Maternal undernutrition during late gestation-induced intrauterine growth restriction in the rat is associated with impaired placental GLUT3 expression, but does not correlate with endogenous corticosterone levels

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Abstract

Fetal intrauterine growth restriction (IUGR) is a frequently occurring and serious complication of pregnancy. Infants exposed to IUGR are at risk for numerous perinatal morbidities, including hypoglycemia in the neonatal period, as well as increased risk of later physical and/or mental impairments, cardiovascular disease and non-insulin-dependent diabetes mellitus. Fetal growth restriction most often results from uteroplacental dysfunction during the later stage of pregnancy. As glucose, which is the most abundant nutrient crossing the placenta, fulfills a large portion of the fetal energy requirements during gestational development, and since impaired placental glucose transport is thought to result in growth restriction, we investigated the effects of maternal 50% food restriction (FR50) during the last week of gestation on rat placental expression of glucose transporters, GLUT1, GLUT3 and GLUT4, and on plasma glucose content in both maternal and fetal compartments. Moreover, as maternal FR50 induces fetal overexposure to glucocorticoids and since these hormones are potent regulators of placental glucose transporter expression, we investigated whether putative alterations in placental GLUT expression correlate with changes in maternal and/or fetal corticosterone levels.

At term (day 21 of pregnancy), plasma glucose content was significantly reduced ($P<0.05$) in FR50 mothers. Fetuses from FR50 mothers showed reduced body weight ($P<0.001$) but higher plasma corticosterone levels ($P<0.05$). Adrenalectomy (ADX) followed by corticosterone supplementation of the mother prevented the FR50-induced rise in maternal plasma corticosterone at term. Food restriction performed on either sham-ADX or ADX mothers induced a similar reduction in the body weight of the pups at term ($P<0.01$). Moreover, plasma corticosterone levels were increased in pups from sham-ADX FR50 mothers ($P<0.01$) and in pups from ADX control mothers ($P<0.01$). Western blot analysis of placental GLUT proteins showed that maternal FR50 decreased placental GLUT3 protein levels in all experimental groups at term ($P<0.05$ and $P<0.01$), but did not affect either GLUT1 or GLUT4 protein levels. Northern blot analysis of placental GLUT expression showed that both GLUT1 and GLUT3 mRNA were not affected by the maternal feeding regimen or surgery.

We concluded that prolonged maternal malnutrition during late gestation decreases maternal plasma glucose content and placental GLUT3 glucose transporter expression, but does not obviously affect fetal plasma glucose concentration. Moreover, the present results are not compatible with a role of maternal corticosterone in the development of growth-restricted rat fetuses.


Introduction

Intrauterine growth restriction (IUGR) is a frequently occurring and serious complication of pregnancy. Infants exposed to IUGR are at high risk for numerous perinatal morbidities as well as physical and/or mental impairments in later life (Cunningham et al. 1997). Fetal growth restriction can be symmetrical or asymmetrical.
(Cunningham et al. 1997). Asymmetrical growth restriction most often results from uteroplacental dysfunction during the later stage of pregnancy, such as impaired placental glucose transfer (Cunningham et al. 1997).

The placenta is not only a passive transport vehicle or a mechanical barrier, it facilitates and regulates bidirectional transfer processes, modifies maternal nutrients destined for the fetus, and has its own active energy metabolism to support these activities (Sibley & Boyd 1988, Hay 1991). Efficient placental (maternal-to-fetal) transfer of glucose, the primary substrate for fetal oxidative metabolism, is crucial to sustain the normal development and survival of the fetus in utero, since it is not capable of producing appreciable amounts of glucose until late in gestation (Girard et al. 1992). This process is brought about by facilitated diffusion along a concentration gradient rendering substrate entry about 10 000 times faster than calculated for diffusion across the lipid membrane layer. The transport facilitators are about 500 amino acids in length and belong to a growing superfamily of integral membrane glycoproteins with 12 membrane-spanning domains that presumably form a channel through which glucose can move in one or more association–dissociation steps (Mueckler 1994). The genes of these glucose transporters have been designated GLUT1–GLUT11 in the order in which they were identified. Among them, the three high-affinity isoforms GLUT1, GLUT3 and GLUT4 have been identified so far in both human and rodent placentas (Hahn et al. 1995, Xing et al. 1998, Knipp et al. 1999, Korgun et al. 2001). Because of their low Michaelis constant (Km), these transporters function at rates close to maximal velocity. Thus, their level of cell surface expression greatly influences the rate of glucose uptake into the cells, and thereafter the transplacental glucose transfer.

Numerous animal and epidemiological studies have indicated that malnutrition is a frequent cause of asymmetrical IUGR (Cunningham et al. 1997), and may even lead to cardiovascular diseases and non-insulin–dependent diabetes mellitus in the adult (Barker et al. 1993a,b, Phillips 1998). As a first step to identifying the underlying molecular mechanisms, we studied the effect of maternal food restriction on rat placental expression of GLUT1, GLUT3 and GLUT4 and on plasma glucose content in both the maternal and fetal compartments.

In addition, a growing body of evidence suggesting that an inadequate glucocorticoid environment in utero represents the missing link between restricted fetal growth and some adulthood diseases (Seckl 1994), together with data from our own laboratories showing that maternal food restriction during late gestation induces fetal overexposure to glucocorticoids (Lesage et al. 2001) and that these hormones inhibit the expression of placental glucose transporters (Hahn et al. 1999), prompted us to investigate whether putative alterations in placental GLUT expression correlate with changes in maternal and/or fetal corticosterone levels.

Materials and Methods

Animals and housing conditions

Wistar rats (200 g) were purchased from IFFA-CREDO (L’Arbresle, France) and housed five per cage in a room with a controlled light cycle (12h light:12h darkness; lights on at 07:00 h) and temperature (22 ± 2 °C), with free access to food (regular rat chow no. 113, containing 22% protein, 5% fat and 53% carbohydrate; UAR, Villemoisson sur Orge, France) and tap water. After 8 days of acclimation, females were mated with a male for 1 night. The next day was taken as day 0 of pregnancy if spermatozoa were found in the vaginal smears. Pregnant females were then transferred to individual cages. Animal use accreditation by the French Ministry of Agriculture (no. 04860) has been granted to our laboratory for experimentation with rats.

Adrenalectomy and substitutive treatment

At day 13 of gestation (E13), pregnant females were weighed and assigned to one of the three following experimental groups of equal average body weight: intact, adrenalectomized (ADX) and sham-ADX females (n=8–12 animals/group). At E14, some females were either adrenalectomized or sham-operated between 0900 and 1200 h under ether anesthesia via the dorsal approach. ADX females were then implanted s.c. with a 100 mg corticosterone pellet (mixture of 50% corticosterone and 50% cholesterol), which has been reported to provide stable and basal levels of corticosterone (Lesage et al. 2001). ADX mothers were given saline (0-9% NaCl) as drinking water. Intact females were left undisturbed in their home cage.

Feeding regimens

At E14, each group of pregnant females (intact, ADX and sham-ADX) was divided into two subgroups (n=4–6 animals/subgroup). The females of the first subgroup were fed ad libitum (control) until E21. The females of the second subgroup (FR50) were fed daily with 12 g commercial rat chow, which represents about 50% of the daily intake of pregnant intact dams, from E14 to E21. The pregnant females exposed to undernutrition were fed every day at 1800 h to allow food intake at the usual time (night). Tap water was available ad libitum.

Decapitation, plasma and tissue collections

Pregnant females at E21 were rapidly weighed and killed by decapitation between 1000 and 1200 h. Each litter usually contained between eight and 12 fetuses, which were collected by Cesarean section and immediately killed by decapitation. Pups were weighed rapidly just before
death, and sex was determined by examination of the genitals. Trunk blood samples of mothers and litter-mate fetuses were collected after decapitation and put into polyethylene tubes pre-rinsed with EDTA. The blood samples were centrifuged at 4000 r.p.m. for 10 min at 4 °C. Plasma samples were kept at −30 °C until corticosterone assay. Blood glucose was measured after decapsulation using a glucometer (One Touch II; Lifescan, Roissy, France). Placentas were weighed, divided into two pieces and immediately frozen on dry ice and stored at −70 °C.

**SDS-PAGE and Western blotting**

Cellular proteins from two half placentas were solubilized in Laemmli sample buffer (Sigma Chemical Co., St Louis, MO, USA) supplemented with complete protease inhibitor cocktail (Boehringer Mannheim, Mannheim, Germany). Insoluble material was removed by centrifugation at 100 000 g for 1 h at 4 °C. Samples were either used immediately or stored for up to 10 days at −70 °C. Before electrophoresis, samples were boiled for 3 min at 100 °C. Equal amounts of protein, determined according to the method of Lowry et al. (1951), were subjected to SDS-PAGE on 8–18% gradient gels (ExcelGel; Pharmacia Biotech, Uppsala, Sweden) using SDS buffer strips (ExcelGel; Pharmacia Biotech). Samples were run for 150 min at a constant 600 V, 50 mA and 30 W. Proteins were transferred onto nitrocellulose membranes (Pharmacia Biotech) by semidy electroblotting in a buffer containing 0·2 mol/l glycine, 25 mmol/l Tris and 20% methanol for 45 min at 30 V, 100 mA and 6 W. Successful transfer was confirmed by Ponceau S (Sigma Chemical Co.) staining of the blots. The membranes were blocked for 12 h with 5% non-fat dry milk (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and 0·1% Tween-20 (Sigma Chemical Co.) in 0·14 mol/l Tris–buffered saline, pH 7·2–7·4, at 4 °C. The same solution was used for subsequent washings and as diluent for the antibodies. The blotting membranes were incubated for 1 h at room temperature with rabbit antiserum against the C-terminal sequences of GLUT1 (CGLFHPGLGADSQV), GLUT3 (NSMQPVKEPGNA) and GLUT4 (CTELEYLGPEND) (all from Chemicon, Temecula, CA, USA). Antiseras were diluted 1:10 000 (GLUT1), 1:3000 (GLUT3) and 1:3000 (GLUT4). After washing, the membranes were further incubated with goat anti-rabbit IgG horseradish peroxidase conjugate (Bio-Rad Laboratories, Inc.) diluted 1:10 000 (GLUT1), 1:5000 (GLUT3) or 1:3000 (GLUT4) for 1 h at room temperature. After three washings in Tris-buffered saline, pH 7·2–7·4, the immunolabeling was visualized using the chemiluminescence-based SuperSignal CL–HRP Substrate System (Pierce Chemical Co., Rockford, IL, USA) according to the manufacturer’s instructions. Membranes were exposed to Hyperfilm (Amersham International plc, Amersham, Bucks, UK) which was subsequently analyzed using an Eagle Eye II gel documentation unit (Stratagene, Cambridge, Cambs, UK). Control blots were incubated with antisera preadsorbed with the corresponding oligo-peptide sequences (10 µg/ml; Pichem, Graz, Austria) used for the immunization of the antibody-generating rabbits.

**Northern blot analysis**

Total RNA from two half placental labyrinths was isolated and 15 µg total RNA was separated by electrophoresis on a 1·2% agarose gel and transferred to a Hybond N+ membrane (Amersham International plc). Human GLUT1 and GLUT3 cDNA fragments were labeled using a random-primed DNA labeling kit (Multi prime DNA Labelling Systems; Amersham International plc) and were used as probes as previously described (Hahn et al. 1999). Probes for GLUT1 and GLUT3 mRNAs were prepared from near full-length human cDNA clones. The pGEM-3 plasmid containing a 2·47 kb GLUT1 cDNA and the pBSII plasmid containing a 2·60 kb GLUT3 cDNA were obtained from the American Type Culture Collection (nos 59631 and 61614 respectively; Bethesda, MD, USA). These human probes show more than 90% homology with rat sequences. Signals were detected by autoradiography with Hyperfilm MPX-ray (Amersham International plc). The same membranes were probed sequentially with GLUT1, GLUT3 and rat glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The optical density of the hybridized signal was measured using a GS-700 densitometer coupled with a computer-assisted image analysis using Multi-Analyst software (Bio-Rad Laboratories, Inc.).

**Radioimmunoassay**

Corticosterone assay in plasma samples was preceded by an extraction in ethylacetate after delipidation in iso-octane. The percent recovery of a known amount of corticosterone was 66%. Corticosterone levels were determined by RIA, using a highly specific corticosterone antiserum (UCB-Bioproducts, Paris, France), as previously described (Bernet et al. 1994), with a detection threshold of 1 ng/ml. The intra- and interassay variations were 2·4% and 4·4% respectively.

**Statistical analysis**

All data are presented as the means ± s.e.m. Statistical analysis was performed using multiple ANOVA, followed by Dunnett’s test. Unpaired Student’s t-test was also used when appropriate. P<0·05 was considered significant.

**Results**

**Maternal and fetal plasma glucose content**

At E21, plasma glucose content was significantly reduced (P<0·05) in mothers subjected to FR50 during the last
that maternal food restriction during the last week of pregnancy decreased placental GLUT3 protein levels at term in all experimental groups (Figs 2 and 3A). In contrast, maternal FR50 did not affect either GLUT1 or GLUT4 proteins levels in any of the experimental groups (data not shown). Northern blot analysis of placental GLUT expression showed that neither GLUT1 (data not shown) nor GLUT3 mRNA were affected at E21 by maternal feeding regimen or surgery (Fig. 3B).

Discussion

Proper fetal development is dependent on sufficient nutrient supply to the fetus. Since substrate transport, including glucose, is directed from the maternal to the fetal circulation under physiological conditions, the placenta’s ability to facilitate this transfer is of critical importance for the development of a healthy fetus (Knipp et al. 1999). In humans, fetal hypoglycemia in IUGR is not due to a decrease in placental GLUT1 glucose transporter density (Jansson et al. 1993). In the present study, in considering the increasing nutrient demands of the developing fetus with progressing gestation, we addressed the question of whether disturbances of the placental glucose transporter systems (including GLUT1, GLUT3 and GLUT4 transporters) could contribute, in rats, to the restricted fetal growth observed with maternal malnutrition.

It was not surprising to observe, in our experimental system, that food restriction during the last week of gestation decreased the maternal plasma glucose content. As a consequence, the maternal-to-fetal glucose concentration gradient, which represents the general driving force for facilitative glucose diffusion across the placenta, is impaired and transplacental glucose transport may be reduced. One of the most important outcomes of this study was that, under these conditions, placental GLUT3 glucose transporter expression was diminished. Nevertheless, GLUT3 gene expression was not significantly affected by the maternal feeding regimen. Such a discrepancy between GLUT3 gene expression and protein level could suggest disturbances of the translational process. Because of its high substrate affinity, GLUT3 is the transporter isomorph characteristic of cells with high glucose requirements such as neurons or tumor cells (Hahn et al. 1998). The $K_m$ value of GLUT3 is so small that it may be saturated even at hypoglycemic levels. Therefore, provided that endogenous factors released in response to malnutrition do not directly affect $K_m$, GLUT3-mediated changes in transmembrane glucose uptake can only be brought about by translocation of cellular carriers or changes in expression levels. In the placenta, the specific expression of GLUT3 in the labyrinth, which specializes in nutrient transfer, suggests that GLUT3 may be important for the placental transfer of glucose (Zhou & Bondy 1993).

Maternal and placental characteristics

Food restriction of pregnant rats during late gestation reduced, at E21, the maternal body weight by 17% (untreated mothers), 12% (sham-ADX) and 12% (ADX) but did not affect placental weight in all experimental groups (Table 1). Plasma corticosterone concentration was significantly increased in untreated and sham-operated FR50 mothers. ADX followed by corticosterone supplementation of the mother prevented the FR50-induced rise in plasma corticosterone at E21 (Table 1).

Fetal characteristics

Fetuses from untreated FR50 mothers showed body weight reduced by 15% ($P<0.001$) but higher plasma corticosterone levels ($P<0.05$) than controls (Table 1). Food restriction performed on either sham-ADX or ADX mothers induced a similar reduction in body weight of pups at term of 7% and 6-3% respectively ($P<0.01$). Plasma corticosterone levels were increased in pups from sham-ADX FR50 mothers ($P<0.01$) in comparison with the respective controls, as well as in pups from ADX control mothers ($P<0.01$) in comparison with sham-ADX controls (Table 1).

Placental GLUT protein and mRNA expression levels

Western blot analysis of placental GLUT proteins showed that maternal food restriction during the last week of pregnancy decreased placental GLUT3 protein levels at term in all experimental groups (Figs 2 and 3A). In contrast, maternal FR50 did not affect either GLUT1 or GLUT4 proteins levels in any of the experimental groups (data not shown). Northern blot analysis of placental GLUT expression showed that neither GLUT1 (data not shown) nor GLUT3 mRNA were affected at E21 by maternal feeding regimen or surgery (Fig. 3B).
To the best of our knowledge, the present data are unique in that they demonstrate a down-regulation of placental GLUT3 associated with a reduction in the maternal plasma glucose concentration. So far, it has been commonly observed that glucose deprivation increases cellular hexose transporter content in various tissues (Kahn & Flier 1990, Klip et al. 1994). Furthermore, it is intriguing to note that placental GLUT1 and GLUT4 expression remained virtually unaffected by maternal malnutrition, since it has been reported that at least GLUT1 is more responsive to modulation by glucose deprivation than GLUT3 (Vannucci et al. 1997). On the other hand, one cannot rule out the possibility that the effectiveness of GLUT1 and GLUT4 is subject to regulation by the hypoglycemic state in a manner aiming to transport more glucose molecules per unit of time, independently of a constant number of transporters in the plasma membrane. It has been reported that functional GLUT4 and probably also GLUT1 exist in at least two stable configurations. Similar to gated ion channels, they can undergo a change of state from one configuration to the other, in which their ability to transport glucose is increased in response to insulin and perhaps to other stimuli (Zierler 1999).

The unexpected finding that fetal plasma glucose content was not reduced upon maternal malnutrition may also point to the existence of effective (fetal) counter-regulatory mechanisms in order to restore euglycemia at term. We can postulate that the potential decreased ability of the placenta to transport glucose, as a result of the down-regulation of GLUT3 glucose transporter expression, could be a protective measure to limit glucose transport out of the placenta. Indeed, since facilitated diffusion is driven by a concentration gradient, there should be a backflux of glucose (Thomas et al. 1990) in the fetal to maternal direction under this condition of maternal hypoglycemia. Nevertheless, it would be interesting to observe whether the fetus is hypoglycemic at earlier time-points. In humans, hormones like glucagon, catecholamines, cortisol and growth hormone are known to be released in response to hypoglycemia (Garber et al. 1976). Thus, we can postulate that putative endocrine disturbances in fetuses could have accounted for the restoration of euglycemia.

In addition to changes in the intrinsic activity of transporter molecules, glucose levels in the fetus could have been maintained by release from placental glycogen stores or by an increase in fetal gluconeogenesis. This could

<table>
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<th>Maternal treatment</th>
<th>Maternal weight (g)</th>
<th>Maternal plasma corticosterone content (ng/ml)</th>
<th>Placental weight (mg)</th>
<th>Fetal weight (g)</th>
<th>Fetal plasma corticosterone content (ng/ml)</th>
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<td>342·5 ± 5·5</td>
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<td>71·0 ± 4·5*</td>
<td>537·4 ± 12·5</td>
<td>4·35 ± 0·08a</td>
<td>55·9 ± 3·5*</td>
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<td>360·0 ± 10</td>
<td>27·9 ± 2·2</td>
<td>547·3 ± 11·9</td>
<td>5·05 ± 0·10</td>
<td>33·0 ± 3·0</td>
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<tr>
<td>FR50</td>
<td>318·4 ± 6·5b</td>
<td>59·0 ± 6·3b*</td>
<td>545·8 ± 9·8</td>
<td>4·70 ± 0·03b</td>
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<td>Control</td>
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<td>543·1 ± 10·2</td>
<td>5·07 ± 0·07</td>
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<td>10·5 ± 2·7</td>
<td>552·0 ± 13·4</td>
<td>4·75 ± 0·06b</td>
<td>52·6 ± 2·5</td>
</tr>
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</table>

*P<0·05, **P<0·01, ***P<0·001 FR50 group vs respective control group; ****P<0·01 ADX control vs sham-ADX control (multiple ANOVA followed by Dunnett’s post-hoc test).

Table 1 Effects of maternal FR50 from E14 to E21 on maternal and fetal characteristics at E21. Data are the means ± S.E.M.; n=4–6 mothers/subgroup; n=12–16 placentas or fetuses/subgroup.

![Figure 2](https://www.endocrinology.org)
compensate for the diminished maternal substrate supply and serve to meet the metabolic needs of the fetus. Indeed, in the rat, placental glycogen is amenable to mobilization by hormonal stimuli causing phosphorylase activation (Barash & Shafrir 1990). However, a putative increase in fetal gluconeogenesis is unlikely as gluconeogenesis develops significantly after birth in the rat (Girard et al. 2001). Moreover, we also found that maternal food restriction reduces the expression of placental 11β-hydroxysteroid dehydrogenase (Lesage et al. 2001), which converts physiological glucocorticoids to inactive 11-keto products (Murphy et al. 1974, Brown et al. 1996), thus protecting the fetus from excess maternal glucocorticoids. Numerous data suggest that, in both humans and other animals, relatively moderate glucocorticoid excess during pregnancy induces IUGR (Seckl 1994). Excessive glucocorticoid exposure in utero exerts tissue-specific effects to restrict fetal growth (Reinisch et al. 1978, Mosier et al. 1982, Novy & Walsh 1983), and may increase or decrease placental size, depending upon the dose or timing of exposure in pregnancy (Gunberg 1957). Within the rat placenta, evidence has been provided that glucocorticoids down-regulate GLUT1 and GLUT3, leading to the speculation that such an effect could contribute to restrict fetal and placental growth. However, the experimental data do not support this hypothesis since fetuses from ADX, sham-ADX or intact mothers developed a similar hypotrophy despite huge differences in maternal and fetal circulating corticosterone levels.

An increase in corticosterone level is shown in the plasma of control fetuses from ADX mothers. Such data are in agreement with activation of the fetal hypothalamo–pituitary–adrenal axis activity in response to maternal adrenalectomy which withdraws negative feedback exerted by maternal corticosterone (Dupouy et al. 1975, Chatelain et al. 1980). This suggests that, under our experimental conditions, the supplying of corticosterone to ADX mothers was unable to completely restore a basal maternal plasma corticosterone level. Moreover, differences in maternal plasma corticosterone levels between untreated control mothers and sham-ADX control ones could be related to the long-lasting effects of maternal surgery.

In conclusion, prolonged maternal malnutrition during late gestation in the rat decreases maternal plasma glucose and placental GLUT3 glucose transporter expression, but does not affect fetal plasma glucose obviously. Moreover, present results are not compatible with a role of maternal corticosterone in the development of growth-restricted rat fetuses.

Figure 3 (A) Quantitative analysis of GLUT 3 protein in rat term placentas from mothers fed ad libitum (control, C) or FR50 from E14 to E21; in sham controls, sham FR50, ADX controls and ADX FR50 rats. Data are the means ± s.e.m. (n=5 placentas/subgroup). *P<0·05, **P<0·01, FR50 subgroup vs respective control subgroup (multiple ANOVA followed by Dunnett’s post-hoc test).
(B) Semi-quantitative analysis (mRNA level/G3PDH mRNA level) of GLUT3 mRNA in rat term placentas as above. Data are the means ± s.e.m. (n=6 placentas/subgroup). No significant differences were noted between subgroups.
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References


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