Regulation of ADRP expression by long-chain polyunsaturated fatty acids in BeWo cells, a human placental choriocarcinoma cell line

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Abstract Transplacental transfer of maternal fatty acids is critical for fetal growth and development. In the placenta, a preferential uptake of fatty acids toward long-chain polyunsaturated fatty acids (LCPUFAs) has been demonstrated. Adipose differentiation-related protein (ADRP) is a lipid droplet-associated protein that has been ascribed a role in cellular fatty acid uptake and storage. However, its role in placenta is not known. We demonstrate that ADRP mRNA and protein are regulated by fatty acids in a human placental choriocarcinoma cell line (BeWo) and in primary human trophoblasts. LCPUFAs of the n-3 and n-6 series [arachidonic acid (20:4n-6), docosahexaenoic acid (22:6n-3), and eicosapentaenoic acid (20:5n-3)] were more efficient than shorter fatty acids at stimulating ADRP mRNA expression. The fatty acid-mediated increase in ADRP mRNA expression was not related to the differentiation state of the cells. Synthetic peroxisome proliferator-activated receptor and retinoic X receptor agonists increased ADRP mRNA level but had no effect on ADRP protein level in undifferentiated BeWo cells. Furthermore, we show that incubation of BeWo cells with LCPUFAs, but not synthetic agonists, increased the cellular content of radiolabeled oleic acid, coinciding with the increase in ADRP mRNA and protein level.█ These studies provide new information on the regulation of ADRP in placental trophoblasts and suggest that LCPUFA-dependent regulation of ADRP could be involved in the metabolism of lipids in the placenta.—Tobin, K. A. R., N. K. Harsem, K. T. Dalen, A. C. Staff, H. I. Nebb, and A. K. Duttaroy. **Regulation of ADRP expression by long-chain polyunsaturated fatty acids in BeWo cells, a human placental choriocarcinoma cell line.** *J. Lipid Res.* 2006. **47:** 815–823.

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Adequate placental transport of fatty acids to the fetus is crucial for normal fetal development and growth. Long-chain polyunsaturated fatty acids (LCPUFAs) are important as cell membrane components as well as precursors of eicosanoids for cellular signaling (1, 2). The essential fatty acids linoleic acid (18:2n:6) and α-linolenic acid (α-LN; 18:3n-3) and their LCPUFA metabolites arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3) play a particularly important role in fetal development because of the high content of AA and DHA in the brain and retina (1–4). These LCPUFAs have been shown to be present at higher levels in fetal than in the maternal circulation, suggesting an active placental transfer in favor of the fetus (5, and references therein). An insufficient supply of LCPUFAs could lead to neural and vascular complications (6). However, the underlying processes for placental fatty acid transfer are still not clear.

The exact mechanism by which PUFAs exert their effects in cellular lipid metabolism is still not fully understood. PUFAs have been shown to mediate their effects through interaction with transcription factors in the nuclear receptor family, such as the peroxisome proliferator-activated receptors (PPARs) PPARα, -δ, and -γ and liver X receptor (LXR) (7, 8). Together with their heterodimeric partner, retinoic X receptor (RXR), PPAR/RXR and LXR/RXR heterodimers bind to specific response elements in the promoter region of target genes (7). The PPARs are central in the regulation of lipid homeostasis in several tissues. In the placenta, both PPARδ and PPARγ play pivotal roles in the development of murine trophoblasts; both PPARδ and PPARγ knockout mice are embryonic lethal as a result of placental malformations (9, 10).

Abbreviations: AA, arachidonic acid; ADRP, adipose differentiation-related protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FCS, fetal calf serum; hCG, human chorionic gonadotropin; LCPUFA, long-chain polyunsaturated fatty acid; LD, lipid droplet; α-LN, α-linolenic acid; LXR, liver X receptor; OA, oleic acid; PAT, perilipin, ADRP, TIP-47; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RXR, retinoic X receptor; TIP-47, tail-interacting protein of 47 kDa.

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PPARγ has also been shown to be involved in differentiation and the invasion of human placental trophoblasts, depending on the ligand (11, 12).

Most mammalian cells are able to store neutral lipids in intracellular lipid droplets (LDs). In addition to serving as lipid storage depots, LDs appear to participate in lipid homeostasis, cell signaling, intracellular vesicle trafficking, and disease processes (13–17). The structure of the LDs is similar to that of lipoproteins: a neutral lipid core surrounded by a phospholipid monolayer and a protein coat (14). The best studied proteins include perilipin and adipose differentiation-related protein (ADRP; the human ortholog is called adipophilin) (18). They belong to a group of PAT family proteins (for perilipin, ADRP, and TIP-47), which share a high degree of sequence similarity (19). Perilipin expression is restricted to adipose tissue and steroidogenic cells and is essential for lipolysis-stimulated translocation of hormone-sensitive lipase to the LD surface upon protein kinase A activation (20, 21). Whereas the role of perilipin seems to be well defined, the roles of ADRP and TIP-47 (for tail-interacting protein of 47 kDa) are still unclear. ADRP is expressed in a wide variety of cells and tissues in which lipids are synthesized or accumulated, including placenta (22, 23). The exact function of ADRP is unknown, but it has been proposed to be involved in LD formation (19, 24, 25), fatty acid uptake (26), and milk lipid secretion (27). In addition, overexpression of ADRP led to an accumulation of lipids in cultured cells (13, 26).

The expression of ADRP has been shown to be regulated by PPARγ activators in placental trophoblasts (22). Previous studies suggest that fatty acids are able to induce ADRP mRNA expression in adipocytes and monocytes (28, 29). This fatty acid regulation, however, has not been investigated in detail, or in placental cells. In this study, we investigated the effects of fatty acids and PPAR, as well as PPAR and RXR agonists, on ADRP expression in the placental trophoblast cell line BeWo, and in primary trophoblasts, and the consequential effects of the uptake of oleic acid (OA) into the cells.

MATERIALS AND METHODS

Materials

Fatty acids were purchased from Cayman Chemicals, whereas [1-^{14}C]OA and [α-^{32}P]dCTP was from NEN® (Perkin-Elmer, Boston, MA). Wy14643 was purchased from Sigma, T0901317 was from Alexis (Lausanne, Switzerland), and GW501516 and LG100268 were kindly provided by J. Lehmann (CareX). BRL49653 (rosiglitazone) was a kind gift from K. Bamberg (AstraZeneca, Mölndal, Sweden).

Cell culture

Term placentas were obtained by cesarian section after uncomplicated pregnancies and were processed within 40 min of delivery. Approval for the use of placentas was given by the Regional Committee for Medical Ethics in Norway, and written informed consent was obtained from all participants.

Placental cytotrophoblast cells were isolated by the method of Kliman et al. (30) with modifications (31). Placental villous material was dissected and subjected to three sequential 30 min trypsin/DNase I digests (0.25% trypsin; Roche, Mannheim, Germany). After each digest, the supernatant was removed, layered over heat-inactivated newborn calf serum (Sigma), and centrifuged at 1,100g for 10 min. The resultant pellets were resuspended in DMEM (Sigma), layered on discontinuous Percoll gradients (10–70%; Sigma), and centrifuged at 1,800g for 30 min. Cytotrophoblast cells band between 35% and 55% Percoll, and these bands were collected from each gradient, pooled, and centrifuged at 1,800g for 10 min. The cytotrophoblast pellet was then resuspended in 2 ml of culture medium [50:50 DMEM-Ham's F12 (Sigma), 10% fetal calf serum (FCS; Invitrogen, Paisley, UK), 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM glutamine, and 0.1% gentamycin (Sigma)], and the cells were plated out in P35 culture dishes at 3×10^6 cells per dish. Cells were maintained at 37°C in 5% CO_2 and 95% humidity, and the medium was changed every day.

BeWo cells (ATCC CCL-98, P194) were grown in Ham's F12 with 10% FBS supplemented with 2 mM L-glutamine and 1% antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin). The cells were routinely maintained at 37°C in a 5% CO_2 atmosphere. At confluence, they were subcultured using a trypsin-EDTA solution to suspend the cells.

Cell viability was determined by measuring lactate dehydrogenase activity (Roche).

Preparation of fatty acids

Stock solutions of 6 mM fatty acids were complexed with fat-free BSA. The fatty acid was dissolved in 0.1 M NaOH for 10 min before adding prewarmed (37°C) 6% fat-free BSA in Ham's F-12. The fatty acid-BSA solution was incubated at 37°C for 10 min to allow complex formation.

RNA analysis

Total RNA was extracted with Trizol® reagent (Invitrogen). RNA (10 or 20 μg) was separated on a 1% agarose formaldehyde/MOPS gel, blotted, and hybridized as described (32). Probes were generated by radiolabeling of cDNAs with [α-^{32}P]dCTP by use of the Multiple DNA Labeling System (Amersham Biosciences, Buckinghamshire, UK). Northern blots were visualized by Hyperfilm MP, scanned with Personal Densitometer SI, and analyzed using ImageQuant™ software (Amersham Biosciences). Partial or full-length cDNAs were generated by RT-PCR as described previously (23). 36B4 (acidic ribosomal phosphoprotein PO) mRNA was used as a loading control (33).

Western blot analysis

Cells were washed and scraped in ice-cold phosphate-buffered saline and then resuspended in lysis buffer consisting of 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 2% Nonidet P-40, 1 mM EDTA, pH 8.0, 20 mM sodium fluoride, 30 mM sodium pyrophosphate, 0.2% SDS, 0.5% sodium deoxycholate, 1 mM DTT, 1 mM sodium vanadate, and protease inhibitor cocktail (20 μl/ml; Sigma P8340). Samples were incubated on ice with frequent vortexing for 15 min. Protein content was quantified using the Bio-Rad colorimetric assay system using BSA as a protein standard (Bio-Rad Laboratories, Hercules, CA).

Proteins (50 μg) were denatured by boiling for 5 min and resolved on a 10% Tris-HCl SDS gel (Criterion™ Precast Gel; Bio-Rad) and transferred to a 0.45 μm nitrocellulose membrane (Criterion; Bio-Rad) by electrotransfer (2.5 mM Tris-HCl, 19 mM glycine, and 20% methanol). Five microliters of Precision Plus Protein™ Standard (Bio-Rad) was used as a molecular weight

marker. The membrane was blocked (PBS, 5% nonfat dry milk, and 0.1% Tween) for 1 h at room temperature before incubation with primary antibody (mouse anti-adipophilin diluted 1:250; Research Diagnostics) overnight at 4°C. The membrane was washed three times (PBS containing 0.1% Tween) followed by incubation with secondary antibody (horseradish peroxidase-labeled goat anti-mouse diluted 1:6,000; Southern Biotech, Birmingham, AL) for 1 h. Finally, the membranes were washed again and developed using enhanced chemiluminescence (Chemilucent; Chemicon, Hampshire, UK) and visualized with Hyperfilm MP (Amersham Biosciences). To ensure equal loading of protein, the membranes were stripped in 0.2 M NaOH for 5 min and reprobed with an antibody against β-actin (diluted 1:10,000; Sigma).

Cell fusion and human chorionic gonadotropin secretion

BeWo cells were seeded on six-well plates (100,000 cells/well). The next day, ∼40% confluence was reached, and the medium was then changed to one containing 50 μM forskolin (Sigma) or vehicle (DMSO), followed by further incubation for the indicated times at 37°C with change of medium every 24 h. The conditioned medium was collected and stored at −20°C until use. Human chorionic gonadotropin (hCG) secretion was determined by measuring its concentration in the conditioned medium with an immunoassay kit that specifically detects the β-chain of hCG (EIA-1793; DRG Instruments).

Fatty acid uptake studies

BeWo cells were seeded on six-well plates and grown until confluence. The day before uptake studies were performed, the cells were stimulated with fatty acids or synthetic ligands at the concentrations indicated in the figure legends. Control cells received vehicle. After 24 h of stimulation, the cells were washed with PBS/BSA to remove remaining fatty acids. The cells were incubated with 100 μM [1-^{14}C]OA (specific activity 1,000–2,000 cpm/nmol) for 2 h. Fatty acid uptake was stopped by the addition of an ice-cold solution of 0.5% BSA, and the cells were washed three times to remove any surface-bound fatty acid. Total lipids were extracted by the method of Folch, Lees, and Sloane Stanley (34), and the incorporation of [1-^{14}C]OA into total lipids was determined by liquid scintillation. Protein content was quantified using the Bio-Rad colorimetric assay system with BSA as the protein standard.

Statistical analysis

Relative ADRP mRNA levels were calculated as the ratio between ADRP and 36B4 signal intensities. The effects of different incubations are expressed relative to control values, which were assigned a value of 1. Statistical significance, where indicated, was determined using a two-tailed Student's t-test. Statistical significance was defined as $P < 5\%$.

RESULTS

Induction of ADRP by LCPUFAs

The BeWo cell line is a human placental choriocarcinoma cell line that has been used to study lipid metabolism and has maintained several of the characteristics of the natural trophoblasts (35). Therefore, we used this cell line as a model system to mimic the effects of fatty acids in trophoblasts; in addition, we compared the effects in BeWo cells using isolated primary human trophoblasts.

BeWo cells were stimulated with 100 μM fatty acids bound to BSA for 24 h in medium containing 10% FCS. The LCPUFAs eicosapentaenoic acid (EPA), AA, and DHA, as well as the shorter monounsaturated fatty acid OA, were able to induce ADRP mRNA expression (**Fig. 1A**). TIP-47, another member of the PAT family, was not regulated by any of these fatty acids.

Because FCS contains fatty acids by itself, we decided to test whether the same fatty acid effects could be observed in medium without FCS. We used serum-free medium containing 1% BSA, and we observed lower basal mRNA expression (data not shown), but the fatty acid-mediated effect was the same as in serum-containing medium (Fig. 1B). These results indicate a tendency toward LCPUFAs (AA, EPA, and DHA) being more potent at
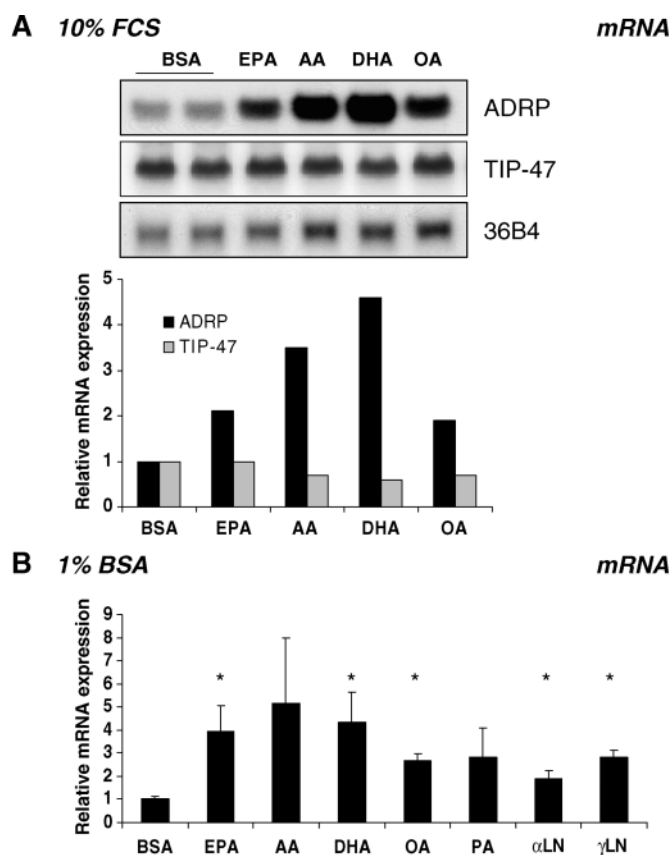
Fig. 1. Fatty acids increase adipose differentiation-related protein (ADRP) mRNA expression in BeWo cells. A: Expression of ADRP and tail-interacting protein of 47 kDa (TIP-47) mRNA in BeWo cells stimulated with 100 μM BSA-bound fatty acids [eicosapentaenoic acid (EPA), arachidonic acid (AA), docosahexaenoic acid (DHA), and oleic acid (OA)] for 24 h in serum-containing medium [10% fetal calf serum (FCS)]. The bar graph shows expression correlated against the 36B4 signal. Results from one of three similar experiments are shown. B: Expression of ADRP mRNA levels in BeWo cells after stimulation with 100 μM fatty acids [EPA, AA, DHA, OA, palmitic acid (PA), α-linolenic acid (α-LN), and γ-linolenic acid (γ-LN)] for 24 h in serum-free medium containing 1% BSA. Data shown represent means ± SD of two to six similar experiments. Statistical differences from controls were evaluated using Student's t-test (* $P < 0.05$).

inducing ADRP mRNA than shorter chain fatty acids (OA, α-LN, and γ-LN) (Fig. 1B).

Dose-dependent effect of fatty acid-mediated ADRP mRNA and protein expression

To test the potency of the PUFAs on induction of ADRP mRNA expression, BeWo cells were stimulated with increasing concentrations of PUFAs for 24 h. ADRP mRNA induction was dose-dependent and increased steadily up to 100 μM AA, EPA, and DHA (**Fig. 2A**). Concentrations of >100 μM were considered toxic, as defined by lactate dehydrogenase values of >5% of control (data not shown).

The effect of fatty acids on ADRP protein expression was subsequent examined by Western blot analysis. Stimulation of BeWo cells for 24 h with increasing concentrations of AA (from 10 to 100 μM) increased ADRP protein expression (Fig. 2B).

Time-dependent stimulation of ADRP mRNA expression after treatment of BeWo cells with LCPUFAs

The BeWo cells were incubated with 50 μM AA, EPA, and DHA for increasing lengths of time up to 48 h. A time-dependent increase in ADRP mRNA expression was observed after stimulation with all fatty acids (Fig. 2C). All fatty acids showed a tendency toward a maximal level of ADRP mRNA expression at 9 h; thereafter, a stabilization or a slow decrease was apparent after 24 and 48 h of fatty acid stimulation.
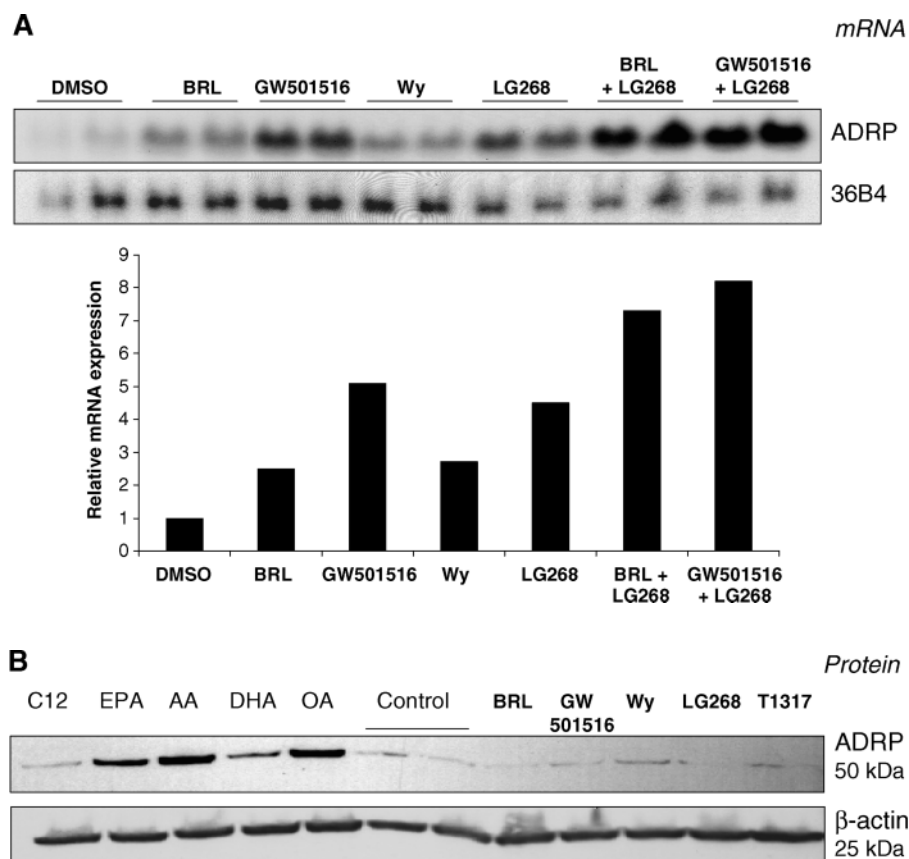
Involvement of nuclear receptors in the regulation of ADRP mRNA expression

Fatty acids have been shown to be natural activators for nuclear receptors, such as the PPARs and RXR (7, 8, 36, 37). We investigated whether PPARα, PPARδ, PPARγ, and their heterodimeric partner, RXR, were involved in the regulation of ADRP in BeWo cells using selective receptor agonists. The synthetic agonists for PPARγ (BRL49653) and PPARα (Wy14643) moderately induced the expression of ADRP mRNA, whereas the PPARδ (GW501516) and RXR (LG100268) agonists markedly induced the ADRP mRNA (**Fig. 3A**). Combination of the RXR agonist with PPARγ or PPARδ resulted in additive effects.

We next investigated the effects of fatty acids and nuclear receptor agonists on ADRP protein expression. In agreement with the ADRP mRNA expression, stimulation of BeWo cells with EPA, AA, and DHA increased ADRP protein levels. Interestingly, OA, which appeared to regulate ADRP mRNA expression only moderately, was as effective as the LCPUFAs at inducing ADRP protein expression. We stimulated BeWo cells for 24 h with synthetic ligands for PPARs and RXR, and we also included a LXR agonist (T0901317), which is a strong stimulator of lipogenesis.
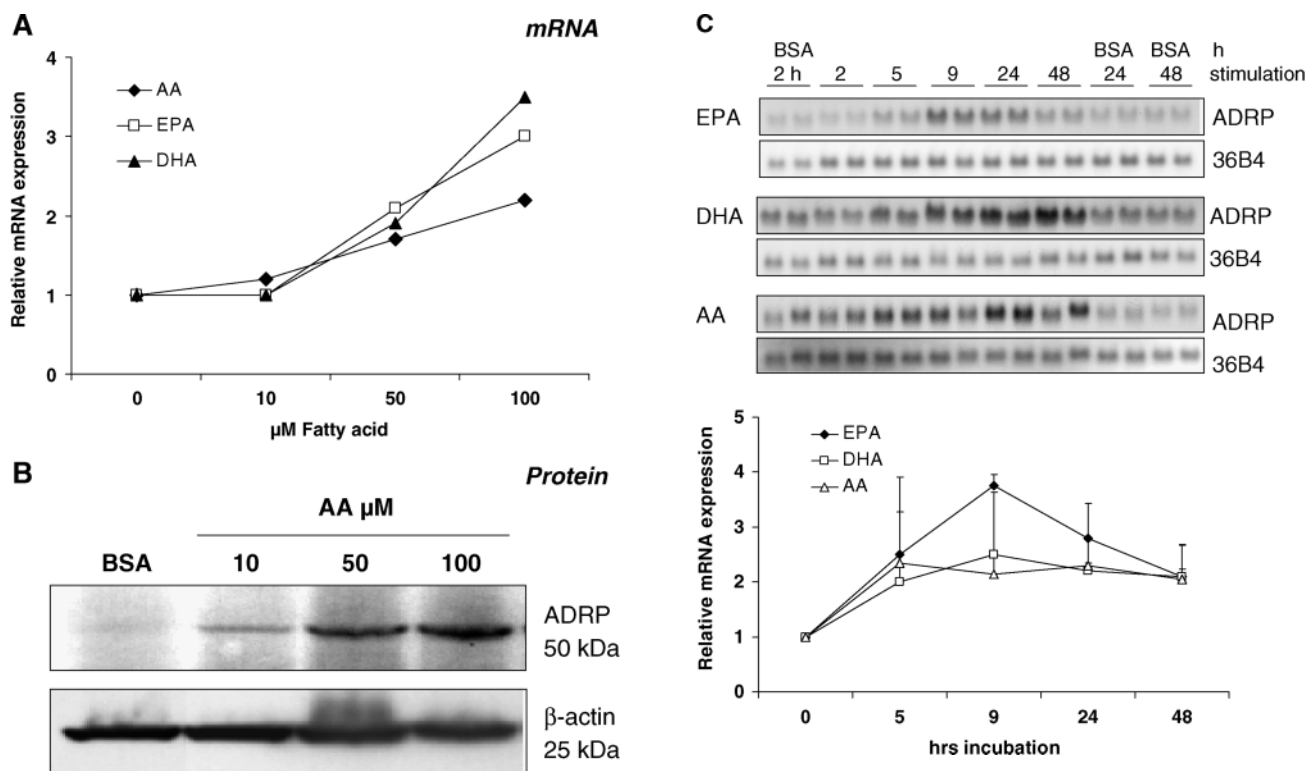
Fig. 2. Dose-dependent effects of fatty acid-mediated ADRP mRNA and protein expression. A: Expression of ADRP mRNA in BeWo cells stimulated with 0, 10, 50, and 100 μM BSA-bound AA, EPA, and DHA for 24 h in serum-free medium containing 1% BSA. The graph shows expression correlated against the 36B4 signal. Results from one of two similar experiments are shown. B: Expression of ADRP protein in BeWo cells incubated with increasing concentrations of AA for 24 h. C: Expression of ADRP mRNA in BeWo cells stimulated with 50 μM EPA, DHA, and AA for the indicated times. The graph shows expression correlated against the 36B4 signal. Data shown represent means ± SD of two experiments performed in duplicate.

Fig. 3. Involvement of nuclear receptors in the regulation of ADRP. A: Expression of ADRP mRNA in BeWo cells cultured in the presence of the synthetic agonists peroxisome proliferator-activated receptor γ [PPARγ (BRL; BRL49653; 1 μM], PPARδ (GW501516; 100 nM), PPARα (Wy; WY14643; 100 μM), and retinoic X receptor α [RXRα (LG268; LG100268; 100 nM)] for 24 h. Data presented represent one of two similar experiments. B: Expression of ADRP protein in BeWo cells cultured in the presence of the synthetic agonists described for A as well as liver X receptor (LXR) ligand (T1317; T0901317; 1 μM), and with 100 μM fatty acids as described in Fig. 1A as well as lauric acid (C12). Data shown represent one of three similar experiments.

Surprisingly, neither of the nuclear receptor agonists had any effect on ADRP protein expression (Fig. 3B).

Effect of differentiation on ADRP mRNA expression level in BeWo cells and in primary human trophoblasts

The syncytiotrophoblast is the multinucleated transporting epithelium of the placenta with microvillous (maternal-facing) and basal (fetal-facing) plasma membranes differentially expressing transport proteins that affect vectorial transcellular transport. Cytotrophoblast cells isolated from the placenta are used as models of syncytiotrophoblasts because they multinucleate after 66 h of incubation in culture medium (30, 31). The BeWo cell line can be induced to differentiate (syncytialize) within 24–48 h using agents that increase the intracellular cAMP levels. We used forskolin to induce differentiation and measured hCG as a biochemical marker of differentiation. An 8-fold increase of hCG protein secretion into the medium was observed from day 1 to day 3 after the addition of 50 μM forskolin, indicating that the cells were in a differentiated state (data not shown). Treatment with forskolin gradually increased ADRP mRNA expression in the BeWo cell line, and by day 3 it reached a 5-fold increase compared with control cells (DMSO) (**Fig. 4**). The mRNA ex-

pression level of TIP-47 did not seem to change during differentiation. The mRNA expression of ADRP and TIP-47 during differentiation was confirmed in primary human trophoblasts (data not shown).

Effect of fatty acids in differentiated BeWo cells and primary human trophoblasts

We next tested whether the differentiation status of the trophoblasts affected PUFA induction of ADRP mRNA. Treatment of differentiated BeWo cells with AA or EPA induced the ADRP mRNA level to a similar extent as in undifferentiated cells (**Fig. 5A**). In contrast to the BeWo cell line, primary trophoblasts spontaneously differentiate in culture, as demonstrated by augmented hCG levels (data not shown). Treatment of primary trophoblasts in an early differentiation state (cultured for 2 days) with DHA, γ-LN, or the synthetic PPARδ agonist (GW501516) for 24 h induced the ADRP mRNA level (data not shown) to a comparable extent as a similar treatment of trophoblasts in a later differentiation state (cultured for 4 days) (Fig. 5B).

Fatty acid uptake by BeWo cells

Because treatment of BeWo cells with LCPUFAs led to an increase in ADRP protein level, we wanted to examine
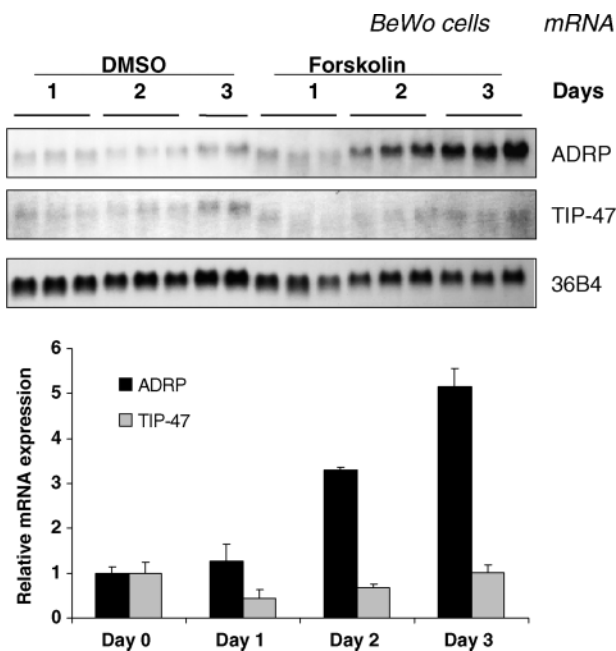
Fig. 4. Effects of differentiation on ADRP mRNA levels in BeWo cells and in primary human trophoblasts. BeWo cells were cultured in the presence of 50 µM forskolin, and control cells received vehicle (DMSO) for up to 3 days. Cells were harvested for mRNA analysis after 1, 2, and 3 days of forskolin treatment. Results shown in the bar graph represent means ± SD of two experiments.

whether such prestimulation of the cells would result in any effect on fatty acid uptake. After prestimulation for 24 h with the indicated fatty acids and nuclear receptor agonists, total fatty acid uptake was examined using radio-labeled OA. Prestimulation by LCPUFAs (DHA, AA, and EPA) resulted in an ∼20% increase in the cellular content of radiolabeled OA in BeWo cells. In contrast, prestimulation by the monounsaturated fatty acid OA (18:1n-9) and the shorter saturated fatty acid lauric acid (12:0) (data not shown) and synthetic ligands for PPARs, LXRs, and RXR had no effect (**Fig. 6**).

DISCUSSION

We have previously shown that members of the family of PAT proteins are highly expressed in placenta (23). The high placental expression was shown for ADRP as well as TIP-47 but not perilipin or S3-12. Here, we demonstrate that ADRP, but not TIP-47, is regulated by fatty acids in the placental cell line BeWo and in primary human trophoblasts. Fatty acid-mediated regulation of ADRP has also been shown previously in adipocytes and monocytes (28, 29). In those studies, the effects on ADRP mRNA in adipocytes and monocytes were unspecific with respect to fatty acid chain length and double bonds. Our studies indicate that the regulation of ADRP in BeWo cells by fatty acids is specific to LCPUFAs such as AA, EPA, and DHA. We earlier showed a preferential uptake of LCPUFAs in BeWo cells (38). These LCPUFAs have been shown to be preferentially transported by the placenta to the fetus from the maternal circulation (5, and references therein).

The effects of fatty acids on ADRP mRNA expression were largely similar to those seen for ADRP protein level. In contrast, differential effects on ADRP mRNA expression versus protein expression were observed after stimulation
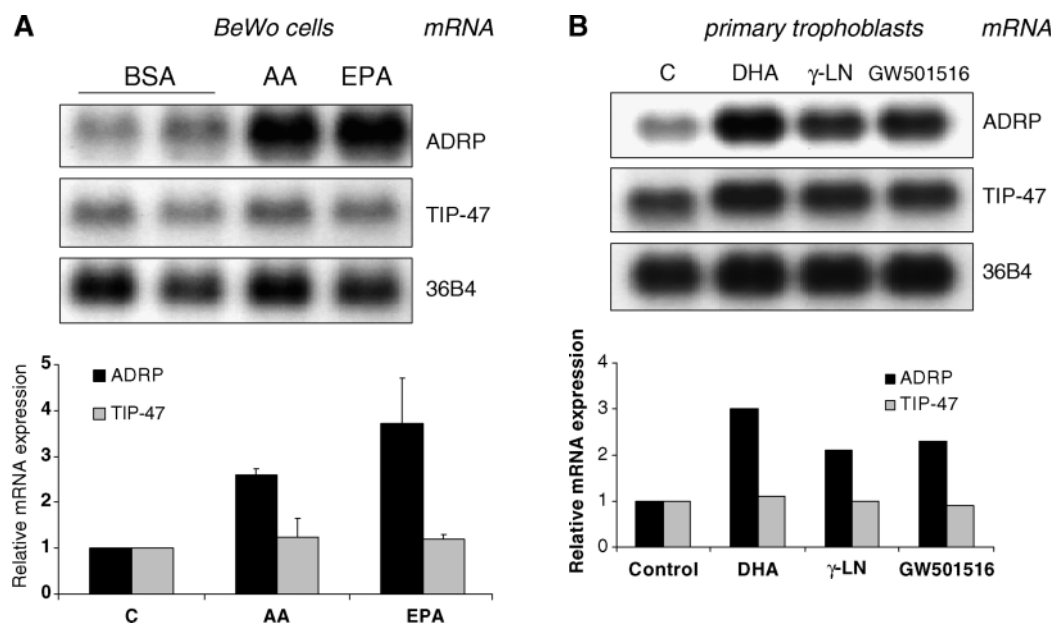
Fig. 5. Effects of fatty acids in differentiated BeWo cells and primary human trophoblasts. A: BeWo cells were cultured in the presence of 50 µM forskolin for 48 h. Fatty acids (100 µM AA and EPA) were added together with forskolin for the last 24 h before harvesting for mRNA analysis. Results shown in the bar graph represent means ± SD of three experiments. C, control. B: Primary human trophoblasts were cultured for 72 h before the addition of fatty acids (100 µM DHA and γ-LN) and 100 nM GW501516 (PPARδ agonist) for the last 24 h. The cells were harvested for mRNA analysis.
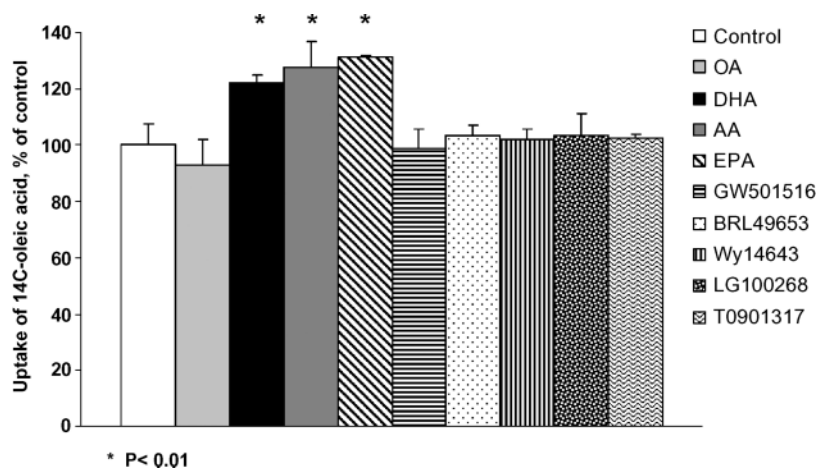
Fig. 6. Effects of long-chain fatty acids on [^{14}C]OA uptake by BeWo cells. Uptake of [^{14}C]OA was measured after BeWo cells had been stimulated with 100 μM fatty acids (OA, DHA, AA, and EPA) or synthetic agonists for PPARγ (BRL49653; 1 μM), PPARδ (GW501516; 100 nM), PPARα (WY14643; 100 μM), LXR (T0901317; 1 μM), and RXRα (LG100268; 100 nM) for 24 h. Uptake was measured 2 h after the addition of 100 μM [^{14}C]OA. Uptake of OA was calculated as picomoles of OA and related to micrograms of protein per well. Data represent means ± SD obtained from three to six separate experiments.

with synthetic agonists for PPAR and RXR. Especially the PPARδ agonist increased ADRP mRNA in BeWo cells, whereas there was no augmented ADRP protein expression after treatment of BeWo cells with synthetic ligands for 24 h. This could be attributable to a different time-dependent response for ADRP protein expression after stimulation with synthetic ligands, compared with fatty acids, minimal translation, or a higher level of protein degradation. It was suggested recently that the ADRP protein is stabilized by association with the LD surface and that the protein is actively degraded by proteasomes when not bound to LDs (39, 40). Our results indicate that synthetic PPAR and RXR agonists are able to induce ADRP mRNA level but not necessarily to increase the lipid load into the cells. Hence, with no increase in lipid load, the ADRP protein will not be stabilized and will undergo degradation.

Whether the PPARs or other transcription factors mediate the fatty acid-dependent increase in ADRP mRNA in BeWo cells or trophoblasts remains to be elucidated. PPAR-mediated regulation of ADRP mRNA in other cell types has been shown to be mediated by a conserved PPAR response element (PPRE) in the ADRP promoter. This response element is able to bind all three PPAR isoforms in in vitro binding assays (23, 41–44), but it is still unclear whether the element is transactivated by all PPAR members in vivo. ADRP mRNA expression has been shown to be stimulated by PPARγ and RXR agonists in cultured primary human trophoblasts (22). In our studies in BeWo cells, PPARγ and PPARα agonists only moderately regulated ADRP mRNA level, whereas PPARδ was a better inducer. With respect to fatty acid activation of PPARs, both PPARα and PPARδ are activated by fatty acids in in vitro assays (7). As PPARδ is expressed at higher levels in human trophoblasts than PPARα (45), our results suggest that PPARδ is a more likely candidate to mediate the fatty acid effect. A more systematic study of the expression level of PPARs in BeWo cells will be required to determine the hierarchy of these receptors on their roles in lipid metabolism and ADRP regulation in this cell line. The RXR agonist LG100268 was also able to induce ADRP mRNA. By being a heterodimeric partner of PPARs, RXRs are able to regulate any PPAR/RXR complex bound to the PPRE

when activated by a RXR activator. Hence, the recent finding that LCPUFAs such as DHA are natural RXR ligands opens the possibility that RXRs might mediate the fatty acid effect on ADRP mRNA transcription (36, 37).

Consistent with other reports, we found that ADRP mRNA expression increased during the differentiation of BeWo cells and primary human trophoblasts (22). We used hCG as a biochemical marker of cell differentiation. Recently, hCG was reported to induce ADRP mRNA and protein expression in granulosa cells, but it required the synergistic action of prostaglandin E$_2$ to do so (46). However, the increasing level of hCG could be part of the mechanism for ADRP induction during differentiation.

We have shown previously that BeWo cells preferentially take up LCPUFAs compared with shorter fatty acids such as OA (38). Here, we show that prestimulation with LCPUFAs, but not OA or a shorter chain fatty acid (lauric acid) (data not shown), led to an increased ability of BeWo cells to accumulate radiolabeled OA. This LCPUFA-mediated effect coincided with an increase in ADRP mRNA and protein expression. One exception is OA, which resulted in increased ADRP protein level but did not have any effect on the radiolabeled OA uptake. Transmembrane fatty acid uptake is dependent on several transport proteins (such as FAT/CD36, FATPs, and p-FABPpm) (38). We speculate that the fatty acid-mediated increase in cellular fatty acid content is attributable to the LCPUFA-specific regulation of these fatty acid transporters; hence, stimulation with a shorter fatty acid such as OA will not result in enhanced fatty acid content in the cells.

Further work involving ADRP ablation or transient siRNA downregulation is necessary to prove whether ADRP is involved in fatty acid uptake, transport, or lipid storage in BeWo cells or primary trophoblasts. In contrast to adipocytes and monocytes, trophoblasts are not lipid-accumulative cells. Hence, the role of ADRP in trophoblasts could be different from its role in other cell types. Our data are consistent with recent reports showing an enhanced uptake of LCPUFAs, but not shorter fatty acids, in COS-7 cells when ADRP is overexpressed (26). Furthermore, in macrophages, stimulation of ADRP expression promoted the storage of triglycerides and cholesterol (13).

In conclusion, this report demonstrates the regulation of ADRP in BeWo cells and primary human placental trophoblasts by LCPUFAs. These LCPUFAs are preferentially transported by the placenta and are critical for fetal growth and development (1, 2, 5). Information on the regulation of additional proteins involved in lipid transport across the placenta may provide insight that will help us understand pregnancy complications involving maternal hyperlipidemia, such as preeclampsia and diabetes mellitus.

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