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Hypoxia regulates the expression of fatty acid binding proteins in primary term human trophoblasts

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Abstract

Objective—Fatty acids (FA) are essential for fetal development. Cellular FA uptake is modulated by fatty acid binding proteins (FABPs). We hypothesized that hypoxia regulates the expression of FABPs in human trophoblasts.

Study design—Primary term human trophoblasts were cultured for 72 h in either standard ($O_2 = 20\%$) or hypoxic ($O_2 < 1\%$) conditions. FABP expression was interrogated using PCR and western immunoblotting. Trophoblast lipid droplets were examined using BODIPY 493/503 staining.

Results—We detected the expression of FABP1, 3, 4, 5 and pm, but not FABP2 or FABP6-9 subtypes in trophoblasts. Exposure to hypoxia markedly increased lipid droplet accumulation in trophoblasts. Consistent with this observation, hypoxia enhanced the expression of FABP1, 3 and 4. Lastly, agonists of peroxisome proliferator activated receptor- γ (PPAR γ) enhanced the expression of FABP1 and 4 in trophoblasts.

Conclusions—Hypoxia enhances the expression of FABP1, 3 and 4 in term human trophoblasts, suggesting that FABPs support fat accumulation in the hypoxic placenta.

Keywords

trophoblasts; hypoxia; lipid droplets FABP; PPAR γ

INTRODUCTION

Normal fetal growth and development depends on placental transfer of fatty acids (FA). This process is particularly important for uptake of FA and their n-6 and n-3 long chain polyunsaturated fatty acid (LCPUFA) derivatives, which cannot be formed *de novo* by mammalian cells, and are supplied by maternal diet.^{1,2} The high levels of LCPUFA in the fetal serum compared to the maternal serum suggests an active trans-placental FA transport.³ Although incompletely understood, transfer of FA is a multi-step process of uptake, transfer and intracellular translocation that is facilitated by several membrane and cytosolic lipid binding proteins.^{1,4} Several groups of proteins are involved in FA transport, including microvillous and basolateral membrane proteins as well as cytosolic, organelle-associated

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membrane proteins. These proteins include: (a) plasma membrane-associated CD36/fatty acid translocase, fatty acid transport proteins (FATPs) and plasma membrane-associated fatty acid binding protein (FABPpm), (b) cytosolic FABPs,⁵ and (c) lipid droplet associated proteins, which include perilipin, adipophilin and TIP-47 (PAT proteins) as well as the recently identified S3-12 protein.^{6,7} Here we focused on the family of FABP proteins. These 15 kD proteins are expressed in diverse tissues, and all bind long chain fatty acids.^{8,9} Genetic ablation of most FABPs, including FABP1 (L-FABP, expressed mainly in the liver), FABP3 (H-FABP, expressed mainly in the heart) or FABP4 (A-FABP, expressed mainly in adipose tissue) reveals an absence of a significant morphological phenotype, suggesting a functional redundancy among FABP family members.⁹ We recently found that hypoxia enhances the accumulation of fat in primary human trophoblasts (PHTs) and surmised that this process is coupled to increased expression of proteins associated with intracellular fat transport, which might alter fat availability to the developing fetus. We therefore tested the hypothesis that hypoxia up-regulates the expression of FABPs in PHTs.

MATERIALS AND METHODS

Trophoblast isolation and culture

The procurement of human tissues for our studies was approved by the Institutional Review Board of Washington University School of Medicine. Placentas were obtained immediately after term uncomplicated singleton pregnancies and deliveries. Placental fragments measuring 0.125 cm³ were sharply dissected at a site located at half the placental radius from the lateral placental margin, at the middle of the placental depth, 8-10 mm from the placental basal plate. Cytotrophoblasts were isolated by the modified trypsin-DNAse, Percoll gradient centrifugation method.^{10,11} Cultures were plated at a density of 300,000 cells/cm² and maintained in Dulbecco's Modified Eagle's (DME) medium (Sigma, St. Louis, MO) containing 10% fetal bovine serum (Hyclone, Logan, UT), 20 mM HEPES (pH 7.4, Sigma) and antibiotics as described.¹¹ Some of the cultures were maintained in Ham's Waymouth's medium (H/W, equal mix of Ham's F-12 and Waymouth, Hyclone) with serum and antibiotics. After 4 h of culture in a standard condition of 20% O₂/5% CO₂, non-adherent cells and syncytial fragments were removed by washing three times with phosphate buffered saline (PBS). Some of the plates were then cultured for 24, 48 or 72 h in a hermetically enclosed incubation chamber (Thermo Electron, Marietta, OH) that provided hypoxic atmosphere defined as < 1% O₂ (5% CO₂, 10% H₂, 85% N₂), with a continuous oxygen monitor at 37 °C. The medium was replaced every 24 h. Where indicated, cultures were exposed to cobalt chloride (CoCl₂, Sigma), or to the peroxisome proliferators activated receptor gamma (PPAR γ) agonist GW1929 (Calbiochem, La Jolla, CA) in the presence or absence of the retinoic X receptor (RXR) agonist LG268 (a generous gift from Ligand Pharmaceuticals, San Diego, CA), all added after 4 h in culture.

RT-PCR and RT-quantitative PCR (RT-qPCR)

RNA was purified using TriReagent (Sigma) according to the manufacturer's instructions. DNase I (DNA-free, Ambion, Austin, TX) was added to the purified RNA for 20 min at 37° C to remove contaminating DNA. Control human RNA from diverse tissues was purchased from Ambion. Complementary DNA (cDNA) was prepared from 1 μ g RNA using the TaqMan Gold RT-PCR kit with the supplied random hexamer primers (Applied Biosystems, Foster City, CA). Standard PCR was performed on synthesized transcripts using primer pairs listed in Table 1. All sequences were checked for specificity using BLAST analysis. For quantitative PCR (qPCR), cDNA was prepared using TaqMan RT-PCR kit (Applied Biosystems) in a 50 μ l reaction mix that contained 1 μ g of total RNA, 1 $\times$ RT buffer, 5.5 mM MgCl₂, deoxy-NTP mixture (500 μ M of each dNTP), 2.5 μ M random hexamers, 0.4 units/ μ l RNase inhibitor, and 1.25 units/ μ l Multi-Scribe reverse transcriptase at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. PCR was performed using 3 μ l RT product, produced with 0.3 μ M of each primer

pairs listed in the Table 2 in duplicate with SYBR Green PCR Master Mix (Applied Biosystems) in a total reaction volume of 25 μ L. Reactions were analyzed using Applied Biosystems Geneamp 5700 or 7300 System. Dissociation curves were run on all reactions to ensure amplification of a single product with the appropriate melting temperature. Fold change was computed relative to control using the $2^{-\Delta\Delta C_T}$ method.¹² Samples were normalized to YWHA expression¹³, determined in parallel reactions.

Western immunoblotting and immunoprecipitation

PHTs were rinsed with PBS and lysed with 150 μ L of cold lysis buffer with protease inhibitors, as we previously described.¹⁴ The plates were scraped, and the lysate sonicated three times for 10 sec each time on ice. The lysates were centrifuged, and 100 μ g of protein/lane were resolved using 15% SDS-PAGE at 100 V for 2.5 h. The proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore Corp., Bedford, MA) overnight at 4°C. Each blot was blocked using 5% nonfat dry milk in TBST (tris buffered saline¹⁴ with 0.05% Tween 20) for 1 h, then incubated overnight in 5% non-fat dry milk in TBST at 4°C with the following antibodies: mouse monoclonal anti-FABP1 (stock 0.1 mg/ml, diluted 1:100), mouse monoclonal anti-FABP3 (stock 0.1 mg/ml, diluted 1:250) or rabbit polyclonal anti-FABP4 (stock 1 mg/ml, diluted 1:500, all from Abcam Inc., Cambridge, MA), or goat polyclonal anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA) for control. Each blot was then washed and incubated for 2 h with either horseradish peroxidase-conjugated donkey anti-mouse IgG, goat anti-rabbit IgG or rabbit anti-goat IgG (1:1500 dilution, Santa Cruz Biotech) at room temperature, washed, and processed for chemiluminescence using a Western Lightning ECL kit (Perkin Elmer, Boston, MA). For immunoprecipitation, protein lysates were prepared as described above. The protein sample (100 μ g) was precleared by incubating with 50 μ L protein G Agarose beads (Upstate, Charlottesville, VA) on a rotating wheel for 30 min at 4°C. The beads were pelleted by centrifugation and the supernatant collected. The precleared supernatant was mixed with antibodies to FABP1 and 3 (described above) and incubated overnight at 4°C. Protein G agarose beads (50 μ L) were then added and incubation continued for 2 h. The samples were centrifuged, the beads washed three times, and then resuspended in 50 μ L sample buffer and processed for electrophoresis and western immunoblotting as described.

Lipid accumulation assay

After two washes in PBS the cells were fixed in 2% paraformaldehyde for 20 min at room temperature. The cells were washed and permeabilized with 1 mL 0.1% Triton-X-100 in PBS for 5 min followed by staining for 15 min with 10 μ g/ml BODIPY 493/503 (Invitrogen/Molecular Probes, Eugene, OR) diluted in PBS from a 10 mg/ml stock in ethanol. The cells were washed four times with PBS, and lipid droplets visualized using Nikon E800 microscope, equipped with epifluorescence optics. For quantitative fluorometry, the cells were subsequently washed in PBS and lysed with 150 μ L of cold lysis buffer (1% Nonidet P-40, 0.5% deoxycholate, and 0.1% SDS in PBS). Fluorescence was measured using a fluorospectrometer (ND3300, NanoDrop Technologies, Wilmington, DE).

Hormone assays

Culture supernatants were assayed for hCG in duplicates by enzyme-linked immunosorbent assay (ELISA, DRG International, Mountainside, NJ) according to the manufacturer's instructions. Inter- and intra-assay variability were 6.6% and 3.4%, respectively. Values represent mean hormone levels released to the media over a 24 h period, normalized to cellular protein.

Statistical analysis

All experiments were repeated at least three times. Representative experiments are shown in the figures. The statistical analysis was performed on all experiments. The fold change in transcript expression was calculated using a geometric mean of individual assays. The ordinal data were analyzed using the non-parametric Kruskal-Wallis analysis and post-hoc Dunnett test for multiple comparisons or the Mann-Whitney test, using Primer of Biostatistics software (McGraw-Hill NY, NY). Significance was determined at $p < 0.05$.

RESULTS

We initially sought to identify the FABP subtypes that are expressed in term PHTs. Using PCR and appropriate RNA control samples we found that FABP1, 3, 4, 5 and FABPpm are expressed in the term placenta and in term PHTs (Fig. 1A). We also used RT-qPCR to examine the expression of FABP1, 3, 4 and FABPpm during the 72 h culture period (Fig. 1B). Except for FABP1, the expression of all other FABPs that were detected in placenta trophoblasts was enhanced during the culture period (Fig. 1B). We recently found that hypoxia enhances the accumulation of lipid droplets in hypoxic PHTs. Because FABPs have a role in FA trafficking we surmised that hypoxia regulates the expression of FABPs in PHTs. We initially sought to validate our experimental system, and determine the influence of hypoxia on several transcripts that are known to exhibit altered expression in hypoxic trophoblasts.^{15,16} As expected, we found that hypoxia reduced hCG expression, increased erythropoietin and erythropoietin receptor transcripts levels and reduced transferrin receptor expression (Fig. 2A). We used BODIPY fluorophore 493/503, which specifically stains neutral fat, to demonstrate that hypoxia increased the number of lipid droplets in term PHTs (Fig. 2B). We confirmed these results using quantitative determination of BODIPY fluorescence, and found that hypoxia led to a 6-fold increase in neutral fat levels in hypoxic PHTs. These data were confirmed using Nile Red and oil-red-O staining (not shown).

We next examined the influence of hypoxia on the expression of FABPs in PHTs. As shown in Figure 3A-B, hypoxia enhanced the expression of mRNA for FABP1, 3 and 4, but not FABP5 or FABPpm. The effect was time-dependent (Fig. 3B), and was recapitulated using exposure to the hypoxia-mimetic CoCl (Fig. 3C). To buttress our findings we analyzed the effect of hypoxia on FABP proteins. We used a whole cell lysate for detection of FABP4 and immunoprecipitation for detection of the less abundant FABP1 and FABP3, both expressed below the detection level when a whole cell lysate was used for immunoblotting. As shown in Fig. 3D, the expression level of FABP1, 3 and 4 was increased in PHTs exposed to hypoxia for 48 h. Because hypoxia inhibits cellular differentiation in culture¹¹ we sought to confirm that the up-regulation of FABPs in hypoxia could not be attributed to diminished differentiation. We cultured PHTs in either DME medium, which supports trophoblast differentiation, or in H/W medium, which inhibits differentiation of cultured trophoblasts.¹⁷ Whereas media hCG levels confirmed the predicted state of cell differentiation (Fig. 4A), we detected no statistically significant change in the expression of FABPs (Fig. 4B) indicating that the enhanced expression of FABP 1, 3 and 4 should be attributed to hypoxia and not to an altered state of trophoblast differentiation.

We recently showed that PPAR γ ligands diminish the impact of hypoxic injury to cultured trophoblasts.¹⁸ We therefore examined the influence of ligand-activated PPAR γ , along with its heterodimeric nuclear receptor partner RXR, on expression of FABPs in PHT. As shown in Fig. 5, we found that PPAR γ and RXR agonists enhanced the expression of FABP1 and 4, but not FABP3. Interestingly, there was no change in the expression of FABP5 and FABPpm (data not shown). These results suggest that activation of PPAR γ /RXR signaling would not attenuate the effect of hypoxia on trophoblast FABPs.

COMMENT

We found that the term human placenta expresses several members of the FABP family of proteins, namely FABP1, 3, 4, 5 and FABPpm. These observations are consistent with previous reports that focused on selected FABPs.^{19,20} Importantly, our data indicate that hypoxia enhances the expression of FABP1, 3 and 4 in PHTs. This increase is associated with accumulation of lipid droplets in hypoxic trophoblasts, suggesting a role for these FABP subtypes in uptake or accumulation of lipids during hypoxic stress. Interestingly, The expression of FABP1, 3 and 4 is prominent in the liver, heart and adipose tissue, respectively, which are known to participate in fatty acid uptake. Our findings suggest a shared mechanism for fat uptake between these tissues and trophoblasts.

We also found that cell differentiation had a relatively minor influence on the expression of FABPs. Using a time-course of 96 h, Daoud *et al*²¹ showed that the expression of FABP1 and 3 increases during the culture period. Unlike our data, these researchers reported a decrease in the expression of FABP1 and 3 in cells exposed to CoCl₂, likely reflecting a markedly longer exposure time (96 h) to CoCl₂ in their protocol. Our shorter exposure to CoCl₂ is consistent with the influence of hypoxia on FABP mRNA and protein. Although the mechanism of hypoxia-induced FABP stimulation was not pursued in the present work, our findings of increased FABP expression by hypoxia at the RNA and protein level, which we re-capitulated using the hypoxia-mimetic CoCl₂, might point to a transcriptional effect, likely mediated by HIF-1 α .²²

The nuclear protein PPAR γ and its hetero-partner RXR enhance placental fatty acid transport and storage.^{23,24} PPAR γ /RXR also facilitate the differentiation of trophoblasts.¹⁴ Whereas our previous data indicate that PPAR γ activation attenuates the negative impact of hypoxia on trophoblast differentiation¹⁸, it is clear that the effect of PPAR γ /RXR activation on fat accumulation predominates, with enhanced expression of FABP1 and 4 by specific PPAR γ /RXR ligands. Our data is consistent with that of Schachtrup *et al*²⁵, who demonstrated that the promoters for FABP1 and 4, but not FABP3, are activated by PPAR γ /RXR. The possibility of a cross talk between PPAR γ signaling and hypoxia in stimulation of trophoblast cell lipid accumulation is currently pursued in our laboratory.

It is known that the affinity of diverse fatty acids to different members of the FABP family of proteins is not uniform.^{5,9} While exceeding the scope of this paper, this information and the selective effect of hypoxia on FABP proteins reported here suggest a unique role for each FABP protein in fat uptake and accumulation. Whereas our studies were performed using primary human trophoblasts, it is clear that *in vitro* culture conditions do not capture the diverse influences on cellular behavior that might occur *in vivo*, such as alteration in blood perfusion, extracellular matrix and tissue architecture. Therefore, the potential contribution of FABPs to placental dysfunction and fetal growth restriction remains to be established.

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REFERENCES

1. Haggarty P. Placental regulation of fatty acid delivery and its effect on fetal growth--a review. *Placenta* 2002;23(Suppl A):S28–38. [PubMed: 11978057]
2. Herrera E, Amusquivar E. Lipid metabolism in the fetus and the newborn. *Diabetes Metab Res Rev* 2000;16:202–10. [PubMed: 10867720]

3. Coleman RA. The role of the placenta in lipid metabolism and transport. *Semin Perinatol* 1989;13:180–91. [PubMed: 2665097]
4. Stahl A. A current review of fatty acid transport proteins (SLC27). *Pflugers Arch* 2004;447:722–7. [PubMed: 12856180]
5. Veerkamp JH, van Moerkerk HT, Prinsen CF, van Kuppevelt TH. Structural and functional studies on different human FABP types. *Mol Cell Biochem* 1999;192:137–42. [PubMed: 10331668]
6. Londos C, Sztalryd C, Tansey JT, Kimmel AR. Role of PAT proteins in lipid metabolism. *Biochimie* 2005;87:45–9. [PubMed: 15733736]
7. Wolins NE, Quaynor BK, Skinner JR, Schoenfish MJ, Tzekov A, Bickel PE. S3-12, adipophilin, and TIP47 package lipid in adipocytes. *J Biol Chem* 2005;280:19146–55. [PubMed: 15731108]
8. Hanhoff T, Lucke C, Spener F. Insights into binding of fatty acids by fatty acid binding proteins. *Mol Cell Biochem* 2002;239:45–54. [PubMed: 12479567]
9. Haunerland NH, Spener F. Fatty acid-binding proteins--insights from genetic manipulations. *Prog Lipid Res* 2004;43:328–49. [PubMed: 15234551]
10. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF 3rd. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology* 1986;118:1567–82. [PubMed: 3512258]
11. Nelson DM, Johnson RD, Smith SD, Anteby EY, Sadovsky Y. Hypoxia limits differentiation and up-regulates expression and activity of prostaglandin H synthase 2 in cultured trophoblast from term human placenta. *Am J Obstet Gynecol* 1999;180:896–902. [PubMed: 10203658]
12. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* 2001;25:402–8. [PubMed: 11846609]
13. Meller M, Vadachkoria S, Luthy DA, Williams MA. Evaluation of housekeeping genes in placental comparative expression studies. *Placenta* 2005;26:601–7. [PubMed: 16085039]
14. Schaiff WT, Carlson MG, Smith SD, Levy R, Nelson DM, Sadovsky Y. Peroxisome proliferator-activated receptor- γ modulates differentiation of human trophoblast in a ligand-specific manner. *J Clin Endocrinol Metab* 2000;85:3874–81. [PubMed: 11061552]
15. Zamudio S, Baumann MU, Illsley NP. Effects of chronic hypoxia in vivo on the expression of human placental glucose transporters. *Placenta* 2006;27:49–55. [PubMed: 16310037]
16. Roh CR, Budhraja V, Kim HS, Nelson DM, Sadovsky Y. Microarray-based identification of differentially expressed genes in hypoxic term human trophoblasts and in placental villi of pregnancies with growth restricted fetuses. *Placenta* 2005;26:319–28. [PubMed: 15823618]
17. Douglas GC, King BF. Differentiation of human trophoblast cells in vitro as revealed by immunocytochemical staining of desmoplakin and nuclei. *J Cell Sci* 1990;96(Pt 1):131–41. [PubMed: 2165075]
18. Elchalal U, Schaiff WT, Smith SD, Rimon E, Bildirici I, Nelson DM, et al. Insulin and fatty acids regulate the expression of the fat droplet-associated protein adipophilin in primary human trophoblasts. *Am J Obstet Gynecol* 2005;193:1716–23. [PubMed: 16260216]
19. Das T, Sa G, Mukherjee M. Characterization of cardiac fatty-acid-binding protein from human placenta. Comparison with placenta hepatic types. *Eur J Biochem* 1993;211:725–30. [PubMed: 8436130]
20. Campbell FM, Bush PG, Veerkamp JH, Dutta-Roy AK. Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta. *Placenta* 1998;19:409–15. [PubMed: 9699962]
21. Daoud G, Simoneau L, Masse A, Rassart E, Lafond J. Expression of cFABP and PPAR in trophoblast cells: effect of PPAR ligands on linoleic acid uptake and differentiation. *Biochim Biophys Acta* 2005;1687:181–94. [PubMed: 15708366]
22. Kaelin WG Jr. How oxygen makes its presence felt. *Genes Dev* 2002;16:1441–5. [PubMed: 12080083]
23. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, et al. PPAR γ is required for placental, cardiac, and adipose tissue development. *Mol Cell* 1999;4:585–95. [PubMed: 10549290]
24. Schaiff WT, Bildirici I, Cheong M, Chern PL, Nelson DM, Sadovsky Y. Peroxisome proliferator-activated receptor- γ and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts. *J Clin Endocrinol Metab* 2005;90:4267–75. [PubMed: 15827101]

25. Schachtrup C, Emmeler T, Bleck B, Sandqvist A, Spener F. Functional analysis of peroxisome-proliferator-responsive element motifs in genes of fatty acid-binding proteins. *Biochem J* 2004;382:239–45. [PubMed: 15130092]

Figure 1.

The expression of FABP subtypes in PHTs. (A) RT-PCR was used to examine the expression of nine FABP subtypes in intact human placental fragments and in cultured PHTs (48 h in culture, representing cytotrophoblasts and syncytiotrophoblasts). Transcript expression in other human tissues serves as a negative or positive control. (B) Quantitative analysis of FABP transcript expression in cultured term PHTs. Cells were cultured for 24-72 h in standard conditions, and mRNA expression determined in duplicates using RT-qPCR (* denotes $p < 0.05$, Kruskal-Wallis, $n = 12$).

Figure 2.

The effect of hypoxia ($O_2 < 1\%$) on lipid droplet accumulation in PHTs. Cells were cultured in either $O_2 = 20\%$ or $O_2 < 1\%$ for 48 h or for the time points indicated. (A) Hypoxia influences trophoblast hCG and transcripts for erythropoietin (Epo), erythropoietin receptor (EpoR) and transferrin receptor (TfR). Medium hCG and mRNA expression were determined as described in Methods. (* denotes $p < 0.05$, $n = 3$, Mann-Whitney or Kruskal-Wallis for multiple comparisons). (B) Hypoxia enhances the accumulation of lipid droplets in trophoblasts. Upper panels depict light microscopy, and lower panels depict fluorescence from BODIPY fluorophore 493/503 in PHTs cultured for 48 h in either $O_2 = 20\%$ (a;c) or $O_2 < 1\%$ (b;d). Shown is a representative experiment ($n = 3$). Scale bar, 30 μM .

Figure 3.

The influence of hypoxia on the expression of FABP1, 3 and 4 in PHTs. Cells were cultured for 48 h (A) or for 24-72 h (B) in either $O_2 = 20\%$ or $O_2 < 1\%$. FABP expression was determined using RT-qPCR (* denotes $p < 0.05$, $n = 3-5$, Kruskal-Wallis). (C) The influence of the hypoxia-mimetic CoCl on FABP expression in PHTs. Cells were cultured for 48 h in the presence or absence of 0.4 mM CoCl (* denotes $p < 0.05$, $n = 3$, Mann-Whitney). (D) The influence of hypoxia (48 h) on FABP proteins. The expression of FABP1 and FABP3 was determined using immunoprecipitation followed by western immunoblotting, and FABP4 or actin using western immunoblotting with a whole cell lysate. The blots represent three experiments.

Figure 4.

Trophoblast differentiation does not alter FABP expression. (A) PHTs were cultured in either DME or H/W medium for 72 h in standard conditions, and medium hCG determined daily as described in Methods (* denotes $p < 0.05$, $n = 3$, Kruskal-Wallis). (B) The expression of mRNA for FABPs, determined in duplicates using RT-qPCR ($p = \text{NS}$, $n = 3$, Mann-Whitney).

Figure 5.

The influence of PPAR γ /RXR agonists on FABP expression in trophoblasts. PHTs were cultured in DME for 48 h in the absence or presence of the PPAR γ agonist GW1929 (2 μ M), RXR agonist LG268 (0.1 μ M) or both. The expression of mRNA for FABPs was determined in duplicates using RT-qPCR (* denotes $p < 0.05$, $n = 3$, Kruskal-Wallis).

Table 1

Primer sequences used for RT-PCR

Name	Accession no.	Direction / location	Direction and site sequence
FABP1	NM_001443	F 154-173 R 355-332	GTGTCGGAATCGTGCAGAA TGAGTTCGGTCACAGACTTGATGT
FABP2	NM_000134	F 1069-1096 R 1271-1246	GATAAACTAAAAGCATAGGCTGCATATG TCAAAATCAGAATGGCAATTATCTCT
FABP3	NM_004102	F 42-61 R 108-87	TCAGCCTAGCCCAGCATCAC TCTTGCTGTCCACTAGCTTCCA
FABP4	NM_001442	F 388-406 R 463-444	TGGTGGTGGAATGCGTCAT GGTCAACGTCCCTTGGCTTA
FABP5	NM_001444	F 93-115 R 293-273	CAGCAAAGGCTTTGATGAATACA TTTCTGCCATCAGCTGTGGTT
FABP6	NM_001445	F 74-93 R 276-256	AGCACCACCCATTCTCCTCA AAGTGAAGTCCTGCCATCCT
FABP7	NM_001446	F 171-192 R 372-350	CCCACCTCTTTCCAAATAAGA CACGCCTAGAGCCTTCATGTACT
FABP8	NM_002677	F 2247-2277 R 2447-2425	AATAGAAATCCATGAATCCATACTGATAT TCCAGAACTCCTTCCACTCACAT
FABPpm	NM_002080	F 1859-1878 R 2064-2043	CCGCACTGCAGAAATGATGT GGAGTTGATGGTCAATCAAGCA

Table 2

Primer sequences used for RT-q PCR

Name	Accession no.	Direction / location	Direction and site sequence
FABP1	NM_001443	F 5-23 R 80-59	CCGCAGGTCAGTCGTGAA G TCCTGGCTCTGCAGTTGGTACT
FABP3	NM_004102	F 42-61 R 108-87	TCAGCCTAGCCCAGCATCAC TCTTGCTGTCCACTAGCTTCCA
FABP4	NM_001442	F 388-406 R 463-444	TGGTGGTGGAATGCGTCAT GGTCAACGTCCCTTGGCTTA
FABP5	NM_001444	F 53-72 R 131-105	CCACAGTTCAGCAGCTGGAA ACTCCTAGCTCCTTCATGTATTCATCA
FABPpm	NM_002080	F 2221-2237 R 2294-2276	TCTGCCCCGGTTG GACCT GCCGCTGGACTCACAGTGT
Epo	NM_000799	F 1023-1042 R 1108-1088	ACAGAGCCATGCTGGGAAGA CAGGTAAATCGCTCCAAAGC
EpoR	NM_000121	F 1356-1374 R 1469-1450	CATCTGCTTTGGCCTCGAA GCTCAGGGCACAGTGTCCAT
TtR	NM_003234	F 213-235 R 377-356	TGCTGTGATCGTCTTTTCTTGA TCATCCCAATATAAGCGACGTG
YWHA	NM_145690	F 1133-1156 R 1226-1207	ACTTTTGGTACATTGTGGCTTCAA CCGCCAGGACAAACCAGTAT