Activation of Peroxisome Proliferator–Activated Receptor Gamma and Retinoid X Receptor Results in Net Depletion of Cellular Cholesteryl Esters in Macrophages Exposed to Oxidized Lipoproteins


Objective—Peroxisome proliferator–activated receptor gamma (PPARγ), a ligand-activated transcription factor, has pleiotropic effects, including regulation of macrophage differentiation and lipid homeostasis. The PPARγ ligands, thiazolidinediones (TZDs), attenuate atherosclerosis in mice by uncertain mechanisms. The objective of this study was to determine whether activation of PPARγ or its obligate heterodimer, retinoid X receptor (RXR), modulates macrophage foam cell formation induced by oxidized (ox) lipoproteins.

Methods and Results—Incubation of THP-1 macrophages with oxHTG-VLDL, oxREM, or oxLDL increased cellular cholesteryl ester over 6-fold. Preincubation with the TZD, ciglitazone, the RXR-specific ligand, 9-cis retinoic acid (9cRA) or the combination reduced CE mass accumulation by up to 65%. Ciglitazone and 9cRA increased CD36 mRNA (up to 4-fold); however, uptake of [125I]oxLDL was only modestly enhanced (up to 1.8-fold) because of a concomitant PPARγ:RXR–induced decrease in SRAI/II activity (up to 40%). This suggested that PPARγ:RXR activation inhibited cholesteryl ester accumulation by enhancing cholesterol efflux. Ciglitazone and 9cRA were found to increase the expression of ATP-binding cassette proteins A1 and G1, resulting in enhanced cholesterol efflux to lipoprotein-deficient serum, apoAI and HDL.

Conclusions—PPARγ and/or RXR activation inhibit foam cell formation through enhanced cholesterol efflux despite increased oxLDL uptake. These observations explain the reduced atherosclerosis in TZD-treated mice and may extend the therapeutic implications of these ligands. (Arterioscler Thromb Vasc Biol. 2003;23:475-482.)

Key Words: macrophages ■ oxidized lipoproteins ■ PPARγ ■ atherosclerosis
potentially increasing oxLDL uptake.\textsuperscript{17,18} Consequently, PPAR\(\gamma\) activation was predicted to promote macrophage foam cell formation and by extension atherogenesis.

However, PPAR\(\gamma\) activation by rosiglitazone or troglitazone reduces atherosclerotic lesions in LDL receptor (LDLR)-null\textsuperscript{19,20} and apoE-null mice,\textsuperscript{21} despite an increase in macrophage CD36 expression.\textsuperscript{19,21} Furthermore, increased lesions were observed in macrophage-specific PPAR\(\gamma\)-null mice, generated by transplantation of bone marrow from PPAR\(\gamma\)-null mice to LDLR-null mice.\textsuperscript{22} One explanation for the protective effect of PPAR\(\gamma\) activation is that enhanced CD36 expression in adipocytes could provide a relatively benign sink for potentially deleterious oxLDL.\textsuperscript{23} Other potential mechanisms have been proposed, including attenuation of hyperinsulinemia and/or hyperglycemia; increase of plasma HDL cholesterol concentration;\textsuperscript{23} and a reduction in adhesion molecules, chemokine receptors, and chemokines, such as monocyte chemotactic protein.\textsuperscript{8,20} Still uncertain in these animal models is the role of macrophage foam cell formation in the PPAR\(\gamma\)-induced inhibition of atherogenesis.

The results of in vitro studies are inconclusive as to whether PPAR\(\gamma\) activation increases oxLDL uptake.\textsuperscript{7,15} In THP-1 macrophages, PPAR\(\gamma\) and RXR ligands increased CD36 expression, leading to enhanced DiI-labeled oxLDL uptake,\textsuperscript{7} whereas in wild-type embryonic stem cells (ES), \textsuperscript{125}\textsubscript{I}oxLDL uptake was unaffected by a PPAR\(\gamma\) or RXR ligand alone, although a 20% decrease with both ligands together was observed.\textsuperscript{15} However, oxLDL uptake does not specifically measure CD36 activity but rather the activity of all scavenger receptors that recognize oxLDL, including SRAI/II. PPAR\(\gamma\) ligands do not appear to affect SRAI/II mRNA expression; however, protein levels were reduced in troglitazone-treated wild-type ES cells,\textsuperscript{15} a result that would predict a decrease in oxLDL uptake. Nevertheless, the impact of PPAR\(\gamma\) activation on macrophage expression of both CD36 and SRAI/II and how this influences net ox lipoprotein uptake remains to be clearly established. Furthermore, the link between macrophage PPAR\(\gamma\) activation, oxidized lipoprotein-induced CE accumulation and subsequent foam cell formation has yet to be fully elucidated.

PPAR\(\gamma\) activators also modulate cholesterol efflux by regulating the gene expression of ATP-binding cassette proteins A1 (ABCA1) and G1 (ABCG1).\textsuperscript{22,24,25} The integral role of ABCA1 in regulating cellular cholesterol transport is exemplified by loss-of-function mutations causing Tangier disease, which is characterized by significant cholesterol accumulation in macrophages.\textsuperscript{26,27} ABCA1 expression is upregulated by PPAR\(\gamma\) ligands through a transcriptional cascade mediated by the nuclear hormone receptor, liver X receptor (LXR\(\alpha\)).\textsuperscript{22} The LXR\(\alpha\) gene contains a PPRE, so that on PPAR\(\gamma\) activation, LXR\(\alpha\) expression is induced. Ligand-bound LXR\(\alpha\) heterodimerizes with RXR and binds the LXRRE in the ABCA1 promoter, thereby increasing its expression and enhancing cellular cholesterol efflux to HDL and apoAI. PPAR\(\gamma\) activation in macrophages stimulates ABCA1 expression and promotes cholesterol efflux from lipid-loaded cells, a result that has been confirmed by the lack of effect of PPAR\(\gamma\) ligands in PPAR\(\gamma\)-null cells.\textsuperscript{25} Thus, the overall consequence of PPAR\(\gamma\) ligands on macrophage cholesterol ester (CE) accumulation and hence foam cell formation would be the net effect on the competing processes of lipoprotein uptake and cholesterol efflux.

Therefore, our objectives were to examine the impact of PPAR\(\gamma\) and/or RXR ligands on cellular cholesterol accumulation induced by ox lipoproteins in cultured macrophages and to elucidate the mechanisms involved. Our results demonstrate that macrophage CE accumulation induced by ox lipoproteins is significantly reduced by TZDs. In addition, the RXR ligand, 9cRA, reduced CE accumulation, and this effect was amplified when combined with ciglitazone. Ciglitazone alone or in combination with 9cRA significantly enhanced both expression of ABCA1 and ABCG1 and efflux of cholesterol to lipoprotein-deficient serum (LPDS) apoAI and HDL\(_3\). Despite a marked induction in CD36 expression, uptake of \textsuperscript{125}\textsubscript{I}oxLDL was only modestly increased. This was caused by a decrease in SRAI/II activity by PPAR\(\gamma\) and RXR ligands. On balance, PPAR\(\gamma\) and RXR activation reduces the CE accumulation in macrophages induced by ox lipoproteins as the result of an overriding impact on ABCA1-mediated cholesterol efflux.

**Methods**

Please see online data supplement (which can be accessed at http://atvb.ahajournals.org) for materials and methods.

**Results**

**OxHTG**

VLDL and their remnants induced lipid accumulation in THP-1 human macrophages: Incubation of THP-1 human macrophages with oxHTG-VLDL and oxREM induced CE mass by 6.2- and 6.4-fold (both \(P<0.05\)) and triglyceride (TG) mass by 2.7- and 3.0-fold (both \(P<0.05\)), respectively (Figure 1A and B). Similarly, oxLDL and AcLDL induced cellular CE accumulation by 5.8- and 11-fold (\(P<0.05\)), with neither lipoprotein inducing TG mass accumulation. Cellular free cholesterol (FC) mass was significantly enhanced by all modified lipoprotein preparations (1.5- to 2.2-fold; Figure 1C), a result consistent with the observation that cholesterol derived from ox lipoproteins is retained within the lysosomal compartment for an extended period.\textsuperscript{28}

**Ligand Activation of PPAR\(\gamma\) Inhibited CE Mass Induced by Modified Lipoproteins**

Pretreatment of THP-1 macrophages with ciglitazone significantly inhibited cellular CE mass induced by oxHTG-VLDL, oxREM, or oxLDL by –40%, –38%, and –47% (all, \(P<0.05\)), respectively, compared with lipoprotein alone (Figure 1A). The effect of ciglitazone on CE mass was dose dependent (Figure 2). In addition, another TZD, troglitazone, inhibited cellular CE accumulation induced by oxHTG-VLDL in a dose-dependent manner from –42% to –68% (\(P<0.05\); Figure 2).

In contrast, TG mass induced by either lipoprotein preparation was unaffected by pretreatment of cells with either TZD (Figure 1B and data not shown). Also, in the absence of ox lipoprotein the TZDs had no significant effect on cellular CE content.
Ox Lipoproteins Markedly Reduce CE Accumulation Induced by Modified LDL

The addition of both ciglitazone and 9cRA together did not further reduce CE accumulation in either cell type.

Ligand Activation of RXR Inhibited CE Mass Induced by Modified Lipoproteins

Pretreatment of macrophages with 9cRA significantly inhibited oxHTG-VLDL-, oxREM-, and oxLDL-induced CE mass by −36%, −33%, and −44% (all $P<0.05$), respectively, compared with lipoprotein alone (Figure 1A). The effect of 9cRA was dose dependent, with inhibition of CE mass induced by oxHTG-VLDL ranging from −15% to −50% ($P<0.05$, Figure 2). In contrast, 9cRA did not affect TG mass induced by any ox lipoproteins tested (Figure 1B).

Combined RXR and PPARγ Activation Markedly Reduced CE Accumulation Induced by Ox Lipoproteins

To further enhance the activity of the PPARγ:RXR heterodimer, THP-1 cells were pretreated with ciglitazone and 9cRA together. These ligands significantly reduced CE accumulation induced by oxHTG-VLDL, oxREM, or oxLDL by −69%, −52% and −63% (all $P<0.05$, Figure 1A), respec-

tively, compared with lipoprotein alone. This decrease in CE mass was markedly enhanced compared with the inhibition observed by either PPARγ or RXR ligand alone, suggesting a synergistic effect. In contrast, TG accumulation induced by ox lipoproteins was unaffected by the combination of ligands (Figure 1B).

In addition, the increases in FC mass induced by ox lipoproteins alone were significantly reduced by pretreatment of macrophages with both ciglitazone and 9cRA to levels similar to those observed in control cells (Figure 1C).

PPARγ and RXR Activation Inhibited oxHTG-VLDL–Induced CE Accumulation in LDLR-Null and Wild-Type MPMs

TZD treatment of LDLR-null mice inhibits formation of early atherosclerotic lesions in vivo. To determine whether this decrease in atherosclerosis may be mediated through a PPARγ-induced inhibition of macrophage foam cell formation, MPMs were isolated from both LDLR-null and wild-type mice. OxHTG-VLDL induced CE accumulation in both wild-type and LDLR-null MPMs by approximately 20-fold. Pretreatment of MPMs with ciglitazone significantly inhibited CE accumulation induced by oxHTG-VLDL by −35% and −20% in wild-type and LDLR-null MPMs, respectively (both $P<0.05$; see online Figure I, which can be accessed at http://atvb.ahajournals.org). Preincubation with 9cRA also significantly inhibited oxHTG-VLDL–induced CE accumulation in both wild-type and LDLR-null MPMs by 23% and 27%, respectively ($P<0.05$). The concentration of 9cRA required to achieve this effect was higher than that used in THP-1 cells. This was most likely caused by the low level of expression of PPARγ in nonthioglycollate-stimulated MPMs. The addition of both ciglitazone and 9cRA together did not further reduce CE accumulation in either cell type.

PPARγ and RXR Activation Regulate CD36 and SRAI/II Activity

Macrophage uptake of oxHTG-VLDL is mediated by both CD36 and SRAI/II. As demonstrated in the Table, CD36...
mRNA was increased 2-fold by either ciglitazone or 9cRA alone and 4-fold by the combination of ligands. Consequently, \textsuperscript{[125]}IoxLDL binding, uptake, and degradation was increased by 1.3 to 1.8-fold (P<0.05; Figure 3) in cells treated with ciglitazone and 9cRA either alone or in combination. However, this enhanced oxLDL uptake was attenuated compared with the extent of PPAR\(\gamma\) and RXR-induced CD36 mRNA upregulation. This is because oxLDL is also a ligand for SRAI/II as confirmed by the attenuated increase in CE accumulation induced by oxHTG-VLDL in MPMs from SRAI/II-null (12-fold) mice versus the wild-type MPMs (18-fold; Figure I). Therefore, we determined the contribution of SRAI/II expression to the PPAR\(\gamma\)-mediated decrease in CE accumulation induced by ox lipoproteins by isolating MPMs from SRAI/II-null mice and their controls and pre-treating them with PPAR\(\gamma\) ligands.

In control cells, ciglitazone and 9cRA alone or in combination significantly inhibited oxHTG-VLDL–induced CE accumulation by −27% to −36% (P<0.05, Figure 1). However, in the absence of SRAI/II expression, these ligands alone or in combination, only reduced CE accumulation induced by oxHTG-VLDL by −15% to −22% (P<0.05). Therefore, decreased SRAI/II activity mediates, in part, the reduction in CE mass induced by PPAR\(\gamma\) and RXR ligands.

Consistent with these findings is the effect of PPAR\(\gamma\) and RXR ligands on cellular uptake of \textsuperscript{[125]}IAcLDL, a modified lipoprotein with ligand specificity for SRAI/II. In THP-1 cells, \textsuperscript{[125]}IAcLDL binding, uptake, and degradation were significantly inhibited by ciglitazone by −35% and −24%, respectively (P<0.05; Figure 3). In addition, 9cRA inhibited \textsuperscript{[125]}IAcLDL binding, uptake and degradation by −23% and −21%, respectively (P<0.05), an effect slightly enhanced by combining the ligands. This result explains the modest increase in oxLDL uptake in that the increased CD36 expression is offset by a decrease in SRAI/II activity. Furthermore, AcLDL-induced cellular CE accumulation in THP-1 cells was significantly inhibited by ciglitazone and 9cRA alone or in combination by −40% to −70% (P<0.05, Figure 1). Collectively these finding indicate that decreased SRAI/II activity contributes to the inhibition of foam cell formation induced by ligands for PPAR\(\gamma\) and RXR.

**PPAR\(\gamma\) and RXR Activation Enhanced Macrophage Cholesterol Transport Genes and Efflux**

Although ciglitazone and 9cRA increased ox lipoprotein uptake, CE mass accumulation induced by these lipoproteins was significantly reduced by both ligands. Therefore, we addressed the effects of PPAR\(\gamma\) and RXR activation on cholesterol efflux.

ABCA1 and ABCG1 transporters are responsible for the removal of cellular cholesterol stores. Preincubation of macrophages with ciglitazone induced ABCA1 and ABCG1 mRNA expression by 1.7- and 1.8-fold (P<0.05; Table), respectively. Similarly 9cRA induced ABCA1 and ABCG1
Ciglitazone and 9cRA–Mediated Induction of Cholesterol Efflux Is Mediated Through PPARγ and LXR

To demonstrate directly that ciglitazone and 9cRA mediate cholesterol efflux through activation of PPARγ and subsequently LXR, the effects of known antagonists of PPARγ (bisphenol A diglycidyl ether; BADGE) and LXR (5α,6α-epoxycholesterol-3-sulfate, ECHS) were examined in the cholesterol efflux assay. Incubation of macrophages with BADGE alone had no significant effect on cholesterol efflux to apoAI (Figure 4B). However, co-incubation of BADGE and ciglitazone completely blocked the ciglitazone-induced 30% increase in cholesterol efflux to apoAI. In contrast, the co-incubation of BADGE and 9cRA had no effect on the 9cRA-induced increase in cholesterol efflux to apoAI, suggesting that 9cRA enhances efflux through a mechanism independent of PPARγ. Therefore, when cells were incubated with ciglitazone, 9cRA, and BADGE, the amount of cholesterol efflux to apoAI was reduced by 18% (P<0.05), a value similar to that observed in the presence of 9cRA alone plus BADGE.

In contrast with BADGE, the incubation of macrophages with the LXR antagonist (ECHS) alone showed a significant 20% decrease in cholesterol efflux to apoAI (Figure 4B). This result is most likely because of the inhibition of the effects of endogenous LXR ligands. Co-incubation of cells with ciglitazone and ECHS completely blocked the 30% induction of cholesterol efflux to apoAI by ciglitazone. This finding indicates that stimulation of macrophage cholesterol efflux by PPARγ activation occurs through LXR. ECHS also completely blocked the enhancement of cholesterol efflux to apoAI induced by 9cRA alone or in combination with ciglitazone, suggesting that 9cRA increases cholesterol efflux through stimulation of the LXR:RXR dimer. The combination of BADGE and ECHS in the presence of either ligand alone or together did not further reduce cholesterol efflux to ECHS alone. Therefore, PPARγ and RXR ligands mediate enhanced cholesterol efflux to apoAI through a PPARγ:RXR-sensitive pathway.

To further demonstrate the importance of the PPARγ-LXR:ABCA1 pathway on foam cell formation, we also used the LXR antagonist in the lipid mass assay. As shown in Figure 5, ECHS completely blocked the ciglitazone- and 9cRA-induced reduction in CE mass in macrophages incubated with oxHTG-VLDL. This suggests that the primary pathway mediating the PPARγ inhibition in CE accumulation induced by ox lipoproteins is enhancement of cholesterol efflux through a PPARγ:LXR:ABCA1 transcriptional cascade.

Discussion

In this report we demonstrate that PPARγ ligands, namely the TZDs troglitazone and ciglitazone, and an RXR ligand, namely 9cRA, significantly reduced macrophage CE accumulation induced by atherogenic lipoproteins, namely ox-HTG-VLDL, their remnants, and LDL. To our knowledge this is the first report to demonstrate that PPARγ and RXR ligands alone or in combination markedly decrease macrophage CE accumulation, thereby inhibiting foam cell forma-
enhancement in macrophage [125I]oxLDL uptake induced by 
protein uptake.
increased CD36 scavenger receptor expression and ox li-
for any increase in the cellular cholesterol pool as a result of 
mediated cellular cholesterol efflux, more than compensating 
MPMs that SRAI/II contributes to only 30% of the overall 
teins we have previously demonstrated in SRAI/II-null 
effects on cholesterol efflux,7,15,24,25 the outcome of ciglita-
involved in macrophage lipid homeostasis.25 Furthermore, 
apparent; however, ES cells may not faithfully reflect either 
and LG268. The reasons for these disparities are not readily 
formation. 19

The role of the PPARγ-LXR-ABCA1 pathway in the 
cliglitazone-induced inhibition of cellular CE mass induced by 
Lipid homoeostasis is not well understood. However, it was evident from in vivo mouse studies that 
PPARγ and RXR ligands decrease atherosclerotic lesion 
formation in vitro. Our findings support a mechanism whereby 
PPARγ and RXR ligands enhance ABCA1- and ABCG1-
mediated cellular cholesterol efflux, more than compensating for any increase in the cellular cholesterol pool as a result of 
increased CD36 scavenger receptor expression and ox lipoprotein uptake.

Since the discovery that PPARγ ligands upregulate CD36 expression,7 there has been great interest as to whether this leads to enhanced foam cell formation, especially because TZDs are widely prescribed to diabetic patients, a population at high risk of atherosclerosis.11 However, because of inconsistent reports on oxLDL uptake and uncertainty regarding effects on cholesterol efflux,7,15,24,25 the outcome of cigitazone and 9cRA treatment on lipoprotein-induced cholesterol mass accumulation in macrophages was difficult to predict. However, it was evident from in vivo mouse studies that PPARγ and RXR ligands decrease atherosclerotic lesion formation.19–21,25,33 In this study, we show only a modest enhancement in macrophage [125I]oxLDL uptake induced by cigitazone and 9cRA treatment, a finding consistent with Tontonoz et al.7 However, Moore et al.3 reported a decrease in [125I]oxLDL binding, in ES cells treated with troglitazone and LG268. The reasons for these disparities are not readily apparent; however, ES cells may not faithfully reflect either qualitatively or quantitatively the expression of key genes involved in macrophage lipid homeostasis.25 Furthermore, differences in lipoprotein preparation and the extent of oxidation may affect the affinity of oxLDL for various scavenger receptors.34 Using our preparation of ox lipoproteins we have previously demonstrated in SRAI/II-null MPMs that SRAI/II contributes to only 30% of the overall increase in ox lipoprotein-induced CE mass accumulation.30 This implies that in our studies, CD36 is the major scavenger receptor for ox lipoproteins and hence the reason we observe a net increase in [125I]oxLDL uptake in the face of the PPARγ-induced decrease in SRAI/II activity. Nevertheless, despite the increase in ox lipoprotein uptake, PPARγ ligands significantly decrease macrophage CE accumulation induced by ox lipoproteins and hence foam cell formation. Therefore, ox lipoprotein uptake alone can be a poor predictor of macrophage CE accumulation or by extension foam cell formation.

Our results showing inhibition in modified LDL-induced CE accumulation by TZDs differ from the findings of Chinetti et al.24 Those authors demonstrated that in THP-1 macrophages and human primary macrophages, rosiglitazone treatment did not affect cholesterol accumulation induced by AcLDL or oxLDL. It is unclear whether this difference is attributable to rosiglitazone use rather than cigitazone or troglitazone or to some other mechanism. This lack of effect of rosiglitazone on oxLDL- and AcLDL-induced macrophage cholesterol accumulation is surprising, because in separate experiments, ABCA1 expression and apoAI-mediated cholesterol efflux from cholesterol-loaded cells were significantly increased, a result that concurs with our findings. The possible absence of a cholesterol acceptor in the culture medium could have prevented cholesterol efflux during incubations of macrophages with AcLDL or oxLDL. In the present study incubations of macrophages with either oxLDL or AcLDL included LPDS, which we have previously demonstrated to be an effective acceptor of cellular cholesterol.30 In addition to modulating scavenger receptor expression, PPARγ and RXR are important regulators of macrophage cholesterol efflux.24,25,35 Cholesterol efflux to acceptors is mediated by both specific energy-requiring processes and nonspecific passive diffusion.36 Our results clearly demonstrate that cigitazone and 9cRA, either alone or in combination, can stimulate the expression of both ABCA1 and ABCG1, resulting in enhanced efflux of cellular cholesterol to apoAI and HDLc. A key observation of this study was that cigitazone and 9cRA enhanced cholesterol efflux to LPDS. This likely explains the inhibition of CE accumulation in THP-1 and MPMs incubated with ox lipoproteins. Despite enhanced ox lipoprotein uptake with increased CD36 expression, more cholesterol was being effluxed to LPDS (probably because of its content of apoAI30), in response to cigitazone and 9cRA. Our results agree with those of others who demonstrated that rosiglitazone alone or in combination with the RXR ligand LG268 stimulated expression of ABCA1 and ABCG1 and cholesterol efflux to apoAI from THP-1 cells, MPMs, or human monocyte–derived macrophages.22,24 However, those authors did not demonstrate that this specifically decreases ox lipoprotein-induced cellular CE accumulation. Our findings contrast those of Akiyama et al.25 who found that in thioglycollate-elicited MPMs, troglitazone decreased the expression of ABCA1 and reduced cholesterol efflux to HDL. Furthermore, those authors showed that, unexpectedly, cigitazone, rosiglitazone, and pioglitazone had no effect on ABCA1 expression.25 Reasons for such disparities between studies are not readily apparent but may be related to cell type. Again, the effect of these TZDs on foam cell formation was not directly determined.

Several lines of experimental evidence have shown that PPARγ regulates ABCA1 and ABCG1 expression through a
transcriptional cascade involving LXR.\textsuperscript{22,25} We wanted to demonstrate that this cascade applied to THP-1 cells and, importantly, to establish that ABCA1/ABCG1-mediated cholesterol efflux was the predominant mechanism underlying reduced foam cell formation. By using antagonists for both PPAR\(\gamma\) and LXR,\textsuperscript{31,32} we showed that either antagonist completely blocks cigitazone-enhanced cholesterol efflux to apoAI. This confirms that PPAR\(\gamma\) activation of ABCA1-mediated cholesterol efflux requires LXR. However, the PPAR\(\gamma\) antagonist could not inhibit the effect of 9cRA. Macrophage ABCA1 expression is upregulated by both PPAR\(\gamma\) and LXR agonists (Table).\textsuperscript{34,37} However, RXR is the obligate partner for both PPAR\(\gamma\) and LXR. Therefore, it was not known whether 9cRA mediated enhanced cholesterol efflux to apoAI through activation of the PPAR\(\gamma\) or LXR dimer. Our results demonstrate conclusively that the 9cRA-induced increase in cholesterol efflux to apoAI was specific to the LXR antagonist, indicating that RXR ligands enhance efflux primarily through an LXR sensitive pathway. Evidence that the PPAR\(\gamma\)-LXR:ABCA1 pathway regulates foam cell formation in vitro comes from demonstrating the reversal of the cigitazone-induced CE mass reduction by an LXR antagonist (Figure 5).

Our observation that PPAR\(\gamma\) and RXR ligands can inhibit foam cell formation induced by ox lipoproteins specifies an important mechanism underlying the reduced atherosclerosis in mice treated with TZDs or RXR ligands.\textsuperscript{20,21,33} Although PPAR\(\gamma\) and RXR ligands tend to stimulate lipoprotein uptake, their ability to stimulate ABCA1- and ABCG1-mediated cholesterol efflux predominates in our macrophage model. These observations imply that the normal physiological response of arterial wall macrophages to PPAR\(\gamma\) ligands derived from ox lipoproteins is to increase CD36-mediated uptake of these lipoproteins, thereby relieving the arterial wall of the immunological pressure induced by ox lipoproteins. Simultaneously, this would fuel a mechanism whereby the excess intracellular cholesterol is redirected for efflux. In this case, cholesterol esterification would only be stimulated as a protective effect when cholesterol efflux pathways were saturated.\textsuperscript{38} Nonetheless, our study demonstrates that the addition of exogenous PPAR\(\gamma\) and/or RXR ligands over and above those ligands derived from oxidized lipoproteins would further stimulate cholesterol efflux through the reverse cholesterol transport pathway. Ultimately, this mechanism would contribute to a reduction in atherosclerosis.

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Figure I

Expressed as % of Control (ug CE/mg cell protein)

- oxHTG- VLDL+ + + + + + + +
- Ciglitazone - + - + - + - +
- 9cRA - - + + - - + +

C57Bl/6
LDLR-null
CD1
SRAI/II-null