Atherosclerosis and Lipoproteins

In Vivo Evidence for a Role of Adipose Tissue SR-BI in the Nutritional and Hormonal Regulation of Adiposity and Cholesterol Homeostasis

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Objectives—This study examines the role of insulin and angiotensin II in high-density lipoprotein (HDL) metabolism by focusing on the regulation and function of scavenger receptor type-BI (SR-BI) in adipose tissue.

Methods and Results—Insulin or angiotensin II injection in wild-type mice induced a decrease in circulating HDL and it was associated with the translocation of SR-BI from intracellular sites to the plasma membrane of adipose tissue. Refeeding upregulated adipose HDL selective cholesteryl esters uptake and SR-BI proteins through transcriptional and posttranscriptional mechanisms. This occurred along with a decrease in serum HDL and an increase in adipose cholesterol content. Similar results were obtained with transgenic mice overexpressing locally angiotensinogen in adipose tissue. In adipose 3T3-L1 cell line, HDL induced lipogenesis by increasing liver X receptor binding activity. This mechanism was dependent of insulin and angiotensin II.

Conclusions—Our results raise the possibility that adipose tissue SR-BI translocation might be a link between adipose tissue lipid storage and HDL clearance. (Arterioscler Thromb Vasc Biol. 2007;27:1340-1345.)

Key Words: adipose tissue ■ angiotensin II ■ high-density lipoprotein ■ insulin

A adipose tissue has a central role in the energy metabolism adaptation to the nutritional environment because of its ability to store energy as triglycerides. Besides its role in triglyceride storage, adipose tissue is also the body’s largest pool of cholesterol store, representing ≈25% of whole-body cholesterol in human.1 A particular interest of adipocytes is that these cells accumulate cholesterol proportionally to triglycerides. Because of an extremely low cholesterol de novo synthesis,2 adipocytes must acquire cholesterol from exogenous sources. Interestingly, clinical observations have found a correlation between obesity and low levels of high-density lipoprotein (HDL) cholesterol,3 which is the reflect of an increased incidence of atherosclerosis.4 There is also growing evidence that altered insulin sensitivity or increased angiostin II concentrations, which occur along with obesity, play a crucial role in the acceleration of atherosclerosis but mechanisms are not yet fully deciphered.5,6

The scavenger receptor type-BI (SR-BI) has been identified as a membrane transporter involved in the selective cholesteryl esters (CEs) uptake from HDL.7 The pivotal role of SR-BI in lipoprotein metabolism and cholesterol transport in steroidogenic tissues and liver has been well-established.8 SR-BI is also expressed in adipocytes, but little is known about its function and regulation in these cells.9 Recently, studies from our laboratory have reported that SR-BI provide an important source of cholesterol from HDL in adipose cell lines and that insulin and angiotensin II induce the translocation of this receptor leading to an increase in cholesterol influx and storage.10,11 However, the nutritional and hormonal regulation of this translocation and its potential consequences on plasma HDL have not been yet explored in vivo.

Because cholesterol plays a role in the regulation of signal transduction and gene expression, we further investigated the consequence of the cholesterol influx induced by SR-BI in adipocytes. Recently, the nuclear liver X receptor (LXR), whose ligands are the oxysterols, derived from cholesterol, has been reported to regulate lipid storage in adipocytes on ligand activation.12,13 LXR activates the transcription of numerous genes involved in lipogenesis and lipid storage, especially the sterol regulatory element-binding protein-1c (SREBP-1c) transcription factor.14 SREBP-1c has been previously identified as the insulin and angiotensin II-responsive transcription factor inducing adipose lipogenesis.15,16 These findings have recently led to propose that LXR through oxysterol activation is an indirect mediator for some of insulin actions on its target gene expression.17,18

The purpose of the present study was to test in vivo the hypothesis that insulin and angiotensin II could be involved

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1340
in HDL homeostasis in part through their actions in the regulation of SR-BI in adipose tissue. We then investigated the contribution of this receptor in the well-known function of insulin and angiotensin II as lipogenic hormones.

Materials and Methods
For detailed descriptions of the Materials and Methods, please see supplemental material available online at http://atvb.ahajournals.org.

[^H] Cholesteryl Oleyl Ether Uptake
For the incorporation of HDL-associated[^H]Ceth (4×10^5 cpm/mg protein), wild-type mice (Charles River Laboratories, Wilmington, Mass) were divided into 3 groups: mice were fasted for 12 hours and then intravenously injected with reconstituted HDL (2×10^6 cpm), followed 10 minutes later by an intraperitoneal injection of saline or insulin (Actrapid; 0.01 U/mouse). In another group, mice were refeed for 12 hours and injected with the reconstituted HDL. One hour after tracer injection, mice were euthanized, and blood was collected by cardiac punctation. Epididymal fat pads and liver were harvested, and tissue content of[^H]Ceth radioactivity was measured after lipid extraction. The total uptake attributed to each organ was expressed as the radioactivity recovered in that organ over the total radioactivity injected. Assuming that epididymal adipose tissue contributes to one-fifth of total adipose tissue, and reflects the metabolic behavior of all adipose tissue, the total[^H] cholesteryl oleyl ether uptake attributed to total fat was calculated and expressed as percent of injected dose.

Results
HDL Cholesterol Uptake by Adipose Tissue
We first determined in vivo the ability of adipose tissue to bind apolipoproteins and, thus, to capture cholesterol from HDL. Supplemental Figure IA (please see http://atvb.ahajournals.org) depicts apolipoproteins distribution in adipose cells membranes (supplemental Figure IA) and cholesteryl-Bodipy into the cells (supplemental Figure IB) 1 hour after intravenous injection of labeled HDL in fed male mice, with no colocalization between these 2 fluorescent dyes (supplemental Figure ID). These results confirm in vivo that uptake and routing of sterols from HDL in adipose tissue were functional.

Insulin and Angiotensin II Induce the Translocation of SR-BI in Adipose Tissue and Decrease Plasma HDL
To evaluate whether insulin and angiotensin II could affect HDL cholesterol uptake in adipose tissue, these hormones were intraperitoneally injected in 2-hour fasted wild-type mice and SR-BI expression was determined. One hour after injection, the short insulin or angiotensin II exposure had no effect on SR-BI mRNA expression (data not shown). However, immunoblot of subcellular membrane fractions revealed that insulin rapidly induced the SR-BI abundance in the plasma membrane (2-fold increase) to the detriment of SR-BI in the high-density microsome fraction (2-fold decrease; Figure 1A). Little SR-BI immunoreactivity was observed in the low-density microsomes fraction (Figure 1A). Angiotensin II intraperitoneal injection revealed similar findings because SR-BI was increased by >50% in the adipose tissue plasma membrane fraction and decreased by ∼60% in the high-density microsome fraction (Figure 1B). These results prompted us to evaluate the consequence of insulin and angiotensin II intraperitoneal injection on plasma HDL. Both insulin and angiotensin II rapidly decreased plasma HDL cholesterol since the first hour, leading to ∼30% decrease after 120 minutes as compared with saline injection (Figure 1C). Therefore, acute action of insulin and angiotensin II
Ether in Epididymal Adipose Tissue sensitive to nutritional and hormonal regulation, HDL labeled

Accumulation of HDL [3H] Cholesteryl Oleyl Ether in Epididymal Adipose Tissue

To confirm that HDL cholesterol uptake in adipose tissue was sensitive to nutritional and hormonal regulation, HDL labeled with [3H]CEt was injected in fasted mice, fasted mice injected with insulin, or 12-hour refed mice. After 1 hour, insulin tented to significantly decrease the plasma radioactivity by 25%, and refed decreased the plasma radioactivity by 35% (Table 2). In fasted mice, based on the fact that 0.5% of the injected dose of HDL-associated [3H]CEt appeared in epididymal fat pads, we estimated that total fat mass incorporated 2.5% (see Materials and Methods and Table 2). This amount was increased 3-fold in fasted mice injected with insulin and 7.5-fold in 12-hour refed mice (Table 2). As

Table 1. Effect of Refeeding and Adipose Angiotensinogen Overexpression on Physiological Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fasted WT</th>
<th>Refed WT</th>
<th>Fed WT</th>
<th>Fed Ovex</th>
</tr>
</thead>
<tbody>
<tr>
<td>N and sex</td>
<td>5, male</td>
<td>5, male</td>
<td>5, male</td>
<td>5, male</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>22.4±0.6</td>
<td>25.5±0.7*</td>
<td>25.9±0.4</td>
<td>27.3±0.4*</td>
</tr>
<tr>
<td>Plasma HDL cholesterol (mg/dL)</td>
<td>53.2±4.6</td>
<td>30.1±1.4*</td>
<td>34.8±3.2</td>
<td>33.9±2.4*</td>
</tr>
<tr>
<td>Epididymal fat weight (g)</td>
<td>0.16±0.01</td>
<td>0.25±0.03*</td>
<td>0.21±0.01</td>
<td>0.29±0.04*</td>
</tr>
<tr>
<td>Cholesterol content (mg/E adipose tissue)</td>
<td>0.87±0.06</td>
<td>1.66±0.24*</td>
<td>1.51±0.24</td>
<td>2.03±0.15*</td>
</tr>
<tr>
<td>Triglyceride content (mg/E adipose tissue)</td>
<td>49.3±3.1</td>
<td>86.1±3.1*</td>
<td>73.4±4.2</td>
<td>92.1±5.5*</td>
</tr>
<tr>
<td>Plasma insulin (μU/mL)</td>
<td>37.5±2.4</td>
<td>52.4±6.3*</td>
<td>49.5±7.2</td>
<td>62.5±6.1</td>
</tr>
<tr>
<td>Plasma angiotensinogen (nmol)</td>
<td>202.8±8.4</td>
<td>191.6±14</td>
<td>181.5±9.5</td>
<td>226.2±8.6*</td>
</tr>
</tbody>
</table>

Each value represents the mean±SEM. All the animals were 16 weeks old at the time of euthanization and were analyzed for plasma parameters and epididymal (E) adipose tissue.

*p<0.05, refed mice (high carbohydrate diet) vs fasted mice or transgenic mice (Ovex) vs wild-type (WT) mice.

Figure 2. Effect of refeeding and angiotensinogen overexpression on the subcellular distribution and gene expression of SR-BI in adipose tissue. Immunobots of SR-BI were performed from adipose tissue plasma membrane (PM) and high-density microsome (HDM) of fasted and refed wild-type (WT) mice (A) or WT and transgenic mice (Ovex) overexpressing angiotensinogen in adipose tissue (B). A representative autoradiogram is shown at the top, and quantitative results are expressed as % over PM and HDM levels of fasted mice or WT mice, respectively. SR-BI mRNA expression in fasted/refed mice (C) and WT/Ovex mice (D). Results were normalized to ribosomal 18S amount. Values are means±SEM of 3 independent experiments.

*p<0.005, significant difference vs WT mice or vs Ovex mice, respectively.
expected, the liver accounted for a large amount of radiolabeled HDL cholesterol uptake as compared with adipose tissue (∼15%; Table 2). However, this amount was slightly but not significantly increased after insulin injection (1.2-fold) and increased after refeeding to a lesser extent than adipose tissue (∼1.5-fold). These results confirm that adipose tissue plays a crucial role in HDL cholesterol homeostasis.

HDL Induces Triglyceride Storage by a LXR-Dependent Pathway Through SR-BI

The increased adipose tissue cholesterol content in refed mice or in Ovex mice along with the triglycerides content (Table 1) raised the question of the potential role of SR-BI in the control of triglyceride metabolism. Figure 3A also reveals that during the differentiation of 3T3-L1 cell line with formation of large intracellular triglyceride droplets, a strong induction of SR-BI mRNA expression occurred and positive correlations were observed with cholesterol accumulation ($R^2=0.93$). Interestingly, addition of HDL (20 μg protein/mL) in lipoprotein-deficient serum medium increased cell triglyceride content by 20% within 5 hours and by almost 40% after 24 hours. This accumulation was completely inhibited by the presence of a blocking SR-BI antibody (Figure 3B). Moreover, 5-hour HDL treatment significantly increased the mRNA content of genes involved in lipogenesis pathway in a SR-BI-dependent fashion (Figure 3C). We reported that expression of ABCA1 was also increased by HDL and prevented by the blocking SR-BI antibody (Figure 3C). These genes are well-known targets of the nuclear LXR. We then assessed the potential LXR binding activity in 3T3-L1 adipocytes cultured in presence of HDL by EMSA using the LXRE-binding site of the SREBP-1c promoter. In cells cultured in lipoprotein-deficient serum medium, no LXR binding activity was detected (Figure 3D). By contrast, a strong induction of LXR binding activity was observed when the cells were cultured in presence of HDL in the medium. This was prevented by the presence of blocking SR-BI antibody (Figure 3D). The specificity of the LXR binding activity was confirmed by unlabeled DNA competition of the HDL-stimulated condition. Therefore, HDL triggers lipid storage through an LXR nuclear activation.

Insulin and Angiotensin II Stimulate HDL-Induced LXR Target Genes

We next investigated the roles of insulin and angiotensin II in the induction of LXR target genes in the presence of HDL. We reported that neither insulin nor angiotensin II alone was able to stimulate ABCA1 expression in fully differentiated 3T3-L1 cells (Figure 4A). Nevertheless, in presence of HDL, both insulin and angiotensin II significantly increased the amount of ABCA1 mRNA by ∼60% as compared with HDL-treated cells (Figure 4A). These inductions were prevented by the presence of blocking SR-BI antibody (Figure 4A). In contrast to what is observed with ABCA1, SREBP-1c mRNA was significantly increased by insulin alone (40%) and tended to be increase by angiotensin II alone after a 5-hour treatment (Figure 4B). In addition, insulin and angiotensin II stimulated HDL-induced SREBP-1c mRNA levels by ∼1.8-fold and 2-fold, respectively, as compared with HDL-treated cells, and a blocking SR-BI antibody abolished this additional effect (Figure 4B). Thus, we propose SR-BI as a new candidate for insulin- and angiotensin II-enhanced lipogenesis in adipocytes.

**Discussion**

Adipocytes abundantly express SR-BI, and it is likely that these cells capture significant amounts of CEs through the classical HDL delivery mechanism in vitro. Our data reveal that the selective uptake of CE from HDL in adipose

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**TABLE 2.** $[^{3}H]$CEt HDL Distribution in Plasma, Total Adipose Tissue, and Liver From Fasted, Insulin-Injected, and Refed Mice

<table>
<thead>
<tr>
<th></th>
<th>Fasted WT</th>
<th>Fasted WT + Insulin</th>
<th>Refed WT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td>66.6±7.2</td>
<td>51.0±5.8</td>
<td>44.6±6.2*</td>
</tr>
<tr>
<td><strong>Total adipose tissue</strong></td>
<td>2.5±1.0</td>
<td>7.5±1.6*</td>
<td>18.0±5.5*</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>14.7±1.6</td>
<td>17.7±2.6</td>
<td>22.8±2.3*</td>
</tr>
<tr>
<td><strong>Total recovery</strong></td>
<td>83.8±5.8</td>
<td>76.2±7.5</td>
<td>85.4±12.0</td>
</tr>
</tbody>
</table>

*Value are means±SEM of 4 individual animals in each group.*

*P<0.05, significant difference vs fasted mice.

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**Figure 3.** Implication of SR-BI in triglyceride storage and LXR binding activity in 3T3-L1 cells. A, 3T3-L1 fibroblasts were induced to differentiate and were harvested every 2 days for cholesterol content determination and RNA isolation. SR-BI transcript level was normalized to ribosomal 18S RNA amount. Results are expressed as % of day 0 value, the time at which the differentiation procedure was initiated. B, Fully differentiated 3T3-L1 adipocytes (8 days) cultured for the last 24 hours in lipoprotein-deficient serum medium (LPDS) were treated by HDL and HDL plus blocking SR-BI antibody during 5 or 24 hours before triglyceride content determination. The 5-hour-treated cells were also used for RNA isolation and nuclear extracts. C, Glut-4, FAS, SREBP-1c, and ABCA1 mRNA levels were quantified and expressed as % over basal level corresponding to the untreated 3T3-L1 cells. Values are means±SEM of 3 independent experiments. *P<0.05, **P<0.005, ***P<0.001, significant difference versus untreated cells. D, EMSA with nuclear extracts; 4 μg nuclear extracts were incubated in presence of 0.1 ng of the radiolabeled LXRE-binding site in absence or in presence of 0.5 ng of an unlabeled LXRE-binding site. Arrow shows the position of the LXR-specific complex. This EMSA is representative of 2 independent experiments.
Shetty and al22 revealed that insulin increased cell surface insulin- and angiotensin II-induced HDL clearance. Recently, associated CE in fasted rats. However, little attention has been placed on the metabolism of HDL cholesterol in different conditions leading to an increase in plasma insulin or angiotensin II. After refeeding, the decreased plasma HDL along with the increased adipose tissue cholesterol content are consistent with previous studies reporting that adipose tissue cholesterol levels are dependent on nutritional conditions and that plasma cholesterol could be in equilibrium with adiposity.2,25 Interestingly, hepatic HDL-selective CE uptake was also sensitive to refeeding, but the mechanism remains to be clarified. We also report for the first time that overexpression of angiotensinogen specifically in adipose tissue (Ovex mice) are conditions leading to an increase in plasma insulin or angiotensin II. After refeeding, the decreased plasma HDL along with the increased adipose tissue cholesterol content are consistent with previous studies reporting that adipose tissue cholesterol levels are dependent on nutritional conditions and that plasma cholesterol could be in equilibrium with adiposity.2,25 Interestingly, hepatic HDL-selective CE uptake was also sensitive to refeeding, but the mechanism remains to be clarified. We also report for the first time that overexpression of angiotensinogen specifically in adipose tissue leads to a similar phenotype. In these conditions, SR-BI proteins are not only regulated through posttranscriptional mechanisms but also at transcriptional levels. Interestingly, insulin and angiotensin II have been previously reported to increase SR-BI mRNA expression in 3T3-L1 adipocytes or in other cells type.26,27 We thus propose a mechanism in which increased insulinemia that occurs during refeeding or increased angiotensin II in Ovex mice could account for the induction of SR-BI expression and adipose tissue plasma membrane translocation. This regulation could contribute to the increased adipose tissue cholesterol storage in conjunction with the insulin- and angiotensin II-related stimulation of lipogenesis.

Cholesterol homeostasis plays a crucial role in the regulation of signal transduction in adipocytes and could be involved in the process of triglyceride storage.28 Adipose tissue cholesterol content is increased in refed mice or in Ovex mice along with triglyceride. In addition, a strong induction of cholesterol storage along with SR-BI expression occurred during the course of adipocyte differentiation and triglyceride storage. Previous works, in which 3T3-L1 cells were treated during the course of differentiation, revealed that HDL increased triglyceride storage.29 The present study demonstrates that treatment of fully differentiated 3T3-L1 adipocytes with HDL also induce triglyceride storage, and this occurs in SR-BI-dependent fashion. Further information should be provided by the generation of transgenic mice overexpressing SR-BI in adipose tissue. Thus, these results suggest that cholesterol from HDL could be a signal that triggers lipid storage.

The LXR is a nuclear receptor for which endogenous ligands are oxysterols (intermediates of cholesterol metabolism).20 This nuclear receptor is involved in adipocyte lipid metabolism by inducing several lipid-related genes and lipogenesis.12,13 Our study reveals that SR-BI, which mediates the selective uptake of cholesterol from HDL, seems to be a
determinant step in the induction of LXR binding activity in adipose tissue. As expected, insulin and angiotensin II on their own are able to stimulate SREBP-1c expression in 3T3-L1 adipocytes.15,16 However, we reported that both insulin and angiotensin II are able to stimulate the action of HDL on LXR target genes, SREBP-1c and ABCA1, in SR-BI–dependent fashion. This suggests a new mechanism action for the lipogenic hormones that could have major interest in vivo in a context where adipose tissue is exposed to circulating lipoproteins such as HDL. The fact that refeeding and angiotensinogen overexpression increase adipose tissue cholesterol content in vivo is in line with our results and point out new insight into how insulin and angiotensin could govern lipid metabolism in vivo in adipose tissue.

In conclusion, our observations provide the first in vivo evidence that insulin and angiotensin II can participate in HDL homeostasis through action on the translocation of SR-BI in adipose tissue. Such a role of insulin and angiotensin II might be physiologically important in the control of adiposity and raise a potential new link between obesity, insulin-resistance, and atherosclerosis.

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Disclosures
None.

References
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MATERIALS AND METHODS

Materials. NB 400-104 antibody against SR-BI and blocking SR-BI antibody, NB400-113 were from Novus Biologicals (Littleton, CO). Insulin was purchased from NovoNordisk (Actrapid®, NovoNordisk, Copenhagen), angiotensin II from Sigma and human plasma HDL, for in vitro study, from Calbiochem (La Jolla, CA).

Animals. For the fasting/refeeding experiments, C57BL/6 male mice were fasted 24h and then refed for 12h with a standard chow diet (UAR, AO3, France). The transgenic mice overexpressing adipose tissue angiotensinogen (termed Ovex mice) were generated as previously described.⁠¹⁠ Epididymal fat pads of 16-weeks old mice were used in this study. Animal studies were conducted according to the French Guidelines for Care and Use of Experimental Animals.

Fluorescent Immunochemistry. Human plasma HDL were separated by ultracentrifugation in density 1.063-1.21 and then, were labelled with cholesteryl-Bodipy® 542/563 C11 (Red) (Molecular Probes, Eugene, OR).² HDL apolipoproteins were labelled with Alexa Fluor® 488 Protein Labeling Kit (Molecular Probes, Eugene, OR). The protein concentration of labelled HDL was determined using Bradford assay. To assess the uptake HDL-derived accumulation of cholesteryl-Bodipy, wild-type mice were intravenously injected with the previous preparation (250 µg equivalent human HDL proteins). Cholesteryl-Bodipy (red) and apoliproteins-labeled (green) were observed on adipose tissue frozen sections with a Zeiss laser scanning confocal microscope (LSM 500).
[^3]H cholesteryl oleyl ether HDL preparation. Mice plasma HDL was delipidated using potato starch (Sigma) and then reconstituted with 200μCi[^3]H cholesteryl oleyl ether ([^3]HCEt) (Amersham), 2mg of the corresponding cholesterol ester and 0.1mg of free cholesterol as previously described. Unincorporated lipids were removed by ultracentrifugation in density 1.063 and HDL preparation was exhaustively dialyzed against PBS.

Insulin and angiotensin II i.p. injection. Food was removed for 2h between 9:00 and 11:00 A.M. Insulin (Actrapid®, 0.01 units/ mouse), and angiotensin II (1μg/ mouse) were administered by intraperitoneal injection in wild-type mice, and blood samples were collected by tail bleeding at the time points indicated.

Blood parameters. Plasma HDL-cholesterol were determined using a commercial kit (Randox labs, Crumlin, U.K). Plasma angiotensinogen was cleaved by mouse renin and radioimmunoassay of angiotensin I was carried out.

Cell culture conditions. 3T3-L1 mouse preadipocytes were differentiated into adipocytes as previously described. Fully differentiated adipocyte cells were shifted to a 10% lipoprotein-deficient serum (LPDS) during 24h before treatment. Then, cells were treated with no additions as a control, 100nM insulin, 100nM angiotensin II, 20µg protein/ml HDL or blocking SR-BI antibody (1:100) (added 1 hour before experiments), in various combinations as described in the figure legends. The blocking SR-BI antibody has been previously tested for his efficiency to inhibit cholesterol uptake from HDL in 3T3-L1 adipocytes.

Membrane protein preparation, SDS-PAGE, Western Blot analysis. 2h-fasted male mice were administered insulin i.p. (Actrapid®, 0.4units/ mouse),
angiotensin i.p. (1µg/ mouse) or saline. After 1 hour, adipose tissue was homogenized in TES buffer (20mM Tris, pH 7.4, 1 mM EDTA, 250mM sucrose, 1mM PMSF). The homogenate was fractionated by differential centrifugation to isolate plasma membranes (PM), low-density microsomes (LDM) and high-density microsomes (HDM). The protein contents of each subcellular fraction were determined using the Bradford assay. SDS-PAGE and Western Blot analysis were performed as described previously.

Electromobility shift assay. Nuclear proteins preparation and LXR electromobility shift assay (EMSA) were performed as previously described.

RNA analysis. Total RNA extraction, RT-PCR and PCR reaction were performed as previously described. Ribosomal 18S RNA expression was used to account for variability in the initial quantities of mRNA.

Lipid determination. Total lipids were extracted with chloroform/methanol according to Bligh and Dyer from total cell or tissue lysates. Determination of triglyceride and cholesterol was performed using a commercial kit (Sigma, St. Louis, MO and Randox labs, Crumlin, U.K).

Statistical analysis. Data are given as means ± S.E.M. Comparison of mean values between groups was evaluated by student’s two-tailed unpaired t test. Differences were considered significant at $P < 0.05$. 
REFERENCES


Supplemental Fig. I. **Intracellular localization of cholesteryl-Bodipy in adipose tissue.** Wild-type mice were i.v. injected with dually labelled HDL, Alexa Fluor apolipoproteins (green) and cholesteryl-Bodipy (red) for 1 h. Immunofluorescent labelling of apolipoproteins in adipose cell membranes (A) and intracellular cholesteryl-Bodipy (B) was obtained with a Zeiss laser scanning microscope from adipose tissue section. Phase-contrast microscopy of epididymal adipose tissue section (C). Colocalization (D).
Supplemental Figure I