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ORIGINAL ARTICLE

Low amino acids affect expression of 11β-HSD2 in BeWo cells through leptin-activated JAK-STAT and MAPK pathways

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Abstract Maternal protein restriction diminishes placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity and causes fetal growth restriction in mammals. However, it is unknown whether such effect was caused directly by nutrient deficiency, or indirectly through the mediation of maternal hormones. In the present study, a human placental cell line (BeWo) was cultured in F12K as control and F12 as low amino acids (LAA) media for 48 h to investigate the effects of amino acids deficiency on 11β-HSD2 expression and activity. Despite a significant up-regulation of 11β-HSD2 mRNA expression in LAA cells, 11β-HSD2 activity and protein content were decreased by 38 and 54%, respectively ($P < 0.05$), indicating a mechanism of post-transcriptional regulation. Among 5 miRNAs targeting 11β-HSD2, miR-498 was expressed significantly higher in LAA cells. Leptin concentration was significantly lower ($P < 0.01$) in LAA medium. The mRNA expression of both isoforms of leptin receptor was significantly higher in LAA cells, although no difference was detected at protein level. To further clarify whether leptin is involved in mediating the effect of LAA on 11β-HSD2 activity, leptin was supplemented to LAA medium, whereas three specific inhibitors of leptin signaling pathways, WP1066 for JAK-STAT, PD98059 for MAPK and LY294002 for PI3K, respectively were added to control medium. Leptin restored the diminished 11β-HSD2 activity in LAA cells, whereas WP1066 (5 nM) and PD98059 (50 nM) significantly decreased 11β-HSD2 activity in control cells. In conclusion, the present results indicate that LAA diminishes 11β-HSD2 expression and activity in BeWo cells through leptin-activated JAK-STAT and MAPK pathways.

Keywords 11β-HSD2 · Leptin · miRNA · Low amino acids · BeWo cells

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Introduction

Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol to inactive cortisone to protect the fetus from over-exposure to maternal glucocorticoids. Disruption of placental 11β-HSD2 activity can cause permanent adverse effects on fetus (Jansson and Powell 2007). Maternal low protein diet causes diminished expression and/or activity of placental 11β-HSD2 (Bertram et al. 2001; Langley-Evans et al. 1996; Stocker et al. 2004), which is presumed to be mediated by the elevated maternal or fetal cortisol secretion caused by maternal nutrition deficiency (Cottrell and Seckl 2009; Reynolds 2010). However, not all the reports support this hypothesis. Glucocorticoids have been reported to increase, rather than decrease, 11β-HSD2 expression and/or activity in human and baboon models in vivo (Clifton et al. 2006; Ma et al. 2003) or in vitro (van Beek et al. 2004). Furthermore, glucocorticoid level was unchanged in either maternal or fetal circulation under maternal protein restriction (Fernandez-Twinn et al. 2003). Therefore, it remains unclear whether the decreased 11β-HSD2 activity was caused directly by nutrient deficiency, or indirectly through the mediation of glucocorticoids or other hormones.

Leptin concentration in maternal blood increases 2–3 times in rat and human, and 25 times in mouse during pregnancy, which is contributed primarily by leptin secreted from placenta (Ashworth et al. 2000; Masuzaki et al. 1997). Under the maternal low protein diet, maternal serum leptin concentration was reported to decrease in the pregnant rat (Fernandez-Twinn et al. 2003; Jansson et al. 2006). Leptin administration to pregnant rats prevented the decrease in placental 11β-HSD2 activity induced by maternal low protein diet (Stocker et al. 2004). Two forms of leptin receptor are expressed in the placenta, the long form leptin receptor (Ob-Rl) and the short form leptin receptor (Ob-Rs) (Bajoria et al. 2002; Ebenbichler et al. 2002), which indicates that placenta is not only a source of leptin (Hassink et al. 1997), but also a target for the action of leptin. Leptin has been reported to act on placental cells to prevent apoptosis (Magarinos et al. 2007; Perez-Perez et al. 2008), stimulate protein synthesis (Perez-Perez et al. 2009, 2010), or promote amino acid transport (Jansson et al. 2003; von Versen-Hoynck et al. 2009), through different signaling pathways, including MAPK, JAK-STAT or PI3K. It is speculated that leptin secreted by placenta per se might participate in linking maternal nutrition and placental 11β-HSD2 expression and activity. Nevertheless, it has not been elucidated how maternal low protein diet affects placental leptin secretion and through what signaling pathways leptin affects the expression and activity of 11β-HSD2 in the placenta.

It has been shown that maternal low protein diet or the maternal nutrient restriction causes significant decrease in free amino acid concentrations in maternal circulation (Bhasin et al. 2009; Kilberg et al. 2009; Kwon et al. 2004; Rees et al. 2006). Branched-chain amino acids (BCAAs), particularly leucine, stimulate leptin production by rat adipocytes in vivo (Lynch et al. 2006) and in vitro (Roh et al. 2003). It is likely that amino acid deficiency caused by maternal low protein diet is responsible for diminished leptin secretion from placenta, and subsequently results in disrupted expression and activity of 11β-HSD2 in the placenta via leptin-activated pathways.

Human choriocarcinoma cells display many of the biochemical and morphological characteristics for in utero invasive trophoblast cells and have been widely used as a model in placenta research (Myren et al. 2007; Sarkar et al. 2001; Jones et al. 2006). Here we use BeWo cells, a human choriocarcinoma cell line, as a model to investigate the effects of low amino acids medium on leptin secretion and 11β-HSD2 expression and activity. To further elucidate the possible role of leptin in mediating the effect of low amino acids, leptin and the specific inhibitors of three different leptin signaling pathways were added, respectively, to low amino acids and control media and 11β-HSD2 activity of the BeWo cells was determined.

Materials and methods

Cell culture

BeWo cells were obtained from the Chinese Academy of Medical Sciences and Peking Union Medical College. The cells were maintained in 25 cm^2 flasks at 37°C under 5% CO_2 and cultured in the basal medium F-12 Kaighn's (F12K, GIBCO, USA) with 10% fetal calf serum (FCS, LanZhou National Hyclone Bio-Engineering CO., Ltd China). The medium was changed every 2 days and cells were passaged every 4 days. Cells used were between passages 5–18. At 80% confluency, flasks of cells were separated randomly into two groups, one group of cells being cultured in F12K as control (Con) and the other in F12 (GIBCO, USA) as low amino acids (LAA). The amino acid concentrations in the F12 medium are approximately half of those in F12K. The formulas are shown in the following links. F12: http://www.invitrogen.com/site/us/en/home/support/Product-Technical-Resources/media_formulation.64.html, F12K: http://www.invitrogen.com/site/us/en/home/support/Product-Technical-Resources/media_formulation.65.html. After 48 h, the cells and the media were collected for further analysis.

Leptin (398-LP, R&D, USA) was added at 5 ng/mL to the LAA medium after 24 h of seeding, whereas leptin signal pathway inhibitors (Sigma, USA), WP1066 (JAK-STAT), PD98059 (MAPK), and LY294002 (PI3K) were added to the control medium at the doses of 5 and 50 nM. After 48 h, 11β-HSD2 activity was determined.

Radioimmunoassay of leptin and glucocorticoids in media

The culture media were lyophilized for leptin analysis. Cortisol and leptin were measured with commercial RIA kits purchased from Beijing North Institute of Biotechnology, Beijing, China. The detection limit for leptin was 0.45 ng/mL and the intra- and inter-assay coefficients of variation were 5 and 10%, respectively. The detection limit for cortisol was 2 ng/mL and the intra- and inter-assay coefficients of variation were 10 and 15%, respectively.

Reverse transcription and real-time RT-PCR

A 2 μg of total RNA extracted from BeWo cells was reverse transcribed following the standard protocol, and 2 μL of 5–20 folds diluted RT products was used for PCR in a final volume of 25 μL containing 12.5 μL SYBR Green Real-time PCR Master Mix (TOYOBO Ltd., Japan) and 0.2–0.8 μM of each forward and reverse primers specific for Ob-Rs, Ob-Rl, 11β-HSD2 (Table 1). Human GAPDH was used as a reference gene for normalization

purposes (no marked difference in GAPDH mRNA was detected between Con and LAA). The method of $2^{-\Delta\Delta Ct}$ was used to analysis the real-time RT-PCR data (Livak and Schmittgen 2001). All samples were included in the same run of RT-PCR and repeated at least for three times.

Western blotting

Whole cell lysates were prepared in cold lysis buffer (Sato et al. 2008) containing Mini EDTA-free protease inhibitors (11697498001, Roche, USA) for Western blot analysis as described previously (Yuan et al. 2009). Monoclonal mouse antibody against human leptin receptor (1:400, MAB867, R&D, USA), rabbit polyclonal antibody against human 11β-HSD2 (1:500, sc-20176, Santa Cruz, USA), and mouse monoclonal antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:10000, KC-5G4, KangChen, China) were used, respectively, as primary antibodies and followed by goat anti-mouse (1:5000, GGHL-90P, Univ-bio, China) or goat anti-rabbit (1:5000, GGHL-15P, Univ-bio, China) horse radish peroxidase conjugated secondary antibodies. The blots were then washed and detected by enhanced chemiluminescence (ECL) using the LumiGlo substrate (Super Signal West Pico Trial Kit, Pierce, USA). ECL signals recorded on X-ray film were scanned and analyzed with Kodak 1D Electrophoresis Documentation and Analysis System 120 (Kodak Photo Film Co. Ltd., USA). The band densities of leptin receptors and 11β-HSD2 were normalized with that of GAPDH.

11β-HSD2 activity: radiometric conversion assay

[1,2,6,7-^{3}H(N)]-Cortisol (72.4 Ci/mmol) was purchased from Perkin Elmer Life and Analytical Science, Boston, USA. Polyester-backed thin-layer chromatography (TLC, GF254) plates were obtained from Qingdao Haiyang Chemical Co., Ltd, China. Non-radioactive cortisol and cortisone were products of Sigma, USA.

At the end of treatment, cells were washed three times with respective serum-free medium, F12K or F12, to remove hormones and serum residues. 11β-HSD2 activity was determined by measuring the rate of cortisol to cortisone conversion, as described previously (Tremblay et al. 1999). Briefly, the cells were incubated for 1 h at 37°C in 1–2 ml of serum-free medium containing approximately 50,000 cpm [^{3}H]-cortisol and 100 nM unlabeled cortisol. At the end of incubation, the medium was collected, and steroids were extracted with ethyl acetate, including cortisol and cortisone (each 40 μg). The extracts were dried, and the residues were resuspended with 100 μL ethyl acetate. A fraction of the resuspension were spotted on a thin-layer chromatography plate, and outspreaded in chloroform/methanol (9:1, vol/vol). The bands containing the labeled cortisol and cortisone were identified by UV light and cut out into the ethyl acetate to extract. After drying, the radioactivity was counted with Multi-Purpose Scintillation Counter (LS 6500, BerckMan Coulter, USA). The rate of cortisol to cortisone conversion was calculated, and the blank values (defined as the amount of conversion in the absence of cells) were subtracted and the 11β-HSD2

Table 1 Nucleotide sequences of specific primers

Target gene name	Primer	PCR products (bp)	GenBank accession	References
11β-HSD2	5′-GGTCAAGGTCAGCATCATCCAG-3′	153	NM000196.3	
	5′-CCATGCAAGTGCTCGATGTAGTC-3′			
Ob-Rs	5′-GTAAGAGGCTAGATGGACTGGGATAT-3′	115	NM002303	(Meissner et al. 2005)
	5′-ATTCTCCAAAATTCAGGTCCTCTCA-3′			
Ob-Rl	5′-TGTTCCTGGGCACAAGGACTTA-3′	177	NM002303	
	5′-CACAGTTGTTGGCATCATCTCATC-3′			
GAPDH	5′-GCACCACCAACTGCTTAGCA-3′	118	NM002046.3	(Murthi et al. 2008)
	5′-GTCTTCTGGGTGGCAGTGATG-3′			
7HSL scRNA	5′-ATCGGGTGTCCGCACTAAGTT-3′	126	×04248	(Galiveti et al. 2010)
	5′-CAGCACGGGAGTTTTGACCT-3′			
miR-101	5′-TACAGTACTGTGATAACTGAA-3′			
miR-515-5p	5′-TTCTCCAAAAGAAAGCACTTTCTGAA-3′			
miR-195	5′-TAGCAGCACAGAAATATTGGCAA-3′			
miR-498	5′-TTTCAAGCCAGGGGGCGTTTTTCAA-3′			
miR-155	5′-TTAATGCTAATCGTGATAGGGGTAA-3′			
Poly(T)Adapter	5′-TAGAGTGAGTGTAGCGAGCACAGAATTAATACGACTCACTATAGGTTTTTTTTTTTTTTTVN-3′			
Reverse primer	5′-TAGAGTGAGTGTAGCGAGCA-3′			

activity was expressed as picomoles of cortisone formed per 1.0×10^7 cells per hour.

Quantitation of miRNAs targeting 11β-HSD2

Total RNA was isolated from the cells using TRIzol® reagent (Invitrogen, USA) and treated with RNase-free Dnase I (Takara Biotechnology, China). The treated total RNA (4 μg) was polyadenylated by poly(A) polymerase (PAP) at 37°C for 1 h in a 20 μL reaction mixture following the manufacturer's directions for the Poly(A) Tailing Kit (AM1350, Ambion, USA) (Shi and Chiang 2005). After phenol–chloroform extraction and ethanol precipitation, the RNAs were dissolved in DEPC-treated water and reverse transcribed with 25 μL mixture consisting of 1× RT buffer (Promega, USA), 100 U Moloney Murine Leukemia Virus reverse transcriptase (M-MLV) (Promega, USA), 8 U RNase inhibitor (Promega, USA), 5.3 μmol/L Oligo dT-Adaptor Primer (TaKaRa Biotechnology, China) and 0.8 mmol/L dNTP (TaKaRa Biotechnology, China). The miRNAs for 11β-HSD2 are predicted as described previously (Brown and Sanseau 2005; Rajewsky 2006). Five miRNAs were selected for quantification and the primer sequences are shown in Table 1. Human 7SL scRNA was used as a reference gene for normalization purposes (no marked difference in 7SL scRNA expression was detected between Con and LAA). Real-time PCR was performed for the five miRNAs. All samples were included in the same run of RT-PCR and repeated at least for three times.

Statistical analysis

All data are presented as mean ± SEM, and were analyzed using SPSS 13.0 for Windows. Values of mRNA abundance and protein content are expressed as the fold change relative to that of control. The data were analyzed by one-way ANOVA followed by LSD test or t test for independent samples. $P < 0.05$ was considered significant.

Results

11β-HSD2 expression and activity

As shown in Fig. 1a, 11β-HSD2 mRNA expression increased significantly in LAA cells when compared with the control. However, the protein content and the activity of 11β-HSD2 were decreased in LAA cells by 54 and 38%, respectively ($P < 0.05$, Fig. 1b, c) when compared with the control suggesting a post-transcriptional regulation.

miRNAs targeting 11β-HSD2

Among 5 miRNAs predicted to target 11β-HSD2, miR-498 was expressed significantly higher in cells cultured in LAA medium than that in control medium ($P < 0.05$, Fig. 2).

Cortisol and leptin concentrations in media

Cortisol was detected in the media of both control (7.1 ± 1.1 ng/ml) and LAA (7.2 ± 1.4 ng/ml) groups. Cortisol contents in both groups were lower than that in the fresh medium in the absence of cells, no difference in cortisol concentration was found between two groups. Leptin was detected in both LAA and control media, with significantly reduced concentration in LAA medium compared with control after 48 h of culture ($P < 0.01$, Fig. 3).

mRNA and protein expression of Ob-Rs, Ob-Rl

Both Ob-Rs and Ob-Rl mRNA abundances were found to be significantly up-regulated in LAA cells as compared to the control ($P < 0.05$, Fig. 4). However, Western Blotting analysis failed to detect significant alterations in the protein contents of either Ob-Rs or Ob-Rl (Fig. 5).

Leptin and leptin signaling pathway inhibitors on 11β-HSD2 activity

As shown in Fig. 6, leptin supplementation to LAA medium prevented the decrease of 11β-HSD2 activity ($P < 0.05$) in LAA medium, whereas administration of 5 nM WP1066, an inhibitor for JAK-STAT pathway, and 50 nM PD98059, an inhibitor for MAPK pathway, decreased 11β-HSD2 activity of cells cultured in control medium ($P < 0.05$, Fig. 7).

Discussion

It is reported that maternal nutrient restriction (50% of NRC nutrient requirement) in ewes resulted in 17–35% of decrease in the concentrations of 16 free amino acids on day 78 of gestation, including the 8 essential amino acids, in the maternal plasma (Kwon et al. 2004). Maternal protein restriction (60 g protein/kg diet) throughout pregnancy in rats decreased maternal serum concentration of the branched chain amino acids (valine, isoleucine and leucine) by 29–36%, and the threonine concentration was reduced by 80% compared with the control group fed on 180 g protein/kg diet (Rees et al. 1999). Similar results have been reported in rats showing significantly decreased maternal serum concentrations of the branched-chain amino acids and/or the total essential amino acids under

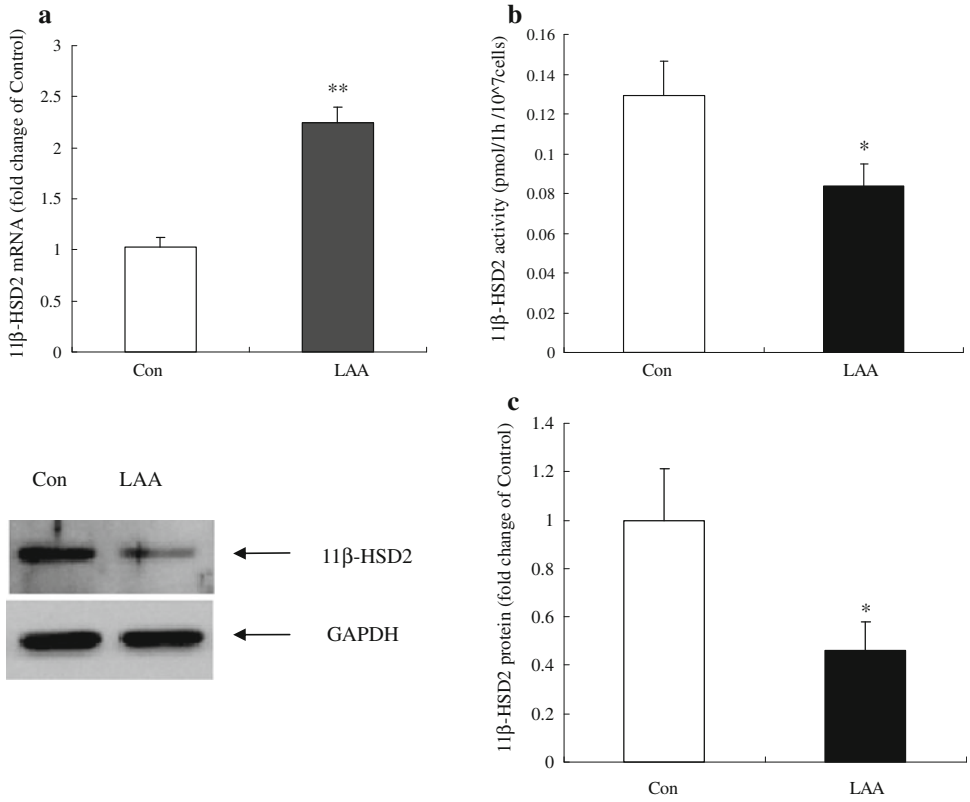

Fig. 1 Effect of low amino acids on 11β-HSD2 mRNA and protein expression and activity in BeWo cells. **a** 11β-HSD2 mRNA, **b** 11β-HSD2 protein, **c** 11β-HSD2 activity. Values are mean ± SEM, $n = 6$–8/group for the mRNA and protein, $n = 12$–14/group for the enzyme activity. *Con* control group, *LAA* low amino acids group. *$P < 0.05$, **$P < 0.01$ compared with control

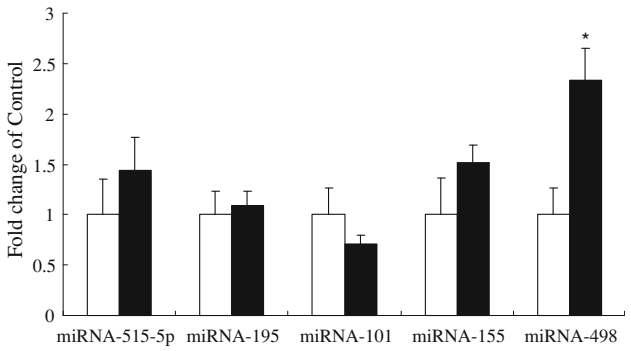

Fig. 2 Expression of miRNAs in BeWo cells. Values are mean ± SEM, $n = 5$–6/group. *Con* control group, *LAA* low amino acids group. *$P < 0.05$ compared with control

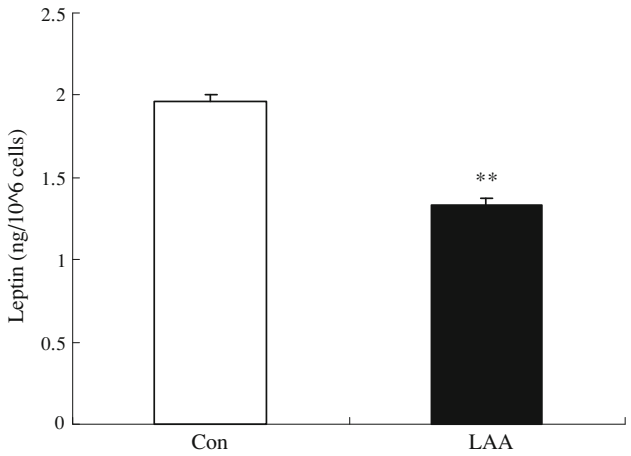

Fig. 3 Leptin contents in the supernatant of BeWo cell culture. Values are mean ± SEM, $n = 5$–6/group. *Con* control group, *LAA* low amino acids group. **$P < 0.01$ compared with control

maternal protein restriction (Bhasin et al. 2009; Parimi et al. 2004; Rees et al. 2006). BeWo cells have been used to study the effects of amino acid restriction on amino acid transport (Jones et al. 2006), in which DMEM Glutamax 1 was used as control, while the amino acid deprivation medium was a balanced salt solution containing no non-essential, and 50% essential amino acids used in MEM. This may be suitable for investigating the short-term (within 6 h) effects on amino acid transport, but may not be suited for long-term effects. As we needed to maintain the BeWo cells in healthy condition for at least 48 h to investigate the effects of amino acid deficiency on the expression and activity of 11β-HSD2, we chose F12K and F12 media in the present study. The F12K is a standard medium recommended for BeWo cell culture, containing 20 amino acids including the 8 essential amino acids. The formula of F12 is similar to that of F12K in amino acid composition, except that the amino acid concentrations are approximately half of the F12K. No significant difference was observed in the viability of the BeWo cells between control and LAA media over 48 h.

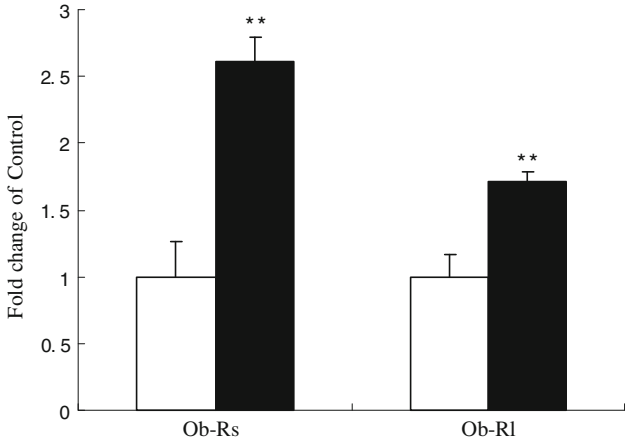

Fig. 4 Expression of Ob-Rs and Ob-Rl mRNA in BeWo cells. Values are mean ± SEM, $n = 5$–6/group. *Con* control group, *LAA* low amino acids group. $^{**}P < 0.01$ compared with control

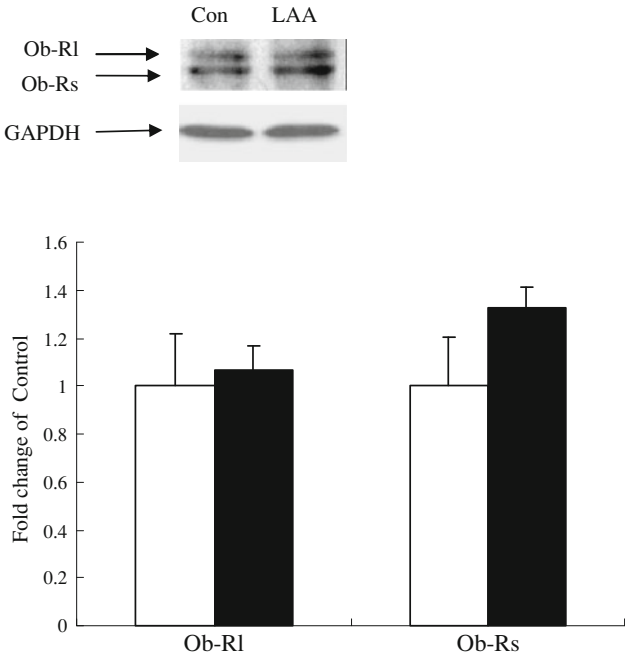

Fig. 5 Expression of Ob-Rl and Ob-RS protein in BeWo cells. Values are mean ± SEM, $n = 6$–8/group. *Con* control group, *LAA* low amino acids group

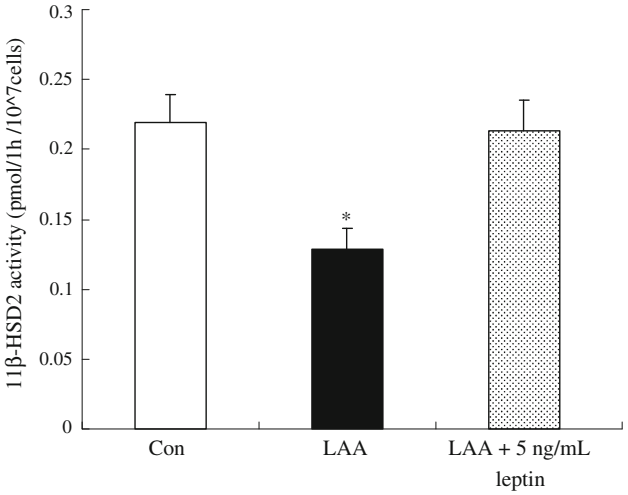

Fig. 6 Leptin restored 11β-HSD2 activity of BeWo cells cultured in LAA medium. Values are mean ± SEM, $n = 10$–12/group. *Con* control group, *LAA* low amino acids group, *LAA + leptin* 5 ng/mL of leptin added to the LAA medium. $^{*}P < 0.05$ compared with control and LAA + leptin groups

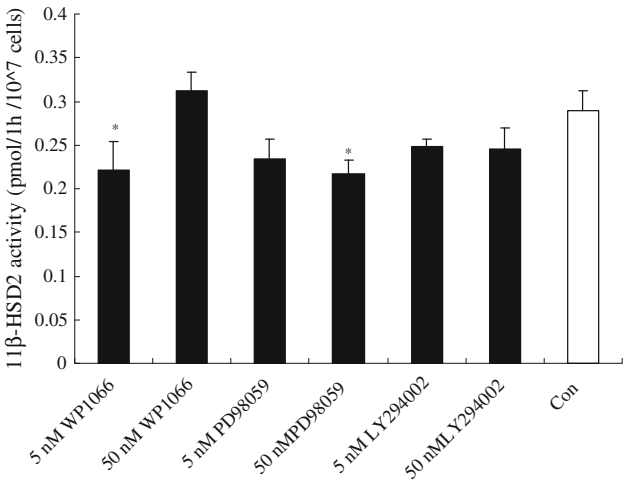

Fig. 7 Leptin signal pathway inhibitors decreased 11β-HSD2 activity of BeWo cells cultured in control medium. Values are mean ± SEM, $n = 10$–12/group. $^{*}P < 0.05$ compared with control

To our knowledge, this is the first study regarding the effect of amino acid restriction on 11β-HSD2 expression and activity in placental cells in vitro. In agreement with the in vivo studies on maternal protein restriction (Langley-Evans et al. 1996; Stocker et al. 2004), we found amino acid restriction significantly decreased 11β-HSD2 protein expression and activity in BeWo cells in vitro. However, opposite changes were observed in 11β-HSD2 mRNA abundance which was significantly increased in LAA group. Recently, microRNAs (miRNAs), small non-coding RNAs of 22 nt in average, are identified to play important roles in post-transcriptional regulation by binding to the complementary sequences of the targeting genes resulting in mRNA cleavage or translational repression (Bartel 2004). The mismatch of mRNA and protein abundances could be induced by miRNA-mediated post-transcriptional regulation. We found a significant up-regulation of miRNA-498, one of the five miRNAs predicted to target 11β-HSD2, in LAA group, indicating a possible role of miRNA-498 in translational repression of 11β-HSD2 mRNA in BeWo cells subjected to amino acid deficiency. The effects of amino acids on miRNA expression have been reported previously in human skeletal muscle (Drummond et al. 2009). Nevertheless, further studies are

required to validate the post-transcriptional regulation and/or translational repression functions of miRNA-498 on 11β-HSD2 under low amino acids challenge.

The present in vitro study demonstrated the direct effects of low amino acids on 11β-HSD2 expression and activity. Despite the fact that in vitro studies allow single factor analysis compared to the in vivo studies in which multiple factors interact, it remains a question whether the effects are elicited directly by amino acids or mediated by the hormones secreted from the BeWo cells per se.

Placenta is lacking enzyme P450c17 and not capable of synthesizing cortisol de novo from cholesterol (Jeschke et al. 2007). However, trophoblasts cells express predominately 11β-HSD2 and are able to convert cortisol to inactive cortisone. Cortisol was detected in both control and LAA media as they contain 10% of FCS. After 48 h of culture, the cortisol concentration in the supernatant was lower than that in the fresh media used as blank control, indicating the role of 11β-HSD2 under basal condition. However, the cortisol concentrations in the supernatant did not show significant difference between two groups, probably because the cortisol in the media was too low to saturate the activity of 11β-HSD2 in BeWo cells under both control and LAA media. The radiometric conversion assay allows more sensitive and reliable determination of 11β-HSD2 activity in BeWo cells.

Maternal protein restriction was reported to decrease maternal serum leptin concentration in rats (Fernandez-Twinn et al. 2003; Jansson et al. 2006). However, it was not possible to clarify which of the major sources of leptin in the pregnant mother, adipose tissue or placenta, or both, are affected. In agreement with the previous reports (Grosfeld et al. 2001; Nuamah et al. 2004; Wyrwoll et al. 2005; Yura et al. 1998), we confirmed that BeWo cells are able to produce and secret leptin. Moreover, we observed, for the first time, reduced leptin secretion from BeWo cells under the challenge of amino acid deficiency.

Stocker et al. (2004) reported that treating the pregnant rats with leptin prevented the reduction in placental 11β-HSD2 activity induced by maternal low protein diet. Consistent with the in vivo finding, leptin supplementation prevented the decrease of 11β-HSD2 activity in LAA medium. BeWo cells express both isoforms of leptin receptor, the long form Ob-Rl and the short form Ob-Rs (Magarinos et al. 2007). Leptin affects placental amino acid transport by activating JAK-STAT signaling pathway (von Versen-Hoynck et al. 2009), while leptin's anti-apoptotic effects on human trophoblastic cells are mediated by MAPK pathway (Perez-Perez et al. 2008). Recently, it was reported that leptin stimulates protein synthesis in the human trophoblastic cells via the activation of MAPK and PI3K pathways (Perez-Perez et al. 2009, 2010). In the present study, WP1066 and PD98059 supplemented to the control medium decreased the 11β-HSD2 activity of BeWo cells, indicating that JAK-STAT and MAPK pathways are involved in mediating the effects of amino acids on 11β-HSD2 activity in BeWo cells.

In conclusion, the present study provides the first evidence that low supply of amino acids reduces leptin secretion from BeWo cells and diminishes 11β-HSD2 activity in BeWo cells through leptin-activated JAK-STAT and MAPK pathways.

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References

Ashworth CJ, Hoggard N, Thomas L, Mercer JG, Wallace JM, Lea RG (2000) Placental leptin. Rev Reprod 5:18–24

Bajoria R, Sooranna SR, Ward BS, Chatterjee R (2002) Prospective function of placental leptin at maternal–fetal interface. Placenta 23:103–115

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Bertram C, Trowern AR, Copin N, Jackson AA, Whorwood CB (2001) The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 2 11beta-hydroxysteroid dehydrogenase: potential molecular mechanisms underlying the programming of hypertension in utero. Endocrinology 142:2841–2853

Bhasin KK, van Nas A, Martin LJ, Davis RC, Devaskar SU, Lusis AJ (2009) Maternal low-protein diet or hypercholesterolemia reduces circulating essential amino acids and leads to intrauterine growth restriction. Diabetes 58:559–566

Brown JR, Sanseau P (2005) A computational view of microRNAs and their targets. Drug Discov Today 10:595–601

Clifton VL, Rennie N, Murphy VE (2006) Effect of inhaled glucocorticoid treatment on placental 11beta-hydroxysteroid dehydrogenase type 2 activity and neonatal birthweight in pregnancies complicated by asthma. Aust N Z J Obstet Gynaecol 46:136–140

Cottrell EC, Seckl JR (2009) Prenatal stress, glucocorticoids and the programming of adult disease. Front Behav Neurosci 3:19

Drummond MJ, Glynn EL, Fry CS, Dhanani S, Volpi E, Rasmussen BB (2009) Essential amino acids increase microRNA-499, -208b, and -23a and downregulate myostatin and myocyte enhancer factor 2C mRNA expression in human skeletal muscle. J Nutr 139:2279–2284

Ebenbichler CF, Kaser S, Laimer M, Wolf HJ, Patsch JR, Illsley NP (2002) Polar expression and phosphorylation of human leptin receptor isoforms in paired, syncytial, microvillous and basal membranes from human term placenta. Placenta 23:516–521

Fernandez-Twinn DS, Ozanne SE, Ekizoglou S, Doherty C, James L, Gusterson B, Hales CN (2003) The maternal endocrine environment in the low-protein model of intra-uterine growth restriction. Br J Nutr 90:815–822

Galiveti CR, Rozhdestvensky TS, Brosius J, Lehrach H, Konthur Z (2010) Application of housekeeping npcRNAs for quantitative expression analysis of human transcriptome by real-time PCR. RNA 16:450–461

Grosfeld A, Turban S, Andre J, Cauzac M, Challier JC, Hauguel-de Mouzon S, Guerre-Millo M (2001) Transcriptional effect of hypoxia on placental leptin. FEBS Lett 502:122–126

Hassink SG, de Lancey E, Sheslow DV, Smith-Kirwin SM, O'Connor DM, Considine RV, Opentanova I, Dostal K, Spear ML, Leef K, Ash M, Spitzer AR, Funanage VL (1997) Placental leptin: an important new growth factor in intrauterine and neonatal development? Pediatrics 100:E1

Jansson T, Powell TL (2007) Role of the placenta in fetal programming: underlying mechanisms and potential interventional approaches. Clin Sci (Lond) 113:1–13

Jansson N, Greenwood SL, Johansson BR, Powell TL, Jansson T (2003) Leptin stimulates the activity of the system A amino acid transporter in human placental villous fragments. J Clin Endocrinol Metab 88:1205–1211

Jansson N, Pettersson J, Haafiz A, Ericsson A, Palmberg I, Tranberg M, Ganapathy V, Powell TL, Jansson T (2006) Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. J Physiol 576:935–946

Jeschke U, Richter DU, Mobius BM, Briese V, Mylonas I, Friese K (2007) Stimulation of progesterone, estradiol and cortisol in trophoblast tumor BeWo cells by glycodelin A N-glycans. Anticancer Res 27:2101–2108

Jones HN, Ashworth CJ, Page KR, McArdle HJ (2006) Expression and adaptive regulation of amino acid transport system A in a placental cell line under amino acid restriction. Reproduction 131:951–960

Kilberg MS, Shan J, Su N (2009) ATF4-dependent transcription mediates signaling of amino acid limitation. Trends Endocrinol Metab 20:436–443

Kwon H, Ford SP, Bazer FW, Spencer TE, Nathanielsz PW, Nijland MJ, Hess BW, Wu G (2004) Maternal nutrient restriction reduces concentrations of amino acids and polyamines in ovine maternal and fetal plasma and fetal fluids. Biol Reprod 71:901–908

Langley-Evans SC, Phillips GJ, Benediktsson R, Gardner DS, Edwards CR, Jackson AA, Seckl JR (1996) Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. Placenta 17:169–172

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408

Lynch CJ, Gern B, Lloyd C, Hutson SM, Eicher R, Vary TC (2006) Leucine in food mediates some of the postprandial rise in plasma leptin concentrations. Am J Physiol Endocrinol Metab 291:E621–E630

Ma XH, Wu WX, Nathanielsz PW (2003) Gestation-related and betamethasone-induced changes in 11beta-hydroxysteroid dehydrogenase types 1 and 2 in the baboon placenta. Am J Obstet Gynecol 188:13–21

Magarinos MP, Sanchez-Margalet V, Kotler M, Calvo JC, Varone CL (2007) Leptin promotes cell proliferation and survival of trophoblastic cells. Biol Reprod 76:203–210

Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K (1997) Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med 3:1029–1033

Meissner U, Spranger R, Lehner M, Allabauer I, Rascher W, Dotsch J (2005) Hypoxia-induced leptin production in human trophoblasts does not protect from apoptosis. Eur J Endocrinol 153:455–461

Murthi P, Fitzpatrick E, Borg AJ, Donath S, Brennecke SP, Kalionis B (2008) GAPDH, 18S rRNA and YWHAZ are suitable endogenous reference genes for relative gene expression studies in placental tissues from human idiopathic fetal growth restriction. Placenta 29:798–801

Myren M, Mose T, Mathiesen L, Knudsen LE (2007) The human placenta—an alternative for studying foetal exposure. Toxicol In Vitro 21:1332–1340

Nuamah MA, Yura S, Sagawa N, Itoh H, Mise H, Korita D, Kakui K, Takemura M, Ogawa Y, Nakao K, Fujii S (2004) Significant increase in maternal plasma leptin concentration in induced delivery: a possible contribution of pro-inflammatory cytokines to placental leptin secretion. Endocrinol J 51:177–187

Parimi PS, Cripe-Mamie C, Kalhan SC (2004) Metabolic responses to protein restriction during pregnancy in rat and translation initiation factors in the mother and fetus. Pediatr Res 56:423–431

Perez-Perez A, Maymo J, Duenas JL, Goberna R, Calvo JC, Varone C, Sanchez-Margalet V (2008) Leptin prevents apoptosis of trophoblastic cells by activation of MAPK pathway. Arch Biochem Biophys 477:390–395

Perez-Perez A, Maymo J, Gambino Y, Duenas JL, Goberna R, Varone C, Sanchez-Margalet V (2009) Leptin stimulates protein synthesis-activating translation machinery in human trophoblastic cells. Biol Reprod 81:826–832

Perez-Perez A, Gambino Y, Maymo J, Goberna R, Fabiani F, Varone C, Sanchez-Margalet V (2010) MAPK and PI3K activities are required for leptin stimulation of protein synthesis in human trophoblastic cells. Biochem Biophys Res Commun 396:956–960

Rajewsky N (2006) microRNA target predictions in animals. Nat Genet 38(Suppl):S8–S13

Rees WD, Hay SM, Buchan V, Antipatis C, Palmer RM (1999) The effects of maternal protein restriction on the growth of the rat fetus and its amino acid supply. Br J Nutr 81:243–250

Rees WD, Hay SM, Antipatis C (2006) The effect of dietary protein on the amino acid supply and threonine metabolism in the pregnant rat. Reprod Nutr Dev 46:227–239

Reynolds RM (2010) Corticosteroid-mediated programming and the pathogenesis of obesity and diabetes. J Steroid Biochem Mol Biol 122:3–9

Roh C, Han J, Tzatsos A, Kandror KV (2003) Nutrient-sensing mTOR-mediated pathway regulates leptin production in isolated rat adipocytes. Am J Physiol Endocrinol Metab 284:E322–E330

Sarkar S, Tsai SW, Nguyen TT, Plevyak M, Padbury JF, Rubin LP (2001) Inhibition of placental 11beta-hydroxysteroid dehydrogenase type 2 by catecholamines via alpha-adrenergic signaling. Am J Physiol Regul Integr Comp Physiol 281:R1966–R1974

Sato K, Chisaka H, Okamura K, Challis JR (2008) Effect of the interaction between lipoxygenase pathway and progesterone on the regulation of hydroxysteroid 11-beta dehydrogenase 2 in cultured human term placental trophoblasts. Biol Reprod 78:514–520

Shi R, Chiang VL (2005) Facile means for quantifying microRNA expression by real-time PCR. Biotechniques 39:519–525

Stocker C, O'Dowd J, Morton NM, Wargent E, Sennitt MV, Hislop D, Glund S, Seckl JR, Arch JR, Cawthorne MA (2004) Modulation of susceptibility to weight gain and insulin resistance in low birthweight rats by treatment of their mothers with leptin during pregnancy and lactation. Int J Obes Relat Metab Disord 28:129–136

Tremblay J, Hardy DB, Pereira LE, Yang K (1999) Retinoic acid stimulates the expression of 11beta-hydroxysteroid dehydrogenase type 2 in human choriocarcinoma JEG-3 cells. Biol Reprod 60:541–545

van Beek JP, Guan H, Julan L, Yang K (2004) Glucocorticoids stimulate the expression of 11beta-hydroxysteroid dehydrogenase type 2 in cultured human placental trophoblast cells. J Clin Endocrinol Metab 89:5614–5621

von Versen-Hoynck F, Rajakumar A, Parrott MS, Powers RW (2009) Leptin affects system A amino acid transport activity in the human placenta: evidence for STAT3 dependent mechanisms. Placenta 30:361–367

Wyrwoll CS, Mark PJ, Waddell BJ (2005) Directional secretion and transport of leptin and expression of leptin receptor isoforms in human placental BeWo cells. Mol Cell Endocrinol 241:73–79

Yuan L, Ni Y, Barth S, Wang Y, Grossmann R, Zhao R (2009) Layer and broiler chicks exhibit similar hypothalamic expression of orexigenic neuropeptides but distinct expression of genes related to energy homeostasis and obesity. Brain Res 1273:18–28

Yura S, Sagawa N, Ogawa Y, Masuzaki H, Mise H, Matsumoto T, Ebihara K, Fujii S, Nakao K (1998) Augmentation of leptin synthesis and secretion through activation of protein kinases A and C in cultured human trophoblastic cells. J Clin Endocrinol Metab 83:3609–3614