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The Lipid Droplet-Associated Protein Adipophilin Is Expressed in Human Trophoblasts and Is Regulated by Peroxisomal Proliferator-Activated Receptor-γ/Retinoid X Receptor

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Uptake and transplacental transfer of fatty acids is essential for fetal development. Human adipophilin and its murine ortholog adipocyte differentiation-related protein are lipid droplet-associated proteins that are implicated in cellular fatty acid uptake in adipocytes. The nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ) stimulates lipid uptake by adipocytes and enhances differentiation of placental trophoblasts. We therefore hypothesized that adipophilin is expressed in human trophoblasts and that its expression is regulated by PPARγ. We initially determined that adipophilin is expressed in human villous trophoblasts and that adipophilin expression is enhanced during differentiation of cultured primary term human trophoblasts. We also found that exposure of cultured human trophoblasts to the PPARγ ligand troglitazone resulted in a concentration-dependent increase in adipophilin expression. We observed a similar increase with LG268, a ligand for retinoid X receptor (RXR), the heterodimeric partner of PPARγ. Lastly, we demonstrated that ligand-activated PPARγ and RXR stimulated the transcriptional activity of adipophilin promoter in CV-1 cells and in the placental JEG3 cell line. We conclude that the expression of adipophilin is enhanced during trophoblast differentiation and is up-regulated by ligand-activated PPARγ/RXR. Enhanced adipophilin expression may contribute to fatty acid uptake by the placenta. (*J Clin Endocrinol Metab* 88: 6056–6062, 2003)

MATERNAL SUPPLY OF nutrients is obligatory for intrauterine fetal development and growth. Among the essential nutrients, lipids from lipoproteins are used as substrates for the growing fetus, an energy source, and precursors for steroid hormone synthesis. Nutrients transported from the maternal to the fetal circulation cross the villous trophoblast. The trophoblast consists of two surface membranes: a microvillous membrane that faces the maternal blood space and a basal membrane that faces the fetal blood vessels within the villous core. Although triglycerides do not directly cross the placental barrier (1), the presence of lipoprotein lipase activity in trophoblast cells allows placental hydrolysis and uptake of maternal plasma triglycerides (2–4). In contrast, fatty acids can cross trophoblast surface membranes by simple diffusion and facilitated transport, mediated by membrane-bound and cytosolic fatty acid binding proteins (5–7).

Adipophilin is the human ortholog of murine adipose differentiation-related protein (ADRP), a 50-kDa protein that was initially cloned from a mouse adipocyte cDNA library (8). The N terminus of ADRP is highly homologous to the N-terminal end of perilipin A and TIP47/pp17 (9–11). Another recently identified protein, S3–12 (12), contains a 33-amino acid repeat sequence that exhibits strong homology to ADRP. Previous studies showed that ADRP mRNA is expressed primarily in adipose tissue and is induced early during adipocyte differentiation (13, 14). Adipophilin colocalizes with the surface of neutral lipid droplets in adipocytes and in a wide range of cells and tissues that store or synthesize lipids, including milk-secreting mammary epithelial cells, testicular Leydig and Sertoli cells, adrenal cortex cells, and hepatocytes with fatty changes associated with alcoholism (15–17). Unlike adipophilin, perilipin expression is more restricted and localizes primarily to triacylglycerol-rich lipid droplets in adipocytes and the cholesterol ester-rich droplets in steroidogenic cells (18, 19). Adipophilin expression is enhanced on incubation of preadipocytes with long-chain fatty acids (17). By increasing the initial uptake rate, adipophilin facilitates the uptake of long-chain free fatty acids in COS-7 cells, supporting the role of adipophilin as a fatty acid carrier protein (20).

Peroxisome proliferator-activated receptor-γ (PPARγ) is a member of the steroid receptor superfamily of nuclear receptors. PPARγ is expressed predominantly in adipose tissue (21, 22), monocytes, macrophages (23, 24), and the placenta (25–28). Known ligands for PPARγ include fatty acids, oxidized low-density lipoprotein derivatives, the prostaglandin metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, and the antidiabetic thiazolidinediones (24, 29–33). Stimulation of PPARγ activity by natural or synthetic ligands results in adipogenesis in target tissues through the induction of genes mediating fatty acid uptake, metabolism, and storage (25, 31, 34). PPARγ is also indispensable for murine placental development. The placentas of PPARγ-deficient murine embryos

Abbreviations: ADRP, Adipose differentiation-related protein; Ct, threshold cycle; ΔCt, Ct values; PPARγ, peroxisome proliferator-activated receptor-γ; RXR, retinoid X receptor.

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exhibit failed maturation of labyrinthine trilaminar trophoblast epithelium along with vascular defects. These defects compromise maternal-fetal exchange and result in embryonic death by E10.5 (25, 26). The placental labyrinth in PPARγ-deficient mice also expresses fewer and markedly smaller lipid droplets, compared with wild type (25). We demonstrated that PPARγ modulates differentiation of human cytotrophoblast into syncytiotrophoblast in a ligand-specific manner and that the activity of PPARγ is enhanced by oxidized lipids (27, 35). Taken together, these data suggest that PPARγ may regulate the expression of fatty acid transporters in human trophoblasts. In a recent microarray screen for PPARγ targets in trophoblasts, we found that adipophilin is markedly up-regulated by ligand-activated PPARγ (36, and Roh, C. R. and Y. Sadovsky, manuscript in preparation). We therefore hypothesized that adipophilin is expressed in human trophoblasts and that its expression is influenced by PPARγ. Here we demonstrate that adipophilin is expressed in primary term human trophoblasts and that ligand-activated PPARγ enhances the expression of adipophilin in trophoblasts and the activity of adipophilin promoter in the trophoblast cell line JEG3.

Materials and Methods

Cell culture

Our study was approved by the human studies committee of Washington University in St. Louis. Primary human cytotrophoblasts were prepared from normal term human placentas using the trypsin-deoxyribonuclease-dispase/Percoll method as described by Kliman *et al.* (37) with previously published modifications (27, 38). Cultures were plated at a density of 300,000 cells/cm^2 and maintained in Earl's medium 199 containing 10% fetal bovine serum (HyClone Laboratories, Inc., Logan, UT), 20 mmol/liter HEPES (pH 7.4), 0.5 mmol/L-glutamine (Sigma, St. Louis, MO), penicillin (10 U/ml), streptomycin (10 μg/ml), and fungizone (0.25 μg/ml). All cultures were maintained at 37 C in a 5% CO_2 atmosphere. Medium was changed 4 h after initial plating and every 24 h thereafter. Where indicated, cultures were supplemented with 10 μM troglitazone (Biomol, Plymouth Meeting, PA) and/or 0.1 μM LG268 (a gift from Ligand Pharmaceuticals, San Diego, CA). These ligands were added either after 4 h in culture or at the time points indicated. The time point of 4 h, selected to allow cells to adhere, was defined as time 0. In ligand time course immunoblotting experiments all cells were harvested for western analysis after 72 h. The time points indicate the period of ligand exposure such that 24 h indicates ligand exposure from 48–72 h in culture, 48 h indicates ligand exposure from 24 to 72 h in culture, and 72 h indicates ligand exposure for the entire culture period. The concentrations of PPARγ and retinoid X receptor (RXR) ligands in the culture media were previously optimized to yield maximal responses *in vitro* and were consistent with published reports (24, 39). Overall, more than 15 freshly isolated placental preparations contributed to the results.

Immunohistochemistry and Western immunoblotting

Placental biopsies from term uncomplicated pregnancies were collected immediately after delivery, fixed for 2–4 h in 10% neutral buffered formalin, and embedded in paraffin. Five-micron sections were deparaffinized in xylene and rehydrated in an ethanol gradient. Endogenous peroxidase activity was quenched by incubating the specimens in 3% H_2O_2 in methanol for 30 min. After equilibrating for 5 min in distilled water, the samples were heated in a microwave at maximum power for 10 min. The slides were washed, blocked for 30 min with normal goat serum, and incubated for 2 h at room temperature in the absence or presence of antiadipophilin guinea pig polyclonal antibody (1:500, Research Diagnostics Inc, Flanders, NJ). After washes, the slides were incubated for 30 min with a biotinylated goat antiguinea pig secondary antibody (1:2000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), followed by 30-min incubation with avidin/biotin-horseradish peroxidase complex (Vector Laboratories, Inc., Burlingame, CA) and 3-min incubation with diaminobenzidene (Zymed Laboratories, Inc., South San Francisco, CA). The slides were rinsed and counterstained with Mayer's hematoxylin (Sigma), dehydrated in ethanol, cleared with xylene, and mounted with glass cover slips using Histomount (Zymed Laboratories, Inc.). The results were verified using similarly prepared slides stained with antiadipophilin mouse monoclonal antibody (Research Diagnostics Inc.) according to the manufacturer's protocol or isotype matched IgG_1. After washes the slides were incubated for 30 min with horseradish peroxidase-conjugated donkey antimouse antibody (1:400, Santa Cruz Biotechnology).

Cell lysis, sonication, electrophoresis, and transfer for Western immunoblotting were performed as previously described (27). Membranes were incubated overnight at 4 C with antiadipophilin mouse monoclonal antibody (1:1000, Progen Biotechnik, Heidelberg, Germany). The blot was subsequently washed, incubated for 1 h with horseradish peroxidase-conjugated donkey antimouse antibody (1:3000, Santa Cruz Biotechnology), washed, and processed for luminescence using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Arlington Heights, IL). Differences in protein expression were quantified using densitometry.

RNA extraction and real-time quantitative PCR

Total RNA was prepared from cultured trophoblasts using TriReagent (MRC, Cincinnati, OH) as recommended by the manufacturer. RNA was digested with DNase I, quantified by absorbance at 260 nm, and quality determined by electrophoresis. For reverse transcription, 2 μg total RNA were added into reverse transcriptase buffer, 5.5 mM $MgCl_2$, 2.5 μM random hexamers, 500 μM deoxynucleotide triphosphate mixture, 20 U Rnasin, and 50 U MultiScribe reverse transcriptase in a final volume of 50 μl. Reverse transcriptase reactions were performed by incubation for 10 min at 25 C, 30 min at 48 C and inactivation for 5 min at 95 C. Relative expression of adipophilin RNA was assessed using the SYBRGreen I assay on the GeneAmp 5700 sequence detection system (Applied Biosystems, Foster City, CA). Each PCR was performed in duplicates with 50-μl volumes of 2 μl cDNA, 25 μl SYBRGreen PCR master mix (Applied Biosystems), and 0.3 μM of each primer, including forward, GGCAGAGAACGGTGTGAAGAC, and reverse, TCTGGATGATGGGCAGAGC. PCR was conducted using the following cycle parameters: 2 min at 50 C, 10 min at 95 C, and 40 two-step cycles of 15 sec at 95 C and 1 min at 60 C. Analysis was carried out using the GeneAmp 5700 sequence detection software, which calculates the threshold cycle (Ct) for each reaction. A difference in Ct values (ΔCt) was calculated by subtracting the mean Ct of the reference 18S for each Ct of duplicates, and then ΔΔCt was calculated by subtracting the reference mean Ct from each ΔCt value for each condition.

Adipophilin promoter and transient transfection

We used human adipophilin coding sequences (NM001122, GenBank, National Center for Biotechnology Information) (14) to probe the human genome database and identified contig NT037733.1 (chromosome 9p21.3) that harbors sequences identical to adipophilin. Using PCR primers forward 5′-TGGCGCAACTTGTCTGCTCAAATAA-3′ and reverse 5′-CTGCAATCAAAGTAGGGAGGGTATG-3′, we amplified genomic sequences from nt 538846 to 536349 within contig NT037733.1, which correspond to promoter sites −2381 to +117 of adipophilin. We cloned this promoter fragment, which includes adipophilin's first intron, between *Kpn*I and *Xho*I sites upstream of luciferase in pGL-Basic vector (Promega, Madison, WI).

JEG3 or CV-1 cells were plated in 12-well plates (50,000 cells/well) 1 d before transfection. Transfection was performed using the calcium-phosphate coprecipitation method, as previously described (27, 40). Cells in each well were transfected with 0.5 μg of adipophilin-Luc promoter plasmid, 0.2 μg of pCDNA3.1 expression vector, or pCDNA3.1-PPARγ, which expresses human PPARγ1 (a gift from Tom Mariani, Washington University) as well as 0.05 μg cytomegalovirus-β-galactosidase reporter plasmid (incorporated to normalize for cell viability and transfection efficiency). Ligands were added after overnight transfection, and luciferase assay was performed 24 h later. Cells were lysed and luciferase activity determined using a Lumistar luminometer (BMG Lab Technologies, Durham, NC), and β-galactosidase

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assay performed using a plate reader (Anthos htIII, Salzburg, Austria) as we previously described (27, 35). All experiments were performed in duplicate and repeated at least three times. Results (mean ± SD) were expressed as relative luciferase units, corrected to β-galactosidase.

Differences were analyzed using either *t* test or one-way ANOVA and post hoc Student-Newman-Keuls test, with $P < 0.05$ determined significant.

Results

We initially examined the expression of adipophilin in the term human placenta. For this purpose we prepared sections of placental villi derived from uncomplicated term deliveries. Using immunohistochemistry, we detected adipophilin expression in villous trophoblasts (Fig. 1), with highest expression along the microvillous membrane (Fig. 1C). A lower level of adipophilin expression was also detected in villous stromal cells and endothelial cells. Using Western immunoblotting, we confirmed the expression of adipophilin in cultured primary term human trophoblasts. As shown in Fig. 2, adipophilin expression in trophoblasts was enhanced over 72 h of culture, corresponding to differentiation of these cells into syncytium, as we have previously shown (38). We concluded that adipophilin is expressed in villous trophoblasts, and its expression increases during cytotrophoblast differentiation into syncytiotrophoblasts.

Ligand activation of PPARγ enhances adipogenesis in undifferentiated fibroblasts and other tissues (31, 34). In addition, activation of PPARγ by thiazolidinediones enhances differentiation of cytotrophoblasts into syncytiotrophoblasts (27). We therefore examined the role of the thiazolidinedione troglitazone in regulation of adipophilin expression in cultured trophoblasts. We have previously shown that PPARγ is expressed in human villous trophoblasts *in vivo* and plated term human trophoblasts *in vitro* and that expression of PPARγ in cultured trophoblasts is unchanged between 24 and 72 h of culture (27). As shown in Fig. 3A, 24 h of troglitazone was sufficient to enhance adipophilin. Similarly, addition of troglitazone to the culture medium of plated trophoblasts resulted in a concentration-dependent increase in adipophilin expression (Fig. 3B). Because RXR heterodimerizes with PPARγ (41, 42) and RXRα is expressed in term human trophoblasts (43–45), we examined the effect of RXR activation on adipophilin expression. As shown in Fig. 4, LG268, a selective RXR agonist, enhanced the expression of adipophilin. This enhancement was concentration dependent, with maximum effect observed at 0.01–0.1 μM, and declined at a higher concentration (Fig. 4B). Taken together,

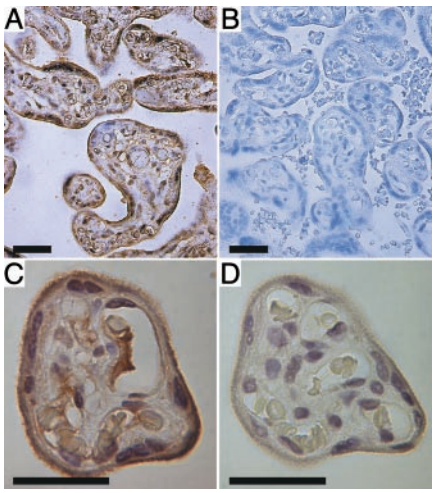

FIG. 1. Adipophilin is expressed in human villous syncytiotrophoblasts. Formalin-fixed sections of normal term human placenta were stained for adipophilin (A, ×400, and C, ×1000) and counterstained with Mayer's hematoxylin. Adipophilin expression in A was detected using antiadipophilin guinea pig polyclonal antibody as described in *Materials and Methods*, and B was similarly stained, but the primary antibody was omitted. Adipophilin expression in C was detected using antiadipophilin mouse polyclonal antibody, and D was similarly stained with isotype matched nonspecific IgG as a primary antibody (*bar*, 100 μM). Results represent three independent experiments.

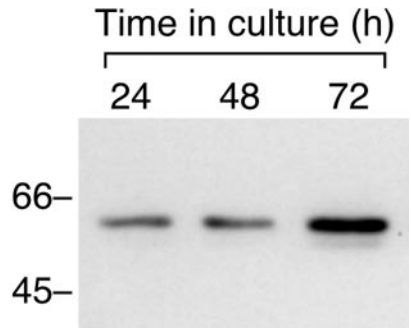

FIG. 2. Western immunoblotting depicting the expression of adipophilin in differentiating trophoblasts *in vitro*. Primary trophoblasts were cultured for the indicated times, with adipophilin detected as described in *Materials and Methods*. Results represent three independent experiments, in which maximum adipophilin increase by 72 h (1.9-fold) was significantly different from the other two time points ($P < 0.05$).

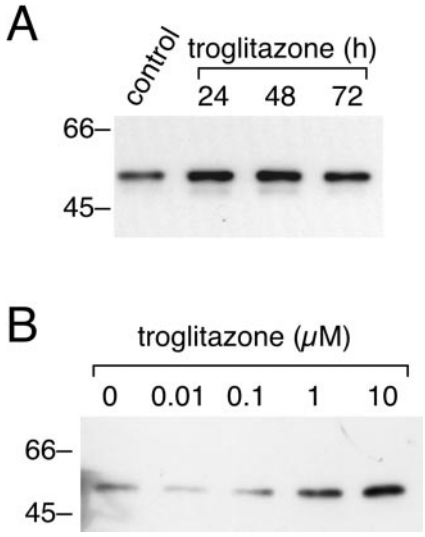

FIG. 3. The PPARγ agonist troglitazone enhances the expression of adipophilin in cultured term human trophoblasts. Adipophilin expression was assessed using Western immunoblotting. Results represent three independent experiments. A, Trophoblasts were cultured for 72 h and exposed to troglitazone (10 μM) for the last 24, 48, or the entire 72 h of culture, as described in *Materials and Methods*. Adipophilin expression was similar among troglitazone-exposed cultures and was significantly different from control (2-fold, $P < 0.05$). B, Primary trophoblasts were cultured for 72 h in the presence of troglitazone (0.01–10 μM) and adipophilin expression determined using Western immunoblotting, as described in *Materials and Methods*.

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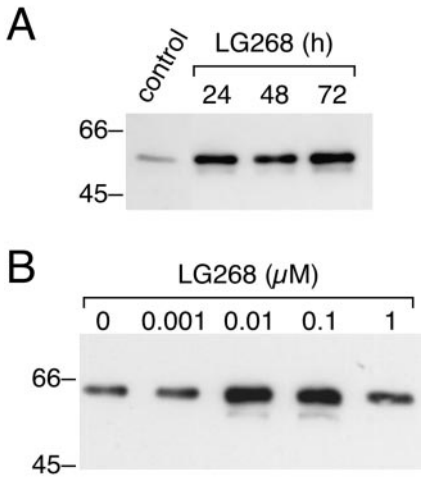

FIG. 4. The RXRα agonist LG268 enhances the expression of adipophilin in cultured term human trophoblasts. A, Trophoblasts were cultured for 72 h and exposed to LG268 (0.1 μM) for the last 24, 48, or the entire 72 h of culture, as described in *Materials and Methods*. Adipophilin expression was assessed using Western immunoblotting. Results represent three independent experiments. Adipophilin expression was similar among LG268 exposed cultures and was significantly different from control (3-fold, $P < 0.05$). B, Primary trophoblasts were cultured for 72 h in the presence of LG268 (0.001–1 μM) and adipophilin expression determined using Western immunoblotting, as described in *Materials and Methods*.

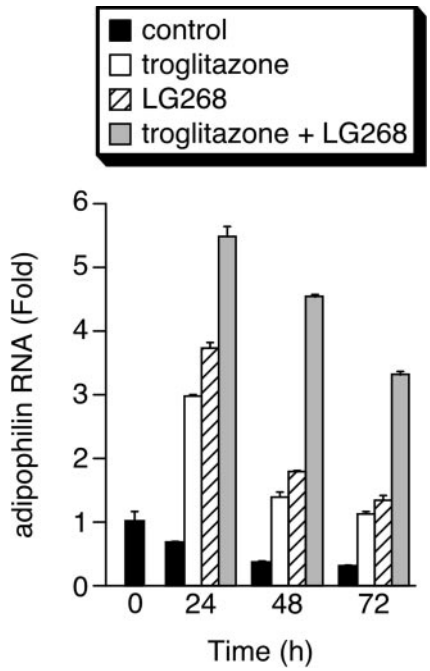

FIG. 5. Regulation of adipophilin RNA by PPARγ and RXR agonists. Trophoblasts were plated in the presence or absence of troglitazone (10 μM) and/or LG268 (0.1 μM). Ligands were added after 4 h in culture (defined as 0 h) and cells harvested after either 24, 48, or 72 h time points. RNA was isolated and quantified using real-time quantitative PCR as detailed in *Materials and Methods*. Each experiment was performed in duplicate and repeated at least twice. The difference between each ligand and control at each time point was significant at $P < 0.05$.

our data indicate that PPARγ and RXR enhance the expression of adipophilin in term human trophoblasts.

To obtain additional support for the influence of PPARγ/RXR activation on expression of adipophilin in term human trophoblasts, we examined the effect of troglitazone and LG268 on adipophilin RNA expression, assessed using real-time quantitative PCR. Interestingly, basal level of adipophilin RNA gradually decreased during the culture period (Fig. 5). Nevertheless, exposure of cultured cells to troglitazone, LG268, or both ligands markedly increased adipophilin RNA expression, providing further support to the influence of PPARγ/RXR on adipophilin gene expression.

PPARγ regulates the transcriptional activity of diverse genes (46) and increases the level of placental adipophilin mRNA. We therefore sought to examine the effect of PPARγ on adipophilin promoter. For this purpose we used the human genome database (GenBank) to clone the adipophilin promoter from 5′ promoter sequences (−2381/+117) within contig NT037733.1, as described in *Materials and Methods*. This fragment was then cloned into pGL3-basic to generate the adipophilin-luciferase reporter construct. We initially transfected the adipophilin-luc reporter plasmid into CV-1 cells, an undifferentiated line derived from monkey kidney cells, selected because of absent endogenous PPARγ expression. Although basal activity was higher than the activity of the parental pGL3 vector, we found that neither troglitazone nor LG268 influenced reporter activity (Fig. 6A). In contrast, upon cotransfection of PPARγ into CV-1 cells, we observed a greater than 2-fold stimulation of reporter activity by either troglitazone or LG268, with even greater activity when both ligands were present (Fig. 6A). We repeated these experiments in JEG3 cells, which express RXR as well as a low level of PPARγ (43–45). As shown in Fig. 6B, in the absence of cotransfected PPARγ the activity of adipophilin promoter was not stimulated by troglitazone but was stimulated by LG268. Similar to the results with CV-1 cells, cotransfection of PPARγ into JEG3 cells resulted in enhancement of adipophilin reporter activity by either troglitazone or LG268, with a higher degree of activity using both ligands simultaneously (Fig. 6B). These results suggest that PPARγ and RXR up-regulate the transcription of adipophilin promoter.

Discussion

PPARγ plays a pivotal role in differentiation of murine and human placental trophoblasts (25–28). PPARγ also regulates fatty acid metabolism in adipocytes through induction of genes that govern fatty acid uptake, storage, and metabolism (21, 22). Building on these observations, we studied the expression of adipophilin, a lipid droplet-associated protein, in term human trophoblasts, and determined the role of PPARγ in this process. We initially demonstrated that adipophilin is expressed in human placental villous trophoblasts *in vivo* as well as in cultured primary trophoblast cells *in vitro*. This observation adds the trophoblasts to several other steroid-producing cells known to express adipophilin (15, 16). Using immunohistochemical analysis, we localized the highest expression of adipophilin to the trophoblast microvillous membrane. This surface, which is bathed in maternal blood, is positioned to mediate uptake of nutrients, including fatty acids, to the developing fetoplacental unit (47). Because adipophilin and its murine ortholog ADRP are associated with

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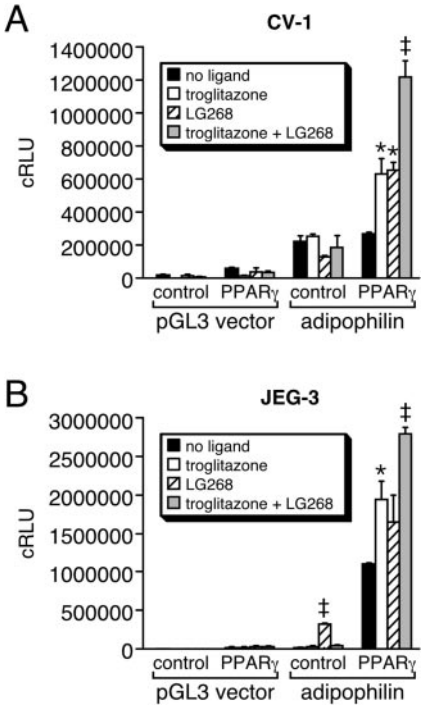

FIG. 6. Regulation of adipophilin promoter by PPARγ and RXR agonists. A, CV-1 cells were transfected with the reporter plasmid pGL3 or pGL3-adipophilin-Luc, as well as pCDNA3.1 or pCDNA3.1-PPARγ, as shown in the figure and described in *Materials and Methods*. Cells were exposed to troglitazone (10 μM) and/or LG268 (0.1 μM) for 24 h before being assayed for luciferase activity. Results (mean ± SD), normalized to β-galactosidase activity, are expressed as relative luciferase activity (cRLU), and represent three to four independent experiments, each performed in duplicate. A, Regulation of adipophilin promoter in CV-1 cells. B, Regulation of adipophilin promoter in JEG3 cells. ‡, $P < 0.05$, compared with all ligands. *, $P < 0.05$, compared with no ligand control.

lipid droplets and implicated in facilitating cellular fatty acid uptake and storage of neutral lipids in adipocytes (16, 17, 20), it is likely that adipophilin plays a role in placental fatty acid uptake and transport. In addition, we found that the expression of adipophilin increased during cytotrophoblast differentiation into syncytiotrophoblast *in vitro*. Consistent with our observation, Tarrade *et al.* (48) demonstrated an increase in accumulation of fatty acids in differentiated syncytiotrophoblasts, compared with cytotrophoblasts, providing further support to the potential role of adipophilin in fatty acid uptake by trophoblasts. Interestingly, the expression of ADRP mRNA is induced very early during adipocyte differentiation (8, 13). We determined that adipophilin mRNA was expressed as early as 4 h of trophoblast culture, and its level was reduced afterward. Whereas differentiation of 3T3-L1 adipocytes was accompanied by a decrease in levels of ADRP protein (15), we found that the expression of adipophilin protein was enhanced during 72 h in culture. This finding may point to regulation of adipophilin protein at the level of translation or protein degradation.

We also demonstrated that troglitazone-activated PPARγ enhances adipophilin expression. We made a similar observation in a microarray screen for PPARγ targets in trophoblasts (36, and Roh, C. R. and Y. Sadovsky, manuscript in preparation). These findings correspond to the central role of PPARγ in adipogenesis, glucose homeostasis, and trophoblast differentiation (25–27, 31, 34, 35) and supported by Buechler *et al.* (49), who demonstrated the induction of adipophilin mRNA by PPARγ agonists in human blood monocytes. The expression pattern of adipophilin in human placental villi largely parallels that of PPARγ, which is expressed primarily in trophoblasts and mostly absent from villous mesenchymal core (27, 28, 48). Interestingly, another member of the PPAR family of proteins, PPARδ, also enhances the expression of adipophilin and other fatty acid transporters in the THP-1 monocytic cell line, and up-regulates uptake of fatty acids in these cells (50).

PPARγ and RXR heterodimers coregulate diverse aspects of placental development and function. For example, PPARγ and RXR modulate invasion of human trophoblasts and upregulate the expression of total human chorionic gonadotropin as well as human chorionic gonadotropin β-subunit (48, 51). Consistent with this notion, we found that the selective RXR ligand LG268 enhances adipophilin expression in cultured human trophoblasts, thus supporting the proadipogenic effect of PPARγ (52, 53). The effect of LG268 was concentration dependent, with maximum effect at 0.01–0.1 μM. It is possible that a high concentration of LG268 exhibit nonspecific influence on trophoblasts, thus mitigating the enhancement of adipophilin expression. Whereas deficiency of either PPARγ or RXRα results in a similar defect in the placental labyrinthine zone, the defect in PPARγ−/− mice manifests before embryonic d 10.5, and that of RXRα−/− mice is observed after embryonic d 12.5 (54–56). Null mutations of both RXRα and RXRβ results in embryonic death by embryonic d 10.5, with a defect similar to that of PPARγ (57). Together, these results point to the functional interaction of PPARγ and RXR in regulation of placental development and to partial redundancy between the RXR isoforms.

The transcriptional mechanisms that regulate adipophilin expression are presently unknown. Although in an initial scan of our promoter fragment we could not identify the presence of canonical PPARγ binding elements, our results indicate that PPARγ and RXR play a role in regulating adipophilin promoter. It is possible that this regulation reflects interaction of PPARγ/RXR with additional DNA binding proteins. In our studies we used CV-1 cells, which express RXR endogenously (58). We also used JEG3 cells, which express a relatively low level of PPARγ as well as a high level of RXR (43–45, 59). Whereas the influence of endogenous RXR on adipophilin promoter in JEG3 was apparent, overexpression of PPARγ was needed to uncover the influence of troglitazone on this promoter. Further confirmation of the role of PPARγ and RXR in regulating adipophilin gene expression awaits detailed promoter analysis in primary trophoblasts.

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