Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet

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Intrauterine growth restriction (IUGR) represents an important risk factor for perinatal complications and for adult disease. IUGR is associated with a down-regulation of placental amino acid transporters; however, whether these changes are primary events directly contributing to IUGR or a secondary consequence is unknown. We investigated the time course of changes in placental and fetal growth, placental nutrient transport *in vivo* and the expression of placental nutrient transporters in pregnant rats subjected to protein malnutrition, a model for IUGR. Pregnant rats were given either a low protein (LP) diet ($n = 64$) or an isocaloric control diet ($n = 66$) throughout pregnancy. Maternal insulin, leptin and IGF-I levels decreased, whereas maternal amino acid concentrations increased moderately in response to the LP diet. Fetal and placental weights in the LP group were unaltered compared to control diet at gestational day (GD) 15, 18 and 19 but significantly reduced at GD 21. Placental system A transport activity was reduced at GD 19 and 21 in response to a low protein diet. Placental protein expression of SNAT2 was decreased at GD 21. In conclusion, placental amino acid transport is down-regulated prior to the development of IUGR, suggesting that these placental transport changes are a cause, rather than a consequence, of IUGR. Reduced maternal levels of insulin, leptin and IGF-1 may link maternal protein malnutrition to reduced fetal growth by down-regulation of key placental amino acid transporters.

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Intrauterine growth restriction (IUGR) constitutes an important clinical problem associated with increased perinatal morbidity, higher incidence of neuro-developmental impairment and increased risk of adult disease, such as diabetes and cardiovascular disease (Barker *et al.* 1993; Barker, 2001; Harding, 2001). The mechanisms underlying IUGR remain to be fully established; however, an abundance of data has recently accumulated describing a number of specific alterations in the expression and activity of placental nutrient and ion transporters in IUGR (Glazier *et al.* 1997; Norberg *et al.* 1998; Jansson *et al.* 2002*a*; Johansson *et al.* 2003; Strid *et al.* 2003). For example, IUGR in human pregnancies is associated with a down-regulation of several important placental amino acid transporters, such as system A, a sodium-dependent transport system mediating the transport of neutral amino acids (Dicke & Henderson, 1988; Mahendran *et al.* 1993). Based on these findings, it has been suggested that changes in expression and activity of nutrient transporters in the placenta represent an important mechanism for regulation of fetal growth as a response to altered maternal nutrition and/or placental nutrient supply (Jansson & Powell, 2000). Whether these changes are primary and therefore contribute to IUGR or secondary due to an already decreased fetal growth remains to be established. If the placental transport alterations contribute directly to the growth failure of the fetus, it would markedly affect our understanding of the regulation of fetal growth and would make placental nutrient transporters an interesting possible target for treatment in efforts to alleviate the development of IUGR.

There are a number of maternal conditions and placental pathologies that are associated with fetal under-nutrition and IUGR. In Western societies, placental

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insufficiency is probably the most common cause underlying the development of IUGR whereas maternal malnutrition is the leading cause of IUGR in developing countries. The relationship between moderate variations in maternal nutrition in pregnancy and birth weight in human pregnancy is complicated; however, some recent studies suggest that in Western settings the balance of macronutrients in a woman's diet can have a significant influence on newborn size (Godfrey *et al.* 1996; Moore *et al.* 2004). In particular, there appears to be a positive correlation between the proportion of calories originating from protein and birth weight (Moore *et al.* 2004). In addition, some evidence suggests that the composition of maternal diet may programme the fetus in the absence of a change in fetal growth (Campbell *et al.* 1996). However, the mechanisms linking maternal nutrition to fetal growth and short- and long-term outcome remains to be established.

A substantial number of animal experimental studies have demonstrated that alterations of maternal diet (typically calorie or protein restriction) during pregnancy affect fetal growth as well as programme the fetus for disease later in life (reviewed in Langley-Evans, 2001; Ozanne, 2001). However, most of these studies have addressed the outcome in response to various dietary manipulations, and the mechanisms signalling altered maternal nutrition to the fetus are not well established. There are a few reports implicating altered placental nutrient transport in the development of IUGR in response to protein malnutrition. In a study by Malandro *et al.* (1996), protein restriction in the pregnant rat was shown to down-regulate the *in vitro* activity of specific placental amino acid transporters (System A and transporters for cationic amino acids). However, it is unclear from these studies if placental transport changes are a cause or a secondary consequence of IUGR.

The system A amino acid transporter is encoded by three members of the slc38 gene family giving rise to three subtypes, SNAT1, SNAT2 and SNAT4, which are all expressed in the placenta (Desforges *et al.* 2006). *In vitro* studies have demonstated that insulin, leptin, cortisol and IGF-I (Karl *et al.* 1992; Kniss *et al.* 1994; Karl, 1995; Jansson *et al.* 2003) stimulate placental system A activity and that hypoxia down-regulates it (Nelson *et al.* 2003). However, the identity of the factors that cause the decrease in placental System A activity in human IUGR or in response to protein malnutrition in the rat is not known. We have proposed that the placenta functions as a nutrient sensor, altering the expression and activity of key nutrient transporters such as System A in response to changes in the ability of the maternal supply line to deliver nutrients and oxygen (Jansson & Powell, 2006). Mammalian cells possess an important nutrient sensing pathway that controls protein synthesis at the level of translation. A key factor in this pathway is mammalian target of rapamycin (mTOR), a phosphatidylinositol kinase-regulated protein kinase (Raught *et al.* 2001). In response to alterations in amino acid availability, in particular branched chain amino acids such as leucine, mTOR controls translation, transcription and protein stability (Jacinto & Hall, 2003). The mTOR signalling pathway has been implicated in regulating individual cell growth in response to nutrient stimuli, and it is possible that mTOR, which is expressed at the mRNA level in the placenta (Kim *et al.* 2002), may represent a mechanism by which altered nutrient availability regulates placental nutrient transporters.

In the current study, we used the maternal protein restriction model for IUGR to test the hypothesis that alterations in placental nutrient transport precede the development of IUGR, which would give support to the idea that placental transporter alterations are a cause rather than an effect of IUGR. Furthermore, we tested the hypothesis that placental mTOR expression is altered in response to maternal protein malnutrition. Pregnant rats were given an isocaloric diet with normal or low protein content from day 2 of pregnancy and studied at day 15, 18, 19 or 21 (term 23). In one subgroup of animals, placental samples were obtained for measurements of the protein expression of SNAT2, an isoform of the system A amino acid transporter, and mTOR. In a second group of animals, placental transport of amino acids and glucose was measured in the awake, chronically catheterized animal. In addition, the effect of protein malnutrition on maternal amino acid concentrations and circulating levels of insulin, IGF-I and leptin was assessed.

Methods

Materials

[^{14}C]Methylaminoisobutyric acid ([^{14}C]MeAIB) and 3-*O*-methyl-D-[^{3}H]glucose were obtained from New England Nuclear Life Science Products (Boston, MA, USA). A polyclonal rabbit antibody against mTOR recognizing the human and rat protein (ab 2732) was purchased from Abcam (Cambridge, UK). Furthermore, we used a polyclonal antibody directed against SNAT2, a system A amino acid transporter isoform, which we have generated in rabbits, as previously described (Ling *et al.* 2001). Rat chow (control and low protein diets) was obtained from Harlan Teklad, UK. All other chemicals were purchased from Sigma (St Louis, MO, USA).

Animals

The local ethical committee for animal research at Göteborg University approved the experiments. Timed pregnant Sprague-Dawley rats were obtained from BK Universal (Uppsala, Sweden). They were delivered to the animal facilities at gestation day (GD) 2. Day 1 of pregnancy was defined as the day immediately following

the night during which males were present. The animals were maintained on a 12 h light–dark cycle at 21°C. The pregnant rats were divided into two experimental groups; in group one, the animals were killed at GD 15, 18, 19 or 21 (term 23) and placentas were homogenized in buffer D (10 mM Tris-Hepes, 250 mM sucorse, 1.6 μM antipain, 0.7 μM pepstatin, 0.5 μg ml^{-1} aprotinin, 1 mM EDTA), frozen in liquid nitrogen and stored at −80°C for later protein expression analysis. In the second experimental group, placental transport measurements were carried out at GD 15, 18, 19 or 21 in awake animals. Placental and fetal weights as well as maternal weight gain and litter size were recorded in both groups.

Diets

Animals had access to water *ad libitum*. In the low protein group (LP), animals were fed a diet containing 4% protein starting at GD 2 and continuing throughout pregnancy. Animals in the control group (C) were fed chow with normal protein concentration (18%). The diets were isocaloric. The LP diet was compensated with sucrose to increase the calorie content, 82% energy from carbohydrate and 13% energy from fat compared to control diet that contained 67% energy from carbohydrate and 13% energy from fat.

Western blot

Placental homogenates were used for analysing protein expression of SNAT2, a system A isoform. For mTOR analyses, the supernatant of the rat placental homogenates was used since mTOR is a cytoplasmic protein. Supernatants were obtained through centrifugation of homogenate samples at 4°C at 10 000 *g* for 15 min. Protein concentrations were determined using the Bradford assay (Bradford, 1976). Western blotting was performed as previously described (Jansson *et al.* 1999). Briefly, samples were loaded (30 μg total protein for System A and 20 μg for mTOR) on a SDS-PAGE gels (10% for SNAT2 and 7% for mTOR) and electrophoresis was run at a constant 200 V. Two samples from GD 21 were used on each gel to normalize density readings between gels. Proteins were transferred onto nitrocellulose membranes (Amersham) by electroblotting overnight at a constant 30 V. Membranes were incubated with primary antibodies (1 : 4000 for SNAT 2 and 1 : 2000 for mTOR), washed and incubated with secondary peroxidase labelled antirabbit IgG (1 : 1000). In order to confirm specificity of the SNAT2 antibody, the antigenic peptide for SNAT2 was used in concentrations 300-fold higher than the antibody. After further washings, the immunolabelling was visualized using ECL detection reagents (Amersham). Membranes were exposed to Hyperfilm (Amersham). The analysis included scanning of films followed by densitometry by means of IPLab gel software (Signal Analysis Corp., Vienna, VA, USA).

Real time RT-PCR

To measure the relative amount of the SNAT1, SNAT2 and SNAT4 mRNAs, separate quantitative real-time PCR analyses were performed with a DNA Engine Opticon 2 system (Bio-Rad Laboratories, Inc., MA, USA) using the detection of SYBR Green I (Applied Biosystems Inc., Foster City, CA, USA). Each RNA sample was measured in duplicate. One hundred nanograms of total RNA was used in each reaction to measure the steady-state mRNA content for SNAT1, SNAT2 and SNAT4. The reactions were first incubated at 50°C for 30 min followed by 95°C for 15 min and then amplification of 35 cycles of each at 95°C for 15 s, 60°C for 60 s (for SNAT2 and SNAT4) and 62.8°C for 60 s for SNAT1. The primers utilized were SNAT1: (forward 5′−3′TCAGCCTGGTACGTCGATGG, reverse 5′−3′CCAGGTTCTTCAAGAGACACAG), SNAT2: (forward 5′−3′AGAGCAATTCCAGTATTAGC, reverse 5′−3′TTAATCTGAGCAATGCGATTGTG) and SNAT4 (forward 5′−3′GGCAGTGGTGTGGAGTACGAAGC, reverse 5′−3′TGGAATCGCGTAGGCCGTG). After PCR, melting curves were acquired by stepwise increase of the temperature from 55°C to 95°C to ensure that a single product was amplified in the reaction.

Implantation of permanent vascular catheters

Transport measurements were carried out as we previously described for the awake pregnant guinea pig (Jansson & Persson, 1990). Animals were anaesthetized with a mixture of xylazine (Rompun, 4.6 mg kg^{-1}) and ketamine hydrochloride (Ketalar, 38.5 mg kg^{-1}) intraperitoneally and given buprenorphine (Temgesic, 0.01 mg kg^{-1}) subcutaneously for analgesia. Local anaesthetics were given subcutaneously and a 2 cm-long midline incision was performed, and the right carotid artery and right jugular vein were localized and catheterized (PE 50 connected to PE 90). Subsequently, catheters were tunnelled subcutaneously to the neck where they emerged, filled with heparinized saline (500 IU ml^{-1}), cut to a length of 2.5 cm and plugged. This surgical procedure lasted 20–30 min. After awakening, animals were returned to the animal facilities where they were left for 3 days to fully recover; during this period the catheters were flushed daily with heparinized saline (500 IU ml^{-1}).

Transport measurements

An arterial blood-sample (1 ml) was withdrawn prior to the infusion of isotopes and transferred to a vial

containing Na_2EDTA (final concentration 1.2 mg ml^{-1}) and centrifuged. Plasma was snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis of plasma hormone and amino acid concentrations. Placental transport was studied by intravenous administration of 3-*O*-methyl-D-[^{3}H]glucose (50 μCi kg^{-1}) and [^{14}C]methylaminoisobutyric acid (10 μCi kg^{-1}). After the administration of isotopes, the rat was placed in a cage where it was free to walk around for 6 min, when another arterial blood-sample (1 ml) was withdrawn for determination of radioactivity in plasma. Seven minutes after the infusion of isotopes, 1 ml pentobarbital (50 mg ml^{-1}) was injected into the venous catheter in order to kill the animal. This time point was chosen based on preliminary experiments demonstrating low feto-maternal backflux of isotopes up to at least 7 min after isotope injection. The placentas and fetuses were weighed individually. Subsequently, fetuses and placentas from each litter were pooled and cut into small pieces and homogenized in three volumes of distilled water. Trichloroacetic acid (20%) was added to homogenates (1 : 3), which were centrifuged 10 min at 12 000 *g*. Twelve millilitres of liquid scintillation fluid (Aquasafe 300 plus, Zinsser Analytic Scintvaruhuset Uppsala, Sweden) was added to 3 ml supernatant. Distilled water (3 ml) was added to 150 μl plasma samples followed by the addition of 12 ml scintillation fluid. Vials were shaken 30 min prior to β-counting in a liquid scintillation counter.

High performance liquid chromatography

Amino acids were determined using *o*-phthalaldehyde derivatization and fluorescence detection essentially as described previously (Lindroth & Mopper, 1979; Sandberg *et al.* 1986). The derivatization solution consisted of *o*-phthalaldehyde (40 mg) dissolved in methanol (400 μl), β-mercaptoethanol (40 μl), borate buffer (2.0 ml, 0.8 M, pH 12) and H_2O (1.6 ml). Amino acids were derivatized in the autosampler before injection (1 : 1, sample : derivatization solution). The chromatographic system consisted of a Varian 5500 HPLC pump, a Waters 717 autosampler and an Applied Biosystems 980 fluorescence detector. The amino acid derivatives were separated on a Nucleosil C_{18} column (200 mm × 4.6 mm; 5 μm particle size; Hichrom, Reading, UK) with a mobile phase consisting of 50 mM NaH_2PO_4 (pH 5.28) and methanol in a gradient from 25 to 95% methanol. A flow rate of 1 ml min^{-1} was used. Detection was carried out by excitation at 333 nm and emission at 418 nm.

Hormone analysis

Maternal plasma samples were obtained from the catheterized animals prior to transport measurements. Commercially available RIA kits were used to measure plasma concentrations of insulin (sensitive rat insulin RIA kit, Linco Research, St Charles, MS, USA), leptin (rat RIA kit, Linco Research) and IGF-1 (Mediagnost, Reutlingen, Germany). Values outside of the linear part of the sigmoidal standard curve were considered as outliers. Whereas maternal plasma concentrations of IGF-I in the control group changed significantly over time in late gestation, there was no gestational effect on insulin and leptin plasma concentrations. Therefore the data for insulin and leptin were pooled from all gestational ages in the control and low-protein groups, respectively.

Data presentation and statistics

Transport data are given as placental dpm per gram placenta (representing placental uptake of isotope), fetal dpm per gram fetus (representing the amount of isotope transported per gram fetus) and fetal dpm per gram placenta (giving a measure of the amount of isotope transported per gram placenta, i.e. the relative transport capacity of the placenta). Transport data are expressed for the LP group in relation to control where control values are arbitrarily assigned a value of 1.

Since observations in individual fetuses and placentas of the same litter are not independent, an average was obtained for each litter, and $n = 1$ therefore represents pooled data from one litter. Data are presented as means ± S.E.M. Differences between the four gestational age groups were analysed statistically by ANOVA, using Dunnett's test *post hoc* and comparing to GD 15. Differences between C and LP groups were evaluated statistically by Student's *t* test for unpaired observations. A P value < 0.05 was considered significant.

Results

Maternal weight gain and fetal and placental weights

Food intake was measured in the two groups and found to be 11% higher ($P < 0.05$) in the LP group (22.9 ± 0.63 g day^{-1}) compared to the control group (20.6 ± 0.39 g day^{-1}). Maternal weight gain was significantly lower in the LP group compared to the control group at GD 18, GD 19 and GD 21 for the non-catheterized dams and at GD 19 and GD 21 in the catheterized dams (Table 1). However, anaesthesia and chronic catheterization did not significantly affect fetal-placental growth (data not shown) and fetal and placental weights from catheterized and non-catheterized animals are therefore presented as one group. In the LP group, fetal and placental weights were significantly reduced at GD 21 compared to controls; however, there were no statistically significant differences in fetal or

Table 1. Maternal weight gain (g)

Gestation	Weight gain	
day	Group 1	Group 2
15 LP	77.1 ± 5.3	24.0 ± 10.9
15 C	66.3 ± 3.8	44.3 ± 5.8
18 LP	61.0 ± 9.4*	41.7 ± 4.6
18 C	99.0 ± 4.5	53.8 ± 5.6
19 LP	81.0 ± 5.9*	54.0 ± 5.5*
19 C	106.9 ± 4.7	76.5 ± 5.7
21 LP	63.0 ± 10.3*	37.3 ± 15.7*
21 C	123.4 ± 6.6	87.1 ± 7.9

Weight gain represents the increase in maternal weight from GD 2 until day of experiment. Group 1 corresponds to animals from which placental samples were obtained, whereas animals in Group 2 were subjected to chronic catherization and *in vivo* transport measurements. Means ± S.E.M. *$P < 0.05$ *versus* control group.

Table 2. Litter size, and fetal and placental weights

Gestation day	*n* litters	litter size (*n*)	fetal weight (g)	placental weight (g)
15 LP	13	11.6 ± 0.42	0.150 ± 0.005	0.183 ± 0.007
15 C	13	11.1 ± 0.58	0.142 ± 0.003	0.172 ± 0.005
18 LP	21	11.1 ± 0.17	0.749 ± 0.018	0.343 ± 0.008
18 C	22	11.6 ± 0.07	0.741 ± 0.020	0.354 ± 0.009
19 LP	18	10.3 ± 0.6	1.270 ± 0.06	0.380 ± 0.008
19 C	15	12.1 ± 0.46	1.167 ± 0.055	0.394 ± 0.008
21 LP	12	11.3 ± 0.52	2.760 ± 0.120*	0.420 ± 0.014*
21 C	16	10.7 ± 0.63	3.500 ± 0.061	0.480 ± 0.013

Means ± S.E.M. *$P < 0.05$ *versus* control group.

placental weights between LP and control animals at GD 15, 18 or 19 (Table 2).

Protein expression of SNAT2 and mTOR

Two distinct bands at approximately 58 and 48 kDa were observed using the SNAT2 antibody in Western blot. Antibody specificity was determined in preadsorption experiments, and both these bands were markedly attenuated (data not shown). Protein expression of SNAT2 (analysing 58 and 48 kDa bands together) in placental homogenates was reduced at GD 21 by 18% ($P < 0.05$) in the LP group compared to control group (Fig. 1*A*).

Using the anti-mTOR antibody, Western blot produced a single band at approximately 237 kDa. There were no significant differences between the LP and control group at GD 15, 18 or 21 when analysing mTOR protein expression (Fig. 1*B*). At GD 18, the low protein diet appeared to be associated with a reduced placental mTOR expression but this difference (−22%) failed to reach statistical significance ($P = 0.09$) (Fig. 1*B*).

Placental mRNA expression of SNAT1, 2 and 4

Placental SNAT1 and SNAT2 mRNA expression increased linearly from GD 15 to GD 21 in the control group,

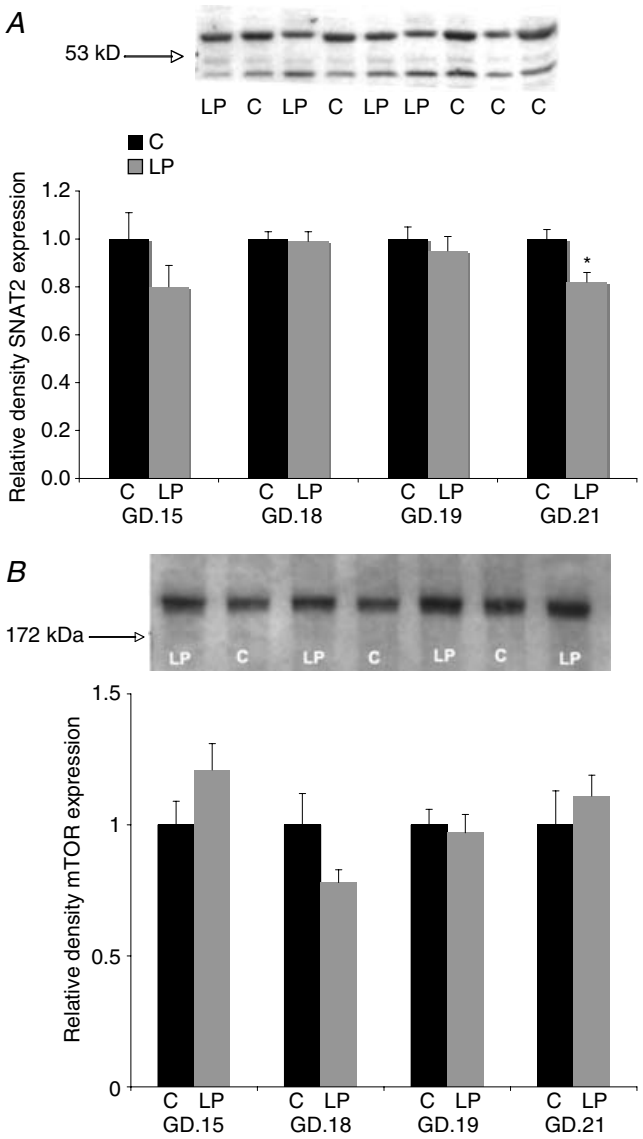

Figure 1. Western blots of SNAT 2 and mTOR in rat placenta
A, Western blot of SNAT 2 in homogenates of rat placenta at GD 21. C = control and LP = low protein diet (top panel). SNAT 2 protein expression as measured by Western blot analysis in homogenates from rat placenta at GD 15 ($n = 10$ C, and $n = 11$ LP), GD 18 ($n = 10$ C and $n = 10$ LP), GD 19 ($n = 11$ C and $n = 10$ LP) and GD 21 ($n = 10$ C and $n = 8$ LP). The protein expression of SNAT 2 was significantly lower ($P < 0.05$) in the LP group at GD 21 compared to control group. *B*, Western blot of mTOR in a cytoplasm-enriched fraction of homogenates from rat placenta at GD 15 C = control and LP = low protein diet (top panel). mTOR protein expression from rat placenta at GD 15 ($n = 10$ C, and $n = 11$ LP), GD 18 ($n = 13$ C and $n = 14$ LP), GD 19 ($n = 11$ C and $n = 11$ LP) and GD 21 ($n = 10$ C and $n = 8$ LP). For *A* and *B*, the mean density of the control group was assigned a value of 1 and the mean density of the LP group was calculated relative to the control group. Values are given as means ± S.E.M. *$P < 0.05$ *versus* control.

whereas SNAT4 mRNA expression in the control group increased until GD19 and declined thereafter (data not shown). Low protein diet did not significantly affect the mRNA expression of SNAT1 and SNAT4 (data not shown). However SNAT2 mRNA expression was significantly reduced at GD 15 in the LP group ($n = 3$) compared to the control group ($n = 3$) (Fig. 2).

Placental uptake and transport of 3-*O*-methyl-d-[^{3}H]glucose

The uptake of glucose by the placenta (dpm placenta/g placenta) and transport to the fetus (dpm fetus/g fetus) were not altered at any of the gestational ages tested. Similarly, the capacity of the placenta to transport glucose (dpm fetus/g placenta) was unchanged by the low protein diet (data not shown).

Placental uptake and transport of [^{14}C]methylaminoisobutyric acid

At GD 15 and GD 18, no changes were observed in placental uptake (dpm placenta/g placenta) of MeAIB or transport to the fetus (dpm fetus/g fetus) in the LP group compared to control. The capacity of the placenta to transport MeAIB (dpm fetus/g placenta) was also unchanged at GD 15 and 18. However, as seen in Fig. 3*A* and *B*, by GD 19 the relative MeAIB transport (dpm fetus/g fetus) and the placental capacity to transport MeAIB (dpm fetus/g placenta) were reduced by 25% ($n = 8$, $P < 0.05$) and 23% ($n = 8$, $P < 0.05$), respectively. As shown in Fig. 3*A*–*C*, all measures of placental MeAIB transport were

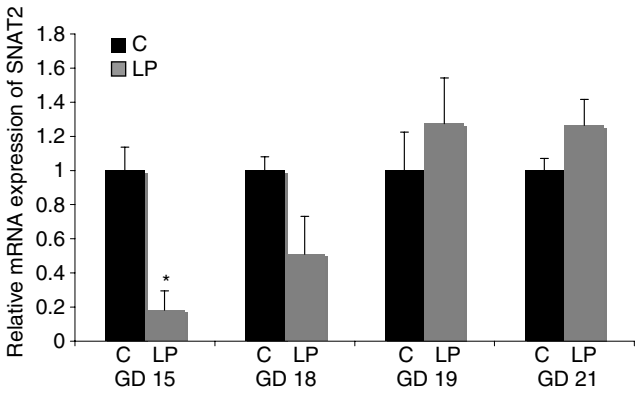

Figure 2. The relative mRNA expression of SNAT2 as in rat placenta at GD 15, 18, 19 and 21
The relative mRNA expression of SNAT2 was measured by real time RT-PCR in rat placenta at GD 15 ($n = 3$ C, control and $n = 3$ LP, low protein), GD 18 ($n = 4$ C, $n = 3$ LP), GD 19 ($n = 5$ C, $n = 5$ LP) and GD 21 ($n = 7$ C, $n = 7$ LP). SNAT2 mRNA expression was significantly lower at GD 15 in the LP group compared to the control group. The mean mRNA expression of the control group was assigned a value of 1 and the mean of the LP group was calculated relative to the control group. Values are given as means ± S.E.M. $^*P < 0.05$ *versus* control.

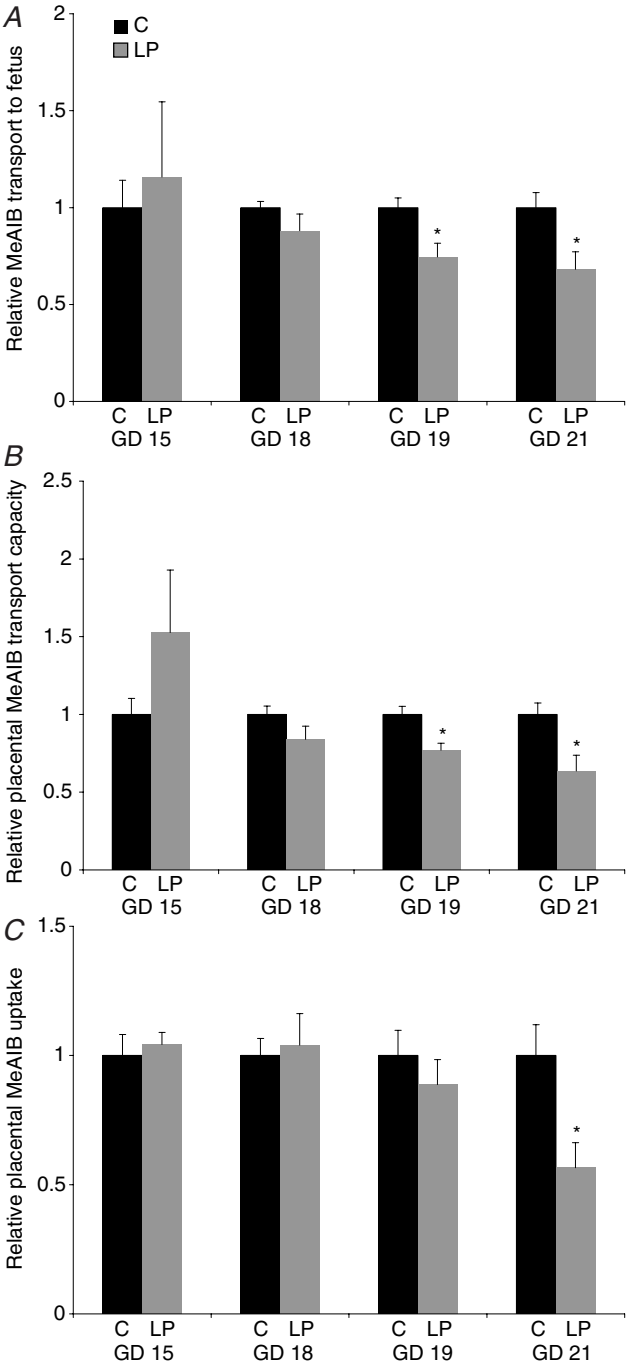

Figure 3. Transport of MeAIB to the fetus at GD 15, 18, 19 and 21
A, transport of MeAIB to 1 g of fetus at GD 15 ($n = 9$ C, control and $n = 7$ LP, low protein), GD 18 ($n = 9$ C and $n = 7$ LP), GD 19 ($n = 8$ C and $n = 10$ LP) and GD 21 ($n = 7$ C and $n = 5$ LP). *B*, amount of MeAIB transported to the fetus per gram placenta at GD 15 ($n = 9$ C, control and $n = 7$ LP, low protein), GD 18 ($n = 9$ C and $n = 7$ LP), GD 19 ($n = 8$ C and $n = 10$ LP) and GD 21 ($n = 7$ C and $n = 5$ LP). *C*, placental uptake of MeAIB at GD 15 ($n = 9$ C, control and $n = 7$ LP, low protein), GD 18 ($n = 9$ C and $n = 7$ LP), GD 19 ($n = 8$ C and $n = 10$ LP) and GD 21 ($n = 7$ C and $n = 5$ LP). For *A*–*C*, transport data are expressed for the LP group in relation to control where control values are arbitrarily assigned a value of 1. Values are given as means ± S.E.M. $^*P < 0.05$ LP *versus* control.

significantly reduced in the LP group ($n = 5$) compared to controls at GD 21 ($n = 7$, $P < 0.05$).

The gestational changes in the placental capacity to transport MeAIB is shown in Fig. 4. In the control group, the placental capacity to transport MeAIB increased markedly and was 11-fold higher ($P < 0.05$) at GD 21 as compared to GD 15 (Fig. 4). Over the same time period transport of MeAIB/g placenta in the LP group increased only 5-fold ($P < 0.05$, Fig. 4).

Maternal plasma hormone concentrations

There were no significant differences in insulin or leptin concentrations between GD 15, 18, 19 and 21 in the control groups (data not shown). Thus, the data were pooled from all gestational ages in the control and low-protein groups, respectively. Maternal plasma leptin concentrations were reduced by 26% in the LP group (2.66 ± 0.42 ng ml^{-1}, $n = 22$, $P < 0.05$) compared to controls (3.59 ± 0.23 ng ml^{-1}, $n = 22$). Similarly, maternal insulin concentrations were 25% lower in the LP group (2.16 ± 0.18 ng ml^{-1}, $n = 17$, $P < 0.05$) as compared to controls (2.90 ± 0.20 ng ml^{-1}, $n = 27$, $P < 0.05$) (Fig. 5*A* and *B*). In contrast to leptin and insulin, maternal plasma IGF-I changed significantly in the control group between GD 15 and 21 and data for individual gestational ages are therefore presented (Fig. 6). Maternal IGF-1 levels were significantly reduced by 55% in the LP group (40.27 ± 9.7 ng ml^{-1} $n = 5$, $P < 0.05$) at GD 21 compared to the control group (89.22 ± 1.99 ng ml^{-1}, $n = 5$) (Fig. 6).

Maternal plasma amino acid concentrations

Data from the all the gestational ages were pooled in both the control and LP group since there were no significant differences between gestational ages in controls. Serine (+73%) and glutamine (+20%) were significantly increased in the LP animals whereas maternal plasma concentrations of tyrosine (−9%), valine (−26%), methionine (−28%), and leucine (−17%), were decreased in the LP group ($P < 0.05$) (Fig. 7).

Discussion

In this study we demonstrate that reduction in placental System A transport, as measured in the awake chronically catheterized animal, occurs prior to the development of intrauterine growth restriction in response to maternal protein restriction in the pregnant rat. These findings are

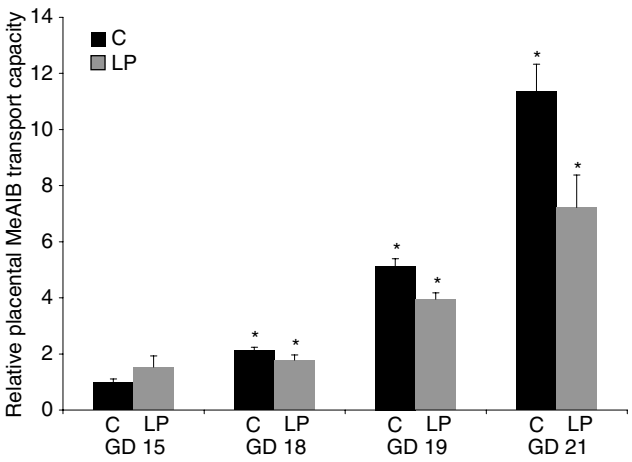

Figure 4. Placental capacity to transport MeAIB in the control and LP group at GD 15, 18, 19 and 21
Placental capacity to transport MeAIB in the control and LP group at GD 15 ($n = 9$ C, $n = 5$ LP), GD 18 ($n = 9$ C, $n = 7$ LP), GD 19 ($n = 8$ C, $n = 10$ LP) and GD 21 ($n = 7$ C, $n = 5$ LP). Transport data for GD 15 control group was arbitrarily assigned a value of 1, and data for all other groups are expressed relative to GD 15 (C). Values are given as means ± S.E.M. *$P < 0.05$ *versus* GD 15 in the control and LP group, respectively.

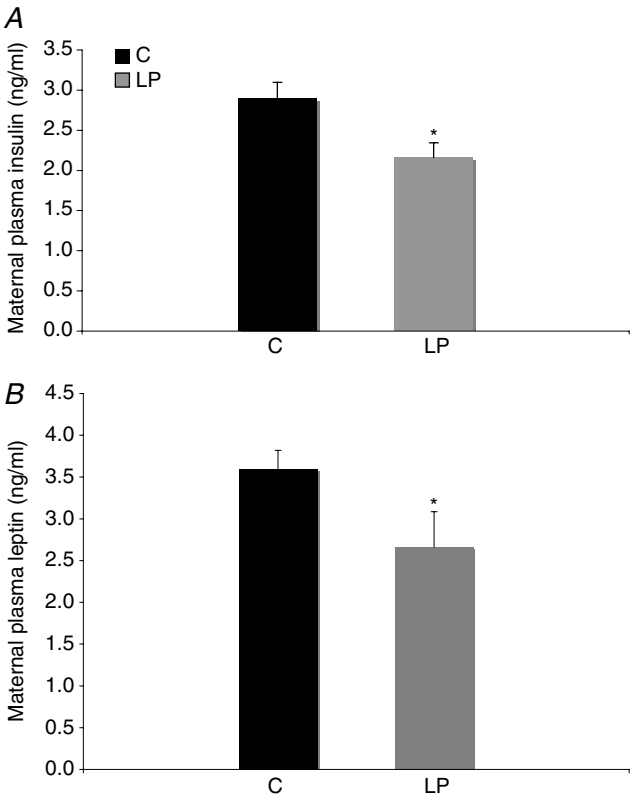

Figure 5. Maternal plasma insulin and leptin concentrations
A and *B*, maternal plasma insulin concentrations ($n = 17$ C, $n = 20$ LP) and plasma leptin ($n = 27$ C, control and $n = 22$ LP, low protein) did not change significantly between GD 15 and 21 and therefore the data were pooled from all gestational ages in each of the control and low-protein groups. Values are given as means ± S.E.M. *$P < 0.05$ *versus* control.

compatible with the hypothesis that placental transport changes in IUGR are a cause, rather than a consequence, of restricted fetal growth. In addition, maternal circulating levels of insulin, leptin and IGF-1, all hormones shown to stimulate placental System A activity, were reduced in response to low protein diet, establishing a possible link between maternal protein malnutrition, down-regulation of key placental amino acid transporters, and reduced fetal growth.

It is well established that the activity and/or expression of placental nutrient and ion transporters are modified in human pregnancies complicated by altered fetal growth (Jansson & Powell, 2000). In IUGR the activity of the placental Na^+/H^+ exchanger, the primary mechanism transporting protons into the maternal circulation, is reduced (Glazier *et al.* 1997; Johansson *et al.* 2002). In addition, IUGR is associated with a reduced placental Na^+,K^+-ATPase activity (Johansson *et al.* 2002), which may diminish the Na^+ gradient constituting the driving force for all Na^+-dependent transport systems, such as system A. The capacity of the placenta to transport glucose is not altered in human IUGR (Jansson *et al.* 1993) but is increased in pregnancies complicated by type-1 diabetes (Jansson *et al.* 1999, 2001), which associated with accelerated fetal growth. In contrast, a number of placental transport systems for essential amino acids, such as transporters for lysine, leucine and taurine (Jansson *et al.* 1998; Norberg *et al.* 1998), are down-regulated in IUGR, whereas placental leucine transport activity is increased in accelerated fetal growth (Jansson *et al.* 2002*a*).

One placental amino acid transport system that has attracted a particular interest in association with altered fetal growth is System A. This amino acid transport system mediates Na^+-dependent uptake of neutral amino acids such as alanine, glycine and glutamine (McGivan & Pastor-Anglada, 1994). System A is ubiquitously expressed and has been cloned and characterized at the molecular level (Hatanaka *et al.* 2000; Sugawara *et al.* 2000). There are three known isoforms of system A present in rat placenta, SNAT1, SNAT2 and SNAT4 (Mackenzie & Erickson, 2004). In both placental and non-placental tissues, system A is highly regulated by nutrients and by hormones such as insulin, cortisol and leptin (Kilberg, 1982; Longo *et al.* 1985; Bonadonna *et al.* 1993; McGivan & Pastor-Anglada, 1994; Jansson *et al.* 2003). It is interesting that many of the hormones known to regulate system A are altered (in terms of plasma levels, secretion or receptor number) in pregnancy complications associated with abnormal fetal and placental growth (Economides *et al.* 1989; Mirlesse *et al.* 1993; Nieto-Diaz *et al.* 1996; Henson & Castracane, 2000).

The activity of system A is reduced in the syncytiotrophoblast microvillous plasma membrane in human IUGR (Glazier *et al.* 1997; Jansson *et al.* 2002*b*). Malandro *et al.* (1996) showed that placental system A was down-regulated at GD 20 (term = 22) in rats fed a low protein diet. Furthermore, Cramer *et al.* (2002) studied the effect of partial inhibition/blocking of system A from GD 7 to GD 20 by systemic administration of MeAIB. Blocking system A in this study caused growth restricted pups at GD 20. These data collectively suggest that alterations in placental system A transport system activity are involved in the pathophysiology of IUGR. However, it remains to be established whether placental system A down-regulation represents a primary event directly contributing to the restricted fetal growth or constitutes a secondary consequence in response to IUGR.

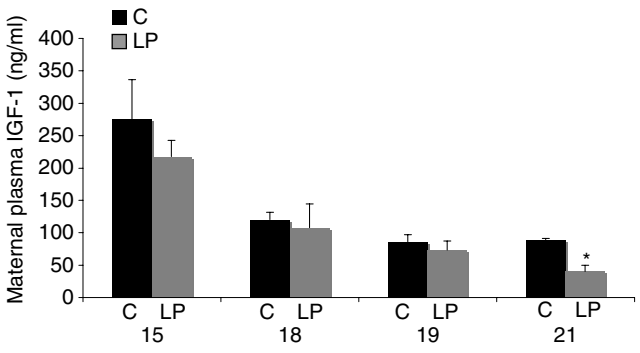

Figure 6. Maternal IGF-1 plasma concentrations in the control and LP groups at GD 15, 18, 19 and GD 21
Maternal IGF-1 plasma concentrations in the control groups at GD 18 ($n = 7$), GD 19 ($n = 9$) and GD 21 ($n = 5$) were significantly decreased compared to GD 15 ($n = 6$). At GD 21 the IGF-1 levels were significantly lower in the low protein group compared with the control group. Values are given as means ± S.E.M. $^*P < 0.05$ *versus* control.

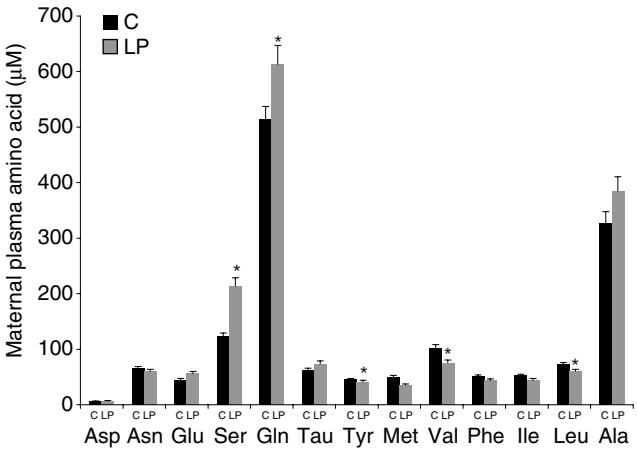

Figure 7. Maternal amino acid plasma concentrations
Maternal amino acid plasma concentrations (μM) ($n = 33$ C, control and $n = 25$ LP, low protein) did not change over time in the control group, and therefore the data were pooled from all gestational ages in the control and low-protein group, respectively. Values are given as means ± S.E.M. $^*P < 0.05$ *versus* control.

In the current study, placental system A transport activity *in vivo* and SNAT2 expression were significantly reduced at GD 21 (term = 23), in agreement with the *in vitro* study of Malandro *et al.* (1996) in which placental system A activity in microvillous membrane vesicles from LP rats was decreased at GD 20 (term = 22). Furthermore, we observed that the *in vivo* placental System A transport activity was decreased in the LP group at GD 19, a gestational age at which placental and fetal weights were maintained. In contrast to System A activity and expression, glucose transport *in vivo* was unaffected in this model of IUGR, findings that are in line with observations in human IUGR (Jansson *et al.* 2002*b*) and models of placental insufficiency in the rodent (Jansson & Persson, 1990). These data strongly indicate that reductions in placental amino acid transport occur before significant reduction in fetal or placental weights can be observed. With a substantial reduction of the placental transport of neutral amino acids at GD 19, the rapid fetal growth (approximately 1 g day^{-1}) at this stage of gestation cannot be sustained, and IUGR is observable 2 days later.

The cellular mechanisms underlying the reduced placental System A transport activity at GD 19 and 21 in the LP group remain to be fully established. The SNAT isoforms are primarily expressed in the labyrinth trophoblast of the rat placenta, suggesting a role for these transporters in the maternofetal transport of neutral amino acids (Novak *et al.* 2006). However, some SNAT expression is also found in other cells, such as marginal giant cells and fetal endothelium, in the rat placenta (Novak *et al.* 2006). Therefore, our measurements of SNAT protein and mRNA expression in placental homogenates may not completely represent the expression in labyrinth trophoblast. This may contribute to explaining the unchanged SNAT mRNA expression at GD 19 and 21, and the unaltered SNAT 2 protein expression at GD 19 despite reduced placental MeAIB transport at these gestational ages. Similarly, measurements of protein expression in homogenates will not detect translocation of transporters from intracellular compartments to the plasma membrane (Jones *et al.* 2006). However, SNAT 2 protein expression in placental homogenates was decreased at GD 21, in line with the *in vivo* transport data, and it is possible that changes in SNAT 1 and 4 protein expression, which we did not measure, may contribute to the observed transport changes at GD 19 and 21. In addition, our finding of a lack of correlation between SNAT 2 mRNA and protein is compatible with previous reports of marked discrepancy between SNAT gene and protein expression, as for example recently reported for SNAT4 in the human placenta (Desforges *et al.* 2006). It is also possible that post-translational modification is an important regulatory mechanism for the System A transporter.

Despite a modest increase in food intake of the low protein fed animals, compared to the control group, the LP group gained less weight during pregnancy. The effect of low protein diet during pregnancy in the rat is first observed as a reduction in maternal weight gain at GD 18 (see Table 1). However significant reductions in fetal weight did not occur until GD 21. The discrepancy in timing between maternal and fetal weight alterations may suggest that the rats given low protein diet have compensated for this successfully in early pregnancy and maintained fetal growth at normal levels. Other models for IUGR such as knockout mice in which the placenta specific insulin-like growth factor 2 (*Igf-2*) gene is deleted show a discrepancy in placental and fetal weight changes. The placental weights in the mutant mice were reduced as early as GD 14 while fetal growth restriction did not occur until GD 19 (mouse gestation is 20 days) (Constancia *et al.* 2002). Previous studies using the low protein diet model for IUGR in rats suggest that the mothers fed a low protein diet enter a catabolic state and maintain plasma amino acid levels that are normal or even higher than control mothers (Malandro *et al.* 1996). In another study (Rees *et al.* 1999) maternal serum concentrations of glutamate and glutamine were increased and concentrations of branched chain amino acids decreased in response to a low protein diet. In general agreement with these results, maternal plasma concentrations of serine and glutamine were moderately higher and concentrations of tyrosine, methionine, leucine and valine lower in our LP animals as compared to the control group. Interestingly, Rees *et al.* (1999) demonstrated a marked decrease in maternal threonine concentrations in LP rats and a decreased litter size in LP dams. In our study we did not measure threonine, precluding comparisons, and the difference in the effect on litter size between the two studies is most likely related to the time point for starting the LP diet; 2 weeks prior to conception in the experiments of Rees *et al.* and after conception in our study. However, even with maternal plasma amino acid concentrations relatively maintained, placental System A activity, and possibly the activity of other amino acid transporters, was decreased by GD 19 and IUGR was observable 2 days later. These data are compatible with the hypothesis that down-regulation of placental amino acid transporters, such as System A, mediates the decreased amino acid supply to the fetus and causes IUGR in maternal protein restriction. The consistent finding of a relationship between placental System A and fetal growth in human pregnancy (Dicke & Henderson, 1988; Mahendran *et al.* 1993) and the IUGR observed as a result of inhibiting placental System A activity (Cramer *et al.* 2002) are observations in support of the important role of placental system A activity in determining fetal growth.

It cannot be excluded that the means by which calories are compensated for in the LP diet, in order to maintain the

diet isocaloric, may have some influence on the maternal metabolic response. However, it appears highly unlikely that the increased content of sucrose, which is metabolized to glucose, in the LP diet is causing the maternal metabolic adaptations and the resulting effects on placental transport and fetal growth that we observe. LP dams in our study enter a catabolic state with breakdown of maternal protein and fat stores as evidenced by lower maternal weight gain, low circulating insulin, IGF-1 and leptin, and generally maintained serum amino acid concentrations despite low intake of protein. This metabolic profile is compatible with a low protein diet, but not a high sucrose intake.

The signals mediating the down-regulation of placental System A in the low protein fed rats are not known. *In vitro*, system A activity is increased in response to low amino acid concentrations, a process known as adaptive regulation (Gazzola *et al.* 1981, 2001), a phenomenon recently shown also for a human placental cell line (Jones *et al.* 2006). If the reverse is true, that high amino acid concentrations down-regulate system A activity, it cannot be excluded that the moderate increase in the maternal plasma concentrations of serine and glutamine, both substrates for the System A transporter, may contribute to the down-regulation of placental System A activity in our study. Furthermore, maternal malnutrition is associated with a reduced placental blood flow (Rosso & Kava, 1980) which may represent a signal for alterations in placental nutrient transporters. Maternal protein malnutrition is also likely to elicit changes in the circulating levels of a number of metabolically active hormones. Indeed, the LP animals showed significantly reduced levels of insulin and leptin, two hormones that we previously have shown to increase the activity of placental system A (Jansson *et al.* 2003). Maternal plasma concentrations of IGF-I is altered in human pregnancies complicated by altered fetal growth (Holmes *et al.* 1997; Lauszus *et al.* 2001) and IGF-I increases the activity of System A (Bloxam *et al.* 1994; Kniss *et al.* 1994; Karl, 1995). In our study the LP group had significantly decreased maternal levels of IGF-1 at GD 21. Thus, we speculate that reduced levels of maternal hormones such as insulin, leptin and IGF-1 link maternal protein malnutrition to reduced fetal growth by down-regulation of key placental amino acid transporters.

It is possible that our proposed model, where a maternal low protein diet elicits changes in metabolic hormones, which down-regulates placental amino acid transporters resulting in decreased amino acid delivery to the fetus and impaired fetal growth, represents an oversimplification. For example, it cannot be excluded that fetal responses to maternal protein malnutrition are initiated prior to the changes in placental nutrient transport, and that these fetal responses mediate the reduced fetal growth rather than the down-regulation of placental amino acid transport. These questions can only be addressed in further studies focusing on the effect of maternal protein malnutrition on key signalling pathways involved in the regulation of fetal growth.

We have suggested that the placenta functions as a nutrient sensor (Jansson & Powell, 2000, 2006), matching fetal growth rate to the ability of the maternal supply line to deliver nutrients by altering placental transport functions. Indeed, the demonstration of down-regulation of placental System A transport in response to maternal protein restriction is compatible with this hypothesis. We speculated that mTOR may represent a signalling pathway for coordinating nutrient sensing functions of the placenta. Notably, placental mTOR protein expression at GD 18 was reduced in the LP group (borderline significance, $P = 0.09$). This is interesting since this is at a time point just before placental system A activity was found to be decreased, compatible with a role for the placental mTOR system in regulating placental function in response to changes in nutrient availability. However, in order to establish the involvement of mTOR in a placental nutrient sensing system, a more detailed analysis is required, including studies of the activation of downstream effectors of mTOR, such as 4E-binding protein-1 and S6-kinase.

References

Barker DJ (2001). The malnourished baby and infant. *Br Med Bull* **60**, 69–88.

Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA & Robinson JS (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet* **341**, 938–941.

Bloxam DL, Bax BE & Bax CM (1994). Epidermal growth factor and insulin-like growth factor I differently influence the directional accumulation and transfer of 2-aminoisobutyrate (AIB) by human placental trophoblast in two-sided culture. *Biochem Biophys Res Commun* **199**, 922–929.

Bonadonna RC, Saccomani MP, Cobelli C & DeFronzo RA (1993). Effect of insulin on system A amino acid transport in human skeletal muscle. *J Clin Invest* **91**, 514–521.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.

Campbell D, Hall M, Barker D, Cross J, Shiell A & Godfrey K (1996). Diet in pregnancy and the offspring's blood pressure 40 years later. *Br J Obstet Gynaecol* **103**, 273–280.

Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, Stewart F, Kelsey G, Fowden A, Sibley C & Reik W (2002). Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* **417**, 945–948.

Cramer S, Beveridge M, Kilberg M & Novak D (2002). Physiological importance of system A-mediated amino acid transport to rat fetal development. *Am J Physiol Cell Physiol* **282**, C153–C160.

Desforges M, Lacey HA, Glazier JD, Greenwood SL, Mynett KJ, Speake PF & Sibley CP (2006). SNAT4 isoform of system A amino acid transporter is expressed in human placenta. *Am J Physiol Cell Physiol* **290**, C305–C312.

Dicke JM & Henderson GI (1988). Placental amino acid uptake in normal and complicated pregnancies. *Am J Med Sci* **295**, 223–227.

Economides DL, Proudler A & Nicolaides KH (1989). Plasma insulin in appropriate- and small-for-gestational-age fetuses. *Am J Obstet Gynecol* **160**, 1091–1094.

Gazzola GC, Dall'Asta V & Guidotti GG (1981). Adaptive regulation of amino acid transport in cultured human fibroblasts. Sites and mechanism of action. *J Biol Chem* **256**, 3191–3198.

Gazzola RF, Sala R, Bussolati O, Visigalli R, Dall'Asta V, Ganapathy V & Gazzola GC (2001). The adaptive regulation of amino acid transport system A is associated to changes in ATA2 expression. *FEBS Lett* **490**, 11–14.

Glazier JD, Cetin I, Perugino G, Ronzoni S, Grey AM, Mahendran D, Marconi AM, Pardi G & Sibley CP (1997). Association between the activity of the system A amino acid transporter in the microvillous plasma membrane of the human placenta and severity of fetal compromise in intrauterine growth restriction. *Pediatr Res* **42**, 514–519.

Godfrey K, Robinson S, Barker DJ, Osmond C & Cox V (1996). Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ* **312**, 410–414.

Harding JE (2001). The nutritional basis of the fetal origins of adult disease. *Int J Epidemiol* **30**, 15–23.

Hatanaka T, Huang W, Wang H, Sugawara M, Prasad PD, Leibach FH & Ganapathy V (2000). Primary structure, functional characteristics and tissue expression pattern of human ATA2, a subtype of amino acid transport system A. *Biochim Biophys Acta* **1467**, 1–6.

Henson MC & Castracane VD (2000). Leptin in pregnancy. *Biol Reprod* **63**, 1219–1228.

Holmes R, Montemagno R, Jones J, Preece M, Rodeck C & Soothill P (1997). Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth. *Early Hum Dev* **49**, 7–17.

Jacinto E & Hall MN (2003). Tor signalling in bugs, brain and brawn. *Nat Rev Mol Cell Biol* **4**, 117–126.

Jansson T, Ekstrand Y, Bjorn C, Wennergren M & Powell TL (2002*a*). Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes* **51**, 2214–2219.

Jansson T, Ekstrand Y, Wennergren M & Powell TL (2001). Placental glucose transport in gestational diabetes mellitus. *Am J Obstet Gynecol* **184**, 111–116.

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 T & Persson E (1990). Placental transfer of glucose and amino acids in intrauterine growth retardation: studies with substrate analogs in the awake guinea pig. *Pediatr Res* **28**, 203–208.

Jansson T & Powell TL (2000). Placental nutrient transfer and fetal growth. *Nutrition* **16**, 500–502.

Jansson T & Powell TL (2006). Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? – A Review. *Placenta* **27** (Suppl A), S91–97.

Jansson T, Scholtbach V & Powell TL (1998). Placental transport of leucine and lysine is reduced in intrauterine growth restriction. *Pediatr Res* **44**, 532–537.

Jansson T, Wennergren M & Illsley NP (1993). Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. *J Clin Endocrinol Metab* **77**, 1554–1562.

Jansson T, Wennergren M & Powell TL (1999). Placental glucose transport and GLUT 1 expression in insulin-dependent diabetes. *Am J Obstet Gynecol* **180**, 163–168.

Jansson T, Ylven K, Wennergren M & Powell TL (2002*b*). Glucose transport and system A activity in syncytiotrophoblast microvillous and basal plasma membranes in intrauterine growth restriction. *Placenta* **23**, 392–399.

Johansson M, Glazier JD, Sibley CP, Jansson T & Powell TL (2002). Activity and protein expression of the Na^+/H^+ exchanger is reduced in syncytiotrophoblast microvillous plasma membranes isolated from preterm intrauterine growth restriction pregnancies. *J Clin Endocrinol Metab* **87**, 5686–5694.

Johansson M, Karlsson L, Wennergren M, Jansson T & Powell TL (2003). Activity and protein expression of Na^+/K^+ ATPase are reduced in microvillous syncytiotrophoblast plasma membranes isolated from pregnancies complicated by intrauterine growth restriction. *J Clin Endocrinol Metab* **88**, 2831–2837.

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.

Karl PI (1995). Insulin-like growth factor-1 stimulates amino acid uptake by the cultured human placental trophoblast. *J Cell Physiol* **165**, 83–88.

Karl PI, Alpy KL & Fisher SE (1992). Amino acid transport by the cultured human placental trophoblast: effect of insulin on AIB transport. *Am J Physiol* **262**, C834–C839.

Kilberg MS (1982). Amino acid transport in isolated rat hepatocytes. *J Membr Biol* **69**, 1–12.

Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P & Sabatini DM (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* **110**, 163–175.

Kniss DA, Shubert PJ, Zimmerman PD, Landon MB & Gabbe SG (1994). Insulinlike growth factors. Their regulation of glucose and amino acid transport in placental trophoblasts isolated from first-trimester chorionic villi. *J Reprod Med* **39**, 249–256.

Langley-Evans SC (2001). Fetal programming of cardiovacular function through exposure to maternal undernutrition. *Proc Nutr Soc* **60**, 505–513.

Lauszus FF, Klebe JG & Flyvbjerg A (2001). Macrosomia associated with maternal serum insulin-like growth factor-I and -II in diabetic pregnancy. *Obstet Gynecol* **97**, 734–741.

Lindroth P & Mopper K (1979). High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde. *Anal Chem* **51**, 1667–1674.

Ling R, Bridges CC, Sugawara M, Fujita T, Leibach FH, Prasad PD & Ganapathy V (2001). Involvement of transporter recruitment as well as gene expression in the substrate-induced adaptive regulation of amino acid transport system A. *Biochim Biophys Acta* **1512**, 15–21.

Longo N, Franchi-Gazzola R, Bussolati O, Dall'Asta V, Foa PP, Guidotti GG & Gazzola GC (1985). Effect of insulin on the activity of amino acid transport systems in cultured human fibroblasts. *Biochim Biophys Acta* **844**, 216–223.

McGivan JD & Pastor-Anglada M (1994). Regulatory and molecular aspects of mammalian amino acid transport. *Biochem J* **299**, 321–334.

Mackenzie B & Erickson JD (2004). Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. *Pflugers Arch* **447**, 784–795.

Mahendran D, Donnai P, Glazier JD, D'Souza SW, Boyd RD & Sibley CP (1993). Amino acid (system A) transporter activity in microvillous membrane vesicles from the placentas of appropriate and small for gestational age babies. *Pediatr Res* **34**, 661–665.

Malandro MS, Beveridge MJ, Kilberg MS & Novak DA (1996). Effect of low-protein diet-induced intrauterine growth retardation on rat placental amino acid transport. *Am J Physiol* **271**, C295–C303.

Mirlesse V, Frankenne F, Alsat E, Poncelet M, Hennen G & Evain-Brion D (1993). Placental growth hormone levels in normal pregnancy and in pregnancies with intrauterine growth retardation. *Pediatr Res* **34**, 439–442.

Moore VM, Davies MJ, Willson KJ, Worsley A & Robinson JS (2004). Dietary composition of pregnant women is related to size of the baby at birth. *J Nutr* **134**, 1820–1826.

Nelson DM, Smith SD, Furesz TC, Sadovsky Y, Ganapathy V, Parvin CA & Smith CH (2003). Hypoxia reduces expression and function of system A amino acid transporters in cultured term human trophoblasts. *Am J Physiol Cell Physiol* **284**, C310–C315.

Nieto-Diaz A, Villar J, Matorras-Weinig R & Valenzuela-Ruiz P (1996). Intrauterine growth retardation at term: association between anthropometric and endocrine parameters. *Acta Obstet Gynecol Scand* **75**, 127–131.

Norberg S, Powell TL & Jansson T (1998). Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. *Pediatr Res* **44**, 233–238.

Novak D, Lehman M, Bernstein H, Beveridge M & Cramer S (2006). SNAT expression in rat placenta. *Placenta* **27**, 510–516.

Ozanne SE (2001). Metabolic programming in animals. *Br Med Bull* **60**, 615–619.

Raught B, Gingras AC & Sonenberg N (2001). The target of rapamycin (TOR) proteins. *Proc Natl Acad Sci U S A* **98**, 7037–7044.

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.

Rosso P & Kava R (1980). Effects of food restriction on cardiac ouput and blood flow to the uterus and placenta in the pregnant rat. *J Nutr* **110**, 2350–2354.

Sandberg M, Butcher SP & Hagberg H (1986). Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycemia: in vivo dialysis of the rat hippocampus. *J Neurochem* **47**, 178–184.

Strid H, Bucht E, Jansson T, Wennergren M & Powell TL (2003). ATP dependent Ca^{2+} transport across basal membrane of human syncytiotrophoblast in pregnancies complicated by intrauterine growth restriction or diabetes. *Placenta* **24**, 445–452.

Sugawara M, Nakanishi T, Fei YJ, Huang W, Ganapathy ME, Leibach FH & Ganapathy V (2000). Cloning of an amino acid transporter with functional characteristics and tissue expression pattern identical to that of system A. *J Biol Chem* **275**, 16473–16477.

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