Dexamethasone stimulates the expression of leptin and 11β-HSD2 in primary human placental trophoblastic cells

Anja Tzschoppe [a,1], Fabian Fahlbusch [a,1], Julia Seidel [a], Helmuth G. Dörr [a], Wolfgang Rascher [a], Tamme W. Goecke [b], Matthias W. Beckmann [b], Ralf L. Schild [c], Ellen Struwe [d], Jörg Dötsch [e,*]

[a] Department of Paediatrics and Adolescent Medicine, University of Erlangen-Nuremberg, Germany
[b] Department of Obstetrics & Gynaecology, University of Erlangen-Nuremberg, Germany
[c] Department of Obstetrics & Gynaecology, Diakonische Dienste Hannover, Germany
[d] Regional Centre for Social Paediatrics, Garmisch-Partenkirchen, Germany
[e] Department of Paediatrics and Adolescent Medicine, University of Cologne, Kerpener Str. 62, D-50937 Cologne, Germany

1. Introduction

Glucocorticoid excess during fetal life has been shown to play an important role in early-life programming after intrauterine growth restriction (IUGR), contributing to the development of adult metabolic, cardiovascular, and neuroendocrine disease [1,2].

The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is expressed in the human placenta and has been suggested to regulate the materno-fetal transfer rate of active cortisol via conversion to inactive cortisone [3,4]. In cultured human trophoblasts, dexamethasone incubation induced 11β-HSD2 activity and mRNA expression, suggesting an autoregulation of 11β-HSD2 by glucocorticoids [5]. This finding is supported by the observation that in healthy pregnancies, increased circulating glucocorticoid levels in pregnant women [6] were paralleled by an increased placental 11-HSD2 expression with advancing gestation [7]. Moreover, in conditions of high maternal glucocorticoid levels like prenatal administration of glucocorticoids to accelerate fetal lung maturation [8], no fetal growth-reducing effects were seen in humans, in contrast to experiments performed in rodents and sheep [9].

In pregnancies complicated by idiopathic IUGR, placental 11β-HSD2 activity and mRNA were decreased, thus exposing the fetus to excess maternal cortisol [2,10,11].

In animal studies, the adverse effects of glucocorticoids on fetal growth and endocrine function have been extensively studied [12–14]. Beside direct influences on the feto-maternal unit, the effects

* Corresponding author. Tel.: +49 221 478 4350; fax: +49 221 478 4635.
E-mail address: joerg.doetsch@uk-koeln.de (J. Dötsch).
[1] These authors equally contributed to this work.

of glucocorticoids on birth weight seemed to be mediated by the placenta, as infusion of betamethasone directly to sheep fetuses did not result in growth restriction [12]. In vitro experiments showed that dexamethasone (10^{-6} M) administration in placental villous tissue altered nutrient transfer capacity by stimulating system A activity, thus promoting uptake of amino acids from maternal to fetal circulation [15].

In view of the fact that glucocorticoid action seems to be modulated by placental enzyme activity, we aimed to investigate which placental systems might be affected by the hypercortisolism in IUGR. In this respect, altered gene and protein expression of various placental endocrine regulators associated with IUGR has already been described, e.g. leptin, and 11β-HSD2 [16–19]. In the present study, we hypothesized that dexamethasone incubation of trophoblastic cells from human healthy placentas might induce altered gene expression of 11β-HSD2 and leptin. This could represent an important safeguard mechanism to protect the fetus from detrimental exposure to elevated levels of maternal glucocorticoids, if these processes also occurred in the human placenta in vivo.

2. Materials and methods

2.1. Placental collection and tissue culture

Two term placentas from women with singleton uncomplicated pregnancies were collected immediately after placental delivery in collaboration with the Department of Obstetrics and Gynecology at the University of Erlangen-Nuremberg. In both cases, caesarean section delivery was performed, and birth weight was >10th percentile according to Voigt et al. [20]. The study was reviewed and approved by the ethics committee of the University of Erlangen-Nuremberg. Written informed consent was obtained from all subjects.

Primary human cytotrophoblasts were isolated from the placentas using the established trypsine-DNAse-dispase/percoll method as initially described by Kliman et al. [21], with additional previously published modifications [22]. Cells were seeded into 6-well plates (Falcon) at a density of 8×10^6 cells/cm^2 and maintained in Earl's Medium 199 (M199, PAA Laboratories, Linz, Austria) supplemented with 10% fetal calf serum (PAA Laboratories), 20 mM Hepes (Sigma–Aldrich Chemie GmbH, Munich, Germany), 0.5 mM L-glutamine (Gibco Invitrogen GmbH, Karlsruhe, Germany), penicillin (10 U/ml), streptomycin (10 mg/ml), and fungizone (0.25 mg/ml) (Sigma–Aldrich Chemie GmbH, Gibco Invitrogen GmbH).

Cultures were grown at 37 °C under normoxia with 95% air, 5% CO_2 in an incubator (Forma Scientific) as described in detail [23]. Medium was changed 2, 5 and 24 h after initial plating to remove non-adherent cells and syncytical debris. After incubation for 24 h, trophoblastic cells were stimulated with 10 μM dexamethasone for 6, 12, 24, 48 and 72 h. Non-dexamethasone-stimulated cells served as vehicle control. Cultured trophoblasts as well as culture supernatants were collected at these time points, snap frozen and stored at −80 °C until further processing. All experiments were assayed in triplicate and were repeated using cells from a different placenta.

2.2. RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from primary human trophoblasts using TRIzol® reagent (Invitrogen GmbH, Karlsruhe, Germany) as recommended by the manufacturer. RNA was quantified by absorbance at 260 nm, and the quality of RNA was confirmed using a 1% agarose gel. After DNase treatment, 1.0 μg RNA was transcribed into cDNA using M-MLV-RT (Promega, Madison, WI, USA) and Oligo dT-primer (MWB-Biotech AG, Ebersberg, Germany). DNase treatment and cDNA synthesis were carried out according to the kit instructions. The cDNA expression level was quantified by real-time PCR.

2.3. TaqMan real-time PCR

To quantify the gene expression (mRNA) of leptin, 11β-HSD2 and IGFBP-1 as markers of potential decidual overgrowth, TaqMan real-time PCR (Perkin Elmer, Foster City, CA) was used. The mRNA expression was normalized to two different house-keeping genes, hypoxanthine guanine phosphoribosyl transferase (HPRT) and β2-microglobulin (β2-MG), that can be detected pseudogene free. Commercial reagents (TaqMan PCR reagent kit, Perkin-Elmer) and conditions were applied according to the manufacturer's protocol. 2.5 μl of complementary DNA (reverse transcription mixture) and oligonucleotides at a final concentration of 300 nmol/L (HPRT, 11βHSD2, IGFBP1 forward) or 600 nmol/L (β2-MG, IGFBP1 reverse, leptin) of primers and 200 nmol/L of TaqMan hybridization probe were analyzed in a 25 μl-volume. All of the primers and probes were purchased from Eurogentec (Belgium) and Sigma (Germany). PCR was conducted using the following thermocycler parameters: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C and 60 °C for 1 min. Serial dilutions of one of the samples served as reference providing relative quantification of the unknown samples.

The following primers and TaqMan probes were used. HPRT: forward 5′-CCGGCTCCGTTATGGC-3′, reverse 5′-GGTCATAACCT GGTTCATCATCA-3′, TaqMan probe 5′(FAM)-CGCAGCCCTGGCGTC GTGATTA-(TAMRA)3′; β2-MG: forward 5′-TGACTTTGTCACAGCC-CAAGATA-3′, reverse 5′-CCAAATGCGGCATCTTC-3′, TaqMan probe 5′(FAM)-TGATGCTGCTTACATGTCTCGATCCCA-(TAMRA)3′; IGFBP-1 forward 5′-CTCTCCATGTCACCAACATCAAA-3′; reverse 5′-GTGCC TTGGCTAAACTCTCTACGA-3′; TaqMan probe 5′(FAM)-AATGGAAG-GAGCCCTGCCGAATAGAACTC-(TAMRA)3′; leptin forward 5′-ACAA TTGTCACCAGGATCAATGAC-3′; reverse 5′-TCCAAACCGGTGACTTT CTGT-3′; TaqMan probe 5′(FAM)-TTTCACACACGCAGTCAGTCTCC TCCA-(TAMRA)3′; 11β-HSD2 forward 5′-CATCACCGGCTGTGACT CTG-3′; reverse 5′-CGGCAGCCGCATGTTAG-3′; TaqMan probe 5′(FAM)-AAAGGAGACAATTTGGCTCTGC-(TAMRA)3′.

2.4. Leptin, β-hCG and LDH concentration determination

Leptin in the culture supernatants was measured using the Human Leptin ELISA Kit (RayBio®, RayBiotech Inc., Norcross, USA) following the manufacturer's instructions. The concentration of β-human chorionic gonadotropine (β-hCG) in the culture medium was determined by the DRG-β-hCG-ELISA Kit (DRG® Diagnostics GmbH, Marburg, Germany) according to the kit protocol. Lactate dehydrogenase (LDH) concentrations were obtained spectrophotometrically by the In Vitro Toxicology Assay Kit Lactate Dehydrogenase based (TOX-7, Sigma–Aldrich Chemie GmbH, Germany). All measurements were assayed in triplicate. Analysis of the results was performed using Ascent Software for Multiscan Vers. 2.6 (©1996–2002 Thermo Labsystems Oy).

2.5. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Differences were assessed using a Kruskal–Wallis one-way ANOVA, where appropriate, with post hoc Bonferroni correction. Statistical analyses were performed using GraphPad Prism® Version 4.0c for Windows, GraphPad Software, San Diego, CA (www.graphpad.com). The limit of significance was set at a p-value of <0.05.

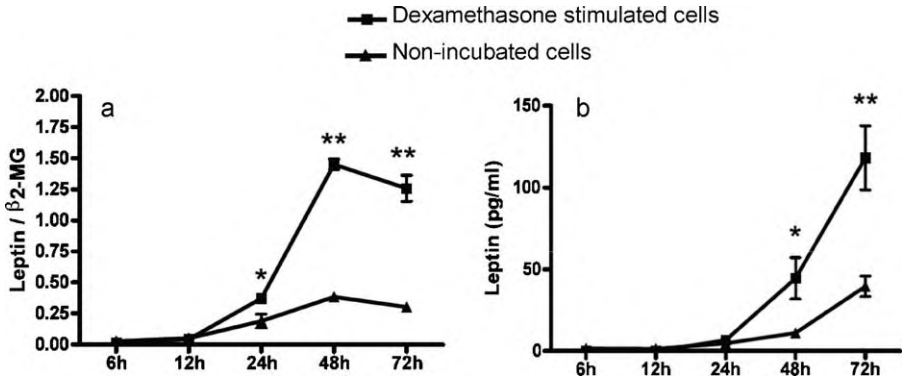

Fig. 1. (a) Leptin gene expression and (b) leptin release in the culture supernatant in human trophoblastic cells in vitro with and without stimulation with 10 μM dexamethasone. There is a significant increase in relative leptin gene expression after 24 h in the dexamethasone stimulated cells. Leptin release in the culture medium was significantly elevated after 48 h of dexamethasone incubation. Gene expression is related to the housekeeping gene β2-microglobulin (β2-MG). Leptin concentration in the culture supernatant is expressed in absolute values (pg/ml). The lines show the mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.001$.

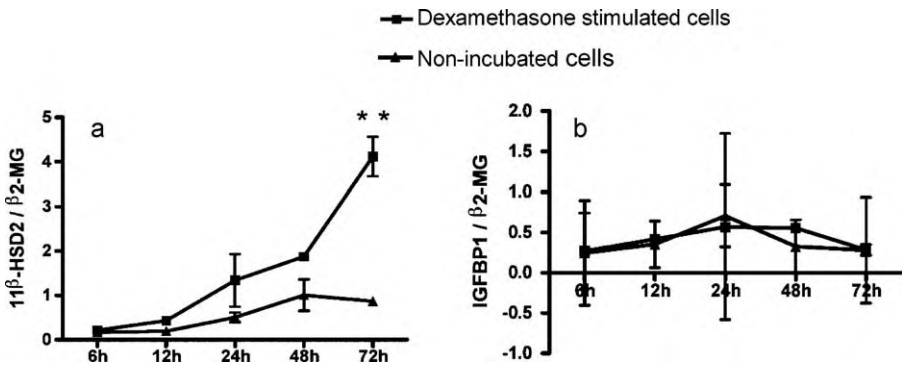

Fig. 2. Gene expression of (a) 11β-HSD2 and (b) IGFBP-1 in human trophoblastic cells in vitro with and without stimulation with 10 μM dexamethasone. There is a significant increase in relative 11β-HSD2 gene expression after 72 h in the dexamethasone stimulated cells. Relative IGFBP-1 mRNA expression did not change during the incubation period in both cell lines. Gene expression is related to the housekeeping gene β2-microglobulin (β2-MG). The lines show the mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.001$.

3. Results

Stimulation of primary placental trophoblasts with 10 μM dexamethasone resulted in a significant increase in leptin gene expression after 24 h compared to the vehicle control (Fig. 1a). When measuring leptin release in the supernatant, we found significantly higher leptin concentrations in the dexamethasone-stimulated trophoblastic cells after 48 h (Fig. 1b).

Dexamethasone incubation significantly increased gene expression of 11β-HSD2, not before 72 h (Fig. 2a). The expression rate of IGFBP1 mRNA as marker for decidual overgrowth in our cell cultures was basal throughout the incubation period in both cell lines (Fig. 2b).

To determine whether the effect of dexamethasone was mediated through alterations in placental cell differentiation or viability, medium was collected at 6, 12, 24, 48 and 72 h during the culture period and assayed for β-hCG secretion as well as LDH release. The secretion of β-hCG in the culture medium as a biochemical fusion marker, which has been shown to correlate with the cell–cell fusion process of cytotrophoblasts into multinuclear syncytia [21,24], increased significantly after 72 h of dexamethasone incubation (Fig. 3). LDH release from placental trophoblasts, a marker of necrosis, remained stable during the incubation period in both dexamethasone-stimulated cells and vehicle controls (Fig. 4).

Results for protein concentration in the culture supernatant are expressed in absolute values (ng/ml or pg/ml). All data for placental gene expression are presented as relative gene expression normalized for β2-microglobulin (Figs. 1 and 2). Similar results were obtained when expression data were normalized to HPRT (data not shown).

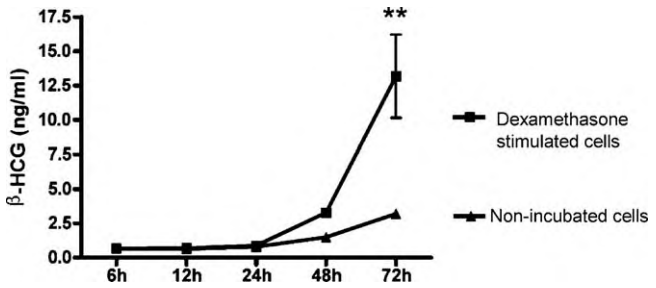

Fig. 3. β-hCG concentration in the culture medium in human trophoblastic cells in vitro with and without stimulation with 10 μM dexamethasone. There is a significant increase in β-hCG levels after 72 h in the dexamethasone stimulated cells. β-hCG concentration in the culture supernatant is expressed in absolute values (ng/ml). The lines show the mean ± SEM. $^{*}p < 0.05$; $^{**}p < 0.001$.

4. Comment

Fetal exposure to high glucocorticoid levels during pregnancy has been shown to result in low birth weight, which predisposes to the development of cardiovascular and metabolic disorders such as hypertension and type 2 diabetes, increased HPA axis reactivity, and increased anxiety-related behaviour in adult life [10,25–29]. In this respect, the central role of the placenta in the early programming process by moderating fetal exposure to maternal factors is well recognized [30].

In the present study, we investigated whether glucocorticoid exposure induced alterations in gene expression of specific endocrine placental regulators in vitro. According to previous

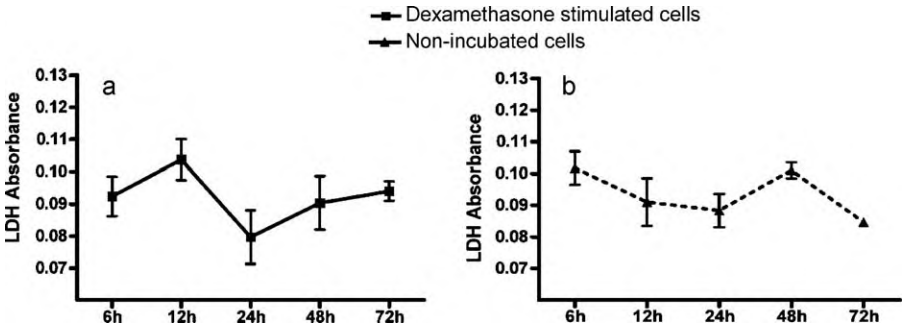

Fig. 4. LDH absorbance in the culture medium in human trophoblastic cells in vitro (a) with and (b) without stimulation with 10 μM dexamethasone. There is no significant increase in LDH absorbance during the incubation period in both cell lines. The lines show the mean ± SEM.

reports, we chose a concentration of 10 μM dexamethasone for trophoblast stimulation [15,31].

Glucocorticoid regulation of maternal and fetal hormonal parameters and metabolism has been well described in ruminants and rodents [32,33]. In this context, maternal dexamethasone administration in pregnant rats induced, for example, an almost three-fold increase in the concentration of the ob-gene product leptin in maternal circulation. In parallel, leptin concentrations in the fetal circulation were shown to be decreased by around 80%, associated with changes in placental leptin receptor expression and reductions in placental content and transfer of leptin [34,35].

During pregnancy, leptin is involved in the regulation of fetal growth, placental angiogenesis and immunomodulation, and mobilization of maternal fat [36]. In vitro experiments showed an upregulation of nitric oxide production and lipolysis by leptin in the human and rodent placenta at term [37,38] with possible consequences for placental blood flow and transfer of free fatty acids to the fetus. Furthermore, in human placental villous fragments at term, leptin stimulated the activity of the amino acid transporter system A, responsible for the placental transfer of neutral amino acids to the fetus [39]. In previous investigations, where IUGR was confirmed by prenatal Doppler ultrasound examination, placental leptin gene expression was significantly increased [18,19].

Thus, leptin could be one of the feto-placental factors altering maternal metabolism to benefit the fetus by mobilizing nutrients. According to placental perfusion studies, 98.4% of placental leptin, mainly produced in the syncytiotrophoblast [40], is released into the maternal circulation [41] and less than 2% into the fetal circulation; placental production, however, contributes significantly to circulating leptin in the human fetus, as demonstrated by the fall in serum leptin concentration in neonatal circulation that occurs after birth [42].

In our study, we observed a stimulatory effect of dexamethasone on leptin mRNA and leptin release in cultured trophoblastic cells. Induction of leptin gene expression in adipose tissue has already been reported after treatment with various glucocorticoids including dexamethasone [43]. In this respect, several glucocorticoid response element consensus binding sites have been found in the proximal promoter of the human leptin gene, indicating a direct transcriptional action of this hormone on the ob-gene [44]. Leptin synthesis could also be increased in vitro by stimuli like dexamethasone, hypoxia and insulin [23,45,46]. Thus, a time- and dose-dependent increase in leptin release and mRNA levels was observed in cultured human trophoblastic cells [45].

We therefore speculate that glucocorticoid exposure in utero might, at least in part, induce leptin synthesis. Whether the increase in leptin expression was a direct effect of dexamethasone on villous trophoblastic cells or secondary to an increased syncytialisation rate requires further investigation. Moreover, it remains speculative whether this process might play a role in the excessive intrauterine glucocorticoid exposure leading to IUGR [10,25].

Our results showed that dexamethasone incubation stimulated 11β-HSD2 gene expression in healthy human term trophoblasts. This is in line with data from the literature, where stimulation of primary cultured cytotrophoblasts with dexamethasone elevated 11β-HSD2 gene expression [5,15,31]. Of note is that human placental trophoblastic cells, grown in primary culture, have been shown to maintain the same pattern of expression of 11β-HSD as in vivo [47].

During normal pregnancy 11β-HSD2 was reported to build a placental barrier regulating the amount of maternal cortisol passing to the fetus [3], thereby facilitating fetal growth [48] and fetal organ maturation [25,49]. In support of this, significant correlations between birth weight and placental 11β-HSD2 activity have been found [16,50].

In IUGR, a reduction in 11β-HSD2 function and a subsequent failure to protect the fetus from rising maternal cortisol concentrations have been implicated to play a key role in determining fetal growth and prenatal programming of adult disease in later life [51,52]. In this respect, placental 11β-HSD-2 activity correlated with cord-blood levels of osteocalcin (a glucocorticoid-sensitive osteoblast product that does not cross the placenta) and other markers of fetal exposure to glucocorticoids [53]. Moreover, variation in placental 11β-HSD2 levels as well as exposure to glucocorticoids correlated with altered expression levels of various glucose and amino acid transporter molecules and growth factors in the placenta [15,54,55]. The effects of glucocorticoids on human placental function, however, in particular the transplacental transfer of nutrients from maternal to fetal circulation, are currently unknown.

Our results suggest that in healthy placentas, locally increased glucocorticoid concentrations may induce 11β-HSD2 gene expression in an autocrine or paracrine fashion to shield the fetus from excessive maternal cortisol. But as the synthetic glucocorticoid dexamethasone is only a poor substrate for 11β-HSD2 and readily crosses the placenta [56], the potential effect of dexamethasone on local placental metabolism of corticosteroids remains speculative. In addition, the rate of syncytialisation secondary to dexamethasone may have a major impact on 11β-HSD2 expression. In a previous study, the incubation of cultured human placental trophoblastic cells with dexamethasone increased 11β-HSD2 activity in a time- and concentration-dependent manner and stimulated gene expression during differentiation by enhancing 11β-HSD2 mRNA stability and the rate of HSD11B2 gene transcription. Moreover, incubation with a glucocorticoid receptor antagonist reversed this effect. Thus, glucocorticoids seemed to autoregulate 11β-HSD2 in the human placenta mediated through the glucocorticoid receptor [5].

The expression rate of IGFBP1 mRNA did not change during the incubation period in our study, neither in the dexamethasone nor in the vehicle control group. IGFBP-1 is mainly synthesized in maternal decidual cells [57], which are the major contaminating cells regarding the method of isolation we used for our experiments. It comprised a maximum rate of 5% contamination with other placental cell lines. As expected, we observed only a basal expression rate and no upregulation of IGFBP-1 gene expression in human trophoblastic cells. There was no sign of decidual overgrowth in our cell cultures.

In this study, the concentration of β-hCG in the culture supernatant increased significantly after 72 h of dexamethasone incubation. A glucocorticoid-dependent regulation of placental hCG has been previously described [15,58]. β-hCG secretion has been shown to correlate with the cell fusion process into multinuclear syncytia [21,24]. In this respect, dexamethasone incubation seemed to induce cytotrophoblast differentiation and maturation in our study, thereby potentially enhancing leptin and 11β-HSD2 gene expression. Besides, glucocorticoid administration did not adversely affect trophoblast viability, as dexamethasone treatment did not significantly increase LDH release. Also in the literature, no effect of glucocorticoids on LDH has been described [15].

In conclusion, our data show that dexamethasone incubation stimulated leptin and 11β-HSD2 gene expression in primary villous trophoblastic cells of healthy human term placentas, while enhancing cytotrophoblast differentiation. This could determine local metabolism of corticosteroids and, thereby, the passage of cortisol via the placental barrier to the fetus, which would protect the fetus from detrimental elevated maternal glucocorticoid exposure. We confirmed the results of our cell culture experiments in two placental cell lines, but there may be variations between trophoblasts from different placentas.

Further experiments are required to investigate the underlying mechanisms in detail and to evaluate whether these are regulatory processes which also occur in the human placenta in vivo. Moreover, it would be interesting to analyze the effect of glucocorticoid incubation on cytokine production. It remains speculative, however, whether these mechanisms might play a role in the pathophysiology of growth restriction and cardiovascular disease after IUGR.

Acknowledgements

The authors wish to thank Novo Nordisk for financial support (Investigator initiated research grant). We also thank Ms. Katrin Bitterer for technical assistance.

References

[1] Barker DJ. The fetal origins of hypertension. J Hypertens Suppl 1996;14(5):S117–20.
[2] Seckl JR. Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 2004;151(Suppl. 3):U49–62.
[3] Benediktsson R, Calder AA, Edwards CR, Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf) 1997;46(2):161–6.
[4] Yang K. Placental 11 beta-hydroxysteroid dehydrogenase: barrier to maternal glucocorticoids. Rev Reprod 1997;2(3):129–32.
[5] van Beek JP, Guan H, Julan L, Yang K. Glucocorticoids stimulate the expression of 11beta-hydroxysteroid dehydrogenase type 2 in cultured human placental trophoblast cells. J Clin Endocrinol Metab 2004;89(11):5614–21.
[6] Dorr HG, Heller A, Versmold HT, et al. Longitudinal study of progestins, mineralocorticoids, and glucocorticoids throughout human pregnancy. J Clin Endocrinol Metab 1989;68(5):863–8.
[7] Schoof E, Girstl M, Frobenius W, et al. Course of placental 11beta-hydroxysteroid dehydrogenase type 2 and 15-hydroxyprostaglandin dehydrogenase mRNA expression during human gestation. Eur J Endocrinol 2001;145(2):187–92.
[8] Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH consensus development panel on the effect of corticosteroids for fetal maturation on perinatal outcomes. JAMA 1995;273(5):413–8.
[9] Newnham JP, Moss TJ. Antenatal glucocorticoids and growth: single versus multiple doses in animal and human studies. Semin Neonatol 2001;6(4):285–92.
[10] Seckl JR. Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. Mol Cell Endocrinol 2001;185(1–2):61–71.
[11] Dy J, Guan H, Sampath-Kumar R, Richardson BS, Yang K. Placental 11beta-hydroxysteroid dehydrogenase type 2 is reduced in pregnancies complicated with idiopathic intrauterine growth restriction: evidence that this is associated with an attenuated ratio of cortisone to cortisol in the umbilical artery. Placenta 2008;29(2):193–200.
[12] Newnham JP, Evans SF, Godfrey M, Huang W, Ikegami M, Jobe A. Maternal, but not fetal, administration of corticosteroids restricts fetal growth. J Matern Fetal Med 1999;8(3):81–7.
[13] Owen D, Matthews SG. Prenatal glucocorticoid exposure alters hypothalamic-pituitary-adrenal function in juvenile guinea pigs. J Neuroendocrinol 2007;19(3):172–80.
[14] Braun T, Li S, Moss TJ, et al. Maternal betamethasone administration reduces binucleate cell number and placental lactogen in sheep. J Endocrinol 2007;194(2):337–47.
[15] Audette MC, Greenwood SL, Sibley CP, et al. Dexamethasone stimulates placental system A transport and trophoblast differentiation in term villous explants. Placenta 2010;31(2):97–105.
[16] Struwe E, Berzl GM, Schild RL, et al. Simultaneously reduced gene expression of cortisol-activating and cortisol-inactivating enzymes in placentas of small-for-gestational-age neonates. Am J Obstet Gynecol 2007;197(1):43. e1–6.
[17] Struwe E, Berzl G, Schild R, et al. Microarray analysis of placental tissue in intrauterine growth restriction. Clin Endocrinol (Oxf) 2010;72(2):241–7. Epub 2009 Jun 22.
[18] McCarthy C, Cotter FE, McElwaine S, et al. Altered gene expression patterns in intrauterine growth restriction: potential role of hypoxia. Am J Obstet Gynecol 2007;196(1):70. e1–6.
[19] McMinn J, Wei M, Schupf N, et al. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 2006;27(6–7):540–9.
[20] Voigt M, Schneider KT, Jahrig K. Analysis of a 1992 birth sample in Germany. 1. New percentile values of the body weight of newborn infants. Geburtshilfe Frauenheilkd 1996;56(10):550–8.
[21] Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss 3rd JF. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. Endocrinology 1986;118(4):1567–82.
[22] Schild RL, Sonnenberg-Hirche CM, Schaiff WT, Bildirici I, Nelson DM, Sadovsky Y. The kinase p38 regulates peroxisome proliferator activated receptor-gamma in human trophoblasts. Placenta 2006;27(2–3):191–9.
[23] Spranger RM, Hartner A, Schild RL, et al. Leptin expression in primary trophoblast cells in response to incubation with the serum of preeclamptic women. Hypertens Pregnancy 2008;27(3):267–77.
[24] Hoshina M, Boothby M, Hussa R, Pattillo R, Camel HM, Boime I. Linkage of human chorionic gonadotrophin and placental lactogen biosynthesis to trophoblast differentiation and tumorigenesis. Placenta 1985;6(2):163–72.
[25] Reinisch JM, Simon NG, Karow WG, Gandelman R. Prenatal exposure to prednisone in humans and animals retards intrauterine growth. Science 1978;202(4366):436–8.
[26] Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CR. Glucocorticoid exposure in utero: new model for adult hypertension. Lancet 1993;341(8841):339–41.
[27] Welberg LA, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 2001;13(2):113–28.
[28] Weinstock M. The long-term behavioural consequences of prenatal stress. Neurosci Biobehav Rev 2008;32(6):1073–86.
[29] Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab 2007;3(6):479–88.
[30] Plagemann A. Perinatal nutrition and hormone-dependent programming of food intake. Horm Res 2006;65(Suppl. 3):83–9.
[31] Xu AQ, Zeng WY, Yang X, Zhou YY, Liao H. Influence of dexamethasone on expression of 11beta-HSD2 in primary cultured cytotrophoblasts from human preterm placenta. Sichuan Da Xue Xue Bao Yi Xue Ban 2009;40(1):116–20.
[32] Forhead AJ, Thomas L, Crabtree J, et al. Plasma leptin concentration in fetal sheep during late gestation: ontogeny and effect of glucocorticoids. Endocrinology 2002;143(4):1166–73.
[33] Ousey JC, Rossdale PD, Dudan FE, Fowden AL. The effects of intrafetal ACTH administration on the outcome of pregnancy in the mare. Reprod Fertil Dev 1998;10(4):359–67.
[34] Smith JT, Waddell BJ. Leptin receptor expression in the rat placenta: changes in ob-ra, ob-rb, and ob-re with gestational age and suppression by glucocorticoids. Biol Reprod 2002;67(4):1204–10.
[35] Smith JT, Waddell BJ. Leptin distribution and metabolism in the pregnant rat: transplacental leptin passage increases in late gestation but is reduced by excess glucocorticoids. Endocrinology 2003;144(7):3024–30.
[36] Hoggard N, Haggarty P, Thomas L, Lea RG. Leptin expression in placental and fetal tissues: does leptin have a functional role? Biochem Soc Trans 2001;29(Pt. 2):57–63.
[37] White V, Gonzalez E, Capobianco E, et al. Modulatory effect of leptin on nitric oxide production and lipid metabolism in term placental tissues from control and streptozotocin-induced diabetic rats. Reprod Fertil Dev 2004;16(3):363–72.

[38] White V, Gonzalez E, Capobianco E, et al. Leptin modulates nitric oxide production and lipid metabolism in human placenta. Reprod Fertil Dev 2006;18(4):425–32.
[39] Jansson N, Greenwood SL, Johansson BR, Powell TL, Jansson T. Leptin stimulates the activity of the system A amino acid transporter in human placental villous fragments. J Clin Endocrinol Metab 2003;88(3):1205–11.
[40] Senaris R, Garcia-Caballero T, Casabiell X, et al. Synthesis of leptin in human placenta. Endocrinology 1997;138(10):4501–4.
[41] Linnemann K, Malek A, Sager R, Blum WF, Schneider H, Fusch C. Leptin production and release in the dually in vitro perfused human placenta. J Clin Endocrinol Metab 2000;85(11):4298–301.
[42] Ertl T, Funke S, Sarkany I, et al. Postnatal changes of leptin levels in full-term and preterm neonates: their relation to intrauterine growth, gender and testosterone. Biol Neonate 1999;75(3):167–76.
[43] Wabitsch M, Jensen PB, Blum WF, et al. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 1996;45(10):1435–8.
[44] Gong DW, Bi S, Pratley RE, Weintraub BD. Genomic structure and promoter analysis of the human obese gene. J Biol Chem 1996;271(8):3971–4.
[45] Coya R, Gualillo O, Pineda J, et al. Effect of cyclic 3′,5′-adenosine monophosphate, glucocorticoids, and insulin on leptin messenger RNA levels and leptin secretion in cultured human trophoblast. Biol Reprod 2001;65(3):814–9.
[46] Meissner U, Spranger R, Lehner M, Allabauer I, Rascher W, Dotsch J. Hypoxia-induced leptin production in human trophoblasts does not protect from apoptosis. Eur J Endocrinol 2005;153(3):455–61.
[47] Sun K, Yang K, Challis JR. Differential regulation of 11 beta-hydroxysteroid dehydrogenase type 1 and 2 by nitric oxide in cultured human placental trophoblast and chorionic cell preparation. Endocrinology 1997;138(11): 4912–20.
[48] Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 1993;341(8841):355–7.
[49] Ward RM. Pharmacologic enhancement of fetal lung maturation. Clin Perinatol 1994;21(3):523–42.
[50] Stewart PM, Rogerson FM, Mason JI. Type 2 11 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid and activity in human placenta and fetal membranes: its relationship to birth weight and putative role in fetal adrenal steroidogenesis. J Clin Endocrinol Metab 1995;80(3):885–90.
[51] McTernan CL, Draper N, Nicholson H, et al. Reduced placental 11beta-hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. J Clin Endocrinol Metab 2001;86(10):4979–83.
[52] Barker DJ. Intrauterine programming of adult disease. Mol Med Today 1995;1(9):418–23.
[53] Benediktsson R, Brennand J, Tibi L, Calder AA, Seckl JR, Edwards CR. Fetal osteocalcin levels are related to placental 11 beta-hydroxysteroid dehydrogenase activity in humans. Clin Endocrinol (Oxf) 1995;42(5):551–5.
[54] Wyrwoll CS, Seckl JR, Holmes MC. Altered placental function of 11beta-hydroxysteroid dehydrogenase 2 knockout mice. Endocrinology 2009;150(3):1287–93.
[55] Jansson N, Pettersson J, Haafiz A, et al. Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. J Physiol 2006;576(Pt. 3):935–46.
[56] White PC, Mune T, Agarwal AK. 11 beta-hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. Endocr Rev 1997;18(1): 135–56.
[57] Matsumoto H, Sakai K, Iwashita M. Insulin-like growth factor binding protein-1 induces decidualization of human endometrial stromal cells via alpha5beta1 integrin. Mol Hum Reprod 2008;14(8):485–9.
[58] Hocker I, Richter DU, Briese V, et al. Investigations on regulation of HCG by cortisol (prednisolon) in trophoblast cells in vitro. Zentralbl Gynakol 2004;126(6):373–7.