Corticotropin-releasing hormone stimulates expression of leptin, 11beta-HSD2 and syncytin-1 in primary human trophoblasts

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Abstract

Background: The placental syncytiotrophoblast is the major source of maternal plasma corticotropin-releasing hormone (CRH) in the second half of pregnancy. Placental CRH exerts multiple functions in the maternal organism: It induces the adrenal secretion of cortisol via the stimulation of adrenocorticotropic hormone, regulates the timing of birth via its actions in the myometrium and inhibits the invasion of extravillous trophoblast cells in vitro. However, the auto- and paracrine actions of CRH on the syncytiotrophoblast itself are unknown. Intrauterine growth restriction (IUGR) is accompanied by an increase in placental CRH, which could be of pathophysiological relevance for the dysregulation in syncytialisation seen in IUGR placentas.

Methods: We aimed to determine the effect of CRH on isolated primary trophoblastic cells in vitro. After CRH stimulation the trophoblast syncytialisation rate was monitored via syncytin-1 gene expression and beta-hCG (beta-human chorionic gonadotropine) ELISA in culture supernatant. The expression of the IUGR marker genes leptin and 11beta-hydroxysteroid dehydrogenase 2 (11beta-HSD2) was measured continuously over a period of 72 h. We hypothesized that CRH might attenuate syncytialisation, induce leptin, and reduce 11beta-HSD2 expression in primary villous trophoblasts, which are known features of IUGR.

Results: CRH did not influence the differentiation of isolated trophoblasts into functional syncytium as determined by beta-hCG secretion, albeit inducing syncytin-1 expression. Following syncytialisation, CRH treatment significantly increased leptin and 11beta-HSD2 expression, as well as leptin secretion into culture supernatant after 48 h.

Conclusion: The relevance of CRH for placental physiology is underlined by the present in vitro study. The induction of leptin and 11beta-HSD2 in the syncytiotrophoblast by CRH might promote fetal nutrient supply and placental corticosteroid metabolism in the phase before labour induction.

Keywords: CRH, leptin, 11beta-HSD2, Syncytin-1, Trophoblast, Syncytiotrophoblast, Placenta

Background

As part of the neuroendocrine system, the hypothalamo-pituitary-adrenal axis controls a wide range of body functions in humans. Hypothalamic corticotropin-releasing hormone (CRH) acts via its two receptors CRH-R1 and CRH-R2 to control stress reaction, autonomic functions, behavioural response, appetite, metabolism and the immune system.

Since its discovery in placental extracts in 1982 [1] it has become evident, that CRH and the related peptide urocortin [2] also exert important functional roles in human reproductive physiology [3,4]. CRH and its receptors are present in ovaries [5], endometrium [6], decidua [7], myometrium [8] and in the placenta (syncytiotrophoblast, chorion and amnion) [9,10]. The placental syncytiotrophoblast is a major source of plasma CRH in the maternal circulation in the second half of pregnancy [11]. Multiple isoforms of the CRH receptors CRH-R1 and CRH-R2 were identified in the placental trophoblast [9,10] and myometrium [12,13] throughout...
gestation. Hence differential local effects on fetal and maternal intra-uterine tissues are conceivable. The effects of placental CRH have been intensively studied indirectly via the action of the cortisol proarys leptin and 11β-HSD2 [14,15] and directly at the placental bed, where it plays an important role in the timing of birth in humans [16]. CRH interacts with progesterone to enhance the contractile response of the myometrium [16,17] and regulates the vascular tonus in the fetoplacental circulation through the nitric oxide (NO)/cGMP pathway [18,19]. Bamberger et al. [20] have recently shown, that CRH inhibits extravillous trophoblast (EVT) invasion by decreasing the expression of CEACAM1 via signalling through CRH-R1. There is growing evidence that a dysregulation of spiral artery invasion by EVT in the first trimester is a process contributing to the vascular resistance observed in the pregnancy complications preeclampsia and intrauterine growth restriction (IUGR) in late pregnancy [21,22]. In line with this finding, we and others have previously shown that placental CRH expression and CRH in maternal plasma are significantly elevated in IUGR [23-26]. IUGR is further pathophysiologicaly characterized by a reduction in trophoblastic syncytialisation rate [27], increased leptin [28] and reduced 11β-HSD2 [29] expression.

Although it is known that the syncytiotrophoblast is a major source of CRH in the second half of pregnancy, the role of CRH on the process of cytotrophoblastic syncytialisation and on endocrine hormone regulation in the syncyciotrophoblast is unknown so far.

To further investigate local actions of CRH on trophoblast function, we sought to determine its influence on the syncytialisation of isolated primary villous trophoblastic cells and on the expression of leptin and 11β-HSD2. We found that CRH induced leptin and 11β-HSD2 expression, without affecting syncytialisation of trophoblastic cells.

**Methods**

**Placental collection and tissue culture**

Six term placentas from women with singleton uncomplicated pregnancies were collected immediately after placental delivery. Elective caesarean section delivery was performed and birth weight was >10th percentile according to Voigt et al. [30]. Primary human cytotrophoblasts were isolated from the placentas using the established trypsin-DNAse-dipase/permoll method as initially described by Kliman et al. [31], with additional previously published modifications [32,33]. The purity of trophoblastic cells was routinely controled by multiple FACS analysis (FACSCalibur, BD Biosciences), as described previously [33], providing at least 90% cytotrophoblasts. In short, we determined that 10–13.3% of the fractions were HLA-A,B,C+ (mainly mononuclear blood cells and fibroblasts) and 86.6–90% HLA-A,B,C negative. Additionally, fractionated cells were 2.4–4.5% CD45+ (mononuclear blood cells) and 95.5–97.6% CK7+ (epithelial marker). Antibodies used: CK7/PE (clone 5 F282), Santa Cruz Bio., Heidelberg, Germany (1:20); HLA-A,B,C/PE (clone W6/32), Biolegend, Uithoorn, Netherlands (1:10); CD45/FITC, Miltenyi Biotec, Berg. Gladbach, Germany (1:10). Hence 86.6–90% of the fractionated cells were trophoblastic cells and 10–13.3% non-trophoblastic cells. Multinucleated fractured syncytial fragments were identified via their DNA-content using propidium iodide staining (Sigma–Aldrich Chemie, Munich, Germany; 50 μg/ml), specific for DNA content. All fractured syncytial cellular fragments, non-adherent cells and debris were removed initially after 4 h and then every 24 h with a medium change [34]. Cells were subsequently seeded into 6-well Falcon plates (Becton Dickinson, Heidelberg, Germany) at a density of 3x10^5 cells/cm^2 and maintained in Earl’s medium 199 (PAA Laboratories, Linz, Austria) supplemented with 10% fetal calf serum (PAA Laboratories), 20 mM Hepes (Sigma–Aldrich), 0.5 mM L-glutamine (Gibco Invitrogen), Karlsruhe, Germany), penicillin (10 U/ml), streptomycin (10 mg/ml), and fungizone (0.25 mg/ml) (Sigma–Aldrich, Gibco Invitrogen, respectively). Cultures were grown at 37 °C under normoxia with 95% air, 5% CO₂ in a humidified atmosphere using a Forma Scientific incubator (Fisher Scientific, Schwerte, Germany) as described in detail previously [33]. After incubation for 24 h, trophoblastic cells were stimulated with 0.5, 1.0 and 2.0 μg/ml (equivalent to 100, 200, 400 nM, respectively) CRH (Bachem, Weil am Rhein, Germany) for 6, 12, 24, 48 and 72 h. This range of concentrations was chosen, as it has been described to exert biological effects on trophoblasts [20]. Our pilot study showed no difference of repetitive stimulation vs. single application of CRH to the cell culture, with significant changes in gene expression between vehicle and CHR-treated groups starting with 48 h (1.0 and 2.0 μg/ml CRH) for the analysed genes. 72 h were chosen as the maximum observational period, because our previous experiments have shown that cytotrophoblast viability steadily decreases afterwards. Hence for illustration of group differences the time-points of 48 h and 72 h and CRH concentrations of 1.0 μg/ml and 2.0 μg/ml are displayed in the results section only. Cultured trophoblasts as well as culture supernatants were collected at the time-points described above, snap frozen and stored at −80°C until further processing. All experiments were assayed in triplicate and were repeated using cells from different placentas.

**Ethics**

The study was reviewed and approved by the Ethics Committee of the Medical Faculty of the University of
Erlangen-Nürnberg (#2625 02/28/02). Written informed consent was obtained from all subjects.

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

Total RNA was isolated from primary human trophoblasts using TRIzol reagent (Gibco Invitrogen) 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 mg RNA was transcribed into cDNA using M-MLV-RT (Promega, Madison, WI, USA) and Oligo dT-primer (MWG-Biotech AG, Ebersberg, Germany). DNase treatment and cDNA synthesis were carried out as previously [33,35].

**SYBR-Green based real-time PCR (2-ΔΔCT – method)**

As previously described [14] the mRNA expression of leptin, 11β-HSD2, Syncytin-1, CRH-R1 and CRH-R2 were quantified by normalising to the house-keeping gene hypoxanthine guanine phosphoribosyl transferase (HPRT) and confirmed with r18S as a second housekeeper, yielding the same results. Commercial reagents (Absolute Blue SYBR Green master mix, ABgene, UK) and conditions were applied according to the manufacturer’s protocol. Serial dilutions of one of the samples served as reference providing relative quantification of the unknown samples. Sequences of primers and probes are listed in Table 1.

**Determination of β-hCG, leptin and LDH concentration in the supernatant**

The concentration of β-human chorionic gonadotropin (β-hCG) in the supernatants of trophoblastic cells was determined by the use of an UniCel Dxi 600 Access Immunoassay System (Beckman Coulter, Krefeld, Germany). The concentration of leptin in the supernatants of trophoblastic cells was determined by the RayBio® Human Leptin ELISA Kit (RayBiotech, Norcross, GA, USA) according to the manufacturer’s instructions. Lactate dehydrogenase (LDH) concentrations were obtained spectrophotometrically [36] by the In Vitro Toxicology Assay Kit Lactate Dehydrogenase based (TOX-7, Sigma–Aldrich). Determination of culture supernatant protein content for protein normalisation was performed with Pierce bicinchoninic acid (BCA) Protein Assay Reagent (Thermo Fisher Scientific, Bonn, Germany). All measurements were assayed in triplicate. Analysis of the results was performed using Ascent Software v2.6 for Multiscan photometer (Thermo Fisher Scientific).

**Statistical analysis**

Results were expressed as mean ± standard error of the mean (SEM). Differences were assessed using the non-parametric Mann–Whitney U test provided with SPSS statistic software (v19.001, IBM, Ehningen, Germany). A p-value of <0.05 was considered significant.

**Results**

**Assessment of primary trophoblastic cell viability and functionality**

At the sequential experimental time-points LDH was assessed spectrophotometrically using the trophoblastic cell supernatants. There was no significant increase observed during trophoblastic cell culture, nor were group differences detected between unstimulated controls and CRH-treated trophoblast cells in terms of viability (Table 2), ruling out a contamination of the supernatant with intracellular β-hCG as a consequence of cell lysis. The β-hCG secretion is a valid parameter of trophoblast syncytialisation rate [31]. The β-hCG content of the trophoblastic cell supernatant, as assessed by ELISA, increased continuously over the time-points investigated in both experimental groups. After 24 h of culture, the increase became significant (p < 0.01) evidencing the progression of syncytialisation of the trophoblastic cells (data not shown). Compared to 6 h, both vehicle and CRH treated primary trophoblastic cells

**Table 1 Primer sequences**

|        | Forward (5´-3´) | Reverse (5´-3´) |
|--------|----------------|----------------|
| HPRT   | CGGCCCTGCCATTGAGC | GGTCTAAACTGGTCA-TCATCA |
| r18S   | GCCATTATCCCACGAGC | GGCCTACTAAACCAT-CCAA |
| Leptin | ACAATTGTCACCAGAATCACATGAC | TCCAAACCGGTAGACC-TCAGT |
| 11β-HSD2 | CATACCGGCTGTGAC-TCTG | CGGACGCCCGCATGAG |
| CRH-R1 alpha | CTACATGCTGTCAGCAG-CGAATCC | GGCAGAACCGGACTGGAAG |
| CRH-R2 | TCCATACAGGAGGAGGTGACTGAA | GGTTGAAATAGATGAA-CAAGTCTG |
| Syncytin-1 | ATGGAGGACAGGGATCAGCAG | AGATCGTGGGCTAGCAG |

**Table 2 LDH absorbance in the culture medium of human trophoblastic cells with and without CRH (1 μg/ml) stimulation**

| Time (hours) | Absorbance levels (rel. units) | p-value |
|--------------|--------------------------------|---------|
|              | Vehicle | CRH | Vehicle | CRH |
|              | mean    | SEM | mean    | SEM |
| 6            | 0.027   | 0.006 | 0.022   | 0.003 | ns   |
| 12           | 0.036   | 0.005 | 0.032   | 0.001 | ns   |
| 24           | 0.026   | 0.017 | -0.004  | 0.003 | ns   |
| 48           | 0.033   | 0.004 | 0.019   | 0.001 | ns   |
| 72           | 0.025   | 0.005 | 0.029   | 0.006 | ns   |
showed a significant increase of β-hCG protein content in the supernatant at 48 h (p < 0.01) and more significantly at 72 h (p < 0.001, data not shown). Stimulation with CRH (1.0 and 2.0 μg/ml) did not influence the amount of β-hCG in the supernatant at 48 and 72 h (Figure 1), indicating that CRH does not alter maturation of trophoblastic cells in vitro. Additionally we measured syncytin-1 (Syn1) expression, as previously described [33,35]. Syn1 is essential for mediating trophoblast cell fusion events [37]. Syn1 expression was significantly induced at 48 h by CRH in a dose-dependent manner (1.0 μg < 2.0 μg, p < 0.029 for both, Figure 1). At 72 h the

| 48h | 72h |
|-----|-----|
| control | 1.0μg CRH | 2.0μg CRH | control | 1.0μg CRH | 2.0μg CRH |
| Leptin gene expression (blue bars) | Leptin protein secretion (red bars) | Syn1 (blue bars) | β-hCG (red bars) | CRH-R1 (blue bars) | CRH-R2 (red bars) | 11β-HSD2 (green bars) |
| displayed are values relative to the control value at the designated time-point as mean ± SEM, * = p < 0.05. | | | | | | |

Figure 1 Overview of gene expression profiles and results of protein detection. Overview of gene expression profiles (RT-PCR) and results of protein detection (ELISA) in cell culture supernatant of vehicle and corticotropin-releasing hormone (CRH) (1.0 and 2.0 μg/ml) treated trophoblasts at 48 and 72 h. Top row: Leptin gene expression (Leptin, blue bars), Leptin protein secretion (Leptin P, red bars). Middle row: Syncytin-1 (Syn1) (blue bars), β-hCG (red bars). Bottom row: CRH-R1 (blue bars), CRH-R2 (red bars), 11β-HSD2 (green bars). Displayed are values relative to the control value at the designated time-point as mean ± SEM, * = p < 0.05.
stimulative effect of CRH on Syn1 expression had subsided (Figure 1).

Leptin expression
Previous experiments have shown a close relation of trophoblast leptin expression to leptin secretion [14]. Leptin expression increased with culture time of trophoblastic cells, irrespective of the stimulation with CRH. This increase was significant after 12 h of culture and peaked after 24 h (Figure 2; p < 0.05). While leptin expression levels in the unstimulated control group declined after 48 h, CRH-treated primary trophoblastic cells showed a more sustained induction of leptin expression (Figure 2). At this time-point, leptin expression was significantly higher in the trophoblastic cells after stimulation with 1.0 and 2.0 μg/ml CRH compared to unstimulated controls (p < 0.05, Figure 1 and Figure 2). After 72 h the leptin expression had returned to a basal level in both groups alike. Leptin protein expression closely matches expressional changes following CRH stimulation (Figure 1). At 48 h CRH dose-dependently increased in leptin secretion into trophoblast culture supernatant. The stimulatory effect of 1.0 μg/ml CRH on trophoblast leptin secretion lasted for 48 h (p < 0.029). After 2.0 μg/ml CRH stimulation, there was still a significant (p < 0.002) induction in leptin secretion detectable at 72 h (Figure 1).

11β-HSD2 expression
Stimulation of trophoblastic cells with 2.0 μg/ml CRH significantly (p < 0.029) induced 11β-HSD-2 expression at 48 h (Figure 1). Stimulation of trophoblastic cells with 1.0 μg/ml CRH did not significantly alter 11β-HSD-2 expression during the observational period (Figure 1).

CRH receptor expression
CRH-R2 gene expression was low (Ct: 32.8 ± 0.35 SEM) in cultured human trophoblasts throughout the duration of the experiment, while CRH-R1 was readily detectable (Ct: 24.89 ± 0.21 SEM). CRH treatment at a concentration of 2.0 μg/ml resulted in an increased CRH-R1 and CRH-R2 expression in these cells (5.05 ± 1.33 SEM, p = 0.029; 4.55 ± 2.05 SEM, p = 0.029, respectively). Interestingly, at 72 h CRH-R1 and CRH-R2 expression levels following CRH treatment (1.0 and 2.0 μg/ml) were not different to expression levels in vehicle treated controls.

Discussion
In the present study, we aimed to clarify the influence of corticotropin-releasing hormone (CRH) exposure on the syncytialisation rate of isolated primary villous trophoblastic cells. Moreover, we investigated whether CRH induces alterations in gene expression of specific endocrine placental regulators in vitro. Our results showed a significantly higher leptin expression in trophoblastic cells, which concomitantly resulted in a significant induction of leptin protein secretion into the supernatant after 48 h of CRH stimulation, compared to unstimulated control cells. Moreover, 11β-HSD2 expression was dose-dependently induced by 2.0 μg/ml CRH after 48 h. Formation of a functional syncytiotrophoblast occurred after 24 h in the CRH-stimulated and the control group to the same degree, as determined by increasing β-hCG secretion without a concomitant increase of cell lysis reflected by constant LDH levels in the supernatant throughout the experiment [31]. Syncytin-1 expression, a key regulator gene of trophoblast syncytialisation

Figure 2 Leptin expression in human trophoblastic cells: Vehicle vs. CRH (1.0 μg/ml) stimulation. A significant (p < 0.05) increase in relative leptin gene expression was observed after 12 h in both groups. CRH treatment significantly (p < 0.05, circle) increased leptin expression above control levels after 48 h. Gene expression is related to the housekeeping gene HPRT. Displayed are means ± SEM, *p < 0.05, **p < 0.01.
[33,35,37], was dose-dependently induced after 48 h of CRH stimulation only.

The finding that leptin expression increases with the progression of trophoblast syncytialisation in both groups is in line with results from Ashworth et al. [38], who showed that placental leptin expression is an exclusive feature of the syncytiotrophoblast (SCT), with a reduced leptin expression in undifferentiated cytotrophoblasts. Likewise, CRH was described as a syncytial peptide [39]. CRH-treated and vehicle stimulated trophoblasts showed an equal increase of β-hCG concentration in the culture supernatant, while cell lysis was low and not different (as determined by LDH). This finding is indicative that the rate of trophoblast syncytialisation gradually increased over time in both groups [40]. The induction of leptin expression after 24 h of culture of CRH- and vehicle treated trophoblastic cells can therefore be attributed to trophoblastic differentiation into functional syncytiotrophoblast. The significant difference in leptin gene expression in CRH-treated and vehicle treated control groups at 48 h and the induction of leptin protein secretion into the cell culture supernatant, however, do not seem to be solely an indirect effect of leptin protein secretion into the cell culture supernatant, while cell lysis was low and not different (as determined by LDH). This finding is indicative that the rate of trophoblast syncytialisation gradually increased over time in both groups [40].

The induction of leptin expression after 24 h of culture of CRH- and vehicle treated trophoblastic cells can therefore be attributed to trophoblastic differentiation into functional syncytiotrophoblast. The significant difference in leptin gene expression in CRH-treated and vehicle treated control groups at 48 h and the induction of leptin protein secretion into the cell culture supernatant, however, do not seem to be solely an indirect effect of leptin expression in undifferentiated cytortrophoblasts. Therefore, we cannot completely rule out that the Syn1 induction measured is derived from EVT. But due to their extremely small fraction in the isolation, a six-fold increase of maternal cortisol more efficiently.

Interesting, higher levels of auto-/paracrine CRH might hypothetically prepare the syncytium to cope with a CRH-triggered increase of maternal cortisol more efficiently.

The above findings regarding leptin are in line with findings from our previous experiments showing a close relationship of trophoblast leptin expression and secretion rate in vitro following dexamethasone stimulation [14]. Interestingly, dexamethasone stimulation (10 μM) produced a more pronounced leptin secretion (~120 pg/ml at 72 h) when compared to CRH (1.0 and 2.0 μg/ml) stimulation (~56-61 pg/ml at 72 h, respectively). However, dexamethasone clearly induced β-hCG secretion. Hence in contrast to CRH the effect of dexamethasone on leptin secretion seems to be partly attributable to an increased rate of trophoblast differentiation and maturation, as also seen by Audette et al. [46]. They were also able to demonstrate a trend to Syn1 induction in placental explants following dexamethasone treatment.

The fact, that we found an increase in Syn1 expression following CRH stimulation without a concomitant increase in β-hCG might point to a differential regulation of the two genes. A common pathway for both Syn1 and β-hCG stimulation is the forskolin triggered induction of cAMP [47]: An activation of adenyl cyclase (AC) raises intracellular CAMP levels and leads to PKA activation via interactions with AKAPs and downstream phosphorylation of p38MAPK and ERK1/2. Accordingly, CRH was found to induce CAMP in human endometrium via CRHR1 triggered protein-kinase A (PKA) [48] and we recently found that Syn1 is induced via the CAMP pathway in endometrial carcinoma [49]. However, as β-hCG was not induced, CRH might either use alternative signalling pathways, such as signalling via PKC [45,50], or the detected Syn1 expression might come from a source other than the cytotrophoblast. However our cytotrophoblast isolation is ~90% pure. Thus, we cannot exclude possible minor fractions of extravillous trophoblasts (EVT) in our cell culture. EVT express CRHR1 [51] and show Syn1 expression [20,52]. Therefore, we cannot completely rule out that the Syn1 induction measured is derived from EVT. But due to their extremely small fraction in the isolation, a six-fold increase in Syn1 expression by EVT following CRH stimulation seems rather unlikely. Another explanation could be, that CRH fosters maintenance fusion events
instead of functional fusion processes, that would be reflected in β-hCG secretion. Importantly, Syn1 is related to trophoblast processes beyond its fusogenic nature. Possible functions of the syncytin proteins are suppression of the maternal immune response against the developing fetus [53] and induction of placental immunity against vertical transmission of retroviral infections [54].

We observed a stimulatory effect of CRH (2.0 μg/ml after 48 h) on the expression of 11β-HSD2 in primary cultured cytotrophoblasts. Like leptin, the induction of 11β-HSD2 by CRH subsided at 72 h, possibly due to CRH receptor internalization, as discussed above. In a previous study using the same in vitro setup, we were able to show that dexamethasone (10 μM) similarly stimulates both leptin and 11β-HSD2 expression in primary trophoblastic cells [14]. 11β–HSD2 gene expression in human placental trophoblasts grown in primary culture has been shown to maintain the same pattern as in vivo [55] and dexamethasone stimulation regularly results in an increase in 11β–HSD2 protein expression in trophoblasts [56]. Upon the finding that CRH induces 11β-HSD2 expression one cannot draw conclusions about the activity of placental glucocorticoid metabolism. Interestingly, Friedberg et al. [57] found a CRH-induced reduction of 11β-HSD1 activity in human adipocytes in vitro. In isolated cytotrophoblasts Sharma et al. [58] were unable to induce 11β-HSD2 activity using CRH concentrations of 1-100 ng/ml, however, they were able to identify the CRH downstream signalling protein p38MAPK [47] as an essential regulator for 11β-HSD2 activity. The fact, that we observed expression changes of 11β-HSD2 following CRH treatment at much higher CRH dosages (2.0 μg/ml) could however imply a possibility of a CRH-driven glucocorticoid induced feed-forward mechanism on 11β–HSD2 activity. Although such a mechanism has not been described for the placenta yet, the subsequent reduction of cortisol availability might be an intriguing regulatory function shielding the fetus of placental CRH-induced maternal glucocorticoids.

Our study focused on the auto- and paracrine effects of CRH on leptin production in isolated trophoblasts, as the placenta co-expresses both leptin (ObR-L) [59] and CRH receptors [9]. We were able to show a significant increase in leptin expression in syncytialised trophoblastic cells following CRH treatment. While the exact auto- and paracrine mechanisms and the functional role of the interaction of CRH and leptin at the level of the syncytiotrophoblast remain to be determined, an increase of endocrine CRH and leptin expression might translate into endocrine signals affecting both fetus and mother, besides their local influence on the trophoblast.

In this respect it is noteworthy, that the major fraction of placental leptin and CRH is secreted into the maternal circulation [60,61]. Nevertheless, the syncytiotrophoblast is also involved in the maintenance of fetal leptin and CRH serum levels [62,63]. Besides its role in fetal organ maturation via cortisol induction, there is in fact evidence, that placental CRH drives parturition via induction of adrenal DHEA-S on the fetal side followed by an increase in placental estrogen secretion [63].

In IUGR, a condition characterised by increased fetal serum CRH levels [25], we found unchanged leptin levels in fetal umbilical cord blood [23], despite an elevated placental leptin mRNA and protein expression [28,64]. Hence, it seems likely that CRH and CRH-induced leptin (as suggested by our results) might interact on the maternal side.

White et al. [65] showed that leptin has lipolytic effects in rat placental tissue in vitro. CRH antagonises lipolysis via down-regulation of 11β-HSD1 in adipose tissue [57]. Hypothetically leptin and CRH might act together in regulating the maintenance of fetal nutrient supply at the placental level.

Conclusions
In summary, our data indicate that CRH stimulation induces leptin secretion in the human syncytiotrophoblast in an auto-/paracrine fashion. Similarly, CRH induced 11β-HSD2 expression. This suggests a short-loop feedback of CRH-induced leptin on CRH action at the fetomaternal interface. Such a putative cross-talk could play an essential role in the regulation of syncytiotrophoblast nutrient supply and cortisol metabolism, besides possible further implications for myometrial contractility, placental bed perfusion and the timing of birth. Furthermore CRH-induced 11β-HSD2 might locally determine placental corticosteroid metabolism and thereby the passage of placental CRH-triggered maternal cortisol via the syncytiotrophoblast to the fetus. This would protect the fetus from detrimental elevated maternal glucocorticoid exposure. The underlying mechanism and the functional role of the interaction of CRH with leptin and 11β-HSD2 at the syncytiotrophoblast remain to be determined.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
FBF contributed to conception and design of the study, analysed and interpreted the data and drafted the manuscript. MR performed the cell culture experiments, including RT-PCRs. GV performed ELISA analysis. RO contributed to acquisition and analysis of the data, AH contributed to interpretation of data and was involved in drafting the manuscript. MR and CM-C were involved in the analysis of data and critically revised the manuscript for important intellectual content. RS contributed to the acquisition and analysis of data, WR critically revised the manuscript for important intellectual content. All authors have given the final approval of the version to be published.
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References
1. Shibasaki T, Odagiri E, Shiizume K, Ling N: Corticotropin-releasing factor-like activity in human placental extracts. J Clin Endocrinol Metab 1982, 55:384–386.
2. Petraglia F, Florio P, Gallo R, Simoncini T, Savoizzio M, Di Blasio AM, Vaughan J, Vale W: Human placenta and fetal membranes express human urocin mRNA and peptide. J Clin Endocrinol Metab 1996, 81:3807–3810.
3. Kalliaridou SN, Makrigiannakis A, Markorakos G, Chrousos GP: Roles of reproductive corticotropin-releasing hormone. Ann N Y Acad Sci 2003, 997:12–135.
4. McLean M, Smith R: Corticotropin-releasing hormone in Human Pregnancy and Parturition. Trends Endocrinol Metab 1999, 10:174–178.
5. Mastorakos G, Scopa CD, Vryonidou A, Friedman TC, Kattis D, Phenekos C, Merino MJ, Chrousos GP: Presence of immunoreactive corticotropin-releasing hormone in normal and polycystic human ovaries. J Clin Endocrinol Metab 1994, 79:1191–1197.
6. Di Blasio AM, Pecorari G, Lino G, Petraglia F, Vignali M, Cavagnini F: Expression of corticotropin-releasing hormone and its R1 receptor in human endometrial stromal cells. J Clin Endocrinol Metab 1997, 82:1594–1597.
7. Petraglia F, Tabanelli S, Galassi MC, Garuti GC, Mancini AC, Genazzani AR, Gupride E: Human decidua and in vitro decidualized endometrial stromal cells at term contain immunoreactive corticotropin-releasing factor (CRF) and CRF messenger ribonucleic acid. J Clin Endocrinol Metab 1992, 74:1427–1431.
8. Rodriguez-Linares B, Linton EA, Phaneuf S: Expression of corticotropin-releasing hormone receptor mRNA and protein in the human myometrium. J Endocrinol 1998, 156:11–21.
9. Gao L, He P, Sha L, Liu C, Dai L, Zhi N, Xi X: Corticotropin-releasing hormone type 1 and type 2 mediate differential effects on 15-hydroxy prostaglandin dehydrogenase expression in cultured human chorion trophoblasts. Endocrinology 2007, 148:3645–3654.
10. Karten E, Grammatopoulos D, Dai Y, Olah KB, Gobharo TB, Eastan AJ, Hillhouse EW: The human placenta and fetal membranes express the corticotropin-releasing hormone receptor talpha (CRH-talpha) and the CRH-C variant receptor. J Clin Endocrinol Metab 1998, 83:1376–1379.
11. Lockwood CJ, Rudunovic N, Nastic D, Petkovic S, Aigner S, Berkowitz GS: Corticotropin-releasing hormone and related pituitary-adrenal axis hormones in fetal and maternal blood during the second half of pregnancy. J Perinat Med 1996, 24:243–251.
12. Grammatopoulos D, Dai Y, Chen J, Karten E, Papadopoulo N, Eastan AJ, Hillhouse EW: Human corticotropin-releasing hormone: differences in subtype expression between pregnant and nonpregnant myometria. J Clin Endocrinol Metab 1998, 83:2539–2544.
13. Stevens MY, Challs JR, Lye SJ: Corticotropin-releasing hormone receptor subtype 1 is significantly up-regulated at the time of labor in the human myometrium. J Clin Endocrinol Metab 1998, 83:4107–4115.
14. Tzschoppe A, Fahrbusch F, Seidel J, Dorr HG, Rascher W, Goecke TW, Beckmann MW, Schild RL, Erwee T, Dotsch J: Dexamethasone stimulates the expression of leptin and 11beta-HSD2 in primary human placental trophoblastic cells. Eur J Obstet Gynecol Reprod Biol 2011, 156(1):50–55. Epub 2011 Feb 2.
15. 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, 89:5614–5621.
16. Grammatopoulos DK, Hillhouse EW: Role of corticotropin-releasing hormone in onset of labour. Lancet 1999, 354:1546–1549.
17. Tyson DK, Smith R: Evidence that corticotropin-releasing hormone modulates myometrial contractility during human pregnancy. Endocrinology 2009, 150:5617–5625.
18. Aggelidou E, Hillhouse EW, Grammatopoulos DK: Up-regulation of nitric oxide synthase and modulation of the guanylate cyclase activity by corticotropin-releasing hormone but not urocin II or urocortin III in cultured human myometrial cells. Proc Natl Acad Sci U S A 2002, 99:3300–3305.
19. Clifton VL, Read MA, Leitch IM, Giles WB, Boura AL, Robinson PJ, Smith R: Corticotropin-releasing hormone-induced vasodilatation in the human fetal-placental circulation: involvement of the nitric oxide-cyclic guanosine 3',5'-monophosphate-mediated pathway. J Clin Endocrinol Metab 1995, 80:2888–2893.
20. Bamberger AM, Minas V, Kalantariou SN, Raddke I, Sadeghian H, Loning T, Charalampopoulos I, Brummer J, Wagener C, Bamberger CM, et al: Corticotropin-releasing hormone modulates human trophoblast invasion through carboxyembryonic antigen-related cell adhesion molecule-1 regulation. Am J Pathol 2005, 168:141–150.
21. Kadyrov M, Kirjons J, Huppler R: Divergent trophoblast invasion and apoptosis in placental bed spiral arteries from pregnancies complicated by maternal anemia and early-onset preeclampsia/intrauterine growth restriction. Am J Obstet Gynecol 2006, 194:557–563.
22. Roberts JM, Taylor RN, Musli TJ, Rodgers GM, Hubel CA, McLaughlin MK: Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989, 161:200–204.
23. Tzschoppe AA, Struwe E, Dorr HG, Goecke TW, Beckmann MW, Schild RL, Dotsch J: Differences in gene expression dependent on sampling site in placental tissue of fetuses with intrauterine growth restriction. Placenta 2010, 31:178–185.
24. Dotsch J, Nuksen KD, Knerr I, Kirschbaum M, Repp R, Rascher W: Leptin and neuropeptide Y gene expression in human placenta: ontogeny and evidence for similarities to hypothalamic regulation. J Clin Endocrinol Metab 1999, 84:2755–2758.
25. Goland RS, Jozaik S, Warren WB, Cornelis IM, Stark RI, Tropper PJ: Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. J Clin Endocrinol Metab 1993, 77:1174–1179.
26. Goland RS, Tropper PJ, Warren WB, Jozaik SM, Cornelis IM, Warren WD: Concentrations of corticotrophin-releasing hormone in the umbilical-cord blood of pregnancies complicated by preeclampsia. Am J Perinatol 1995, 12:1227–1230.
27. Ruenzer MB, Strissel PL, Langbein M, Fahrbusch F, Schild RL, Fachscheider F, Beckmann MW, Strick R: Impaired cell fusion and differentiation in placenta from patients with intrauterine growth restriction correlate with reduced levels of HERV envelope genes. J Mol Med 2010, 88:1143–1156.
28. McCarthy C, Cotter FE, McElwaine S, Twomey A, Moorey EE, Ryan F, Vaughan J: Altered gene expression patterns in intrauterine growth restriction: potential role of hypoxia. Am J Obstet Gynecol 2007, 196(70):e71–e76.
29. 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, 29:193–200.
30. Voigt M, Schneider KT, Jahir H: Analysis of a 1992 birth sample in Germany: 1. New percentile values of the body weight of newborn infants. Geburtshilfe Frauenheilkd 1996, 56:550–558.
31. Kirman HJ, Hestler JS, Semeloff S, Sanger JM, Strauss JS: 3rd Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. Endocrinology 1986, 118:1567–1582.
32. Schild RL, Sonnenberg-Hirche CM, Schaff WT, Bildrich I, Nelson DM, Sadovsky Y: The kinase p38 regulates peroxisome proliferator activated receptor-gamma in human trophoblasts. Placenta 2006, 27:191–199.
33. Ruenzer MB, Strissel PL, Langbein M, Fahrbusch F, Wachtler DL, Fachscheider F, Beckmann MW, Strick R: Impaired cell fusion and...
cytotrophoblasts of the human placenta. J Reprod Immunol 2011, 91:1–8.
5. Mangeney M, Renard M, Schlecht-Louf G, Bouallaga I, Heidmann O, Letzeliter C, Richard A, Ducos B, Heidmann T: Placental syncytins: Genetic
disjunction between the fusogenic and immunosuppressive activity of
retroviral envelope proteins. Proc Natl Acad Sci U S A 2007, 104:20534–20539.
6. Ponferrada V, Mauck BS, Woolley DP: The envelope glycoprotein of
human endogenous retrovirus HERV-W induces cellular resistance to
spleen necrosis virus. Arch Virolog 2003, 148:659–675.
7. Sun K, Yang K, Challs JR: Differential regulation of 11 beta-hydroxysteroid
dehydrogenase type 1 and 2 by nitric oxide in cultured human placentals.
trophoblast and chorionic cell preparation. Endocrinology 1997, 138:4912–4920.
8. Li JN, Ge YC, Yang Z, Guo CM, Duan T, Miyatt L, Guan H, Yang K, Sun K: The
Sp1 transcription factor is crucial for the expression of 11beta-
hydroxysteroid dehydrogenase type 2 in human placental trophoblasts.
J Clin Endocrinol Metab 2011, 96:E899–E907.
9. Friedberg M, Zoumakis E, Hiroi N, Bader T, Chrousos GP, Hochberg Z:
Modulation of 11 beta-hydroxysteroid dehydrogenase type 1 in mature
human subcutaneous adipocytes by hypothalamic messengers. J Clin
Endocrinol Metab 2003, 88:385–393.
10. Sharma A, Guan H, Yang K: The p38 mitogen-activated protein kinase
regulates 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2)
expression in human trophoblast cells through modulation of 11beta-
HSD2 messenger ribonucleic acid stability. Endocrinology 2009,
150:4278–4286.
11. Bodner J, Ebenbichler CF, Wolf HJ, Muller-Holzner E, Stand U, Gander R,
Huter G, Patsch JR: Leptin receptor in human term placenta: in situ
hybridization and immunohistochemical localization. Placenta 1999,
20:677–682.
12. 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, 85:3498–3501.
13. Senaris R, Garcia-Caballero T, Casasell X, Gallego R, Castro R, Considine RV,
Dieguez C, Casanueva FF: Synthesis of leptin in human placenta.
Endocrinology 1997, 138:4501–4504.
14. Ert T, Furie S, Sarkany I, Szabo I, Rascher W, Blum WF, Sulyok Y: Postnatal
changes of leptin levels in full-term and preterm neonates: their relation
to intrauterine growth, gender and testosterone. Biol Neonate 1999,
75:167–176.
15. Smith R, Mesiano S, Chan EC, Brown S, Jaffe RB: Corticotropin-releasing
hormone directly and preferentially stimulates dehydroepiandrosterone
sulfate secretion by human fetal adrenal cortical cells. J Clin Endocrinol
Metab 1998, 83:2916–2920.
16. McMinn J, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, Weksberg R,
Thaker HM, Tycko B: Unbalanced placental expression of imprinted genes
in human intrauterine growth restriction. Placenta 2006, 27:540–549.
17. White Y, Gonzalez E, Capobianco E, Pustovitch C, Martinez N, Higa R, Baier M,
Javenbaum A: Leptin modulates nitric oxide production and lipid
metabolism in human placenta. Reprod Fertil Dev 2006, 18:425–432.