Peroxisome proliferator-activated receptor (PPAR) agonists decrease lipoprotein lipase secretion and glycated LDL uptake by human macrophages

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Abstract Lipoprotein lipase (LPL) acts independently of its function as triglyceride hydrolase by stimulating macrophage binding and uptake of native, oxidized and glycated LDL. Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors expressed in monocyte/macrophages, where they control cholesterol homeostasis. Here we study the role of PPARs in the regulation of LPL expression and activity in human monocytes and macrophages. Incubation of human monocytes or macrophages with PPARα or PPARγ ligands increases LPL mRNA and intracellular protein levels. By contrast, PPAR activators decrease secreted LPL mass and enzyme activity in differentiated macrophages. These actions of PPAR activators are associated with a reduced uptake of glycated LDL and could influence atherosclerosis development associated with diabetes.

Key words: Atherosclerosis; Lipoprotein metabolism; Vascular wall; Diabetes

1. Introduction

Lipoprotein lipase (LPL) is a 58 kDa glycoprotein that plays a central role in lipoprotein metabolism by hydrolyzing triglycerides present in chylomicrons and very low density lipoproteins (VLDLs). LPL, which acts as homodimeric enzyme requires apoCII as cofactor and is produced in a large number of tissues [1]. In most tissues, LPL is synthesized by the parenchymal cells and subsequently transferred to the endothelial surface where, bound to heparan sulfate proteoglycans (HSPGs) [2], it exerts its activity. In the arterial wall, LPL is expressed in macrophages and smooth muscle cells [2].

Through its enzymatic activity, LPL generates remnant lipoprotein particles which are readily taken up by cells via receptors recognizing apoE as ligand [3] and produces free fatty acids and phospholipids [4]. Besides this activity, LPL also acts as a bridging factor which enhances binding to the extracellular matrix [5] and uptake of both native and oxidized LDL by macrophages [6]. It has also been recently reported that in macrophages and fibroblasts, LPL is required for the binding and internalization of glycated LDL (glyLDL), via a pathway independent of the classical LDL receptor pathway [7]. In addition, LPL promotes the adhesion of monocytes to endothelial cells by binding to both monocyte surface proteins and endothelium HSPGs [8]. Taken together these observations indicate that LPL expressed in vascular macrophages may exhibit pro-atherogenic activities.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors which upon heterodimerization with the 9-*cis*-retinoic acid receptor (RXR), bind to specific peroxisome proliferator response elements (PPREs), thus regulating the expression of target genes involved in intra- and extracellular lipid metabolism [9]. PPARs also regulate the expression of several genes implicated in the inflammatory response by interfering with the AP-1, STAT or NFκB signaling pathways [9]. The natural prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15dPG-J2) and the synthetic anti-diabetic glitazones are ligands for PPARγ, while hypolipidemic fibrates and eicosanoids such as leukotriene B4 and 8(*S*)-hydroxyeicosatetraenoic acid (8(S)-HETE) are synthetic and natural ligands respectively for PPARα [9]. PPARα is predominantly expressed in liver, heart, kidney and muscle [10], while PPARγ is adipose tissue specific, where it triggers adipocyte differentiation and lipid storage [11,12]. PPARα is expressed in human monocytes and in fully differentiated macrophages, while PPARγ is exclusively expressed in cells undergoing differentiation into macrophages [13]. In addition, both PPARα and PPARγ are also detected in macrophage-rich areas of human atherosclerotic lesions [14–16]. In macrophages, PPARs inhibit inflammatory cytokine-induced activation [17], promote apoptosis [13] and control lipid homeostasis through their effects on the expression of several key genes including SR-A, CD36, SR-BI and ABCA1 [16,18–20].

Since both LPL and PPARs are expressed in macrophages, and since PPARα and PPARγ regulate the expression of LPL in liver and adipose tissue, respectively [21], the goal of this study was to investigate the role of PPARs in LPL gene regulation in human monocytes and in fully differentiated macro-

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phages. Our results show that LPL expression is upregulated by PPAR activators in human monocytes as well as in differentiated macrophages. However, unexpectedly, the amounts of secreted LPL are reduced after treatment with PPAR activators, an effect which is accompanied by a decrease in LPL enzyme activity. Lowered LPL secretion is accompanied by a reduced uptake of glycated LDL, which may contribute to the beneficial effects of PPAR agonists on macrovascular disease in diabetes.

2. Materials and methods

2.1. Cell culture

Mononuclear cells were isolated from blood of healthy normolipidemic donors by Ficoll gradient centrifugation and cultured as described [13]. Mature monocyte-derived macrophages were used for experiments after 10 days of culture, whereas experiments in monocytes were done using cells isolated after 45 min of adherence to the plastic dishes. For treatment, medium was changed to RPMI 1640 with 1% Nutridoma HU (Boehringer, Mannheim, Germany) containing different activators. Heparin (Choay) (10 U/ml) was added for 24 h at the same time as the activators.

2.2. Isolation of total RNA and Northern blot analysis

After 6 h incubation with the indicated PPAR activators, cells were washed with PBS and used for RNA extraction using the RNA *plus kit* (Bioprobe System, Montreuil, France). For Northern blot analysis, membranes were hybridized with [^{32}P] random prime labeled human LPL [22] or 36B4 control cDNA probes.

2.3. Protein extraction and Western blot analysis

Cells were harvested in ice-cold lysis buffer containing PBS, 1% Triton X-100, and protease inhibitor mixture (ICN, Orsay, France) to which 1 mM phenylmethylsulfonyl fluoride (PMSF) was added. Western blot analysis was performed using rabbit polyclonal antibodies raised against human LPL (amino acids 253–274) or goat polyclonal antibody against β-actin (Santa Cruz) as internal control. Synthetic peptides of LPL (amino acids 253–274) were produced by the classical solid phase synthesis as described by Merrifield [23].

2.4. Quantification of LPL secretion by human macrophages using enzyme-linked immunosorbent assay (ELISA)

LPL mass in culture media was measured by ELISA. After incubation with an anti-LPL polyclonal antibody (chicken IgG, 5 μg/ml in 5 ml PBS, 100 μl/well) for 24 h at 37°C, plates were washed with PBS. Macrophage culture media and a purified human LPL standard (0–40 ng protein/ml) were diluted in 1% BSA–PBS (w/v) and 100 μl was added to each well and incubated overnight at 4°C. Plates were washed and incubated with the mouse monoclonal antibody mAb 5D2 for 3 h at room temperature. After additional washing, 100 μl of anti-mouse peroxidase-conjugated IgG was incubated for 2 h at room temperature. After rinsing, development was carried out with phenylenediamine dihydrochloride (OPD, Dako, Carpinteria, CA, USA) substrate. Using this ELISA technique, both monomer and dimer forms of LPL are detected. Each experiment was performed in triplicate and each triplicate was quantified at three different dilutions.

2.5. LPL catalytic activity assays

LPL was measured in the medium after release by heparin (10 U/ml) as described [24]. LPL catalytic activity was measured using an emulsified [^{3}H]triolein substrate [25]. After incubation of 50–100 μl of sample with 100 μl of substrate for 60 min, released [^{3}H]-fatty acids were separated from the reaction mixture using the method of Belfrage and Vaughan [26]. LPL activity is expressed in mU which corresponds to nmol of fatty acids released per min.

2.6. LDL glycation and acetylation

LDL (1 mg protein per ml) in 10 mM PBS, pH 7.4 containing 0.01% EDTA, 25 μM butylhydroxytoluene (BHT), 50 μM diethylenetriaminepentaacetic acid (DTPA), 12 mg/ml sodium cyanoborohydride and 40 mmol/l glucose was filter-sterilized and incubated at 37°C for 6 days under nitrogen. LDL samples were extensively dialyzed against PBS containing 0.01% EDTA. The amount of glycation was estimated by measuring free NH_2 residues by fluorescamine fluorescence. 20 μl of control or glycated LDL (1 mg of protein/ml) was added to 730 μl of borate buffer (200 mM, pH 8.5) and mixed while 250 μl of fluorescamine (539 μM in acetone) was added. Following incubation at room temperature for 1 h, fluorescence was measured at 475 nm using an excitation wavelength of 390 nm. The fluorescence of treated samples is expressed as percentage of the control sample. To control the integrity of tryptophan residues of apoB, the decrease of the tryptophan fluorescence was measured at 331 nm using an excitation wavelength of 282 nm [27].

Acetylation of LDL was performed as described [28].

2.7. Cholesterol loading experiments

10 days old human macrophages were cholesterol loaded by incubation with glycated or acetylated LDL (50 μg/ml, containing or not [^{3}H]cholesterol) [28] in RPMI 1640 medium supplemented with 1% Nutridoma for 48 h. The PPAR activators rosiglitazone 100 nM and Wy14643 50 μM were added to the culture medium 24 h before cholesterol loading and thereafter every 24 h. After this incubation period, cells were washed in PBS and intracellular lipids were extracted in hexane/isopropanol, dried under nitrogen and total cholesterol subsequently measured by enzymatic assays (Boehringer). In the same experiments, [^{3}H]cholesterol radioactivity was measured by scintillation counting in cellular lipids extracted with hexane/isopropanol. Cellular proteins were collected by digestion in NaOH and measured by the Bradford assay (Bio-Rad).

3. Results

3.1. PPAR activators induce LPL expression in human monocytes

Since PPAR agonists have been shown to regulate LPL expression in liver and adipose tissue, we investigated whether PPAR activation in human monocytes influences LPL expression. Monocytes isolated by 45 min of cell adhesion to culture dishes were incubated with several PPARα and PPARγ ago-

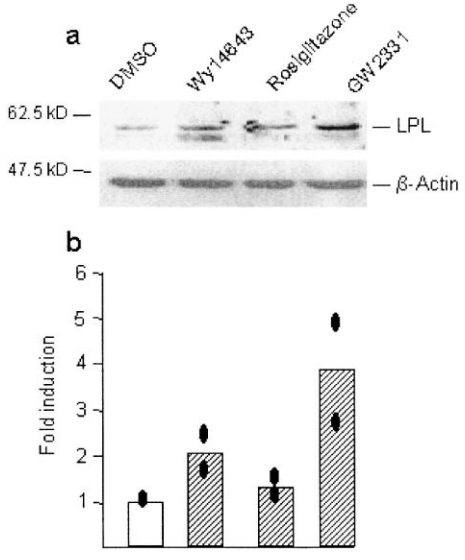

Fig. 1. PPAR activators induce the expression of LPL in human monocytes. Monocytes isolated by 45 min of adhesion to the culture dish, were treated with DMSO (control), Wy14643 (20 μM), rosiglitazone (50 nM) and GW2331 (1 μM) for 24 h. Total cellular protein extracts (25 μg) were used for Western blot analysis as described in Section 2. a: Western blot picture. b: Quantification by optical densitometry of LPL protein bands after normalization to β-actin levels. Results of two independent experiments on different monocyte preparations are shown.

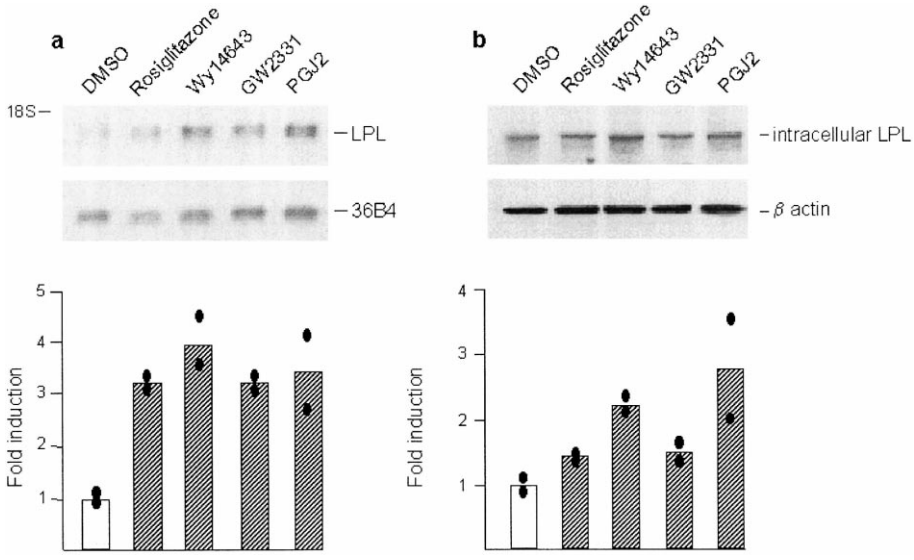

Fig. 2. PPAR activators induce LPL expression in human differentiated macrophages. 10 days old human macrophages were treated for 6 h (for RNA analysis) or for 24 h (for protein analysis) with DMSO (control), Wy14643 (20 μM), rosiglitazone (50 nM), GW2331 (200 nM) and PG-J2 (1 μM). a: Northern blot analysis was performed with 10 μg of total RNA as described under Section 2. Autoradiograph of the Northern blot and quantification by optical densitometry of LPL mRNA levels: values are normalized to internal control 36B4 mRNA and levels are expressed relative to DMSO treated cells set as 1. b: Western blot of intracellular protein levels (25 μg of total cellular proteins). Quantification by optical densitometry of LPL protein bands was performed and values are normalized to β-actin levels and expressed relative to vehicle treated control cells set as 1. Results presented are from experiments performed on two independent cell preparations.

nists during 24 h. Western blot analysis (Fig. 1) showed that LPL protein is slightly expressed in control cells (DMSO). Treatment of cells with the PPARα ligand Wy14643 at low concentration (20 μM), resulted in an approximately 2-fold induction of LPL protein levels. Treatment with the highly specific PPARγ activator rosiglitazone (50 nM) at a concentration activating PPARγ transcription activity without affecting cell viability [13], was without major effect on LPL protein levels, likely due to the low amounts of PPARγ expressed in cells at this step of differentiation [13]. Addition of the mixed PPAR agonist GW2331 (EC_{50} = 50 nM for hPPARα and 300 nM for hPPARγ) at concentrations of 1 μM activating both PPARα and PPARγ, increased LPL expression about 4-fold.

3.2. *PPAR activators induce LPL expression in differentiated human macrophages*

Since PPARs have been shown to activate LPL gene expression at the transcriptional level via a PPRE in its promoter [21], the influence of PPAR agonists on LPL gene expression in human macrophages was evaluated. Northern blot analysis of RNA extracted from differentiated macrophages incubated for 6 h with PPAR ligands was performed. A specific signal with the size of human LPL mRNA [22] was detected in these macrophages (Fig. 2a). Furthermore, LPL mRNA was induced by all PPAR agonists tested (Fig. 2a). The average relative increase of LPL mRNA normalized to control 36B4 mRNA levels, as determined by two independent experiments on different macrophage preparations, was approximately 3-, 4-, 3-, and 3.4-fold for rosiglitazone (50 nM), Wy14643 (20 μM), GW2331 (200 nM) and PG-J2 (1 μM), respectively (Fig. 2a). To determine whether the induction of LPL mRNA expression by PPAR activators in macrophages resulted in an increase of intracellular LPL protein levels, Western blot analysis of total cellular proteins was performed. A marked induction of LPL protein level was observed in cells treated during 24 h with all different PPAR activators tested (Fig. 2b). The increase of the protein expression levels was estimated by densitometric analysis of two independent experiments to be about 1.4-, 2.3-, 1.5-, and 2.8-fold for rosiglitazone (50 nM), Wy14643 (20 μM), GW2331 (200 nM), PG-J2 (1 μM), respectively (Fig. 2b). Furthermore, the effect of GW2331 on LPL induction was weaker than in Fig. 1, since the compound was added at lower concentration. No significant variation in β-actin expression

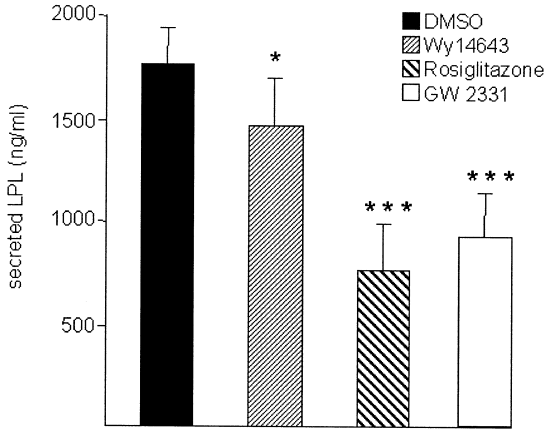

Fig. 3. PPAR activators decrease secretion of LPL in human differentiated macrophages. 10 days old differentiated macrophages were treated with DMSO (control), Wy14643 (20 μM), rosiglitazone (50 nM) and GW2331 (200 nM) after addition of heparin to stabilize LPL. Amounts of secreted LPL were determined by ELISA as described. Results are the mean ± S.E.M. of triplicate determinations, representative of four independent experiments. Statistically significant differences between treatments are indicated (***$P < 0.001$ and *$P < 0.05$ vs. DMSO, as determined by ANOVA followed by Mann–Whitney's test).

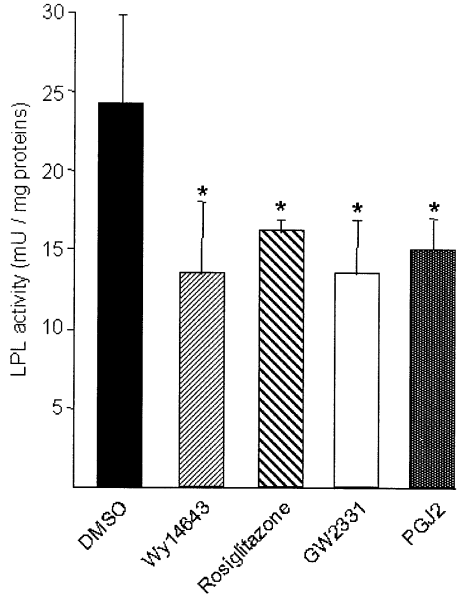

Fig. 4. PPAR activators decrease LPL enzyme activity in human macrophages. 10 days old macrophages were treated for 24 h with DMSO (control), Wy14643 (20 μM), rosiglitazone (50 nM), PG-J2 (1 μM) and GW2331 (200 nM) in the presence of heparin to stabilize LPL. Extracellular LPL activity measurement was performed as described. Results are the mean ± S.E.M. of triplicate determinations, representative of four independent experiments. $*P < 0.05$ vs. DMSO, as determined by ANOVA followed by Mann–Whitney's test.

was observed between control and treated macrophages (Fig. 2b).

3.3. PPAR activators decrease LPL secretion and activity in human differentiated macrophages

To evaluate whether PPARα and PPARγ activators induce LPL secretion by differentiated macrophages, ELISA of LPL levels was performed on culture media. Treatment of differentiated macrophages with PPARα ligands (Wy14643: 20 μM and GW2331: 200 nM) or PPARγ ligand rosiglitazone (50 nM) results in decreased amounts of extracellular enzyme mass (Fig. 3).

Moreover, LPL enzyme activity was markedly reduced by treatment with PPAR activators (Fig. 4) dropping to 53 and 67% of control for Wy14643 (20 μM) and rosiglitazone (50 nM), respectively. In the same experiments, incubation with GW2331 (200 nM) and PG-J2 (1 μM) showed a reduction of LPL activity by 52 and 44%, respectively. These effects were not due to a chemical inhibition of LPL activity by the PPAR activators since incubation of purified LPL with these compounds did not alter its enzymatic activity (data not shown).

3.4. PPAR activators decrease uptake of glycated LDL in human differentiated macrophages

To determine the physiological consequences of inhibition of LPL secretion by PPARs in human macrophages, the effects of PPAR ligands on the uptake of glyLDL, which are characterized by glycation of lysine residues of apoB and taken up by macrophages through an LPL-dependent mechanism were studied [7]. Treatment with either the PPARα ligand Wy14643 or the PPARγ ligand rosiglitazone added 24 h before cholesterol loading and thereafter every 24 h, decreased cholesterol accumulation from glyLDL in these cells (Fig. 5a). In order to demonstrate that the variation of intracellular lipids was not due to the action of PPAR ligands on de novo cholesterol synthesis, human macrophages were loaded with [^{3}H]cholesterol-containing glyLDL and the amounts of [^{3}H]cholesterol were evaluated by scintillation counting of cellular lipid extracts. Wy14643 (50 μM) and rosiglitazone (100 nM) decreased cellular [^{3}H]cholesterol amounts, compared to solvent treated cells (Fig. 5b). As a control, PPARα and PPARγ activators did not influence cholesterol accumulation in differentiated macrophages incubated and treated under the same conditions with acetylated LDL (data not shown). These results indicate that through their actions on LPL regulation, PPAR activation decreases the uptake of glyLDL.

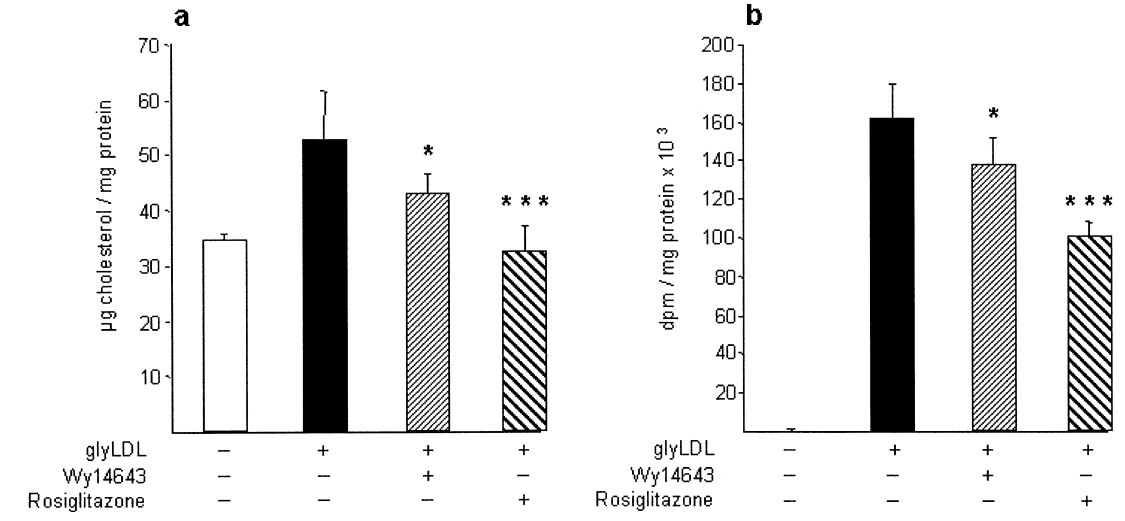

Fig. 5. PPAR activators reduce uptake of glycated LDL by differentiated human macrophages. Differentiated macrophages were incubated for 48 h with 50 μg/ml of [^{3}H]cholesterol-containing glyLDL. Wy14643 (50 μM) or rosiglitazone (100 nM) were added 24 h before cholesterol loading and thereafter every 24 h. a: Intracellular total cholesterol was enzymatically determined. b: Intracellular [^{3}H]cholesterol was determined by scintillation counting. Results are the mean ± S.E.M. of triplicate determinations. $***P < 0.001$ and $*P < 0.05$ vs. DMSO, as determined by ANOVA followed by Mann–Whitney's test.

4. Discussion

Lipid-loaded foam cells, derived principally from monocyte-derived macrophages, are a characteristic feature of atherosclerotic lesions [29]. The transformation of macrophages into foam cells, which is a consequence of cellular uptake of lipids from oxidized LDL and triglyceride-rich lipoproteins and their remnants [30–33], is thought to be an initial step in fatty streak and atherosclerotic lesion formation. Lipid capture by macrophages is mediated by a number of receptors including scavenger receptors [34,35], LDL receptors [36,37], VLDL and TG-rich lipoprotein (TRL) receptors [38], as well as by the interaction and retention of lipoproteins by proteoglycans on the cell surface and by the extracellular matrix. LPL is implicated in the generation of TRL remnants and in their subsequent uptake by macrophages and may thus play a role in the formation of foam cells [39–42]. In addition, a requirement for LPL in the uptake of glycated LDL by macrophages, independently of the LDL receptor pathway, has been recently demonstrated [7].

It is therefore of considerable importance to identify the factors which control LPL expression in atherosclerotic lesion macrophages. Increasing lines of evidence implicate transcription factors of the PPAR family in the regulation of macrophage function and in the control of their transformation into foam cells. Although PPARα and PPARγ have been shown to regulate the expression of the LPL gene in liver and adipocytes respectively [21,43,44], it remained to be determined whether PPAR activators also regulate LPL expression in vascular wall macrophages.

In the present study and for the first time using a model of human monocyte/macrophages, we demonstrate that PPARα and PPARγ activators increase LPL expression. In primary human monocytes, PPARα activation resulted in the induction of LPL expression. By contrast, addition of the PPARγ ligand rosiglitazone did not influence LPL expression. This result is in line with our previous observations that human monocytes express low levels of PPARγ at this step of the differentiation process [13]. In human differentiated macrophages the induction of LPL protein expression levels was observed after treatment with both PPARα and PPARγ ligands, which was associated with increased LPL mRNA levels, in agreement with the presence of high levels of PPARα and PPARγ expression in these cells. These results suggest that PPARα and PPARγ activators upregulate LPL expression in human monocytes and macrophages through a transcriptional mechanism. These conclusions are supported by previous data [21], demonstrating that PPARα and PPARγ ligands induce the expression of LPL in liver and adipocytes respectively via PPAR binding to PPRE site localized at sequences −169 to −157 of the human LPL gene promoter [21]. Although it cannot be excluded that increased LPL mRNA stability also occurs, it appears likely that the regulation of LPL by PPAR agonists in monocytes and macrophages occurs via this positive PPRE. Our data add LPL to the list of genes which are upregulated by PPARs through a PPRE-dependent mechanism in macrophages such as CD36/FAT, a scavenger receptor class B family member and fatty acid transporter [19].

Surprisingly, the increase of LPL gene expression by PPARα and PPARγ agonists is accompanied by a decrease in the mass of LPL secreted by human macrophages as well as lowered extracellular LPL enzyme activity. Although the mechanism of reduced LPL secretion by PPARs is unknown, PPAR activators may cause an inhibition of cellular LPL trafficking resulting in the reduced secretion of LPL. A similar reduction of LPL activity after rosiglitazone treatment was also observed in the 3T3-F442A and 3T3-L1 adipocyte cell lines [45]. These authors furthermore demonstrated that the reduced LPL activity was not due to a chemical inhibition of LPL activity by PPAR activator molecules [45]. In the same line, we could not detect an inhibition of LPL activity by PPAR activation in our LPL lipolysis assays.

Furthermore, our results on the effects of PPAR on reduction of macrophage LPL secretion and activity appear in contrast with previous observations published while this work was in progress [46], demonstrating an increase of extra- and intracellular LPL mass and activity in murine J774 macrophages after treatment with natural fatty acid-derived PPAR activators, such as palmitic acid, stearic acid and linoleic acid [46]. Even the reasons for these discrepancies are still not clear understood, one possible explanation could be that activated PPARs regulate LPL expression and activity in a species-specific manner. In addition, natural and synthetic PPAR ligands could have distinct effects on LPL regulation, which may be due to the low specificity of natural activators compared to the more selective synthetic compounds used in our study. Regulation of macrophage LPL by fatty acids (FAs) may involve other transcription factors such as FA-activated receptor (FFAR) which could recognize the putative PPRE sequence present in the LPL gene. Moreover, FAs could stimulate protein kinase C (PKC) which plays a key role in the regulation of LPL gene [46]. By contrast, in another study using adult rat cardiomyocytes, two synthetic PPARα ligands (Wy14643 and BM-17.0744) reduce LPL mass and activity in culture medium [47].

A major question concerns the role of PPARs in the pathophysiology of atherosclerosis. Here we demonstrate that reduction in LPL secretion and activity leads to a reduced uptake of glyLDL, a naturally occurring modified LDL particle with high atherogenic potential. GlyLDL, which is elevated in diabetes and hypercholesterolemia [48], can enhance chemotaxis and production of superoxide anions in macrophages [48] and increase PAI-1 production [49] and prostaglandins [50] in vascular endothelial cells. Thus, LDL glycation might contribute to the increased risk of atherosclerosis in patients with diabetes mellitus and familial hypercholesterolemia. Our observations identify a novel beneficial role for PPAR in macrophage cholesterol homeostasis, in addition to the previous observation indicating that PPARα and PPARγ display anti-atherogenic effects via the induction of the reverse cholesterol transport pathway in these cells [20].

In addition, the observed decrease of LPL activity could lead to a reduced generation of triglyceride-rich lipoprotein remnants which play a potentially atherogenic role at the endothelium surface and intima of the arterial wall [51]. Taken together our results demonstrate novel roles of PPARα and PPARγ in lipoprotein metabolism in macrophages via their actions on LPL expression and activity. Given the potential pro-atherogenic effect of macrophage LPL in the arterial wall, these results could contribute to the beneficial effects of PPAR activators in atherosclerosis associated with diabetes.

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References

[1] Olivecrona, G. and Olivecrona, T. (1995) Curr. Opin. Lipidol. 6, 291–305.
[2] Goldberg, I.J., Soprano, D.R., Wyatt, M.L., Vanni, T.M., Kirchgessner, T.G. and Schotz, M.C. (1989) J. Lipid Res. 30, 1569–1577.
[3] Gianturco, S.H., Bradley, W.A., Gotto, A.M., Morrisett, J.D. and Peavy, D.L. (1982) J. Clin. Invest. 70, 168–178.
[4] Rutledge, J.C., Woo, M.M., Rezai, A.A., Curtiss, L.K. and Goldberg, I.J. (1997) Circ. Res. 80, 819–828.
[5] Kaplan, M. and Aviram, M. (1997) Biochem. Biophys. Res. Commun. 237, 271–276.
[6] Hendriks, W.L., van der Boom, H., van Vark, L.C. and Havekes, L.M. (1996) Biochem. J. 314, 563–568.
[7] Zimmermann, R., Panzenböck, U., Wintersperger, A., Levak-Frank, S., Graier, W., Glotter, O., Kostner, G.M. and Zechner, R. (2001) Diabetes 50, 1643–1653.
[8] Obunike, J.C., Paka, S., Pillarisetti, S. and Goldberg, I.J. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 1414–1420.
[9] Chinetti, G., Fruchart, J.C. and Staels, B. (2000) Inflamm. Res. 49, 497–505.
[10] Braissant, O., Foufelle, F., Scotto, C., Dauca, M. and Wahli, W. (1995) Endocrinology 137, 354–366.
[11] Tontonoz, P., Graves, R.A., Budavari, A.I., Erdjument-Bromage, H., Lui, M., Hu, E., Tempst, P. and Spiegelman, B.M. (1994) Nucleic Acids Res. 22, 5628–5634.
[12] Tontonoz, P., Hu, E., Graves, R.A., Budavari, A.I. and Spiegelman, B.M. (1994) Genes Dev. 8, 1224–1234.
[13] Chinetti, G., Griglio, S., Antonucci, M., Pineda Torra, I., Delerive, P., Majd, Z., Fruchart, J.C., Chapman, J., Najib, J. and Staels, B. (1998) J. Biol. Chem. 273, 25573–25580.
[14] Ricote, M., Huang, J., Fajas, L., Li, A., Welch, J., Najib, J., Witztum, J.L., Auwerx, J., Palinski, W. and Glass, C.K. (1998) Proc. Natl. Acad. Sci. USA 95, 7614–7619.
[15] Marx, N., Sukhova, G., Murphy, C., Libby, P. and Plutzky, J. (1998) Am. J. Pathol. 153, 17–23.
[16] Chinetti, G., Gbaguidi, G.F., Griglio, S., Mallat, Z., Antonucci, M., Poulain, P., Chapman, J., Fruchart, J.C., Tedgui, A., Najib-Fruchart, J. and Staels, B. (2000) Circulation 101, 2411–2417.
[17] Jiang, C., Ting, A.T. and Seed, B. (1998) Nature (Lond.) 391, 82–86.
[18] Li, A.C., Brown, K.K., Silvestre, M.J., Willson, T.M., Palinski, W. and Glass, C.K. (2000) J. Clin. Invest. 106, 523–531.
[19] Tontonoz, P., Nagy, L., Alvarez, J., Thomazy, V. and Evans, R. (1998) Cell 93, 241–252.
[20] Chinetti, G., Lestavel, S., Bocher, V., Remaley, A.T., Neve, B., Pineda Torra, I., Teissier, E., Minnich, A., Jaye, M., Duverger, N., Brewer, B.H., Fruchart, J.C., Clavey, V. and Staels, B. (2001) Nat. Med. 7, 53–58.
[21] Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.-M., Heyman, R.A., Briggs, M., Deeb, S., Staels, B. and Auwerx, J. (1996) EMBO J. 15, 5336–5348.
[22] Staels, B., Martin, G., Martinez, M., Albert, C., Peinado-Onsurbe, J., Saladin, R., Hum, D.W., Reina, M., Vilaro, S. and Auwerx, J. (1996) J. Biol. Chem. 271, 17425–17432.
[23] Merrifield, R.B. and Stewart, J.M. (1965) Nature (Lond.) 207, 522–523.
[24] Stengel, D., Antonucci, M., Gaoua, W., Dachet, C., Lesnik, P., Hourton, D., Ninio, E., Chapman, M.J. and Griglio, S. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 1172–1180.
[25] Nilsson-Ehle, P. (1978) Lipids 13, 433–437.
[26] Belfrage, P. and Vaughan, M. (1969) J. Lipid Res. 10, 341–344.
[27] Ravandi, A., Kuksis, A. and Shaikh, N.A. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 467–477.
[28] Basu, S.K., Goldstein, J.L., Anderson, G.W. and Brown, M.S. (1976) Proc. Natl. Acad. Sci. USA 73, 3178–3182.
[29] Guyton, J.R. (1994) Curr. Opin. Lipidol. 5, 376–381.
[30] Nordestgaard, B.G., Wootton, R. and Lewis, B. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 534–542.
[31] Chung, B.H., Tallis, G., Yalamoori, V., Anantharamiah, G.M. and Segrest, J.P. (1994) Arterioscler. Thromb. 14, 622–635.
[32] Rapp, J.H., Lespine, A., Hamilton, R.L., Colyvas, N., Chaumeton, A.H., Tweedie-Hardman, J., Kotite, L., Kunitake, S.T., Havel, R.J. and Kane, J.P. (1994) Arterioscler. Thromb. 14, 1767–1774.
[33] Frank, J.S. and Folgelman, A.M. (1989) J. Lipid Res. 30, 967–978.
[34] Brown, M.S. and Goldstein, J.L. (1983) Annu. Rev. Biochem. 52, 223–261.
[35] Endmann, G., Stanton, L.W., Madden, K.S., Bryant, C.M., White, R.T. and Protter, A.A. (1993) J. Biol. Chem. 268, 11811–11816.
[36] Luoma, J., Hiltunen, T., Sarkioja, T., Moestrup, S., Gliemann, J., Kodama, T., Nikkari, T. and Yla-Herttuala, S. (1994) J. Clin. Invest. 93, 2014–2021.
[37] Stanton, L.W., White, R.T., Bryant, C.M., Protter, A.A. and Endmann, G.A. (1992) J. Biol. Chem. 267, 22446–22451.
[38] Takahashi, S., Suzuki, J., Kohno, M., Oida, K., Tamai, T., Miyabo, S., Yamamoto, T. and Nakai, T. (1995) J. Biol. Chem. 270, 15747–15754.
[39] Sofer, O., Fainaru, M., Schafer, Z. and Goldman, R. (1992) Arterioscler. Thromb. 12, 1458–1466.
[40] Seo, T. and St Clair, R.W. (1997) J. Lipid Res. 38, 765–779.
[41] Hendriks, W.L., van der Sman-de Beer, F., van Vlijmen, B.J., van Vark, L.C., Hofker, M.H. and Havekes, L.M. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 498–504.
[42] Whitman, S.C., Sawyez, C.G., Miller, D.B., Wolfe, B.M. and Huff, M.W. (1998) J. Lipid Res. 39, 1008–1020.
[43] Lefebvre, A.M., Peinado-Onsurbe, J., Leitersdorf, I., Briggs, M.R., Paterniti, J.R., Fruchart, J.C., Fievet, C., Auwerx, J. and Staels, B. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 1756–1764.
[44] Staels, B., Schoonjans, K., Fruchart, J.C. and Auwerx, J. (1997) Biochimie 79, 95–99.
[45] Ranganathan, S. and Kern, P.A. (1998) J. Biol. Chem. 273, 26117–26122.
[46] Michaud, S.E. and Renier, G. (2001) Diabetes 50, 660–666.
[47] Carroll, R. and Severson, D.L. (2001) Am. J. Physiol. Heart Circ. Physiol. 281, H888–H894.
[48] Graier, W.F. and Kostner, G.M. (1997) Eur. J. Clin. Invest. 27, 457–459.
[49] Zhang, J., Ren, S., Sun, D. and Shen, G.X. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 1140–1148.
[50] Myers, D.E., Huang, W.N. and Larkins, R.G. (1996) Am. J. Physiol. 271, C1504–1511.
[51] Zilversmit, D.B. (1979) Circulation 60, 472–485.