABCG1−/− adipocytes. Injection IP of labeled 3T3L1-adipocytes resulted in increased HDL-associated ^{3}H-cholesterol in apoA-I transgenic mice but reduced levels in apoA-I −/− animals. Injection IP of labeled ABCA1−/− or SR-BI−/− adipocytes reduced plasma counts relative to their respective controls. TNFα reduced both ABCA1 and SR-BI expression and impaired cholesterol efflux from partially-differentiated adipocytes.

Conclusions—These data suggest a novel metabolic function of adipocytes in promoting cholesterol transfer to HDL *in vivo* and implicate adipocyte SR-BI and ABCA1, but not ABCG1, in this process. Further, adipocyte modulation of HDL may be impaired in adipose inflammatory disease states such as type-2 diabetes.

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DISCLOSURES

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Keywords

cholesterol; lipoproteins; adipocytes; atherosclerosis; inflammation

INTRODUCTION

Low plasma HDL cholesterol (HDL-C) is a key feature of obesity and insulin resistance[1] and has a strong inverse relationship with atherosclerotic cardiovascular disease (CVD)2. Reduced cholesterol-lipidation of nascent HDL results in small, immature lipoprotein particles that are rapidly catabolized and excreted in the kidney. Thus, lipidation of HDL plays a role in supporting the atheroprotective functions of HDL *in vivo*[3].

Lipidation of HDL is determined via a number of cholesterol transporters in several cholesterol-rich tissues. Although macrophage cholesterol efflux to HDL plays a major role in attenuating atherosclerosis, macrophages play a minor role in regulation of HDL-C levels[4]. In contrast, hepatic ATP binding cassette transporter 1 (ABCA1), through lipidation of apoA-I, is required for formation of nascent HDL particles[5]. Indeed, in cholesterol-rich tissues, both hepatic and extrahepatic, ABCA1 has discrete and essential roles in the maintenance of plasma HDL-C6, 7. ABCG1 mediates cholesterol efflux from macrophages to mature HDL particles8, [9] and may play a role in regulating plasma HDL-C levels[10]. In contrast to ABC transporters, SR-BI is a bidirectional transporter that plays a major role in hepatic uptake of HDL cholesterol ester[11, 12]. Although SR-BI is not believed to be important in macrophage cholesterol efflux to HDL[13], a role in lipidating HDL via other peripheral SR-BI-expressing tissues[14] has not been examined. In fact, the relative role of tissues, beyond liver, in HDL lipidation requires further definition.

Adipose tissue contains a very large pool of free cholesterol[15, 16]. In fact, adipocytes are known to support cholesterol efflux to HDL and apoA-I *in vitro*[17, 18]. Recent work shows ABCA1 and SR-BI are expressed in mature adipocytes and adipocyte cholesterol homeostasis may be regulated in a cell-specific manner[19–21]. Adipocyte cholesterol, therefore, represents a uniquely regulated and abundant depot for modulation of HDL-C levels. By extension, adipose inflammatory dysfunction in insulin-resistant states may impair adipocyte HDL lipidation and reduce circulating HDL-C levels in such settings.

In this study, we demonstrate that adipocytes are a regulated source of cholesterol transfer to HDL both *in vitro* and *in vivo*. In contrast to liver and macrophages, adipocyte cholesterol efflux is controlled by ABCA1 and SR-BI, but not ABCG1, and is suppressed, in a differentiation-dependent manner, by TNFα an inflammatory adipocytokine. In summary, we report a novel, adipocyte-dependent mechanism of cholesterol transfer to HDL that may uniquely contribute to reduced plasma HDL-C in adipose inflammatory settings.

MATERIALS AND METHODS

Cell culture

3T3L1 cells were differentiated to adipocytes as described previously[22] (online supplement). Efflux studies were performed on days 0, 5 and 10 post-differentiation. Mouse embryonic fibroblasts (MEF) were isolated from 13.5–14.5 day embryos as described[23] (online supplement). MEFs were grown to confluence prior to addition of differentiation media (as for 3T3L1 except addition of 1μM of PPARγ agonist, GW347845). Human SGBS adipocytes were cultured as previously described[24] (online supplement). Bone-marrow macrophages (BMM) were isolated from mouse femurs and tibias and cultured in DMEM supplemented with 10% FBS and 30% L929 conditioned medium[13].

Cholesterol efflux studies

Fully differentiated ABCA1−/−, ABCG1−/−, SR-BI−/− and littermate, wild-type control MEFs as well 3T3L1 and SGBS adipocytes were labeled with ^{3}H-cholesterol (5μCi/mL) (Perkin-Elmer Analytical Sciences, Boston, MA) for 24h. Wild-type BMM were labeled with 5μCi/ml ^{3}H-cholesterol, and loaded with 25μg/ml acLDL for 24h. After equilibration, ^{3}H-cholesterol efflux from adipocytes and BMM to apoA-I (20μg/ml), HDL3 (50μg/ml), 5% human (for SGBS cells) or 5% mouse (for 3T3L1, MEF and BMM cells) serum and MEM control was assessed over 4h as described previously for macrophages[25]. In MEF-adipocyte studies cells were equilibrated overnight in MEM containing 0.2% BSA ±LXR agonist (10μM). Cells were subsequently co-treated with LXR agonist ±BLT (10μM) or ±probucol (20μM) for 2h prior to efflux. The effect of TNFα (10ng/ml overnight) on cholesterol efflux from 3T3L1 cells was assessed at day 0 (pre), day 5 (~50% differentiated) or day 10 (mature) of adipocyte differentiation. Cell lipid was extracted with isopropanol and total cellular ^{3}H-cholesterol was measured by liquid scintillation counting. Percent efflux to acceptors, minus MEM, is presented.

Adipocyte-transfer of cholesterol to HDL *in vivo*

We employed a modification of our published *in vivo* macrophage to HDL reverse cholesterol transport (RCT) model12, 26. Briefly,] 3T3L1 adipocytes, or MEF-adipocytes derived from SR-BI−/−, ABCA1−/− or littermate wild-type mice, were labeled with 5μCi/mL ^{3}H-cholesterol for 24h. Cells were washed, equilibrated in DMEM containing 0.2% BSA for 6h, lifted off the plate by incubation with EDTA (10mM) for 10min, centrifuged and resuspended in MEM. Adipocyte levels of ^{3}H-cholesterol were measured by liquid scintillation counting. Within individual studies, equal numbers of ^{3}H-cholesterol counts (~1.2×10^{6} cpm in MEF and ~3×10^{6} cpm in 3T3L1) and numbers of adipocytes (~2×10^{6} MEFs and ~7×10^{6} 3T3L1) were injected into each recipient C57BL/6 Wild-Type mouse. Movement of ^{3}H-cholesterol from intra-peritoneal injected adipocytes onto plasma HDL and through liver to bile/feces was monitored as previously described[12, 26] (online supplement). Animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Effects of inflammation on expression of cholesterol transporters in adipose

Female C57BL/6 mice were injected intravenously with 10μg/kg LPS and after 6h mice were sacrificed by cervical dislocation. Adipose tissue was harvested.RNA and protein were isolated, and mRNA and protein expression of cholesterol transporters were assessed by Real-Time PCR and Western blotting respectively (online supplement). General laboratory methods

A description of laboratory methods including lipoprotein analysis, quantitative Real-Time PCR, immunoblot analysis and oil-red staining are presented in online supplement.

Statistical analysis

Data are reported as mean ± SEM. For experiments with multiple treatments, analysis of variance (ANOVA) was used to test for differences in groups means. For mouse experiments with multiple time-points, we performed two-way repeated measures analysis of variance (ANOVA) to test for differences in means between groups. When ANOVA was significant post-hoc Bonferroni corrected t-tests were applied. For comparison of data between two groups at a single time-point (liver, bile, feces, mRNA data), unpaired t-tests were performed. GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA) and Stata 9.0 software (Stata Corp, College Station, TX) were used for statistical analyses. Statistical significance is presented as *$p<0.05$, **$p<0.01$ and ***$p<0.001$ in all figures.

RESULTS

Cholesterol content and cholesterol transporter expression increase during adipocyte differentiation

During 3T3L1 differentiation from fibroblasts to adipocytes (Supplement Figure 1A), cellular cholesterol content increased ~2-fold (Supplement Table 1A), while mRNA expression (reduced delta Ct values relative to β-actin) of ABCA1, ABCG1 and SR-BI all increased (Supplement Table 1B). In fully differentiated adipocytes, SR-BI was abundant with similar expression to ABCA1, but with much lower-levels of ABCG1. Immunoblotting showed increased SR-BI and ABCA1 protein during differentiation, but ABCG1 protein was barely detectable in fully differentiated adipocytes (Supplement Figure 1B&C).

Mature mouse and human adipocytes efflux cholesterol to HDL acceptors

Mature murine (3T3L1) and human (SGBS) adipocytes supported cholesterol efflux to lipid acceptors apoA-I, HDL3 and serum (Supplement Figure 1D-E). Indeed, cholesterol efflux from mature adipocytes was comparable to that from bone-marrow macrophages (BMM) in parallel efflux studies (Supplement Figure 1F). Efflux to MEM from 3T3L1 (0.77±0.01%, n=3) and SGBS (0.42±0.044%, n=3) in the absence of acceptor was minimal.

Adipocyte cholesterol efflux is mediated via ABCA1 and SR-BI transporters

ABCA1−/−, ABCG1−/−, SR-BI−/− and littermate wild-type control MEFs were differentiated into adipocytes (Supplement Figure 2A) and cholesterol efflux studies performed. Initially, the role of individual transporters was probed pharmacologically in wild-type MEF adipocytes. Probucol, an inhibitor of ABCA1-mediated efflux[27], reduced cholesterol efflux from MEF adipocytes to apoA-I and abolished LXR-enhanced cholesterol efflux to apoA-I (Figure 1A). Cholesterol efflux to HDL3 was reduced by BLT, an inhibitor of SR-BI[28], suggesting adipocyte SR-BI mediates lipidation of mature HDL (Figure 1B).

Similar to probucol effects (Figure 1C, left panel), ABCA1 deficiency reduced efflux to apoA-I with complete attenuation of LXR-induced efflux to apoA-I (Figure 1C, right panel). Probucol had no incremental effect on efflux to apoA-I in ABCA1−/− adipocytes. In contrast, absence of ABCA1 had no impact on cholesterol efflux to HDL3 (Figure 1D). These data suggest that (a) ABCA1 plays a central role in adipocyte cholesterol efflux to apoA-I, (consistent with macrophages13 and liver5, 29), (b) LXR-induced adipocyte cholesterol efflux is mediated via ABCA1, and (c) adipocyte ABCA1 plays a minor role in efflux to mature HDL.

SR-BI deficiency had little effect on cholesterol efflux to apoA-I and did not influence inhibition of efflux to apoA-I by probucol (Figure 1E). Lack of adipocyte SR-BI, however, resulted in marked reduction in cholesterol efflux to HDL3 (Figure 1F, right panel); this inhibition was almost identical to the BLT effect on efflux to HDL in wild-type adipocytes (Figure 1F, left panel). BLT had no incremental effect on efflux to HDL in SR-BI−/− adipocytes. These data suggest that (a) SR-BI plays an important role in adipocyte cholesterol efflux to mature HDL particles (in contrast to findings in macrophages13), and (b) SR-BI does not mediate basal or LXR-induced adipocyte cholesterol efflux to apoA-I.

ABCG1 does not regulate adipocyte cholesterol efflux *in vitro*

ABCG1 has been implicated in cholesterol efflux from macrophages[8, 13] and liver[10]. Deficiency of ABCG1 in MEF adipocytes, however, had no effect on cholesterol efflux to any lipid acceptor (Figure 2A). As expected, real-time PCR analysis revealed a marked reduction in ABCG1 mRNA in knock-out cells (Figure 2B). However, ABCG1 protein expression was barely detectable in fully differentiated wild-type MEF adipocytes (Figure

2C). In fact, realtime analysis in MEFs, 3T3L1 adipocytes (Supplement Table 1), human-derived SGBS adipocytes as well as mouse (Supplement Table 2) and human adipose (Supplement Table 3) revealed very low expression of ABCG1 compared with ABCA1 and SR-BI and suggests that ABCG1 plays little role in adipocyte cholesterol homeostasis.

Adipocytes can transfer cholesterol to HDL *in vivo*

We modified our macrophage-to-feces RCT model[12, 26] in order to examine adipocyte-to-HDL transport of cholesterol *in vivo*. We labeled fully differentiated adipocytes with ^{3}H-cholesterol, injected labeled-adipocytes intraperitoneally (IP), and tracked movement of label onto plasma HDL and subsequently into liver and feces. First, we performed studies using 3T3L1 adipocytes injected into apoA-I transgenic (TG), apoA-I deficient or wild-type mice to establish proof-of-concept for this *in vivo* model. Movement of ^{3}H-cholesterol from IP-adipocytes to plasma increased steadily in the first 24h (Figure 3A), tracked with the HDL fraction in each group (Figure 3C&D and Table 1A), and resulted in detectable tracer in liver (not shown) and feces (Figure 3B). Over-expression of apoA-I increased plasma and HDL counts consistent with enhanced adipocyte-to-HDL cholesterol movement whereas absence of apoA-I decreased plasma, HDL and fecal counts reflecting reduced movement of adipocyte-label to HDL. Notably, the time course and extent of ^{3}H-cholesterol movement from adipocytes to HDL and through liver to feces was similar to our published findings for IP injection of macrophage-foam cells in apoA-I transgenic and null mice[26, 30]. These findings suggest that cholesterol movement from adipocytes into plasma *in vivo* is modulated by the circulating levels of HDL acceptor particles.

Adipocyte ABCA1 and SR-BI regulate adipocyte transfer of cholesterol to HDL *in vivo*

Movement of ^{3}H-cholesterol, from IP-injected MEF-adipocytes derived from ABCA1−/− (Figure 4A&B) and SR-BI−/− (Figure 4C&D) mice, into plasma, onto HDL and into feces was significantly reduced compared to IP-injection of adipocytes derived from their wild-type littermates. As expected, the majority of plasma ^{3}H-cholesterol counts was associated with the HDL fraction (Table 1B&C). These studies indicate that adipocytes, acting via functional ABCA1 and SR-BI, can transfer cholesterol to HDL *in vivo*.

Inflammation impairs cholesterol efflux from 3T3L1 adipocytes

We examined whether TNFα, an inflammatory adipocytokine[31], modulated 3T3L1 adipocyte cholesterol efflux in order to explore if adipocyte lipidation of HDL might be reduced during adipose inflammation, a cornerstone of central adiposity and insulin resistance[32, 33]. Because inflammatory modulation of adipocyte functions may be differentiation-dependent[34, 35], we performed studies in pre (day-0), partially (day-5) and fully (day-10) differentiated adipocytes. Protein expression of ABCA1 and SR-BI cholesterol transporters increased during differentiation, whereas ABCG1 protein was barely detectable throughout differentiation (Supplement Figure 1B&C). Pre-adipocytes supported efflux to HDL3 but not to apoA-I (Figure 5A&B). Induction of PPARγ, adiponectin and lipoprotein lipase was suppressed by TNFα particularly in partially and fully differentiated adipocytes (Table 2). TNFα had little effect on expression of transporters and no effect on cholesterol efflux in pre-adipocytes (Figure 5A–C & Table 2). At day-5, however, TNFα inhibited efflux to apoA-I and HDL3 (Figure 5A&B) coincident with attenuation of ABCA1 and SR-BI protein expression (Figure 5C & Table 2). By day-10, there was no significant effect of TNFα on cholesterol efflux, consistent with lesser effect on ABCA1 and SR-BI in fully differentiated adipocytes (Figure 5A–C & Table 2).

Finally, we assessed the effects of inflammation on mouse adipose expression of cholesterol transporters *in vivo*. Endotoxin administration (10μg/kg, IV) down-regulated adipose levels

of SR-BI and ABCA1 mRNA and protein, with little effect on ABCG1 (Supplement Table 2 & Supplement Figure 2D).

DISCUSSION

Adipose tissue harbors a major pool of free cholesterol[18] but its role in regulating circulating HDL-C is poorly understood. In this work, we present the first evidence that adipocytes transfer cholesterol to HDL *in vivo* as well as *in vitro*. We identified a differentiation-dependent role for ABCA1 and SR-BI, but not ABCG1, in adipocyte cholesterol efflux to apoA-I and mature HDL respectively and provide experimental evidence that both ABCA1 and SR-BI can regulate adipocyte cholesterol transfer to HDL *in vivo*. Finally, we show that adipocyte inflammation down-regulates transporters and impairs adipocyte cholesterol efflux to HDL. As adipose inflammation is a hallmark of central obesity and type-2 diabetes, loss of adipocyte-lipidation of HDL may directly contribute to lower HDL-C in these adipose inflammatory states.

Lipidation of HDL particles *in vivo* involves the coordinated effect of several tissues[6, 7] likely involving cell-specific transporter functions. ABCA1 plays a major role in generation of nascent HDL particles[5, 36] and maintenance of plasma HDL-C through integrated hepatic and peripheral tissue actions. Dramatic reductions in HDL-C levels are observed in the absence of ABCA1[6, 7] primarily because of loss of hepatic lipidation of liver-secreted apoA-I. However, peripheral ABCA1 also contributes to HDL-C[6] through intestinal[7] and brain[37] ABCA1. Although macrophage cholesterol efflux to HDL plays a major role in attenuating atherosclerosis, macrophages contain a very small pool of cholesterol and do not regulate circulating HDL-C *in vivo*[4]. Because adipose tissue contains a large pool of free cholesterol[15, 16], we hypothesized that adipocytes may play a unique role in cholesterol transfer to HDL both *in vitro* and *in vivo*. In fact, a role for adipose or involvement of non-ABCA1 transporters in peripheral lipidation of HDL has not been demonstrated.

Our findings support a model of adipocyte-specific regulation of cholesterol efflux to HDL acceptors. We identified a role for ABCA1 and SR-BI transporters in efflux to apoA-I and HDL respectively and demonstrated marked up-regulation of these proteins during adipocyte differentiation. Although ABCG1 promotes macrophage cholesterol efflux to mature HDL[8, 9, 38] we found no evidence that ABCG1 protein is expressed in mature adipocytes or plays a role in adipocyte cholesterol efflux to HDL.

Using a modified version of our published macrophage-to-HDL reverse cholesterol transport model[12, 26], we demonstrate that adipocytes are capable of transferring cholesterol to circulating HDL. Indeed, the timecourse and extent of cholesterol label movement onto HDL was similar to macrophages[26, 39, 40]. Further, cholesterol movement from IP-injected adipocytes to HDL was increased in apoA-I transgenic and reduced in apoA-I null mice. Adipocyte deficiency of ABCA1 or SR-BI reduced tracer movement onto HDL *in vivo*. Overall, our data provide indirect evidence for adipocyte regulation of HDL-C *in vivo* and suggest a role for SR-BI and ABCA1, but not ABCG1, in this process.

Recent studies suggest an underappreciated role for adipocyte cholesterol in adipose function and pathophysiologies[19, 41, 42,43, 44]. Zhao et al[21] showed that primary adipocytes, isolated from rabbits fed a high-cholesterol diet or treated with statins, had altered cholesterol efflux to HDL that correlated with changes in SR-BI expression. They did not prove, however, that SR-BI was causal. Verghese and colleagues[42] demonstrated that enhanced adipocyte cholesterol efflux to HDL occurs during lipolysis without change in SR-BI and ABCA1 expression. It is possible, however, that modulation of transporter function[45–47] or membrane localization[40] rather than change in protein level could mediate

this efflux. Our studies provide novel data that go beyond prior correlative studies. We addressed directly the role of specific transporters and performed *in vivo* studies examining the potential for adipocytes and specific transporters to transfer cholesterol to HDL *in vivo*. Future work with adipose-specific, conditional modulation of ABCA1 and SR-BI in rodent models is required to confirm the importance of these transporters and adipose regulation of HDL cholesterol mass *in vivo*.

Our *in vivo* experimental model does have limitations including its non physiological nature, use of exogenous cells and reliance on cholesterol tracer rather than mass. The peritoneal space is a convenient experimental location in which cells are exposed to extracellular fluid that has many of the characteristics of extracellular fluid in other tissues. Importantly, this model has provided fundamental insights into the macrophage reverse cholesterol transport process[12, 26]. Work by Sehayek and colleagues[48] demonstrates that the subcutaneous administration of macrophages provides a similar pattern of reverse cholesterol transport as the peritoneal cavity arguing against any unique properties for the peritoneum. Although adipocytes do not occur as single cells in the peritoneum, IP-injection of labeled adipocytes resulted in cholesterol-label movement to plasma HDL that was remarkably similar to that published for macrophages. Therefore, we doubt a systematic difference between adipocyes and macrophages in the intraperitoneal model.

We examined the impact of an inflammatory adipocytokine on adipocyte cholesterol efflux in order to explore if loss of adipocyte HDL-lipidation is one possible mechanism for reduced HDL-C in adipose-inflammatory settings[33, 49]. Because adipocyte susceptibility to inflammation[34, 35] depends on adipocyte maturity, we examined TNFα effects during differentiation. TNFα impaired cholesterol efflux most in partially-differentiated adipocytes coincident with greatest suppression of ABCA1 and SR-BI. This is consistent with work by Chung *et al.* who reported that endotoxin impaired glucose transport maximally in partially-differentiated adipocytes[34]. We also found that endotoxemia down-regulated adipose SR-BI and ABCA1 *in vivo*. Thus, despite increased adipose mass and adipose cholesterol in obesity, attenuation of adipocyte-mediated HDL-lipidation may directly contribute to lower HDL-C in metabolic syndrome and type-2 diabetes (Figure 6).

In conclusion, adipocytes support transfer of cholesterol to HDL *in vivo*. This process is mediated by ABCA1 and SR-BI, but not ABCG1, and is attenuated in inflamed adipocytes. Our findings suggest adipocyte-specific cholesterol transporter functions and a role for mature adipose in maintenance of HDL-C levels. Conversely, adipose inflammation may attenuate adipocyte lipidation of HDL leading to lower HDL-C in metabolic syndrome and type-2 diabetes.

SUMMARY

Adipose tissue harbors a major pool of free cholesterol but its role in regulating circulating HDL-C is poorly understood. In this work, we present the first evidence that adipocytes transfer cholesterol to HDL *in vivo* as well as *in vitro*. We identified a differentiation-dependent role for the lipid-transporters ABCA1 and SR-BI, but not ABCG1, in adipocyte cholesterol efflux to apoA-I and mature HDL respectively. We also provide experimental evidence that both ABCA1 and SR-BI can regulate adipocyte cholesterol transfer to HDL *in vivo*. Finally, we show that adipocyte inflammation down-regulates transporters and impairs adipocyte cholesterol efflux to HDL. Our findings suggest a role for mature adipose in directly maintaining HDL-C levels. Conversely, adipose inflammation may attenuate adipocyte lipidation of HDL and may directly contribute to lower HDL-C in adipose inflammatory states such as central obesity and

type-2 diabetes. Thus adipose tissue cholesterol homeostasis may be a direct therapeutic target for modulation of HDL levels *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. Jama 2002;287:356–359. [PubMed: 11790215]
2. Gordon DJ, Rifkind BM. High-density lipoprotein--the clinical implications of recent studies. N Engl J Med 1989;321:1311–1316. [PubMed: 2677733]
3. Brunham LR, Singaraja RR, Duong M, Timmins JM, Fievet C, Bissada N, Kang MH, Samra A, Fruchart JC, McManus B, Staels B, Parks JS, Hayden MR. Tissue-specific roles of ABCA1 influence susceptibility to atherosclerosis. Arterioscler Thromb Vasc Biol 2009;29:548–554. [PubMed: 19201688]
4. Haghpassand M, Bourassa PA, Francone OL, Aiello RJ. Monocyte/macrophage expression of ABCA1 has minimal contribution to plasma HDL levels. J Clin Invest 2001;108:1315–1320. [PubMed: 11696576]
5. Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, Gebre AK, Coutinho JM, Colvin PL, Smith TL, Hayden MR, Maeda N, Parks JS. Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. J Clin Invest 2005;115:1333–1342. [PubMed: 15841208]
6. Singaraja RR, Van Eck M, Bissada N, Zimetti F, Collins HL, Hildebrand RB, Hayden A, Brunham LR, Kang MH, Fruchart JC, Van Berkel TJ, Parks JS, Staels B, Rothblat GH, Fievet C, Hayden MR. Both hepatic and extrahepatic ABCA1 have discrete and essential functions in the maintenance of plasma high-density lipoprotein cholesterol levels in vivo. Circulation 2006;114:1301–1309. [PubMed: 16940190]
7. Brunham LR, Kruit JK, Iqbal J, Fievet C, Timmins JM, Pape TD, Coburn BA, Bissada N, Staels B, Groen AK, Hussain MM, Parks JS, Kuipers F, Hayden MR. Intestinal ABCA1 directly contributes to HDL biogenesis in vivo. J Clin Invest 2006;116:1052–1062. [PubMed: 16543947]
8. Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M, Allikmets R, Schmitz G. 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;97:817–822. [PubMed: 10639163]
9. Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 2005;1:121–131. [PubMed: 16054053]
10. Wiersma H, Nijstad N, de Boer JF, Out R, Hogewerf W, Van Berkel TJ, Kuipers F, Tietge UJ. Lack of Abcg1 results in decreased plasma HDL cholesterol levels and increased biliary cholesterol secretion in mice fed a high cholesterol diet. Atherosclerosis 2009;206:141–147. [PubMed: 19339012]

11. Van Eck M, Twisk J, Hoekstra M, Van Rij BT, Van der Lans CA, Bos IS, Kruijt JK, Kuipers F, Van Berkel TJ. Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. J Biol Chem 2003;278:23699–23705. [PubMed: 12639961]
12. Zhang Y, Da Silva JR, Reilly M, Billheimer JT, Rothblat GH, Rader DJ. Hepatic expression of scavenger receptor class B type I (SR-BI) is a positive regulator of macrophage reverse cholesterol transport in vivo. J Clin Invest 2005;115:2870–2874. [PubMed: 16200214]
13. Wang X, Collins HL, Ranalletta M, Fuki IV, Billheimer JT, Rothblat GH, Tall AR, Rader DJ. Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport in vivo. J Clin Invest 2007;117:2216–2224. [PubMed: 17657311]
14. Altmann SW, Davis HR Jr, Yao X, Laverty M, Compton DS, Zhu LJ, Crona JH, Caplen MA, Hoos LM, Tetzloff G, Priestley T, Burnett DA, Strader CD, Graziano MP. The identification of intestinal scavenger receptor class B, type I (SR-BI) by expression cloning and its role in cholesterol absorption. Biochim Biophys Acta 2002;1580:77–93. [PubMed: 11923102]
15. Krause BR, Hartman AD. Adipose tissue and cholesterol metabolism. J Lipid Res 1984;25:97–110. [PubMed: 6368715]
16. Kovanen PT, Nikkila EA. Cholesterol exchange between fat cells, chylomicrons and plasma lipoproteins. Biochim Biophys Acta 1976;441:357–369. [PubMed: 974088]
17. Angel A, Yuen R, Nettleton JA. Exchange of free cholesterol between low density lipoproteins and human adipocytes. Can J Biochem 1981;59:655–661. [PubMed: 7296348]
18. Prattes S, Horl G, Hammer A, Blaschitz A, Graier WF, Sattler W, Zechner R, Steyrer E. Intracellular distribution and mobilization of unesterified cholesterol in adipocytes: triglyceride droplets are surrounded by cholesterol-rich ER-like surface layer structures. J Cell Sci 2000;113:2977–2989. [PubMed: 10934037]
19. Le Lay S, Robichon C, Le Liepvre X, Dagher G, Ferre P, Dugail I. Regulation of ABCA1 expression and cholesterol efflux during adipose differentiation of 3T3-L1 cells. J Lipid Res 2003;44:1499–1507. [PubMed: 12754274]
20. Zhao SP, Dong SZ. Effect of tumor necrosis factor alpha on cholesterol efflux in adipocytes. Clin Chim Acta 2008;389:67–71. [PubMed: 18155667]
21. Zhao SP, Wu ZH, Hong SC, Ye HJ, Wu J. Effect of atorvastatin on SR-BI expression and HDL-induced cholesterol efflux in adipocytes of hypercholesterolemic rabbits. Clin Chim Acta 2006;365:119–124. [PubMed: 16185675]
22. Elmendorf JS, Chen D, Pessin JE. Guanosine 5'-O-(3-thiotriphosphate) (GTPgammaS) stimulation of GLUT4 translocation is tyrosine kinase-dependent. J Biol Chem 1998;273:13289–13296. [PubMed: 9582374]
23. Zhang Y, Zhao L, Wang C, Lei B. Isolation and culture of mouse embryonic fibroblast. Sichuan Da Xue Xue Bao Yi Xue Ban 2003;34:344–346. [PubMed: 12947735]
24. Wabitsch M, Brenner RE, Melzner I, Braun M, Moller P, Heinze E, Debatin KM, Hauner H. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. Int J Obes Relat Metab Disord 2001;25:8–15. [PubMed: 11244452]
25. McGillicuddy FC, de la Llera Moya M, Hinkle CC, Joshi MR, Chiquoine EH, Billheimer JT, Rothblat GH, Reilly MP. Inflammation impairs reverse cholesterol transport in vivo. Circulation 2009;119:1135–1145. [PubMed: 19221221]
26. Zhang Y, Zanotti I, Reilly MP, Glick JM, Rothblat GH, Rader DJ. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. Circulation 2003;108:661–663. [PubMed: 12900335]
27. Favari E, Zanotti I, Zimetti F, Ronda N, Bernini F, Rothblat GH. Probucol inhibits ABCA1-mediated cellular lipid efflux. Arterioscler Thromb Vasc Biol 2004;24:2345–2350. [PubMed: 15514211]
28. Nieland TJ, Chroni A, Fitzgerald ML, Maliga Z, Zannis VI, Kirchhausen T, Krieger M. Cross-inhibition of SR-BI- and ABCA1-mediated cholesterol transport by the small molecules BLT-4 and glyburide. J Lipid Res 2004;45:1256–1265. [PubMed: 15102890]

29. Yancey PG, Bortnick AE, Kellner-Weibel G, de la Llera-Moya M, Phillips MC, Rothblat GH. Importance of different pathways of cellular cholesterol efflux. Arterioscler Thromb Vasc Biol 2003;23:712–719. [PubMed: 12615688]
30. Moore RE, Navab M, Millar JS, Zimetti F, Hama S, Rothblat GH, Rader DJ. Increased atherosclerosis in mice lacking apolipoprotein A-I attributable to both impaired reverse cholesterol transport and increased inflammation. Circ Res 2005;97:763–771. [PubMed: 16151025]
31. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 1994;43:1271–1278. [PubMed: 7926300]
32. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–1808. [PubMed: 14679176]
33. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 2007;56:16–23. [PubMed: 17192460]
34. Chung S, Lapoint K, Martinez K, Kennedy A, Boysen Sandberg M, McIntosh MK. Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. Endocrinology 2006;147:5340–5351. [PubMed: 16873530]
35. Harkins JM, Moustaid-Moussa N, Chung YJ, Penner KM, Pestka JJ, North CM, Claycombe KJ. Expression of interleukin-6 is greater in preadipocytes than in adipocytes of 3T3-L1 cells and C57BL/6J and ob/ob mice. J Nutr 2004;134:2673–2677. [PubMed: 15465765]
36. Vaisman BL, Lambert G, Amar M, Joyce C, Ito T, Shamburek RD, Cain WJ, Fruchart-Najib J, Neufeld ED, Remaley AT, Brewer HB Jr, Santamarina-Fojo S. ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice. J Clin Invest 2001;108:303–309. [PubMed: 11457883]
37. Karasinska JM, Rinninger F, Lutjohann D, Ruddle P, Franciosi S, Kruit JK, Singaraja RR, Hirsch-Reinshagen V, Fan J, Brunham LR, Bissada N, Ramakrishnan R, Wellington CL, Parks JS, Hayden MR. Specific loss of brain ABCA1 increases brain cholesterol uptake and influences neuronal structure and function. J Neurosci 2009;29:3579–3589. [PubMed: 19295162]
38. Adorni MP, Zimetti F, Billheimer JT, Wang N, Rader DJ, Phillips MC, Rothblat GH. The roles of different pathways in the release of cholesterol from macrophages. J Lipid Res 2007;48:2453–2462. [PubMed: 17761631]
39. Navab M, Anantharamaiah GM, Reddy ST, Hama S, Hough G, Grijalva VR, Wagner AC, Frank JS, Datta G, Garber D, Fogelman AM. Oral D-4F causes formation of pre-beta high-density lipoprotein and improves high-density lipoprotein-mediated cholesterol efflux and reverse cholesterol transport from macrophages in apolipoprotein E-null mice. Circulation 2004;109:3215–3220. [PubMed: 15197147]
40. Rotllan N, Ribas V, Calpe-Berdiel L, Martin-Campos JM, Blanco-Vaca F, Escola-Gil JC. Overexpression of human apolipoprotein A-II in transgenic mice does not impair macrophage-specific reverse cholesterol transport in vivo. Arterioscler Thromb Vasc Biol 2005;25:e128–e132. [PubMed: 15994442]
41. Izem L, Morton RE. Possible role for intracellular cholesteryl ester transfer protein in adipocyte lipid metabolism and storage. J Biol Chem 2007;282:21856–21865. [PubMed: 17522050]
42. Verghese PB, Arrese EL, Soulages JL. Stimulation of lipolysis enhances the rate of cholesterol efflux to HDL in adipocytes. Mol Cell Biochem 2007;302:241–248. [PubMed: 17390217]
43. Le Lay S, Krief S, Farnier C, Lefrere I, Le Liepvre X, Bazin R, Ferre P, Dugail I. Cholesterol, a cell size-dependent signal that regulates glucose metabolism and gene expression in adipocytes. J Biol Chem 2001;276:16904–16910. [PubMed: 11278795]
44. Yvan-Charvet L, Bobard A, Bossard P, Massiera F, Rousset X, Ailhaud G, Teboul M, Ferre P, Dagher G, Quignard-Boulange A. In vivo evidence for a role of adipose tissue SR-BI in the nutritional and hormonal regulation of adiposity and cholesterol homeostasis. Arterioscler Thromb Vasc Biol 2007;27:1340–1345. [PubMed: 17363694]

45. Haidar B, Denis M, Krimbou L, Marcil M, Genest J Jr. cAMP induces ABCA1 phosphorylation activity and promotes cholesterol efflux from fibroblasts. J Lipid Res 2002;43:2087–2094. [PubMed: 12454270]

46. Tang C, Vaughan AM, Oram JF. Janus kinase 2 modulates the apolipoprotein interactions with ABCA1 required for removing cellular cholesterol. J Biol Chem 2004;279:7622–7628. [PubMed: 14668333]

47. Yamauchi Y, Hayashi M, Abe-Dohmae S, Yokoyama S. Apolipoprotein A-I activates protein kinase C alpha signaling to phosphorylate and stabilize ATP binding cassette transporter A1 for the high density lipoprotein assembly. J Biol Chem 2003;278:47890–47897. [PubMed: 12952980]

48. Sehayek E, Hazen SL. Cholesterol absorption from the intestine is a major determinant of reverse cholesterol transport from peripheral tissue macrophages. Arterioscler Thromb Vasc Biol 2008;28:1296–1297. [PubMed: 18420997]

49. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111–2119. [PubMed: 7738178]

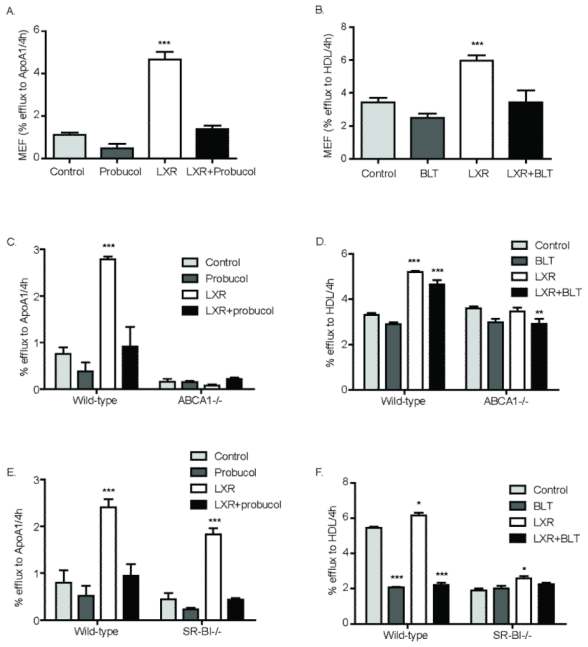

Figure 1.

Mouse embryonic fibroblasts (MEF), derived from wild-type (WT), ABCA1−/− or SRB1−/− mice were differentiated and labeled overnight with ^{3}H-cholesterol (5μCi/mL). Cells were equilibrated and treated overnight±LXR agonist (10μM) and co-treated±LXR agonist, ±BLT (10μM) or ±Probucol (20μM) for 2h. Cholesterol efflux from WT (A and B), WT and ABCA1−/− (C and D), and WT and SR-BI−/− (E and F) MEF adipocytes to apoA-I (20μg/ml) and HDL3 (50μg/ml) over 4h is presented; background efflux to MEM was subtracted. Efflux is presented as % total ^{3}H-cholesterol loaded into cells (n=3–4, *p<0.05, **p<0.01, ***p<0.001 vs. control).

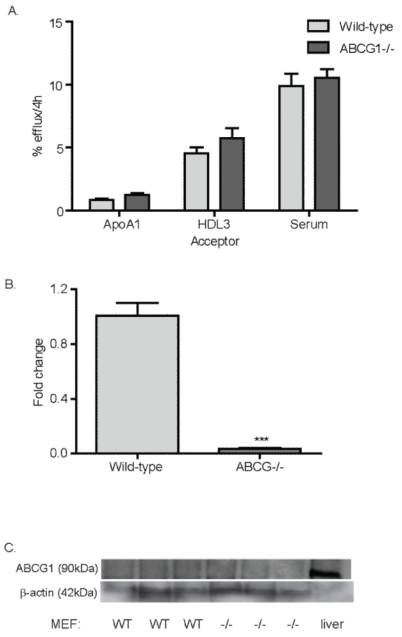

Figure 2.

MEF cells derived from ABCG1 or WT mice were differentiated and labeled overnight with ^{3}H-cholesterol (5μCi/mL), equilibrated and then washed with PBS and efflux to apoA-I (20μg/ml), HDL3 (50μg/ml) or 5% serum was monitored over 4h. RNA and protein was extracted. (A) Efflux from WT and ABCG1−/− to ApoA-I, HDL3 and serum. (B) ABCG1 mRNA levels were markedly reduced in ABCG1−/− cells compared with WT control (n=3, ***p<0.001). (C) ABCG1 protein levels were barely detectable in WT and ABCG1−/− MEF adipocytes compared with positive control mouse liver lysate, positive control.

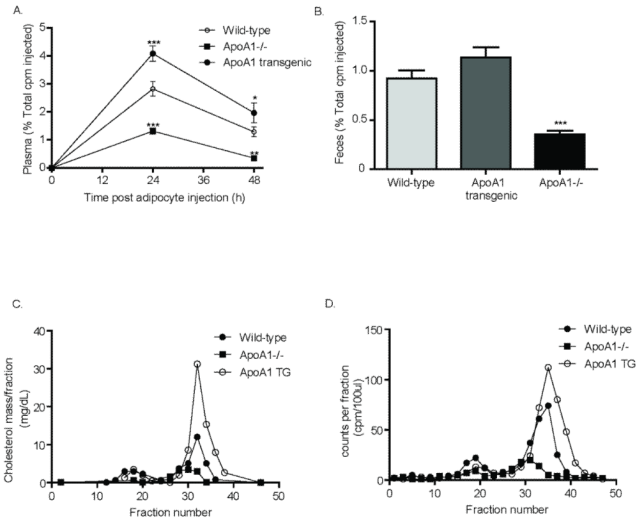

Figure 3.

3T3L1 adipocytes were labeled with ^{3}H-cholesterol (5μCi/mL) overnight and equilibrated for 24h. Equal numbers of adipocytes (in 0.5mL) were intraperitoneally (IP)-injected into apoA-I transgenic (solid circle), apoA-I−/− (solid square) or wild-type (open circle) mice. Movement of ^{3}H-cholesterol from IP-injected 3T3L1 adipocytes into (A) plasma and (B) feces was monitored over 48h (n=6, *p<0.05, **p<0.01, ***p<0.001 vs. wild-type animals). Pooled plasma was separated by FPLC and levels of (C) cholesterol mass and (D) ^{3}H-cholesterol tracer was measured.

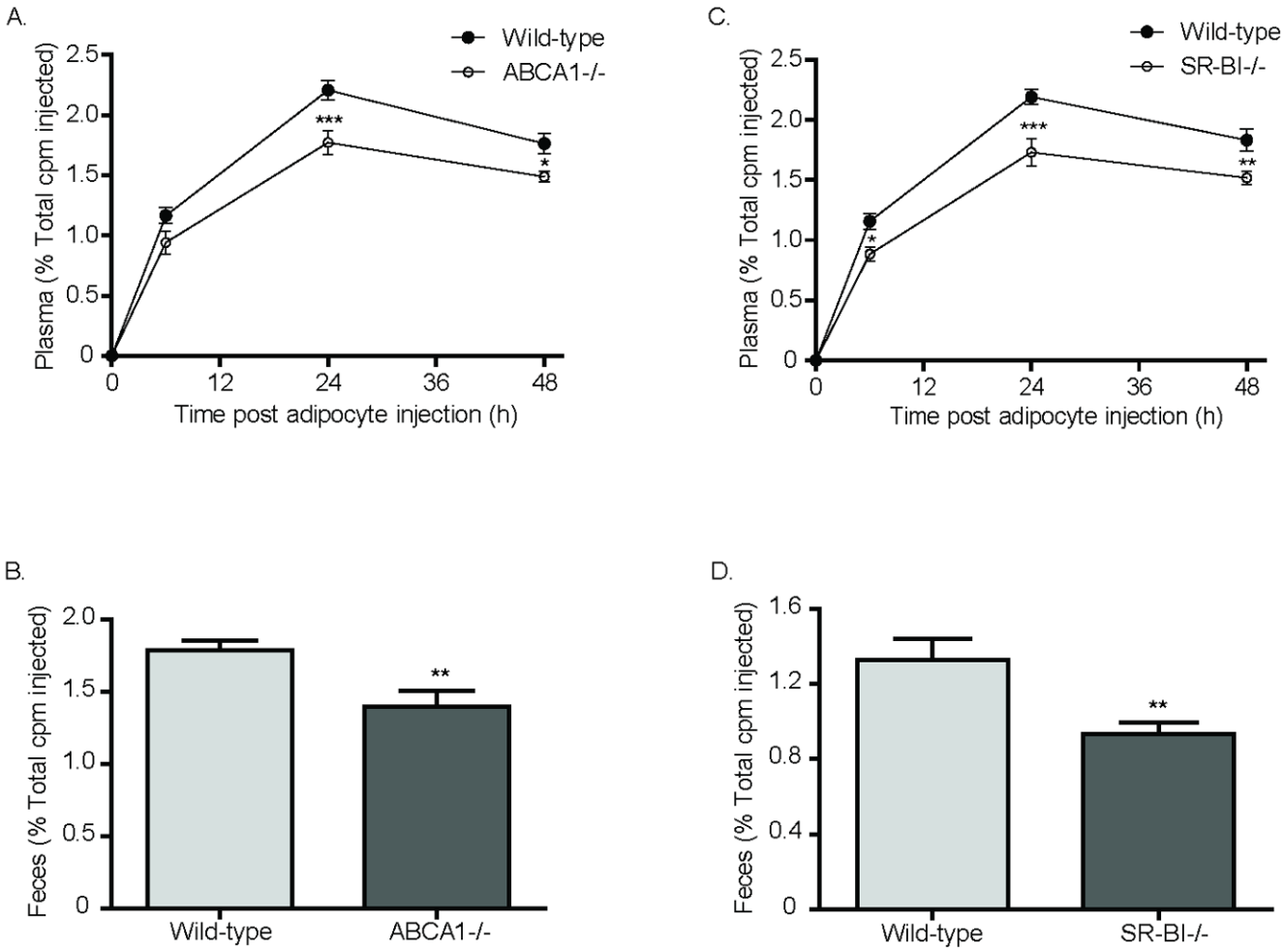

Figure 4.

ABCA1−/− (A&B) or SR-BI−/− (C&D) MEF adipocytes and litter-mate wild-Type (WT) control MEF adipocytes were labeled with ^{3}H-cholesterol (5μCi/mL) overnight and equilibrated. C57BL/6 WT mice were injected IP with WT (solid circle) or ABCA1−/− (open circle) MEF-adipocytes and levels of ^{3}H-cholesterol tracer in (A) plasma and (B) feces was measured over 48h. C57BL/6 WT mice were injected with WT (solid circle) or SR-BI−/− (open circle) MEF-adipocytes and levels of ^{3}H-cholesterol tracer in (C) plasma and (D) feces was measured. Levels of ^{3}H-cholesterol are presented as % total cpm injected (n=12, *p<0.05, **p<0.01 and ***p<0.001 vs. animals injected with WT adipocytes).

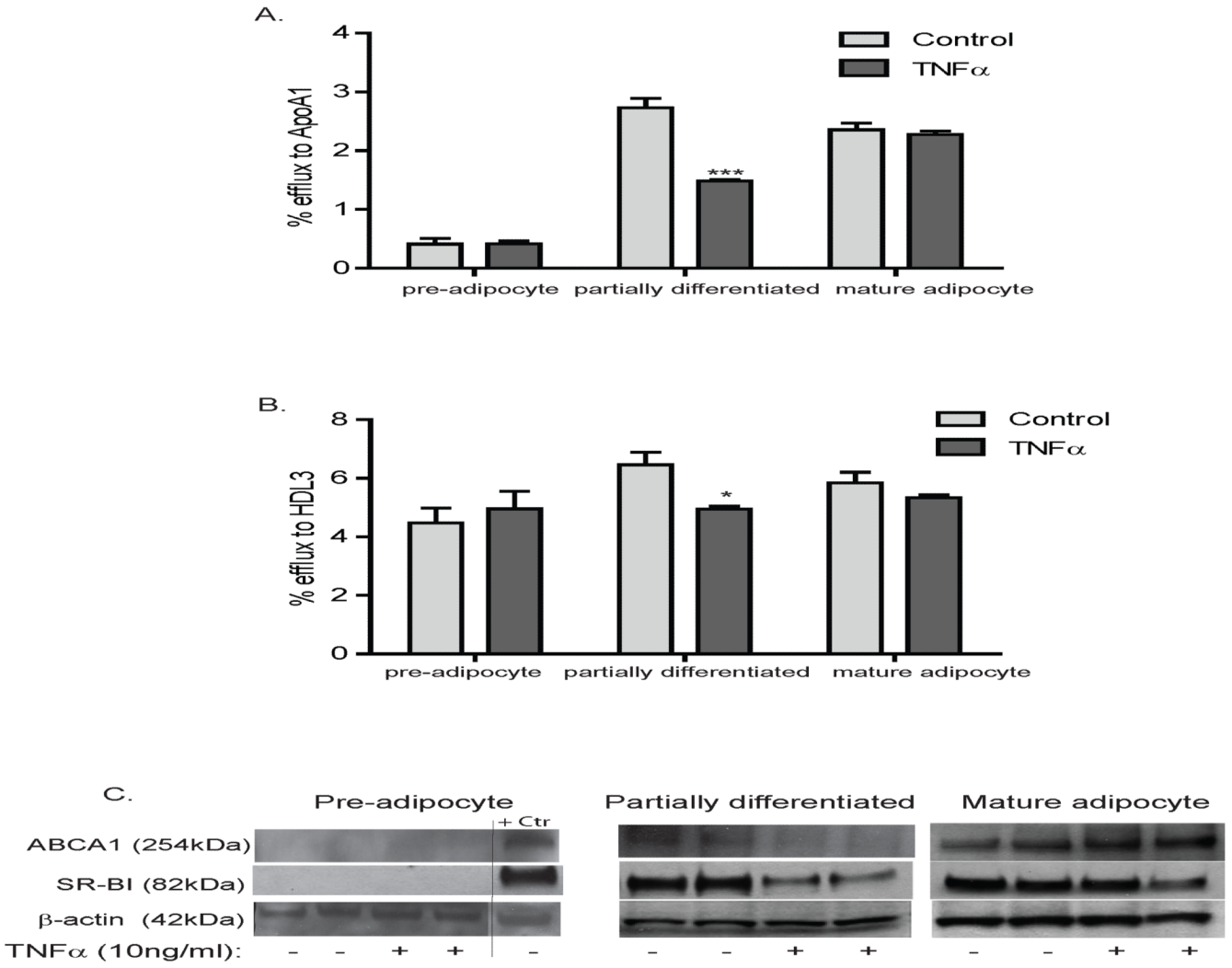

Figure 5.

The effect of TNFα (10ng/ml) on cholesterol efflux from 3T3L1 cells during differentiation was assessed at day 0 (pre-adipocyte), day 5 (partially-differentiated) and day 10 (mature). Cells were labeled with ^{3}H-cholesterol overnight prior to equilibration ±TNFα (10ng/ml). Efflux to (A) apoA-I (20μg/ml) and (B) HDL3 (50μg/ml) at day 0, 5 and 10 ±TNFα are presented. (C) Effects of TNFα vs. saline on ABCA1, ABCG1 and SR-BI protein levels at day 0, 5 and 10.

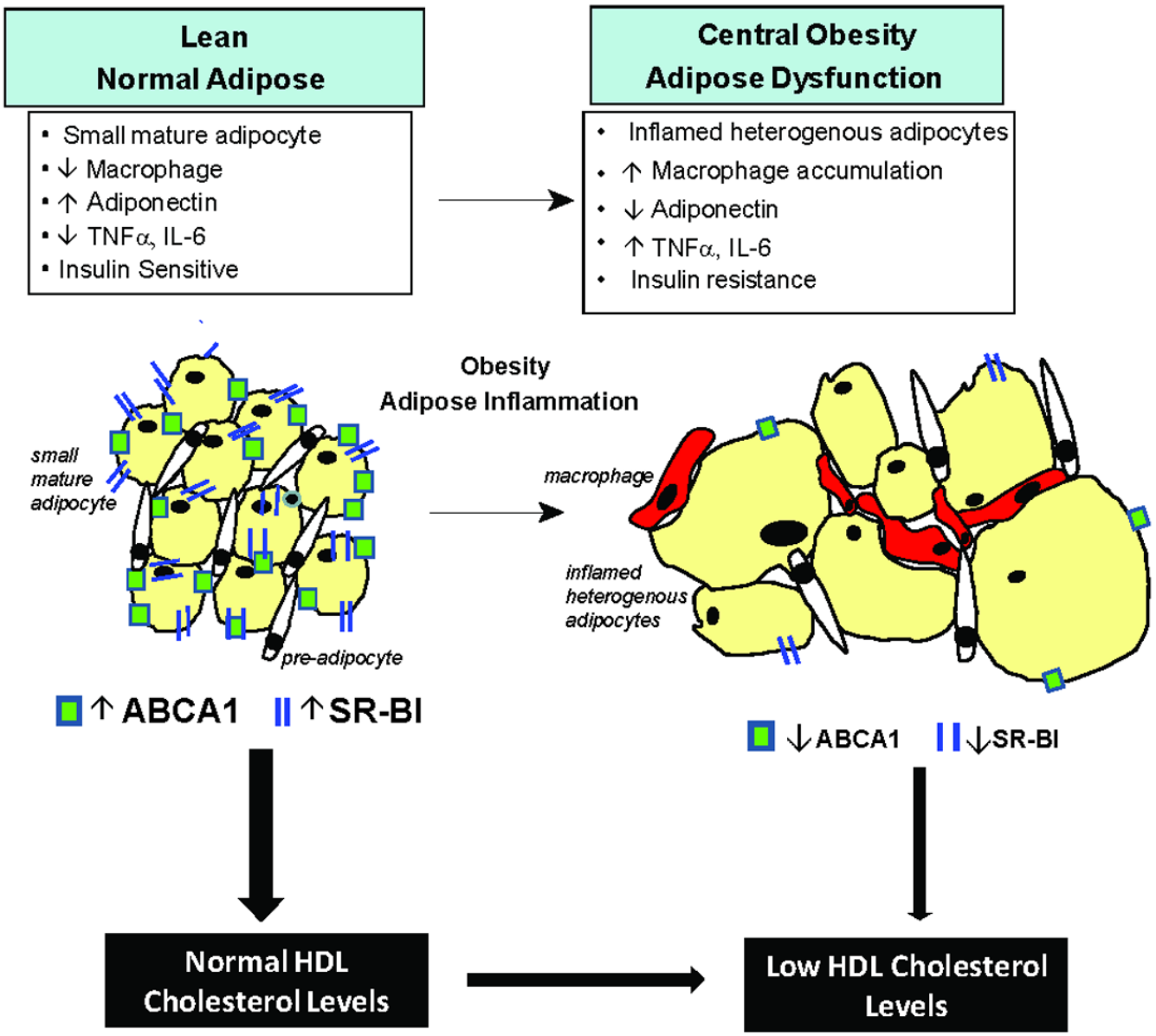

Figure 6.

As adipose inflammation is a hallmark of central obesity and type-2 diabetes, loss of adipocyte lipidation of HDL may directly contribute to lower HDL-C levels in these inflammatory, insulin resistant states. Despite greater adipose mass and cholesterol content in adiposity, adipocyte inflammation is associated with reduced expression of the cholesterol efflux transporters, ABCA1 and SR-BI, and impaired cholesterol efflux to apoA-I and HDL particles.

Table 1

Plasma ^{3}H-cholesterol counts associated with HDL fraction at 48h post adipocyte injection. Plasma was depleted of apoB-containing lipoproteins by PEG precipitation; counts in plasma pre-and post PEG precipitation were determined and the percentage counts in HDL fraction calculated. Data presented as mean ± SEM, n=3 for *in vivo* studies of (A) apoA-I modulation, (B) adipocyte ABCA1 deletion, and (C) adipocyte SR-BI deletion.

Genotype		Percent ^{3}H-cholesterol associated with HDL
(A)	Wild-Type	70.8 ± 1.83
	ApoA-I Transgenic	70.29 ± 5.21
	ApoA-I −/−	54.98 ± 7.17
(B)	WT MEF (ABCA1 study)	76.21 ± 1.14
	ABCA1 −/− MEF	81.63 ± 1.36
(C)	WT MEF (SR-BI study)	82.35 ± 4.06
	SR-BI −/− MEF	81.51 ± 5.83

Table 2

The effect of TNFα on ABCA1, ABCG1, SR-BI, adiponectin, lipoprotein lipase (LPL) and PPARγ mRNA expression in 3T3L1 cells during adipocyte differentiation. The ΔCt value between gene of interest and house-keeping β-actin gene is presented.

ΔCt for gene of interest	Pre-adipocyte			Partially (~50%) differentiated			Mature adipocyte		
	Control	TNFα	P	Control	TNFα	P	Control	TNFα	P
ABCA1	4.7±0.1	4.9±0.1	ns	2.2±0.3	2.6±0.1	ns	1.5±0.2	3.7±0.7	*
ABCG1	9.0±0.3	10.2±0.2	*	5.5±0.1	6.4±0.2	*	4.0±0.1	5.9±0.6	*
SR-BI	5.2±0.2	6.4±0.1	**	0.9±0.1	2.1±0.1	***	0.2±0.2	1.7±0.4	*
Adiponectin	13.1±1.1	14.8±1.4	ns	−3.1±0.4	−1.9±0.1	*	−2.7±0.3	−1.9±0.2	ns
LPL	0.5±0.4	1.9±0.03	*	−4.8±0.2	−3.3±0.1	**	−4.6±0.2	−3.8±0.2	*
PPARγ	6.1±0.1	7.9±0.2	**	1.1±0.1	2.1±0.1	**	0.9±0.1	1.7±0.1	**

The lower the ΔCt value, the greater the mRNA expression (*p<0.05, **p<0.01 and ***p<0.001 vs. control, n=3).