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ORIGINAL ARTICLE

Brain-derived neurotrophic factor is expressed in rat and human placenta and its serum levels are similarly regulated throughout pregnancy in both species

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Summary

Objective Pregnancy is characterized by several metabolic changes that promote fat gain and later onset of insulin resistance. As Brain-derived neurotrophic factor (BDNF) decreases hyperglycaemia and hyperphagia, we aimed to investigate the potential role of placental and circulating BDNF levels in these pregnancy-related metabolic changes in rats and humans.

Design and methods We identified the mRNA and protein expression of placental BDNF and its receptor TrkB using real-time PCR, Western blot and immunohistochemical approaches in both rat and humans. Serum BDNF was measured by ELISA. We also did a longitudinal prospective cohort study in 42 pregnant women to assess BDNF levels and correlations with other metabolic parameters.

Results We found that BDNF and TrkB are expressed in both rat and human placenta. In rat, both placental mRNA and serum levels are increased throughout pregnancy, whereas their protein levels are significantly decreased at the end of gestation. Serum BDNF levels in pregnant women are significantly lower in the first trimester when compared to the second and third trimester ($P < 0{\cdot}0148$, $P < 0{\cdot}0012$, respectively). Serum BDNF levels were negatively correlated with gestational age at birth and fasting glucose levels.

Conclusion Our findings suggest that both BDNF and its receptor TrkB are expressed in rodent and human placenta being regulated during pregnancy. Taken together, these findings support a role of BDNF in the regulation of several metabolic functions during pregnancy.

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Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the family of neurotrophins, which are secretory proteins identified as factors for survival of sympathetic and sensory neurons, synapse formation and plasticity.[1] The biological actions of these neurotrophins are mediated through binding and activation of different members of the tropomyosin-related kinase (Trk) family of tyrosine kinase receptors (TrkA, TrkB and TrkC) and the p75 neurotrophin receptor ($p75^{NTR}$), a member of the tumour necrosis factor receptor superfamily.[1,2] The ligand BDNF binds preferentially to TrkB, while all neurotrophins bind to $p75^{NTR}$.[2]

Brain-derived neurotrophic factor expression has been found in several brain regions related to the homoeostatic regulation of eating behaviour and energy balance control, including the hypothalamus, cortex, hippocampus, amygdala, nucleus of the solitary tract (NTS) and substantia nigra.[3] The TrkB receptor has been also located in these regions of the central nervous system.[4] Additionally, BDNF and TrkB are also expressed in peripheral organs including human placental.[1] BDNF was immunolocalized in the membranous chorion, trophoblast layer and endothelium, whereas TrkB was immunolocalized in the decidua, trophoblast layer and endothelium.[1]

Brain-derived neurotrophic factor has important effects on energy homoeostasis.[5,6] Central and peripheral administration of BDNF decreases appetite, increases energy expenditure, reduces body weight and helps to improve the hyperinsulinaemic and hyperglycaemic conditions in db/db diabetic mice.[3] Genetic mouse models corroborated those pharmacological results and found

that both total[7,8] and selective[9,10] BDNF deletion increase food intake and body weight. Accordingly, the endogenous disruption of TrkB attenuates obesity in BDNF haploinsufficient mice.[11]

Human studies have confirmed preclinical data and linked common single nucleotide polymorphisms in the human *BDNF* gene[12–14] or a mutation in the *TrkB*[15] to hyperphagia and obesity. Moreover, it has also been shown that low circulating levels of BDNF are associated with intake disorders such as anorexia nervosa and bulimia nervosa.[16] Different human studies have also assessed the role of the BDNF/TrkB system throughout gestation. BDNF levels are significantly elevated in patients with pre-eclampsia (PE) and intrauterine growth restriction (IUGR) when compared to the corresponding healthy controls.[1,17] Furthermore, the ratio of maternal/foetal BDNF levels is altered in preterm deliveries with the maternal levels being higher compared with levels in the umbilical cord.[18] The same study showed that placental levels of the TrkB receptor are significantly higher in the case of preterm deliveries. It has also been reported that BDNF levels significantly decrease in maternal serum before and after giving birth.[19] Finally, studies have shown that BDNF plasma levels do not vary during the menstrual cycle.[20] However, their conclusions are limited because of their limited sample size and/or that they were based on transversal studies. This later issue is particularly relevant in studies in human pregnancy because factors such as the socioeconomic status may greatly influence pregnancy progression and outcome.

Based on these findings, we hypothesized that variations in serum levels and BDNF expression in the placenta could contribute to the metabolic regulation in maternal foetal unit during gestation. Thus, in this study, we used molecular and immunological approaches in both rat animal models and humans to determine the expression of BDNF and its receptor in placenta and the regulation of circulating BDNF throughout gestation. Furthermore, to avoid confounding factors, the human study was carried out in a prospective fashion with a longitudinal design.

Materials and methods

Human subjects

A group of normal pregnant women were recruited for a longitudinal prospective cohort, from a low-risk obstetric hospital, Engativá Hospital, associated with the Department of Obstetrics and Gynecology, Universidad Nacional de Colombia in Bogotá. All the women gave written informed consent for participation in the study. The School of Medicine Ethics Committee of the Universidad Nacional approved the study, which was in accordance with the Declaration of Helsinki. The inclusion criterion was normal pregnancy. The patient was included in the early gestational periods between the 10th and 14th week. The gestational ages were determined by a first trimester ultrasound. Exclusion criteria were history of diabetes mellitus, gestational diabetes mellitus, vascular disease, chronic hypertension, renal disease or polycystic ovary syndrome. The nonpregnant women who participated in the study were recruited during the same time period, and they were healthy fertile women.

A total of 42 women delivering at term (37- to 40·5-week gestation), with no medical or obstetrical complications, were studied during early, middle and late pregnancy (11·3th–13·4th, 24th–26·3th and 34th–37·3th weeks), during 2012–2013. For comparison, 16 healthy eumenorrheic women were included in this study, and their serum levels of BDNF were measured during both the follicular (cycle day 4 ± 1) and luteal phase of the menstrual cycle (cycle day 22 ± 1). Menstrual cycle length was determined from the first day of menstrual bleeding to the day before the next bleeding period.

Assays

A venous blood sample was obtained from each woman after an overnight fast; the serum was separated by centrifugation from the clot and stored at −80 °C until BDNF and biochemical analysis. Serum glucose, triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol levels were analysed using enzymatic methods (Spinreact, Naucalpan, Mexico). Additionally, serum insulin levels were measured using chemiluminescent immunoassay (LIAISON® Analyzer, Dia Sorin S.p.A., Saluggia, Italy). Serum progesterone levels of all eumenorrheic fertile women were measured using Roche Elecsys Progesterone Diagnostics Kit (Roche Elecsys 1010 Immunoanalyzer, Boulder, CO, USA) using the methodology previously reported.[21] Homoeostatic model assessment (HOMA) index was calculated as previously described.[21]

Laboratory Animal Models and Strains

Wistar rats (bred in the *Animalario General* USC – Santiago de Compostela, Spain) were housed in a room with controlled conditions for illumination (12-/12-h light/dark cycle), humidity and temperature. Rats were given *ad libitum* access to food and water. Animal studies were reviewed and approved by the Ethics Committee of the University of Santiago de Compostela in accordance with institutional guidelines and the European Union regulation for the care and use of experimental animals.

Experimental setting

Expression analysis of BDNF and TrkB receptor in rat placenta. Brain-derived neurotrophic factor and TrkB receptor mRNA expression in placenta were studied during pregnancy, according to methods described elsewhere.[22] Four groups of rats age matched (12–14 weeks) were randomly assigned to different experimental groups (n = 10 rats/group): (i) three groups of rats, that were sacrificed on gestational days 12, 16 and 21, respectively and (ii) a group of virgin rats (n = 10 rats), that was used as a control group. Animals received food and water *ad libitum* and were sacrificed at 9:00–10:00 in the morning. Virgin rats were sacrificed in parallel with groups of different gestational ages.

Real-time PCR analysis of BDNF and TrkB receptor in placenta. Total RNA from rat tissues was extracted using TRIzol® Plus RNA Purification Kit reagent according to the

manufacturer's instructions (Cat. # 12183-555.; Life Technology, Abcam®, Cambridge, MA, USA). For all samples analysed, 1·5 μg of RNA was reverse-transcribed into cDNA, and the resulting cDNA was used as the template for PCR real-time semi-quantitative analysis using the Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA), as described elsewhere.[22] Rat BDNF and TrkB receptor cDNA expression quantification was performed using TaqMan® Gene Expression Assays Rn01484924_m1 (Cat. # 4331182) and Rn01441749_m1 (Cat. # 4331182), respectively, and hypoxanthine phosphoribosyltransferase (HPRT) was used as the reference gene. Data were normalized by using the $\Delta\Delta C_t$ method as described elsewhere.[23]

Serum analysis of BDNF throughout pregnancy in human and rat. Circulating human BDNF levels were measured using a commercial ELISA Kit, according to the manufacturer's instructions (Cat. # ab99978.; Abcam®). The detection limit of the human BDNF ELISA assay was 80 pg/ml, while the intra-assay and interassay, coefficients of variation were between <10% and <12%, respectively.

In addition, rat BDNF serum levels were determined in duplicate with the commercially available Sandwich ELISA Kit according to the recommended protocols (ChemiKineTM, Cat. # CYT306; Merck Millipore, Billerica, MA, USA). This assay is specific for rat, and the results are expressed in terms of pg/ml. The detection range of rat BDNF is between 7·8 and 500 pg/ml, and the intra-assay and interassay coefficients of variation were ±3·7% and ± 8·5%, respectively.

Immunochemistry of BDNF and TrkB receptor in human and rat placenta. Immunostaining for BDNF and its receptor, TrkB, was performed in human placenta, white adipose tissue and skeletal muscle. Placental samples obtained were from patients at weeks 11, 24 (abortion material) and 38 (term gestation) of gestation. These tissues were obtained from the Pathology Department Services of the School of Medicine at the Universidad Nacional de Colombia. Additionally, both BDNF and TrkB receptor were immunostained in rat placenta at days 12, 16 and 21 of gestation. Tissue block was fixed in 10% neutral buffered formalin, paraffin embedded and immunostaining using a method described elsewhere.[24] Polyclonal rabbit anti-BDNF antibodies (Abcam® – Anti-BDNF antibody – ab6201) and rabbit anti-TrkB antibodies (LS-B8691 IHC-plus™) were employed for the immunostaining, and these antibodies showed reactivity in both human and rat tissues. With respect to the negative control of the technique, there was no incubation with rabbit polyclonal antibodies to BDNF, while the other reagents were applied and the rest of immunostaining process carried out.

Western blot analysis of BDNF and TrkB receptor in rat placenta. Western blots were performed as previously described.[25] Briefly, total protein lysates from placenta (20 μg) were subjected to SDS-PAGE, electrotransferred onto a polyvinylidene difluoride membrane and probed with the indicated antibodies for anti-BDNF and anti-TrkB. For protein detection, we used horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (Amersham Biosciences, Little Chalfont, UK). Six placentas per group were used, and the protein levels were normalized to β-actin for each sample.

Statistical analysis

The data were analysed using Stata 11.0 software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX, USA; StatCorp LP). Data distribution was normal according to the Shapiro–Wilk test for normality. The results are shown as means ± SD (standard deviation from the mean). Categorical data are presented as proportions. Statistical significance was determined by Student's t-test when there was a comparison of two groups or ANOVA when there were more than two groups compared, and Bonferroni *post hoc* test. Correlations using Pearson's correlation coefficient were performed. For all tests, a P value of $<0{\cdot}05$ was considered statistically significant.

Results

Immunochemistry of BDNF and TrkB in rat placenta

Immunoreactivity for BDNF and TrkB in rat placenta was studied in placentas at days 12, 16 and 21 of gestation. An intense immunoreactivity for BDNF was observed in decidual cells as well as in the labyrinth portion of the chorionic plate in placentas at 12 days of gestation; at the same location, moderate immunoreactivity was observed in placenta at day 16 and mild immunoreactivity in placenta at day 21 of gestation (Fig. 1a,c and d, respectively). Furthermore, the study shows intense immunoreactivity for TrkB in decidual cells and in the labyrinth portion in chorionic plate in placentas at days 12 and 16, while by day 21, faint immunoreactivity was observed in the same location (Fig. 1b,d and f, respectively).

Gene expression and protein levels of BDNF and TrkB receptor in placenta throughout rat pregnancy

Our findings demonstrate that placental BDNF mRNA expression increased with advancing gestation, being its expression lowest at day 12 of gestation and highest by day 21 of gestation. There was a significant increase when comparing the levels of day 12 of pregnant rats to days 16 and 21 ($P < 0{\cdot}001$, $P < 0{\cdot}001$, respectively), and a significant difference was also found in the BDNF expression when comparing day 16 to day 21 ($P < 0{\cdot}05$) (Fig. 2a). Regarding TrkB expression analysis in rat placenta, a similar profile to BDNF was seen with a rise in its expression as pregnancy progresses, a expression profile similar to previously studied hormones, such as leptin and prolactin and their respective receptors.[24,26,27] There expression was lowest by day 12 of gestation, and there was an increase by day 16 of gestation, which was maintained to day 21 ($P < 0{\cdot}001$ in both cases) (Fig. 2b). Contrary to gene expression, both BDNF and TrKB protein levels were up-regulated in placentas on day 12 of gestation in comparison with placentas on day 21 of gestation (Fig. 2c).

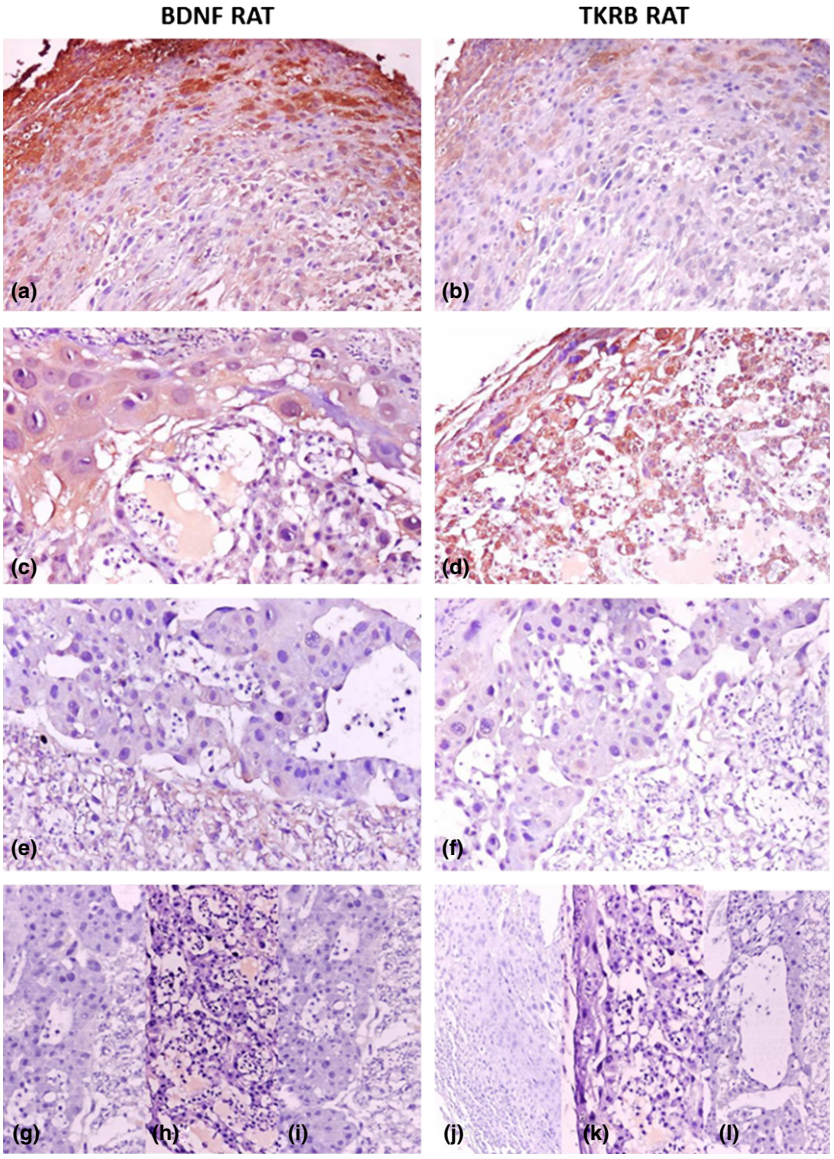

Fig. 1 Immunoreactivity for BDNF and TrkB in rat placenta throughout pregnancy. (a, c and e) correspond to placentas studied at 12, 16 and 21 days, respectively. Intense immunoreactivity for BDNF was observed in decidual cells and in the labyrinthine portion of the chorionic plate by day 12 of gestation, and moderate immunoreactivity was observed by day 16 and faint immunoreactivity by day 21 of gestation in the same location. (b, d and f) correspond to rat placentas of the same gestational ages as above. Intense immunoreactivity for TrkB was observed in decidual cells and the labyrinthine portion at days 12 and 16 of gestation, while by day 21, faint immunoreactivity was observed. (g–l) are the negative controls of the technique, with omission of primary antibody, where (g and j) are rat placentas at day 12, (h and k) are rat placentas at day 16, and (i and l) are rat placentas at day 21 of gestation.

Changes in serum levels of BDNF during rat pregnancy

We next assessed serum BDNF levels in virgin and pregnant rats fed *ad libitum*. The lowest serum BDNF levels were detected at day 12 of gestation when compared with the virgin control group ($P = 0{\cdot}0012$) (Fig. 2d). Serum levels of BDNF are not significantly different between days 16 and 21 of gestation when compared to the virgin rat control ($P > 0{\cdot}05$). Although BDNF levels are low on day 12 of gestation, it is not significantly different when compared to the days 16 and 21 of gestation ($P > 0{\cdot}05$) (Fig. 2d).

Patient characteristics and laboratory parameters

Once we characterized the location and regulation of BDNF in placenta and serum in rodents, we next wanted to investigate the relevance of these results in humans using a similar experimental design, namely evaluation of BDNF levels at different stages of pregnancy. The demographic and biochemical variables of the pregnant women and healthy eumenorrheic women are shown in Table 1. The biochemical parameters had the characteristic behaviour of the physiology of pregnancy. Insulin, total cholesterol, HDL, LDL and triglyceride levels showed significant differences between the two groups.

Immunochemistry of BDNF and TrkB in human placenta

We used human placental samples at 11-week gestation (obtained from abortions), 24-week gestation and 38–week gestation. At 11 weeks, moderate and intense BDNF immunoreactivities were observed in syncytiotrophoblasts, cytotrophoblasts and Hofbauer cells. Furthermore, moderate and intense immunoreactivities were also obtained in the cytoplasm of decidual cells (Fig. 3a). With regard to the immunoreactivity of TrkB during this period of gestation, moderate immunoreactivity was observed in syncytiotrophoblastic cells, while faint immunoreactivity was observed in cytotrophoblasts and Hofbauer cells. Moreover, in decidual

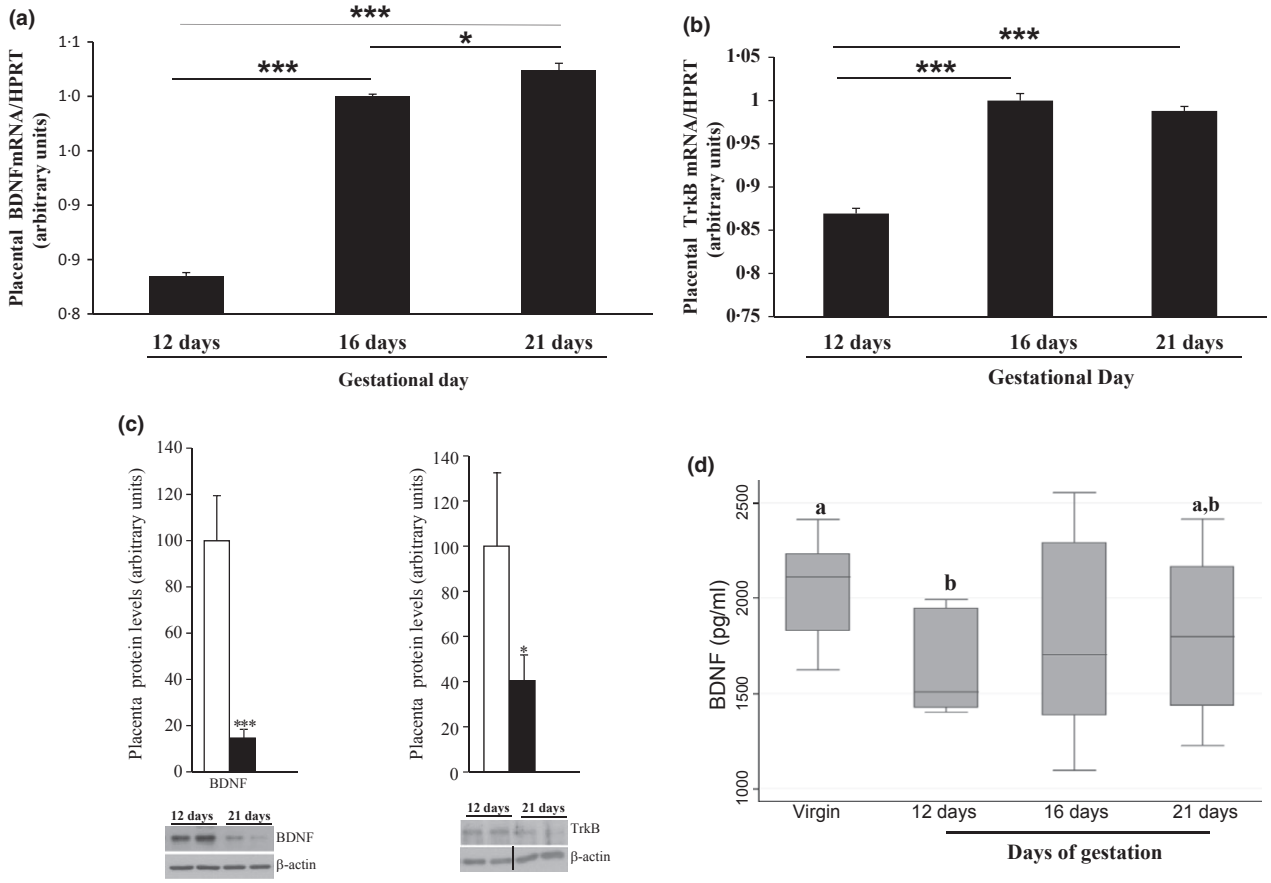

Fig. 2 Analysis of the relative expression of BDNF and TrkB in rat placenta. Differences in BDNF expression were observed between the groups at day 12 when compared against the groups at days 16 and 21 of pregnancy ($P < 0{\cdot}001$ and $P < 0{\cdot}001$) and when comparing day 16 to day 21 ($P < 0{\cdot}05$). (a) Additionally, relative expression of TrkB in the same gestational periods analysed and showed statistically significant differences when comparing the groups at day 12 against groups at days 16 and 21 of gestation ($P < 0{\cdot}05$) (b). Analysis of serum BDNF levels at days 12, 16 and 21 of gestation ($n = 10$/group). (c) Western blot of BDNF and TrkB, BDNF and TrKB protein levels were up-regulated in placentas on day 12 of gestation in comparison with placentas on day 21 of gestation. (d) Each group was compared with the control group of virgin rats ($n = 10$). Values overwritten with different letters are significantly different from each other. All asterisks indicate $P < 0.05$, which was considered statistically significant.

cells, moderate and intense immunoreactivities were observed similar to the results described for BDNF (Fig. 3b). Additionally, immunoreactivity was observed in Hofbauer cells and decidua. It is important to note that the samples studied in this trial were run in duplicate and similar results were found.

In the study of human placentas of 24- and 38-week gestation, moderate and faint BDNF immunoreactivities were observed, respectively, in the cytoplasm of decidual cells as well as in syncytiotrophoblastic cells and cytotrophoblast cells (Fig. 3c,e). Moreover, in these gestational periods, moderate immunoreactivity was observed for TrkB in syncytiotrophoblast, cytotrophoblast and decidual cells (Fig. 3d,f). Figure 3g,h and i show immunoreactivity of negative controls for the BDNF immunohistochemistry technique, and Fig. 3j,k and l show the negative controls of immunoreactivity for TrkB.

Changes in serum levels of BDNF during human pregnancy

Analysis of serum BDNF levels in the group of eumenorrheic healthy women in the follicular and luteal phase of the menstrual cycle did not show statistically significant differences ($P > 0{\cdot}05$) (Supplementary Fig. S1) (Table 1). When comparing circulating levels of BDNF in the group of pregnant women during early, middle and late pregnancy, there was a statistically significant decrease in comparison with the eumenorrheic healthy control group ($P = 0{\cdot}00$, $P = 0{\cdot}0058$ and $P = 0{\cdot}0081$, respectively) (Fig. 4). Throughout gestation, there was also a variation in the circulating levels of BDNF. A marked decrease was identified towards weeks 11–13 (early pregnancy), while at weeks 24–26 (middle pregnancy), there was a significantly increase which was maintained towards 34–37 weeks (late pregnancy) ($P < 0{\cdot}0148$, $P < 0{\cdot}0012$, respectively) (Fig. 4).

Using Pearson's correlation univariate analysis, a negative correlation was observed between serum levels of BDNF at 24–26 weeks of gestation and the gestational age at birth (middle pregnancy, $r = -0{\cdot}3220$, $P = 0{\cdot}0400$) (Fig. 5a). Moreover, a negative correlation between serum levels of BDNF and fasting glucose levels was found ($r = -0{\cdot}1674$, $P = 0{\cdot}0465$) (Fig. 5b). No significant correlations were found between serum levels of BDNF and other anthropometric and biochemical variables analysed (Table S1) (Fig. 5c–f).

Table 1. Demographic and biochemical parameters and BDNF levels of the studied women

Variables	Nonpregnant (n = 16) Eumenorrheic (a)	Pregnant women (n = 42) Early pregnancy (b)	Middle pregnancy (c)	Late pregnancy (d)	P
Age, years (mean ± SD)	23·06 ± 4·12	23·76 ± 6·5			NA
Body mass index, kg/m^2 (mean ± SD)	21·5 ± 2·11	22·21 ± 2·39	24·16 ± 2·15	26·34 ± 2·39	NA
Gestational age, weeks (mean ± SD)		11·69 ± 1·8, 1·3–13·4	24·79 ± 0·65, 24–26·3	34·95 ± 0·92, 34–37·3	NA
Gestational age at delivery, weeks (mean ± SD)				39·25 ± 0·86, 37–40·5	NA
Mode of delivery,%					
Vaginal				73·17%	NA
Caesarean				26·83%	NA
Glucose mg/dl (mean ± SD)	81 ± 5·97	80·55 ± 8·85	73·43 ± 6·43	74·21 ± 6·57	$0·0007^{a,d}$ $0·0004^{b,d}$ $0·0001^{a,c}$ $0·0001^{b,c}$
Insulin UI/ml (mean ± SD)	7·8 ± 5·63	7·68 ± 4·31	10·67 ± 4·36	12·29 ± 4·96	$0·0044^{a,d}$ $0·0000^{b,d}$ $0·0437^{a,c}$ $0·0022^{b,c}$
HOMA (mean ± SD)	1·62 ± 1·31	1·57 ± 0·97	1·95 ± 0·82	2·3 ± 1·04	$0·0432^{a,d}$ $0·0013^{b,d}$
Total cholesterol mg/dl (mean ± SD)	165 ± 22·57	160·74 ± 34·83	218·73 ± 40·94	241·61 ± 35·01	$0·0000^{a,d}$ $0·0000^{b,d}$ $0·0073^{c,d}$ $0·0000^{a,c}$ $0·0000^{b,c}$
HDL cholesterol md/dl (mean ± SD)	44·07 ± 8·46	53·81 ± 12·62	59·83 ± 11·61	58·59 ± 10·43	$0·0000^{a,d}$ $0·0062^{a,b}$ $0·0000^{a,c}$ $0·0255^{b,c}$
LDL cholesterol md/dl (mean ± SD)	112·62 ± 16·42	90·06 ± 28	148·07 ± 44·13	173·72 ± 42·29	$0·0000^{a,d}$ $0·0000^{b,d}$ $0·0080^{c,d}$ $0·0038^{a,b}$ $0·0029^{a,c}$ $0·0000^{b,c}$
Triglycerides md/dl (mean ± SD)	71·83 ± 19·73	96·5 ± 33·19	152·78 ± 47·66	204·94 ± 60·68	$0·0000^{a,d}$ $0·0000^{b,d}$ $0·0000^{c,d}$ $0·0073^{a,b}$ $0·0000^{a,c}$ $0·0000^{b,c}$
BDNF ng/ml (mean ± SD) Follicular Luteal	31·690 ± 8·10 33·1 ± 6·96				>0·05
BDNF ng/ml (mean ± SD)	31·69 ± 8·1	19·36 ± 7·45	23·99 ± 9·48	25·1 ± 8·2	$0·0081^{a,d}$ $0·0012^{b,d}$ $0·0000^{a,b}$ $0·0058^{a,c}$ $0·0148^{b,c}$

Normally distributed data are listed as mean (SD). A P value of <0·05 was considered statistically significant, with superscript letters indicating which columns were compared. Values > 0·05 are not shown in this table.

Discussion

In the present study, BDNF and its receptor have been unambiguously identified in human and rat placentas. The identification of both factors has been accomplished utilizing molecular and immunological approaches. With immunohistochemistry, we were able to demonstrate that BDNF and TrkB seem to be specifically related to some functions because their expression was localized to only some cells of the placenta. Furthermore, we show that serum BDNF presents a similar profile during

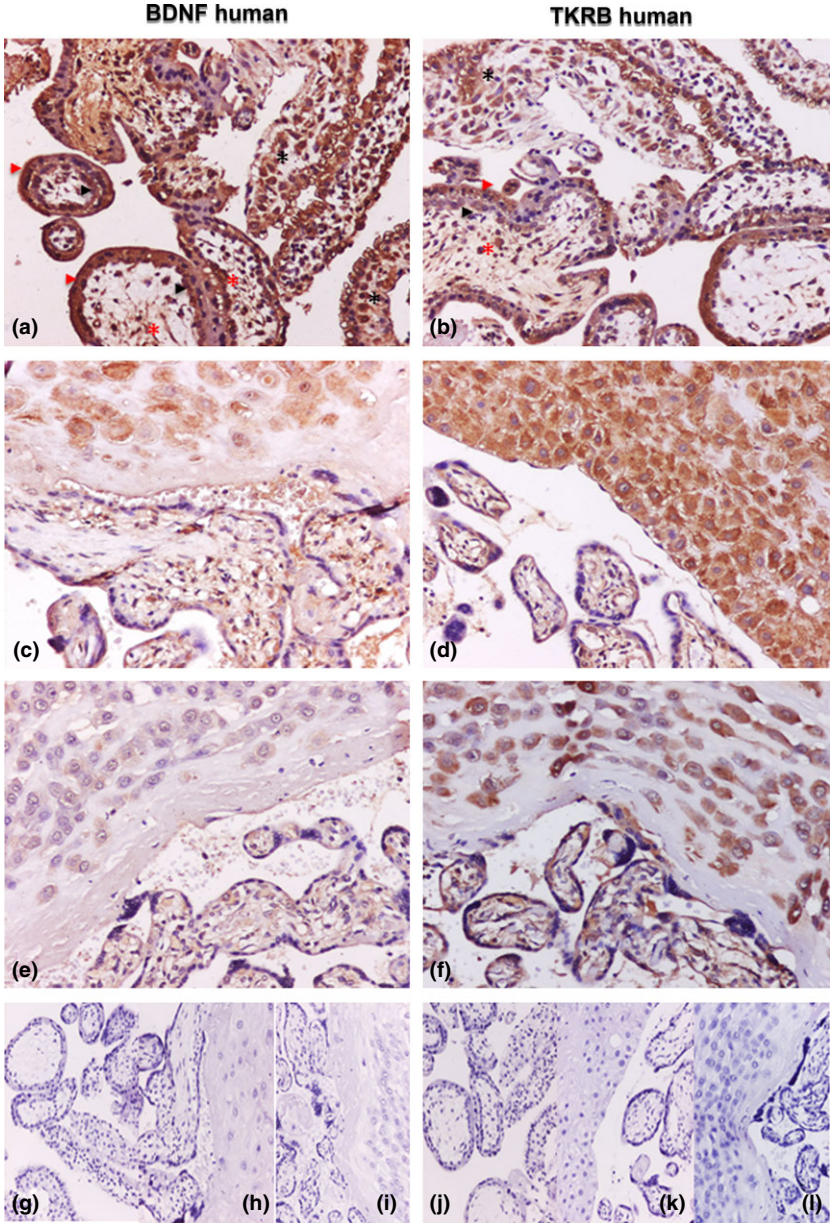

Fig. 3 Immunostaining of BDNF and TrkB in human placenta in the three trimesters of pregnancy. (a) Moderate and intense immunoreactivities were observed for BDNF in syncytiotrophoblastic cells (red arrows), cytotrophoblast (black arrows), Hofbauer cells (red asterisks) and decidual cells (black asterisks) and correspond to placenta at 11 weeks of gestation (abortion material). (b) Moderate immunoreactivity of TrkB was observed in syncytiotrophoblast cells (red arrows) and faint immunoreactivity in cytotrophoblasts (black arrows), whereas Hofbauer cells (red asterisks) showed moderate or intense immunoreactivity. (c and e) correspond to placenta at 24 (abortion material) and 38 (term gestation) weeks of gestation. In these samples, moderate and faint BDNF immunoreactivities in decidual cells, as well as in syncytiotrophoblast and cytotrophoblast cells, were observed. (d and f) correspond to the TrkB immunostaining in human placentas at 24 and 38 weeks of gestation where moderate immunoreactivity was observed in syncytiotrophoblast, cytotrophoblasts and decidual cells. (g–l) correspond to the negative controls of the technique for the study of BDNF and TrkB immunoreactivities, with omission of primary antibody. (g and j) human placenta at 11 weeks of gestation. (h and k) human placenta at 24 weeks of gestation. (i and l) human placenta at 38 weeks of gestation.

gestation in both rats and humans. Of interest, we found a correlation between serum BDNF and gestational age at birth and fasting glucose levels.

Both human and rodent pregnancies are characterized by several metabolic changes that promote adipose tissue accretion in early gestation and later onset of insulin resistance. In rats, from day 10 of pregnancy, glucose-stimulated insulin secretion[28] and food intake[29] are markedly increased, whereas the sensitivity of maternal tissues to insulin decreases.[26] As BDNF decreases hyperglycaemia[30–32] and hyperphagia,[7] we hypothesized that placental and circulating BDNF levels might be involved in these pregnancy-related metabolic changes. Interestingly, we found the lowest serum BDNF levels at day 12, which might reflect the increased glucose-stimulated insulin secretion and food intake at this time of the gestation, and therefore, may contribute to meeting the maternal metabolic requirements during early gestation. Intriguingly, placental BDNF and TrKB gene expression and protein levels showed opposite profiles. Both placental BDNF and TrKB mRNA expression were lower at day 12, whereas protein levels showed higher levels at this time point when compared to placentas at day 21. Unexpectedly, after 12 days of gestation, serum BDNF levels were increased suggesting that BDNF does not modulate food intake or glucose levels in late pregnancy. The present study demonstrates that the post-transcriptional regulation is not co-directional with transcriptional regulation in placenta with respect to BDNF and its receptor, a phenomenon that has been observed in studies of other genes.[33]

To study in humans our findings and the relevance of rodent data, we next performed a longitudinal study to investigate the regulation of placental and circulating BDNF throughout pregnant women. The BDNF/TrkB signalling system has been localized in utero foetal tissue in the placenta, suggesting transient

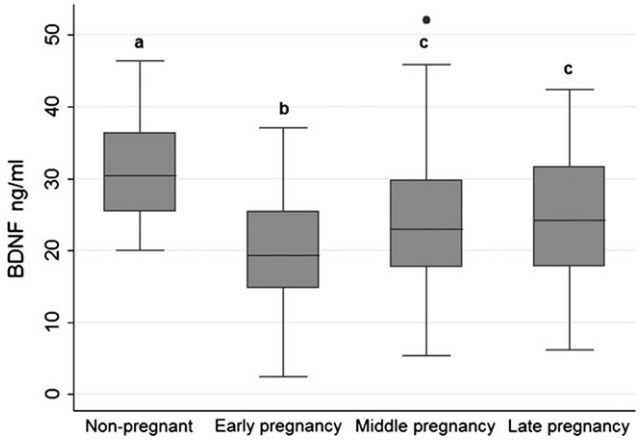

Fig. 4 Analysis of serum BDNF levels during the three periods of gestation in the group of pregnant women ($n = 42$) and a group of nonpregnant women ($n = 16$). Values overwritten with different letters are significantly different from each other.

action of the ligand in these tissues throughout pregnancy.[34] This system has been also detected in the foetal circulation and their levels are high in central and peripheral foetal organs, suggesting that BDNF may act as a nutritional sensor for development efficiency of the placenta and foetal development control.[34] Furthermore, human BDNF levels were significantly elevated in the umbilical cord with increasing gestational age, reflecting the degree of neural maturity in premature infants.[35] In our study, we demonstrate that BDNF is located in human placenta and its placental mRNA expression and serum BDNF profile are identical to the one observed in rats, with lowest levels in the first part of gestation and a decrease thereafter. The placental BDNF/TrkB signalling system has been demonstrated to have a fundamental role in the foeto-placental unit development, and it is therefore tempting to speculate that at the beginning of pregnancy, foetal requirements for neuro-development cause a decrease in maternal BDNF serum levels by transplacental transport.[36,37] Furthermore, as pregnancy progresses, the placenta and foetus might assume a role in the production of this neurotrophin, a phenomenon which could partly explain the serum pattern in both rats and humans and the expression profile of both BDNF and its receptor in rat placenta described in this study.

To gain further knowledge about the potential implications of BDNF in pregnant women, we next investigated the correlation between serum levels of BDNF and other anthropometric and biochemical variables. Our findings showed that serum BDNF levels are negatively correlated with gestational age at birth and fasting glucose levels. Gestational diabetes is one of the most common pregnancy-associated diseases[38] and it has been shown that women with pregestational diabetes have increased rates for small for gestational age (SGA) newborns.[39] The fact that circulating BDNF was negatively correlated with fasting glucose levels suggests that BDNF lacks its ability to reduce hyperglycaemia during pregnancy, and this might be related to some of the disorders caused by high glucose levels.

Another important aspect of BDNF levels that needs to be considered is its relationship with stress and exercise, especially during gestation. The stress system, whose main components are in the central nervous system and consist mainly of corticotropin-releasing hormone (CRH) and noradrenergic neurons, is activated by extrinsic and intrinsic challenges altering body homoeostasis.[40] One of the systems most studied and susceptible to stress is the hypothalamic–pituitary–adrenal (HPA) axis.[41] In turn, this neuroendocrine axis regulates different metabolic processes related to the secretion of glucocorticoids in response to stress and diurnal signals, which lead to alterations in the growth and development of metabolic/endocrine, among others.[40] It has been shown that under conditions of stress levels of neurotrophins are altered, particularly BDNF.[42] The pregestational stress leads to elevated corticosterone levels and reduced BDNF expression in the hippocampus of offspring rats 2 months postnatally, an effect that persists and manifests in adulthood.[43] It has been shown that maternal stress influences the development of the functions of the hippocampus in offspring, which is related to the reduction in the expression of BDNF.[44] On the other hand, a study in maternal generalized anxiety disorder (GAD) during pregnancy, BDNF levels in cord blood in newborns of healthy women were significantly higher compared with infants of mothers with GAD.[45] In stress-induced pregnancy complications, such as pre-eclampsia, BDNF levels are significantly lower compared with normotensive women.[46] Furthermore, it has been shown that chronic exercise helps improve the cognitive impairment induced by stress, by detoxifying mechanisms reactive oxygen species (ROS) in the hippocampus and activating BDNF signalling.[47] Additionally, exercise helps preserve the levels of BDNF, cellular genesis and learning at different stages of development.[48] Maternal rat exercises during pregnancy, decreased maternal deprivation induced anxiety and protects the pups from anxiety, which correlates with increased levels of BDNF in the prefrontal cortex.[49] In this manner, the knowledge obtained from the BDNF serum profiles during pregnancy studied in this proposal could contribute to the understanding of the maternal–foetal physiological response to stress during and after foetal gestation. Indeed, further studies assessing maternal BDNF levels in women that are subjected to stressful pregnancies, such as those with metabolic complications, will be necessary to elucidate this issue.

In conclusion, the present study shows that (i) brain-derived neurotrophic factor and its receptor are expressed in rat and human placentas; (ii) the gene expression of brain-derived neurotrophic factor and its receptor is decreased in early pregnancy in rat placentas; (iii) serum brain-derived neurotrophic factor levels follow the same profile of placental BDNF gene expression and are similarly regulated in rats and humans, being decreased in the initial periods of gestation; and (iv) women serum brain-derived neurotrophic factor levels are negative correlated with gestational age at birth and fasting glucose levels. Overall, our findings suggest that the brain-derived neurotrophic factor/tropomyosin-related kinase type B system might be involved in the regulation of several metabolic functions during pregnancy, which is consistent with the

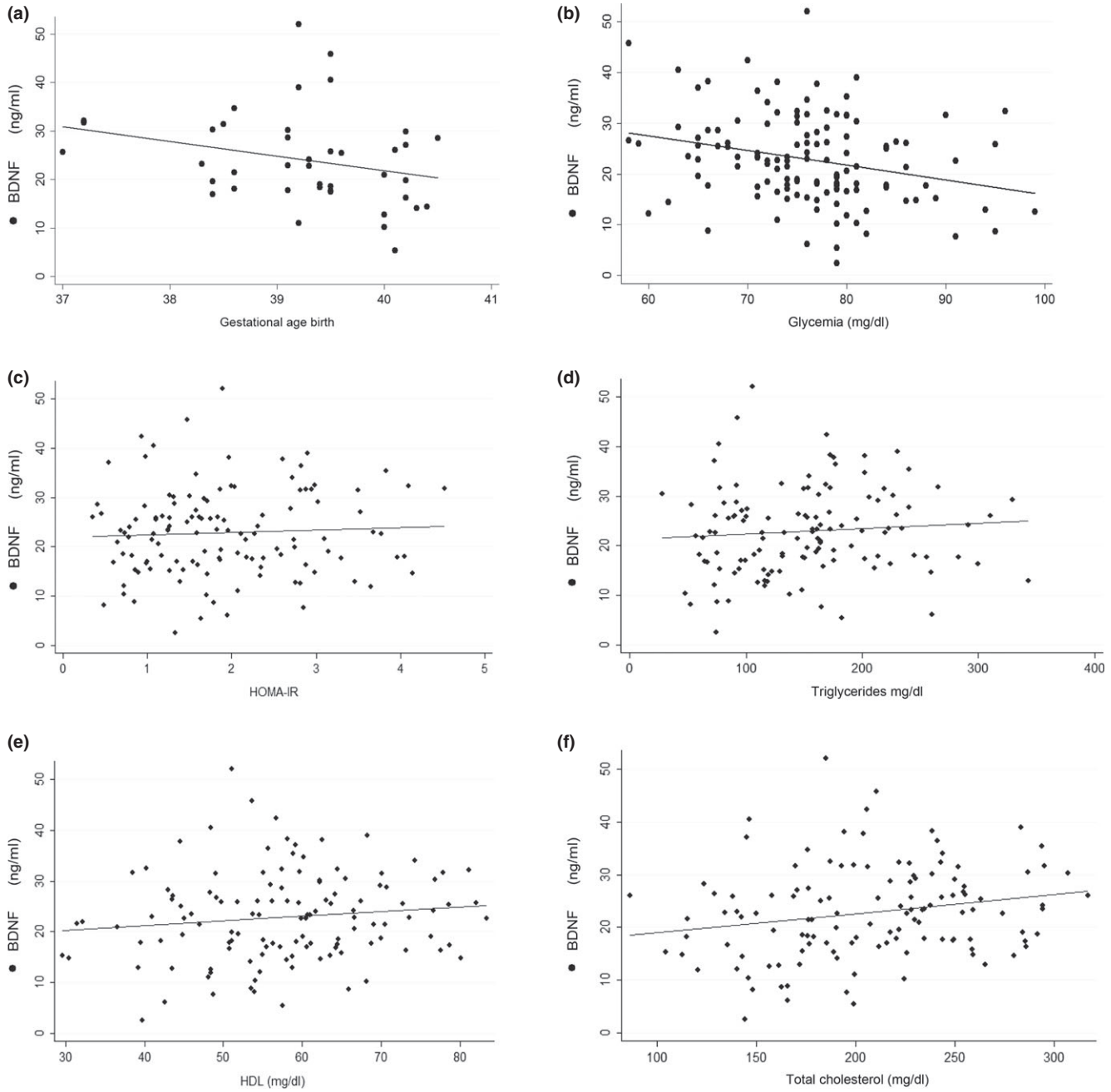

Fig. 5 Correlation coefficients of serum BDNF levels with clinical and biochemical variables in the pregnant group: (a) Gestational age at birth, (b) fasting glycaemia, (c) HOMA index, (d) triglycerides, (e) HDL cholesterol, (f) total cholesterol.

neurotrophic hypothesis proposed by Hristova *et al.*[50] In this manner, variations in the levels of brain-derived neurotrophic factor during pregnancy may in part contribute to the condition of metabolic stress typical to gestation.[51]

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Disclosure statement

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References

1 Fujita, K., Tatsumi, K., Kondoh, E. *et al.* (2011) Differential expression and the anti-apoptotic effect of human placental neurotrophins and their receptors. *Placenta*, **32**, 737–744.

2 Rosas-Vargas, H., Martínez-Ezquerro, J.D. & Bienvenu, T. (2011) Brain-derived neurotrophic factor, food intake regulation, and obesity. *Archives of Medical Research*, **42**, 482–494.

3 Rios, M. (2013) BDNF and the central control of feeding: accidental bystander or essential player? *Trends in Neurosciences*, **36**, 83–90.

4 Spaeth, A.M., Kanoski, S.E., Hayes, M.R. *et al.* (2012) TrkB receptor signaling in the nucleus tractus solitarius mediates the food intake-suppressive effects of hindbrain BDNF and leptin. *American Journal of Physiology. Endocrinology and Metabolism*, **302**, E1252–E1260.

5 Pelleymounter, M.A., Cullen, M.J. & Wellman, C.L. (1995) Characteristics of BDNF-induced weight loss. *Experimental Neurology*, **131**, 229–238.

6 Lapchak, P.A. & Hefti, F. (1992) BDNF and NGF treatment in lesioned rats: effects on cholinergic function and weight gain. *NeuroReport*, **3**, 405–408.

7 Kernie, S.G., Liebl, D.J. & Parada, L.F. (2000) BDNF regulates eating behavior and locomotor activity in mice. *EMBO Journal*, **19**, 1290–1300.

8 Lyons, W.E., Mamounas, L.A., Ricaurte, G.A. *et al.* (1999) Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 15239–15244.

9 Rios, M., Fan, G., Fekete, C. *et al.* (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Molecular Endocrinology*, **5**, 1748–1757.

10 Unger, T.J., Calderon, G.A., Bradley, L.C. *et al.* (2007) Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *Journal of Neuroscience*, **27**, 14265–14274.

11 Carim-Todd, L., Bath, K.G., Fulgenzi, G. *et al.* (2009) Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. *Journal of Neuroscience*, **29**, 678–685.

12 Speliotes, E.K., Willer, C.J., Berndt, S.I. *et al.* (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nature Genetics*, **42**, 937–948.

13 Thorleifsson, G., Walters, G.B., Gudbjartsson, D.F. *et al.* (2009) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nature Genetics*, **41**, 18–24.

14 Chen, Z.Y., Jing, D., Bath, K.G. *et al.* (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, **314**, 140–143.

15 Yeo, G.S., Connie Hung, C.C., Rochford, J. *et al.* (2004) A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nature Neuroscience*, **7**, 1187–1189.

16 Monteleone, P. & Maj, M. (2013) Dysfunctions of leptin, ghrelin, BDNF and endocannabinoids in eating disorders: beyond the homeostatic control of food intake. *Psychoneuroendocrinology*, **38**, 312–330.

17 Mayeur, S., Silhol, M., Moitrot, E. *et al.* (2010) Placental BDNF/TrkB signaling system is modulated by fetal growth disturbances in rat and human. *Placenta*, **31**, 785–791.

18 Dhobale, M., Mehendale, S., Pisal, H. *et al.* (2012) Association of brain-derived neurotrophic factor and tyrosine kinase B receptor in pregnancy. *Neuroscience*, **216**, 31–37.

19 Schulte-Herbrüggen, O., Litzke, J., Hornych, K. *et al.* (2007) Maternal nerve growth factor serum levels in the perinatal period. *Journal of Reproductive Immunology*, **74**, 170–173.

20 Lommatzsch, M., Zingler, D., Schuhbaeck, K. *et al.* (2005) The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiology of Aging*, **26**, 115–123.

21 Garces, M.F., Sanchez, E., Ruíz-Parra, A.I. *et al.* (2013) Serum chemerin levels during normal human pregnancy. *Peptides*, **42**, 138–143.

22 Garces, M.F., Sanchez, E., Acosta, B.J. *et al.* (2012) Expression and regulation of chemerin during rat pregnancy. *Placenta*, **33**, 373–378.

23 González, C.R., Vázquez, M.J., López, M. *et al.* (2008) Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa. *Journal of Molecular Endocrinology*, **41**, 415–421.

24 Jabbour, H.N., Critchley, H.O. & Boddy, S.C. (1998) Expression of functional prolactin receptors in nonpregnant human endometrium: janus kinase-2, signal transducer and activator of transcription-1 (STAT1), and STAT5 proteins are phosphorylated after stimulation with prolactin. *Journal of Clinical Endocrinology and Metabolism*, **83**, 2545–2553.

25 Caminos, J.E., Nogueiras, R., Gallego, R. *et al.* (2005) Expression and regulation of adiponectin and receptor in human and rat placenta. *Journal of Clinical Endocrinology and Metabolism*, **90**, 4276–4286.

26 Ryan, N.K., Van der Hoek, K.H., Robertson, S.A. *et al.* (2003) Leptin and leptin receptor expression in the rat ovary. *Endocrinology*, **144**, 5006–5013.

27 McArdle, C.A., Gorospe, W.C., Huckle, W.R. *et al.* (1987) Homologous down-regulation of gonadotropin-releasing hormone receptors and desensitization of gonadotropes: lack of dependence on protein kinase C. *Molecular Endocrinology*, **1**, 420–429.

28 Koiter, T.R., Poelstra, K., Scheringa, M. *et al.* (1989) Glucose and insulin responses during mixed meals or infusion of glucose in pregnant and lactating rats. *Physiology & Behavior*, **46**, 881–887.

29 Leturque, A., Ferré, P., Satabin, P. *et al.* (1980) In vivo insulin resistance during pregnancy in the rat. *Diabetologia*, **19**, 521–528.

30 Tonra, J.R., Ono, M., Liu, X. *et al.* (1999) Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice. *Diabetes*, **48**, 588–594.

31 Nakagawa, T., Tsuchida, A., Itakura, Y. *et al.* (2000) Brain-derived neurotrophic factor regulates glucose metabolism by

modulating energy balance in diabetic mice. *Diabetes*, **49**, 436–444.
32 Meek, T.H., Wisse, B.E., Thaler, J.P. *et al.* (2013) BDNF action in the brain attenuates diabetic hyperglycemia via insulin-independent inhibition of hepatic glucose production. *Diabetes*, **62**, 1512–1518.
33 Kim, T., Do, M.H. & Lawson, M.A. (2013) Translational control of gene expression in the gonadotrope. *Molecular and Cellular Endocrinology*. [epub ahead of print] doi: 10.1016/j.mce.2013.09.007.
34 Mayeur, S., Lukaszewski, M.A., Breton, C. *et al.* (2011) Do neurotrophins regulate the feto-placental development? *Medical Hypotheses*, **76**, 726–728.
35 Chouthai, N.S., Sampers, J., Desai, N. *et al.* (2003) Changes in neurotrophin levels in umbilical cord blood from infants with different gestational ages and clinical conditions. *Pediatric Research*, **53**, 965–969.
36 Kodomari, I., Wada, E., Nakamura, S. *et al.* (2009) Maternal supply of BDNF to mouse fetal brain through the placenta. *Neurochemistry International*, **54**, 95–98.
37 Mayeur, S., Lukaszewski, M.A., Breton, C. *et al.* (2011) BDNF in feto-placental development. *Médecine Sciences : M/S*, **27**, 251–252.
38 Lain, K.Y. & Catalano, P.M. (2007) Metabolic changes in pregnancy. *Clinical Obstetrics and Gynecology*, **50**, 938–948.
39 Yanit, K.E., Snowden, J.M., Cheng, Y.W. *et al.* (2012) The impact of chronic hypertension and pregestational diabetes on pregnancy outcomes. *American Journal of Obstetrics & Gynecology*, **207**, 333.e1–333.e6.
40 Stratakis, C.A. & Chrousos, G.P. (1995) Neuroendocrinology and pathophysiology of the stress system. *Annals of the New York Academy of Sciences*, **771**, 1–18.
41 Binder, E.B., Bradley, R.G., Liu, W. *et al.* (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *Journal of the American Medical Association*, **299**, 1291–1305.
42 Bath, K.G., Schilit, A. & Lee, F.S. (2013) Stress effects on BDNF expression: effects of age, sex, and form of stress. *Neuroscience*, **239**, 149–156.
43 Huang, Y., Shi, X., Xu, H. *et al.* (2010) Chronic unpredictable stress before pregnancy reduce the expression of brain-derived neurotrophic factor and N-methyl-D-aspartate receptor in hippocampus of offspring rats associated with impairment of memory. *Neurochemical Research*, **35**, 1038–1049.
44 Van den Hove, D.L., Steinbusch, H.W., Scheepens, A. *et al.* (2006) Prenatal stress and neonatal rat brain development. *Neuroscience*, **137**, 145–155.
45 Uguz, F., Sonmez, E.O., Sahingoz, M. *et al.* (2013) Maternal generalized anxiety disorder during pregnancy and fetal brain development: a comparative study on cord blood brain-derived neurotrophic factor levels. *Journal of Psychosomatic Research*, **75**, 346–350.
46 D'Souza, V.A., Kilari, A.S., Joshi, A.A. *et al.* (2013) Differential Regulation of Brain-Derived Neurotrophic Factor in Term and Preterm Preeclampsia. *Reproductive Sciences*, **21**, 230–235.
47 Kwon, D.H., Kim, B.S., Chang, H. *et al.* (2013) Exercise ameliorates cognition impairment due to restraint stress-induced oxidative insult and reduced BDNF level. *Biochemical and Biophysical Research Communications*, **434**, 245–251.
48 Marlatt, M.W., Potter, M.C., Lucassen, P.J. *et al.* (2012) Running throughout middle-age improves memory function, hippocampal neurogenesis, and BDNF levels in female C57BL/6J mice. *Developmental Neurobiology*, **72**, 943–952.
49 Aksu, I., Baykara, B., Ozbal, S. *et al.* (2012) Maternal treadmill exercise during pregnancy decreases anxiety and increases prefrontal cortex VEGF and BDNF levels of rat pups in early and late periods of life. *Neuroscience Letters*, **516**, 221–225.
50 Hristova, M.G. (2013) Metabolic syndrome–from the neurotrophic hypothesis to a theory. *Medical Hypotheses*, **81**, 627–634.
51 Catalano, P.M. (2010) Obesity, insulin resistance, and pregnancy outcome. *Reproduction*, **140**, 365–371.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. BDNF blood levels are not significantly variable during the menstrual cycle ($P > 0{\cdot}05$).

Table S1. Correlation of serum BDNF level with individual variables during pregnancy.