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The influence of ligand-activated LXR on primary human trophoblasts

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Abstract

Introduction—The Liver X Receptors (LXRs) are critical transcriptional regulators of cellular metabolism that promote cholesterol efflux and lipogenesis in response to excess intracellular cholesterol. In contrast, the Sterol Response Element Binding Protein-2 (SREBP2) promotes the synthesis and uptake of cholesterol. Oxysterols are products of cholesterol oxidation that accumulate in conditions associated with increased cellular levels of reactive oxygen species, such as hypoxia and oxidative stress, activating LXR and inhibiting SREBP2. While hypoxia and oxidative stress are commonly implicated in placental injury, the impact of the transcriptional regulation of cholesterol homeostasis on placental function is not well characterized.

Methods—We measured the effects of the synthetic LXR ligand T0901317 and the endogenous oxysterol 25-hydroxycholesterol (25OHC) on differentiation, cytotoxicity, progesterone synthesis, lipid droplet formation, and gene expression in primary human trophoblasts.

Results—Exposure to T0901317 promoted lipid droplet formation and inhibited differentiation, while 25OHC induced trophoblast toxicity, promoted hCG and progesterone release at lower concentrations with inhibition at higher concentrations, and had no effect on lipid droplet formation. The discrepant effect of these ligands was associated with distinct changes in expression of LXR and SREBP2 target genes, with upregulation of ABCA1 following 25OHC and T0901317 exposure, exclusive activation of the lipogenic LXR targets SREBP1c, ACC1 and FAS by T0901317, and exclusive inhibition of the SREBP2 targets LDLR and HMGCR by 25OHC.

Conclusion—These findings implicate cholesterol oxidation as a determinant of trophoblast function and activity, and suggest that placental gene targets and functional pathways are selectively regulated by specific LXR ligands.

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Keywords

LXR; trophoblast; oxysterol; T0901317; SREBP2

Introduction

Fetal growth restriction (FGR) represents the failure of a fetus to reach its predetermined growth potential, and is associated with an increased risk of stillbirth, neonatal morbidity and mortality, and adult metabolic and cardiovascular disease [1–3]. While pathophysiologic mechanisms underlying FGR remain largely unknown, growth-restricted fetuses commonly exhibit diminished fat accretion in the third trimester [4]. The placenta is a metabolically active organ that takes up, synthesizes, stores and transports specific metabolites and nutrients. As the interface between the maternal and fetal circulations, the placenta is a key regulator of nutrient and waste trafficking between mother and fetus. Despite the critical importance of both placental nutrient metabolism and fetal fat accretion, mechanisms and biologic pathways defining placental metabolism of specific lipids are not well delineated.

Cholesterol is critical for diverse cellular functions, including maintenance of membrane fluidity, endocytosis, intracellular trafficking, and steroid hormone synthesis [5]. Importantly, excessive accumulation of cholesterol is cytotoxic [6]. Thus, intricate homeostatic mechanisms have evolved to regulate intracellular cholesterol levels. The Liver X Receptors (LXR α , LXR β) are nuclear receptors for oxysterols, the products of cholesterol oxidation. In contrast to the Sterol-Response Element Binding Protein 2 (SREBP2), a transcription factor which promotes cholesterol uptake and synthesis in response to cholesterol deficiency [7], LXR mediates the cellular response to excess cholesterol through transcriptional activation of genes that promote cholesterol efflux, including ATP-binding cassette proteins ABCA1, ABCG1 and apolipoproteins as well as genes that mediate fatty acid synthesis, such as SREBP1c, acetyl-CoA carboxylase (ACC1), and fatty acid synthase (FAS) [8–10]. Increased expression of these targets diminishes the intracellular pool of metabolically active cholesterol by promoting cholesterol efflux and formation of lipid droplets that sequester cholesterol and fatty acids in the form of cholesteryl esters [11]. Although both LXR isoforms are expressed in the mouse and human placenta [12], their function in the placenta remains unclear.

The distinct functional pathways and gene expression programs that are regulated by LXR, including those controlling cholesterol efflux and lipogenesis, are differentially regulated in a tissue and ligand-specific manner. As an example, mice affected with homozygous deletion of both LXR isoforms demonstrate loss of basal inhibition of ABCA1 expression in intestine and macrophages, but not in liver, striated muscle or embryonic fibroblasts [13]. In human cell lines, the transcriptional response to the synthetic LXR-specific ligand T0901317 varies significantly between cells derived from skeletal muscle, adipose tissue, and liver [14]. In addition to tissue-specific variation, the effect of LXR activation is also ligand dependent. While synthetic LXR ligands promote expression of both ABCA1 and SREBP1-c in cultured human macrophages and 293T cells, endogenous ligands lead to increased expression of ABCA1 only, with no effect on expression of SREBP1-c [15].

Hypoxia and oxidative stress are related insults that impact placental physiology and cholesterol metabolism. Hypoxic injury results from reduced cellular oxygen levels and subsequent loss of oxidative phosphorylation. This process may be further complicated by subsequent reperfusion. Oxidative stress, on the other hand, denotes the cellular injury resulting from unregulated oxidation of lipids, proteins and nucleic acids by reactive oxygen species (ROS) that overwhelm anti-oxidant defenses. With excessive oxidative stress, oxysterols accumulate due to the unregulated oxidation of cholesterol by ROS [16]. Notably, hypoxia precipitates the accumulation of ROS and disruption of the electron transport chain, inducing oxidative stress [17]. Experiments in rodents demonstrating LXR-mediated attenuation of myocardial ischemia-reperfusion injury, splanchnic ischemia and reperfusion injury, and damage resulting from acute brain ischemia, suggest that LXR mediates an adaptive response to hypoxia and oxidative stress [18–20].

Both hypoxia and oxidative stress, separately or concomitantly, are commonly implicated in placental injury [21–23]. However, the impact of these insults on placental cholesterol metabolism, and their subsequent effect on placental function and fetal growth, remain incompletely understood. Aye, *et al.*, demonstrated that primary human trophoblasts (PHT cells) express LXR, and that exposure of these cells to the oxysterols 25-hydroxycholesterol (25OHC), 7-ketocholesterol, 22(R)-hydroxycholesterol or T0901317 inhibited differentiation [24]. We hypothesize that metabolic derangements stemming from excessive cholesterol oxidation could represent one of the mechanistic pathways leading from placental injury to dysfunction and FGR. We aimed to delineate functional pathways and associated patterns of gene expression regulated by oxysterols and/or LXR in the placenta, and to probe and compare the response to endogenous oxysterols and synthetic, LXR-specific ligands.

Materials and Methods

Cell Culture and ligands

PHT cells were purified and cultured as previously published [25]. Briefly, trophoblasts were purified from term human placentas following uncomplicated pregnancy, labor, and delivery at Magee-Womens Hospital of the University of Pittsburgh Medical Center. We excluded placentas from multiple gestations, preterm deliveries, or pregnancies complicated by chorioamnionitis, gestational hypertension, preeclampsia, fetal growth restriction, placental abruption, or diabetes mellitus. For the experiments described, 6 placentas were obtained by the Obstetrical Specimens Procurement Unit under an approved protocol by the Institutional Review Board of the University of Pittsburgh. 3 placentas were obtained from pregnancies resulting in vaginal delivery, and 3 obtained following Cesarean section. Trophoblasts were isolated using Kliman's trypsin-deoxyribonuclease-dispase/Percoll method, with previously published modifications [26, 27]. Cells obtained from individual placentas were cultured separately, without pooling. The cells were initially plated at a density of 350,000 cells/cm² in DMEM (Sigma-Aldrich Corp., St. Louis, MO) supplemented with 10% FBS (HyClone, Logan, UT) and 1% Penicillin/Streptomycin/Fungizone in standard conditions (5% CO₂, 95% air). After 24 h in culture, cells were washed with PBS, culture medium was replaced with serum-free medium, and cells were

exposed to 25OHC or T0901317 as indicated. 100% ethanol was used as the diluent for both 25OHC and T0901317, and all cells, including vehicle-exposed controls, were treated with 0.2% v/v ethanol. After 48 h in culture, cells and media were collected for analysis as described below.

Isolation of RNA and reverse transcriptase-quantitative PCR (RT-qPCR)

RNA isolation and reverse transcription was performed as previously described [28]. Briefly, cells were lysed using Tri-Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instruction. After removal of contaminating DNA using DNA-free (Invitrogen), extracted RNA was quantified and quality was assessed by 260/280 and 260/230 absorbance ratio using a NanoDrop-1000 spectrophotometer (Fisher-Thermo). Samples with a 260/230 ratio below 1 were purified with a silica spin column, and all 260/280 ratios were above 1.9. 1 µg of RNA served as the template for reverse transcription using the SuperScript VILO kit (Invitrogen) according to the manufacturer's instructions. The RT product was used for PCR using 250 nM concentrations for forward and reverse gene-specific primers (Table 1). Reactions were run in duplicate using 384 well plates with 3 µL of cDNA per 10 µL of reaction mixture using SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and analyzed using an Applied Biosystems ViiA 7 Sequence Detection System. Dissociation curves were run on all reactions, and samples were normalized to YWHAZ [29]. The Ct method was used to determine relative gene expression [30].

Progesterone, LDH and hCG assay

ELISA kits were used to quantify medium concentrations of progesterone (Cayman Chemical Co., Ann Arbor, MI), LDH (Roche, Mannheim, Germany) and hCG (DRG International, Mountainside, NJ), according to the manufacturers' instructions. These assays were performed in duplicate for each sample, with intra-assay coefficients of variation consistently below 5%. The LDH assay measures cytotoxicity by quantifying LDH released into cell medium. LDH catalyzes conversion of lactate to pyruvate which leads to the reduction of the tetrazolium salt INT to form the water-soluble dye formazan, which is measured colorimetrically. LDH values are expressed as a percentage, where untreated cells represent 0% cytotoxicity and cells treated with lysis buffer containing Triton X-100 detergent represent 100% cell lysis. The progesterone and hCG assays rely on standards for establishment of optical density-concentration curves. Following a series of titrated dilutions, all quantified progesterone and hCG concentrations fell between the 20th and 80th percentile of standard concentration curves. Assays were performed after 48 h in culture.

Lipid Droplet Staining

Following 72 h in culture and the specified ligand exposure, cells were washed with PBS and fixed in 2% paraformaldehyde for 20 min at room temperature, immersed in 0.1% saponin for 5 min at room temperature to increase permeability, and stained with Bodipy (Invitrogen, Carlsbad, CA) at 10 µg/mL in PBS while shielded from light for 1 h at room temperature. Cell nuclei were counterstained with Hoechst 3342 stain (Invitrogen) at a dilution of 1:2000 in PBS for 3 min.

Statistics

Statistical analyses were performed using Stata (Stata, College Station, TX). Comparison of two means was performed using Student's two-sided t-test, and comparisons in larger numbers of groups was made using ANOVA with the Bonferroni correction for multiple comparisons. Dose-response effects were tested with the non-parametric test for trend developed by Cuzick [31]. This statistical tool is used to test for non-parametric trends in measured variables across three or more ordered groups, and is an extension of the Wilcoxon rank-sum test. A p-value of <0.05 was considered statistically significant.

Results

Cytotoxicity

To compare the effect of endogenous and synthetic ligands on trophoblast toxicity, we quantified the level of LDH in cell medium following exposure to the endogenous oxysterol 25OHC (0, 1, 3, 10, 30 and 100 μM) and the synthetic LXR ligand T0901317 (0, 0.1, 0.3, 1, 3, 10 μM). 25OHC provoked cell toxicity, causing a dose-responsive increase in LDH levels measured in cell medium, reaching 21% and 37% of LDH levels following exposure to lysis buffer at doses of 30 and 100 μM , respectively ($p=0.028$ and $p<0.001$ by ANOVA with Bonferroni correction, Fig 1A). However, increasing concentrations of T0901317 did not cause a significant increase in LDH levels, though there was a trend towards mild toxicity at 10 μM (3.95%, $p=0.092$, Fig. 1B). For all experiments, cells derived from placentas obtained following vaginal delivery or Cesarean section responded comparably to experimental treatments.

Trophoblast differentiation

After quantifying the effect of T0901317 and 25OHC on trophoblast toxicity, we next determined their impact on trophoblast differentiation and syncytialization by measuring concentrations of hCG in culture medium. There were no significant differences in mean hCG levels (normalized to vehicle treated controls from same preparation) following treatment with any concentration of 25OHC (Fig. 2A, left panel). Additionally, no significant trend in levels of hCG released into media with increasing 25OHC was detected using the non-parametric test of trend. However, on evaluation of concentration-response curves of PHTs derived from each of 6 individual placentas, we identified a consistent pattern in which hCG levels increased with increasing 25OHC concentrations up to 3–30 μM , and then declined at higher concentrations. The right panel of Fig. 2A depicts 3 representative concentration-response curves of cells derived from individual placental preparations. While we detected no differences in mean hCG levels with increasing concentrations of T0901317, the pattern of reduced hCG release with increasing concentrations of T0901317 was statistically significant ($p=0.022$ by non-parametric test of trend).

Progesterone release

As a functional measurement of steroid hormone synthesis, we quantified progesterone concentrations in cell culture medium. Similar to the effect on hCG release, we found that at

concentrations up to 3–30 μ M, increasing levels of 25OHC led to increased progesterone concentrations, while concentrations above this threshold reduced levels of progesterone in cell medium. Mean normalized progesterone levels and 3 representative concentration response curves are shown in Fig. 3A. As shown in Fig. 3B, T0901317 had no effect on the release of progesterone to cell medium.

Lipid droplet formation

In subsequent experiments, we exposed PHTs to a single concentration of 25OHC or T0901317. To prevent the detection of non-specific effects of global toxicity, we exposed cells to sub-toxic concentrations of ligand, specifically 10 μ M 25OHC and 1 μ M T0901317. Because LXR promotes cholesterol efflux as well as fatty acid synthesis, we gauged cellular fat storage by staining PHT cells with Bodipy, a lipophilic fluorophore that stains lipid droplets. As shown in Fig. 4, the two LXR ligands had a widely discrepant effect on lipid droplet formation. T0901317 markedly enhanced lipid droplet formation, while 25OHC had no effect.

Gene expression

To identify changes in gene expression that underlie the effect of LXR ligands on lipid accumulation, we used RT-qPCR to quantify gene expression changes in PHT cells exposed to T0901317 and 25OHC. As shown in Fig. 5, T0901317 had no effect on expression of SREBP2 or LXR β , and led to a non-significant increase in LXR α expression. Furthermore, while T0901317 had no effect on the expression of the SREBP2 targets LDL receptor (LDLR) or HMG-CoA reductase (HMGCR), exposure of PHT cells to T0901317 led to increased expression of ABCA1, SREBP1c, ACC1, and FAS. Exposure to 10 μ M of 25OHC elicited different effects on gene expression than T0901317. While 25OHC did not cause significant changes in expression of LXR α or LXR β , it did lead to the selective upregulation of specific LXR targets. We noted increased expression of the cholesterol efflux mediator ABCA1, with no effect on the expression of the lipogenic genes SREBP1c, ACC1, or FAS. Finally, 25OHC inhibited expression of SREBP2 and its targets LDLR and HMGCR.

Discussion

As key regulators of multiple cellular homeostatic functions, the accumulation of oxysterols and activation of LXR have been implicated in the pathogenesis of a wide array of disease processes [11, 32, 33]. Evidence demonstrating an inhibitory effect on trophoblast invasion and differentiation has prompted the emergence of LXR as a potential mediator of trophoblast dysfunction and FGR [24, 34, 35]. Here, we demonstrate that oxysterols influence progesterone and hCG release, as well as induce cytotoxicity in primary human trophoblasts. Additionally, the endogenous oxysterol 25OHC promoted expression of the cholesterol efflux mediator ABCA1, but had no effect on lipid droplet formation or the expression of the lipogenic LXR targets SREBP1c, FAS or ACC1. In contrast, T0901317 exposure led to increased expression of both ABCA1 and lipogenic LXR targets, with robust stimulation of lipid droplet formation. These findings suggest that LXR-driven expression of specific targets and activation of distinct gene expression programs is an important determinant of trophoblast function. Moreover, our findings implicate cytotoxicity and

functional impairments stemming from oxidation of cholesterol as a mechanistic link between oxidative stress and placental dysfunction.

Our observation of concentration-dependent cytotoxicity with 25OHC exposure is consistent with prior work of Aye, *et al* [36]. However, in a prior investigation of the effect of oxysterols and LXR on trophoblast differentiation, this group found that 25OHC diminished multiple markers of trophoblast differentiation, including hCG release [24]. While we did observe evidence of inhibited differentiation with higher concentrations of 25OHC, we also noted enhanced differentiation with lower concentrations. In contrast to the experiments of Aye and colleagues, we cultured PHT cells in serum-free media after 24 hours in culture. The enhanced differentiation observed with lower doses of 25OHC may stem from utilization of 25OHC as sterol substrate and alleviation of cholesterol deficiency in serum-deprived cells [37], while toxicity observed at higher doses may explain the inhibitory effect on differentiation. This concentration-dependent effect may also underlie the similar pattern of progesterone release with increasing concentrations of 25OHC (Fig. 3).

Our findings are consistent with those of Ignatova, *et al.*, who demonstrated that acetylated LDL promoted recruitment of LXRA to LXR response elements (LXREs) in the promoters of both ABCA1 and SREBP-1c, but led to transcription and RNA polymerase II recruitment for ABCA1 only [15]. In addition to binding of LXREs, the extent to which LXR ligands promote transcription of specific gene targets is also determined by the complement of additional activators recruited to specific promoters [13], the differential displacement of corepressors such as NCoR [38], and the stability of conformational changes induced by ligand binding [39]. Future work in our lab will probe determinants of ligand-specific LXR-dependent gene expression and associated functional pathways in the placenta and investigate potential therapeutic targets for the amelioration of FGR caused by placental dysfunction.

The effect of oxysterol accumulation is not limited to LXR-dependent pathways [40]. In addition to the discrepant effect on LXR target transcription, we found that expression of SREBP2, LDLR and HMGCR was inhibited by 25OHC but not affected by T0901317. The disparate functional effects of oxysterols and LXR-specific synthetic ligands is likely not entirely explained by the differential regulation of specific LXR targets, but also by the impact of oxysterols on critical LXR-independent pathways, including the prevention of SREBP cleavage and activation [6, 7]. Pharmacologic inhibition and/or genetic silencing of LXR in trophoblasts exposed to oxidative stress, hypoxic injury and/or oxysterols may clarify the extent to which these injuries are mediated through LXR-dependent pathways.

Mechanistic pathways that define the progression from placental injury to placental dysfunction and fetal growth restriction have not been well delineated. This knowledge gap is manifested in clinical practice, where premature delivery remains the only intervention to alter pathological progression or improve outcomes in pregnancies affected with FGR. The attenuation of injury stemming from acute hypoxia and ischemia-reperfusion by LXR agonists suggests that in certain conditions, LXR mediates an adaptive response to specific injuries [18–20]. Further insight into specific functions of LXR when selectively activated by ligands in the placenta may uncover novel therapeutic applications.

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References

- Bernstein IM, Horbar JD, Badger GJ, Ohlsson A, Golan A. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction. The Vermont Oxford Network. *Am J Obstet Gynecol.* 2000; 182(1 Pt 1):198–206. [PubMed: 10649179]
- McIntire DD, Bloom SL, Casey BM, Leveno KJ. Birth weight in relation to morbidity and mortality among newborn infants. *N Engl J Med.* 1999; 340(16):1234–8. [PubMed: 10210706]
- Barker DJ. Fetal growth and adult disease. *Br J Obstet Gynaecol.* 1992; 99(4):275–6. [PubMed: 1581269]
- Padoan A, Rigano S, Ferrazzi E, Beaty BL, Battaglia FC, Galan HL. Differences in fat and lean mass proportions in normal and growth-restricted fetuses. *Am J Obstet Gynecol.* 2004; 191(4):1459–64. [PubMed: 15507983]
- Cortes VA, Busso D, Maiz A, Arteaga A, Nervi F, Rigotti A. Physiological and pathological implications of cholesterol. *Frontiers in bioscience.* 2014; 19:416–28.
- Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell.* 1997; 89(3):331–40. [PubMed: 9150132]
- Brown MS, Goldstein JL. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc Natl Acad Sci U S A.* 1999; 96(20):11041–8. [PubMed: 10500120]
- Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B. Role of LXRs in control of lipogenesis. *Genes & development.* 2000; 14(22):2831–8. [PubMed: 11090131]
- Costet P, Luo Y, Wang N, Tall AR. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J Biol Chem.* 2000; 275(36):28240–5. [PubMed: 10858438]
- Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, Tontonoz P. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc Natl Acad Sci U S A.* 2001; 98(2):507–12. [PubMed: 11149950]
- Tontonoz P, Mangelsdorf DJ. Liver X receptor signaling pathways in cardiovascular disease. *Mol Endocrinol.* 2003; 17(6):985–93. [PubMed: 12690094]
- Marceau G, Volle DH, Gallot D, Mangelsdorf DJ, Sapin V, Lobaccaro JM. Placental expression of the nuclear receptors for oxysterols LXRalpha and LXRbeta during mouse and human development. The anatomical record Part A, Discoveries in molecular, cellular, and evolutionary biology. 2005; 283(1):175–81.
- Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, Jepsen K, Baek SH, Heyman RA, Rosenfeld MG, Schulman IG, Glass CK. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Molecular and cellular biology.* 2003; 23(16):5780–9. [PubMed: 12897148]
- Hessvik NP, Bakke SS, Smith R, Ravna AW, Sylte I, Rustan AC, Thoresen GH, Kase ET. The liver X receptor modulator 22(S)-hydroxycholesterol exerts cell-type specific effects on lipid and glucose metabolism. *The Journal of steroid biochemistry and molecular biology.* 2012; 128(3–5):154–64. [PubMed: 22051079]
- Ignatova ID, Angdisen J, Moran E, Schulman IG. Differential regulation of gene expression by LXRs in response to macrophage cholesterol loading. *Mol Endocrinol.* 2013; 27(7):1036–47. [PubMed: 23686114]
- Brown AJ, Jessup W. Oxysterols: Sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis. *Molecular aspects of medicine.* 2009; 30(3):111–22. [PubMed: 19248801]

17. Miyata T, Takizawa S, van Ypersele de Strihou C. Hypoxia. 1. Intracellular sensors for oxygen and oxidative stress: novel therapeutic targets. *American journal of physiology Cell physiology*. 2011; 300(2):C226–31. [PubMed: 20980551]
18. Lei P, Baysa A, Nebb HI, Valen G, Skomedal T, Osnes JB, Yang Z, Haugen F. Activation of Liver X receptors in the heart leads to accumulation of intracellular lipids and attenuation of ischemia-reperfusion injury. *Basic research in cardiology*. 2013; 108(1):323. [PubMed: 23266787]
19. Crisafulli C, Di Paola R, Mazzon E, Paterniti I, Galuppo M, Genovese T, Bramanti P, Cappellani A, Cuzzocrea S. Liver X receptor agonist treatment reduced splanchnic ischemia and reperfusion injury. *Journal of leukocyte biology*. 2010; 87(2):309–21. [PubMed: 20028773]
20. Sironi L, Mitro N, Cimino M, Gelosa P, Guerrini U, Tremoli E, Saez E. Treatment with LXR agonists after focal cerebral ischemia prevents brain damage. *FEBS Lett*. 2008; 582(23–24):3396–400. [PubMed: 18789330]
21. Myatt L, Cui X. Oxidative stress in the placenta. *Histochem Cell Biol*. 2004; 122(4):369–82. [PubMed: 15248072]
22. Hracsko Z, Orvos H, Novak Z, Pal A, Varga IS. Evaluation of oxidative stress markers in neonates with intra-uterine growth retardation. *Redox Rep*. 2008; 13(1):11–6. [PubMed: 18284846]
23. Zamudio S, Wu Y, Ietta F, Rolfo A, Cross A, Wheeler T, Post M, Illsley NP, Caniggia I. Human placental hypoxia-inducible factor-1 α expression correlates with clinical outcomes in chronic hypoxia in vivo. *The American journal of pathology*. 2007; 170(6):2171–9. [PubMed: 17525282]
24. Aye IL, Waddell BJ, Mark PJ, Keelan JA. Oxysterols inhibit differentiation and fusion of term primary trophoblasts by activating liver X receptors. *Placenta*. 2011; 32(2):183–91. [PubMed: 21208656]
25. Shi XH, Larkin JC, Chen B, Sadovsky Y. The expression and localization of N-myc downstream-regulated gene 1 in human trophoblasts. *PLoS One*. 2013; 8(9):e75473. [PubMed: 24066183]
26. 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(4):1567–82. [PubMed: 3512258]
27. 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(4):896–902. [PubMed: 10203658]
28. Mishima T, Miner JH, Morizane M, Stahl A, Sadovsky Y. The expression and function of fatty acid transport protein-2 and -4 in the murine placenta. *PLoS One*. 2011; 6(10):e25865. [PubMed: 22028793]
29. Meller M, Vadachkoria S, Luthy DA, Williams MA. Evaluation of housekeeping genes in placental comparative expression studies. *Placenta*. 2005; 26(8–9):601–7. [PubMed: 16085039]
30. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 $\cdot$ (-Delta Delta C(T)) Method. *Methods*. 2001; 25(4):402–8. [PubMed: 11846609]
31. Cuzick J. A Wilcoxon-type test for trend. *Statistics in medicine*. 1985; 4(1):87–90. [PubMed: 3992076]
32. Marwarha G, Rhen T, Schommer T, Ghribi O. The oxysterol 27-hydroxycholesterol regulates alpha-synuclein and tyrosine hydroxylase expression levels in human neuroblastoma cells through modulation of liver X receptors and estrogen receptors--relevance to Parkinson's disease. *Journal of neurochemistry*. 2011; 119(5):1119–36. [PubMed: 21951066]
33. Kiss E, Kranzlin B, Wagenblabeta K, Bonrouhi M, Thiery J, Grone E, Nordstrom V, Teupser D, Gretz N, Malle E, Grone HJ. Lipid droplet accumulation is associated with an increase in hyperglycemia-induced renal damage: prevention by liver X receptors. *The American journal of pathology*. 2013; 182(3):727–41. [PubMed: 23318573]
34. Pavan L, Hermouet A, Tsatsaris V, Therond P, Sawamura T, Evain-Brion D, Fournier T. Lipids from oxidized low-density lipoprotein modulate human trophoblast invasion: involvement of nuclear liver X receptors. *Endocrinology*. 2004; 145(10):4583–91. [PubMed: 15242983]
35. Fournier T, Handschuh K, Tsatsaris V, Guibourdenche J, Evain-Brion D. Role of nuclear receptors and their ligands in human trophoblast invasion. *J Reprod Immunol*. 2008; 77(2):161–70. [PubMed: 17706792]

36. Aye IL, Waddell BJ, Mark PJ, Keelan JA. Placental ABCA1 and ABCG1 transporters efflux cholesterol and protect trophoblasts from oxysterol induced toxicity. *Biochimica et biophysica acta*. 2010; 1801(9):1013–24. [PubMed: 20570635]
37. Panini SR, Gupta A, Sexton RC, Parish EJ, Rudney H. Regulation of sterol biosynthesis and of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in cultured cells by progesterone. *J Biol Chem*. 1987; 262(30):14435–40. [PubMed: 3667583]
38. Phelan CA, Weaver JM, Steger DJ, Joshi S, Maslany JT, Collins JL, Zuercher WJ, Willson TM, Walker M, Jaye M, Lazar MA. Selective partial agonism of liver X receptor alpha is related to differential corepressor recruitment. *Mol Endocrinol*. 2008; 22(10):2241–9. [PubMed: 18669643]
39. Williams S, Bledsoe RK, Collins JL, Boggs S, Lambert MH, Miller AB, Moore J, McKee DD, Moore L, Nichols J, Parks D, Watson M, Wisely B, Willson TM. X-ray crystal structure of the liver X receptor beta ligand binding domain: regulation by a histidine-tryptophan switch. *J Biol Chem*. 2003; 278(29):27138–43. [PubMed: 12736258]
40. Radhakrishnan A, Ikeda Y, Kwon HJ, Brown MS, Goldstein JL. Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. *Proc Natl Acad Sci U S A*. 2007; 104(16):6511–8. [PubMed: 17428920]

- We measured the effects T0901317 and 25-hydroxycholesterol in primary human trophoblasts.
- T0901317 promoted lipid droplet formation and inhibited differentiation.
- Unlike T0901317, 25OHC caused toxicity with a variable effect on hCG and progesterone release.
- These ligands caused distinct changes in trophoblast gene expression.
- Placental gene targets and functional pathways are selectively regulated by specific LXR ligands.

Fig. 1. LXR ligands cause trophoblast toxicity; LDH released into cell medium following exposure to (a) indicated dose of 25OHC; or (b) indicated dose of T0901317; $n=6$ for all experiments. Error bars represent standard deviation (s.d.); * $p=0.028$, ** $p<0.001$ (ANOVA with Bonferroni correction).

Fig. 2.

Effect of LXR ligands on trophoblast differentiation; Relative hCG concentration in cell medium following exposure to (a) indicated dose of 25OHC (left panel); or (b) indicated dose of T0901317 (hCG release from vehicle treated cells set at 1) ; n=6 for all experiments. The right panel of (a) represents measured hCG concentrations (mU/mL) at increasing 25OHC concentrations in 3 representative PHT preparations. Error bars represent s.d.

Fig. 3.

Effect of LXR ligands on progesterone levels in cell medium; Relative progesterone (P4) concentration in cell medium following exposure to (a) indicated dose of 25OHC (left panel); or (b) indicated dose of T0901317 (P4 release from vehicle treated cells set at 1); n=6 for all experiments. The right panel of (a) represents measured P4 (ng/mL) at increasing 25OHC concentrations in 3 representative PHT preparations. Error bars represent s.d.

Fig. 4. Effect of LXR ligands on lipid droplet formation; PHT cells were exposed to vehicle only, 10 μ M 25OHC, or 1 μ M T0901317 prior to fixation and staining.

Fig. 5. Effect of LXR ligands on gene expression in human trophoblasts; RT-qPCR results (n=6, all experiments). *p<0.05 by Student's two-tailed t-test compared to cells exposed to vehicle only. Error bars represent s.d.

Table 1

Primers used for PCR.

Transcript	Accession no.	Direction	Position	Size	Sequence
YWHAZ	NM_001135702.1	F	721–740	148	CTGAAC'TCCCCAGAGAAAAGC
		R	868–849		CCGATGTCCACAATGTCAAG
SREBP2	NM_004599.3	F	308–328	142	TGGGAGAGTTCCCTGACTTGT
		R	469–449		TGAATGACCGTTGCACCTGAAG
LXR α	NM_005693.3	F	678–697	207	ACACCTACATGCGTCGCAAG
		R	904–884		GACGAGCTTCTCGATCATGCC
LXR β	NM_007121.5	F	675–694	126	CAGATGGACGCTTTTCATGCG
		R	805–784		TGCTGTTGTTTCCGAA'TCTTCT
LDLR	NM_000527.4	F	259–279	142	CGACAGATCGGAAAGAAACGA
		R	400–382		CCCGGATTTGCAGGTGACA
ABCA1	NM_005502.3	F	701–721	152	ACATCCTGAAGCCCAATCCTGA
		R	852–833		CTCCTGTCCGATGTCACTCC
SREBP1c	NM_001005291.2	F	358–380	122	TGGATTGCAC'TTTCGAAGACATG
		R	679–658		AGGATGCTCAGTGGCACTGACT
ACCI	NM_198834.2	F	5912–5935	151	TC'TCAACTCTGTCCATTGTGAACA
		R	6062–6037		CTCATTATAGGCCCAATGAGGATTCTC
FAS	NM_004104.4	F	5352–5371	151	GAAC'TCCTTGGCGGAAGAGA
		R	5502–5483		TAGGACCCCGTGGAA'TGTCA