Maternal stress alters endocrine function of the feto-placental unit in rats

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Maternal stress alters endocrine function of the feto-placental unit in rats. Am J Physiol Endocrinol Metab 292: E1526–E1533, 2007. First published January 30, 2007; doi:10.1152/ajpendo.00574.2006.—Prenatal stress (PS) can cause early and long-term developmental effects resulting in part from altered maternal and/or fetal glucocorticoid exposure. The aim of the present study was to assess the impact of chronic restraint stress during late gestation on feto-placental unit physiology and function in embryonic (E) day 21 male rat fetuses. Chronic stress decreased body weight gain and food intake of the dams and increased their adrenal weight. In the placenta of PS rats, the expression of glucose transporter type 1 (GLUT1) was decreased, whereas GLUT3 and GLUT4 were slightly increased. Moreover, placental expression and activity of the glucocorticoid “barrier” enzyme 11β-hydroxysteroid dehydrogenase type 2 was strongly reduced. At E21, PS fetuses exhibited decreased body, adrenal pancreas, and testis weights. These alterations were associated with reduced pancreatic β-cell mass, plasma levels of glucose, growth hormone, and ACTH, whereas corticosterone, insulin, IGF-1, and CBG levels were unaffected. These data emphasize the impact of PS on both fetal growth and endocrine function as well as on placental physiology, suggesting that PS could program processes implied in adult biology and pathophysiology.

prenatal stress; placenta; adrenal; testis; pancreas; glucose; growth hormone; adrenocorticotropin hormone

SUBSTANTIAL EPIDEMIOLOGICAL FINDINGS and experimental studies have emerged associating low birth weight with an increased prevalence of cardiovascular and metabolic disorders in adult life (2–4, 18, 20, 38). In humans, exposure to deleterious environmental factors in utero reduces birth weight and predicts the subsequent occurrence of hypertension, ischemic heart disease deaths, hyperlipidemia, insulin resistance/type 2 diabetes mellitus, and neuroendocrine alterations in adulthood (4, 18, 44). Of the prenatal challenges that might underpin these long-term effects, variations in nutrition and/or glucocorticoid exposure (1, 13, 20, 49, 53, 57) as well as variations in maternal behavior (10, 40) have been proposed as key mediators of developmental programming of adult pathophysiology.

Importantly, exposure of the pregnant rat dam to chronic stress reduces offspring weight at birth and produces long-term metabolic, behavioral, and neuroendocrine changes (35, 39, 46, 65) consistent with a prenatal programming of the adult biology and pathophysiology. Thus, the adult offspring of rat dams exposed to chronic restraint stress during the last week of gestation (40, 62) display metabolic changes, including hyperglycemia (56), altered sensitivity of the cardiovascular system (27), and increased food intake after fasting (35). Prenatal stress (PS) also induced emotional and cognitive disturbances in adult and aged animals including, “anxiety,” depressivelike behavior, and altered reactivity to stress of the hypothalamic–pituitary–adrenal (HPA) axis (11, 37, 39, 41, 55, 60). Collectively, these findings indicate that maternal stress “signals” the developing fetus to adjust multiple facets of its tissue development to alter the adult phenotype. These changes mirror those seen in rats, sheep, and other species prenatally exposed to glucocorticoids (51) and broadly parallel the human phenotype associated with low-birth-weight populations (4).

It has been postulated that the origin of such altered developmental plasticity is the in utero exposure to glucocorticoids, which increased during maternal chronic stress (1), and/or the early postnatal maternal environment (10, 40). However, placental dysfunctions and/or fetal endocrine disturbances could also be implicated.

Maternal-to-fetal transfer of glucocorticoids is predominantly regulated by a placental enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). In the placenta of rats (61) and humans (54), 11β-HSD2 catalyses the conversion of active corticosterone (cortisol in humans) into inert 11-dehydrocorticosterone (cortisone). This enzyme normally protects the fetus from relatively high levels of maternal glucocorticoids. In contrast, placental 11β-HSD type 1 is expressed in decidua and other maternal components of the rat placenta and acts in the reverse (reductase) direction, increasing local glucocorticoid levels (61). It has been hypothesized (13) that variations in the activity of placental 11β-HSD2 may be the physiological equivalent of exposure of the fetus to synthetic glucocorticoids, which are poor substrates for the enzyme. In line with this, placental 11β-HSD2 activity correlates with birth weight in rats (6) and humans (54). However, its sensitivity to maternal stress has never been described, although both glucocorticoids (57) and catecholamines (50), some well-known endocrine

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mediators of stress, alter trophoblast cell 11β-HSD2 expression in vitro.

Fetal growth is also dependent on adequate placental nutrient transfer. A primary nutrient for the developing fetus is glucose, which crosses the placental barrier through facilitated transporters down a maternal-fetal concentration gradient (31). Three high-affinity isoforms of these protein transporters have been identified in both human and rodent placentas: glucose transporter (GLUT)1, GLUT3, and GLUT4 (32, 33). GLUT1 mediates glucose transport into both the placenta and fetus (31, 67), whereas GLUT3 is restricted to cells on the fetal surface of the maternal-fetal barrier (8). GLUT4 expression is very low in rodent placentas but highly expressed in humans (66). To our knowledge, although PS was shown to reduce birth weight of offspring (35), the effect of maternal stress on placental nutrient transfer capacity has never been reported.

This study was undertaken to explore the effects of a prenatal restraint stress on early physiological processes in vivo. For this purpose, we assessed whether chronic maternal stress in late pregnancy alters body weight and food intake of the dams, affects placental 11β-HSD2 and transplacental glucose transfer, and has immediate effects upon fetal growth, pancreatic function, glucose homeostasis, and endocrine functions.

MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Care and Use Committee in accordance with the principles of laboratory animal care (European Communities Council Directive of 1986, 86/609/EEC) and following the Institute for Laboratory Animal Research “Guide for Care and Use of Laboratory Animals.”

Housing conditions. Adult virgin Sprague Dawley female rats (Charles River, L’Arbresle, France) weighing 240 g were housed in groups of five per cage for 2 wk before mating to coordinate their estrous cycles (n = 20). They were then housed overnight separately with a sexually experienced male (400 g). Copulation was determined by detection of sperm, and this was designated as E1. Pregnant females were then divided to PS (n = 10) or control (CTL; n = 10) groups, individually housed in plastic cages, allowed ad libitum access to food (regular rat chow no. 113, containing 22% protein, 5% fat, and 53% carbohydrate; UAR, Villemoisson-sur-Orge, France) and water and maintained on a 12:12-h light-dark cycle (lights on at 0700) with constant temperature and humidity.

In another experiment related to the pregnant females, 12 PS and 12 CTL females were weighed at the beginning and at the end of gestation (day 1 and day 21), and their food intake was monitored during the last week of gestation. Basal feeding behavior was evaluated by measuring consumption of food in the home cages of the animals during each 24-h period from E15 to E21. Food intake was expressed as the weight of the mean daily intake from E15 to E21 (Fig. 1B).

Maternal restraint stress procedure. The stress procedure was performed every day for the last 11 days of gestation according to a previous description (40, 42). Pregnant females were subjected to restraint stress for 45 min three times a day in a transparent plastic cylinder (7 cm in diameter and 19 cm long) exposed to a bright light (650 lux). Stress sessions were conducted during the light phase, but the schedule of sessions was not fixed to reduce a possible habituation to repeated restraint stress (approximate stress exposures were performed around 900, 1200, and 1700). Control females were left undisturbed in their home cages. The offspring were raised by their biological mothers until weaning, 21 days after birth. Only litters of 8–13 pups with similar sex ratio were kept for the study, and the other ones were eliminated to rule out extra stressors such as removal of the pups. Only male fetuses were used, and a maximum of two males per litter were put away in this study.

Tissue collection. On the last day of the stress protocol (day 21 of gestation), females were killed rapidly by decapitation between 1130 and 1300 (corresponding at the time they were usually submitted to restraint stress). The adrenals and thymus of mothers were dissected and weighed. The uterus was quickly removed, fetuses were carefully separated from the placenta, and both were cleaned and weighed. All placentas were frozen in liquid nitrogen. The anogenital distance of the fetuses was measured to determine sex to keep only the litters that had a similar sex ratio (1 ± 0.25). Trunk blood of male fetuses was rapidly collected after decapitation (~500 ul) in tubes prerinised with EDTA. Blood glucose was measured using a glucometer (One Touch II; Lifescan, Roissy, France), and then blood samples were centrifuged at 3,500 g for 10 min at 4°C. Plasma aliquot fractions were kept at −30°C until the day of the assay (RIA).

The fetal adrenals, pancreas, liver, thymus, and testis were dissected and rapidly weighed. All organs except pancreas were frozen in liquid nitrogen and stored at −80°C until analysis.

Placental GLUT levels. Placental protein levels of glucose transporters (GLUT1, GLUT3, and GLUT4) were measured after SDS-PAGE and Western Blotting, as previously described (36). 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.

11β-HSD1, 11β-HSD2, and glucocorticoid receptor mRNA levels. Total RNA was extracted from placenta and fetal liver tissues with TRizol reagent (Gibco BRL, Strasbourg, France) according to the
manufacturer’s instructions. The quality of the total RNA was assessed by determining the 260/280 absorbance ratio and by gel electrophoresis in agarose. The semiquantitative RT-PCR analysis method used here has been described and validated previously (9). Briefly, 3 μg of total RNA were reverse transcribed into complementary DNA (cDNA) using 3 μg of random hexamers and 200 U Moloney murine leukaemia virus RT (Gibco BRL, Strasbourg, France). Semiquantitative PCR was performed with one-thirtieth (1 μl) of the first-strand synthesis reaction in 50 μl volume containing 5 μl of 10× buffer (500 mM KC1, 100 mM Tris·HCl, Triton 1×), 4 μl of MgCl2 (25 mM), 5 μl of dNTP (2.5 mM each), 1 μl (20 μM) of forward and reverse primer, 33.8 μl of H2O, and 0.2 μl of Taq polymerase (1 U) (Qbiogen, Illkirch, France). The cycling parameters were 94°C for 90 s, 60°C for 90 s, and 72°C for 120 s. Negative control RT-PCR reactions were performed by omitting RT from the reaction mixture or by adding H2O instead of template. In the four 25-mer primer pairs, the priming sites were separated by a large intron, thus preventing amplification of any contaminating genomic DNA. For rat glucocorticoid receptor (GR; accession no. M14053), 11β-HSD1 (accession no. NM017080), and 11β-HSD2 (accession no. NM017081) amplification, the forward primer corresponded to the region encoding residues 336–344, 37–45, and 80–88, and the reverse primer was complementary to the region encoding residues 418–426, 180–188, and 255–263, respectively. The predicted size of amplification was 272, 453, and 551 bp, respectively. As a control for the RT-PCR amplification of rat cyclophilin B (accession no. AF071225), a forward primer corresponding to the region encoding residues 45–53 and a reverse primer complementary to the region encoding residues 336–344, 37–45, and 80–88, and the reverse primer was complementary to the region encoding residues 418–426, 180–188, and 255–263, respectively. The predicted size of amplification was 272, 453, and 551 bp, respectively. As a control for the RT-PCR amplification of rat cyclophilin B (accession no. AF071225), a forward primer corresponding to the region encoding residues 45–53 and a reverse primer complementary to the region encoding residues 189–197 were used. The predicted size of the cDNA product was 456 bp. Preliminary experiments allowed us to determine the optimal cycle numbers for each primer pair for linear semiquantitative amplification. Each experiment was performed in triplicate and gave similar results. After amplification, the samples were separated on a 1% gel agarose, visualized by ethidium bromide, and analyzed with a Bio-Rad GS-700 densitometer using the Multi-Analyst software (Bio-Rad Laboratories, Hercules, CA).

11β-HSD1 and -2 activities. 11β-HSD1 and -2 activities in placenta were assayed after homogenization, as previously described (28). The reaction included 0.2 mg/ml protein, 10 mM trитrated corticosterone as substrate, and an excess (400 μM) of the 11β-HSD1- or 11β-HSD2-specific cofactors (NADP or NAD, respectively) as co-substrate. Reactions were within the linear portion of the relationships between product formation, time, and protein concentration. After 6 h for 11β-HSD1 or 50 min for 11β-HSD2 incubation at 37°C, steroids were extracted with ethyl acetate, separated by TLC, identified by migration compared with standards, and quantified with a phosphorimager tritium screening. 11β-HSD1 reductase activity was expressed as the percentage of radioactive corticosterone over the total dehydrocorticosterone added to the radioactive corticosterone. 11β-HSD2 dehydrogenase activity was expressed as the percentage of radioactive dehydrocorticosterone over the total dehydrocorticosterone added to the radioactive corticosterone.

Pancreatic β-cell mass. Pancreases were fixed and processed for immunohistochemistry as previously described (16). Pancreatic β-cells were detected using a polyclonal guinea pig anti-insulin antibody (Dako, Trappes, France), and the β-cell fraction was measured using a Leica DMRB microscope equipped with a color video camera coupled to a Quantimet 500MC computer (16). β-Cell mass was obtained by multiplying the β-cell fraction by total pancreatic mass.

Plasma hormone levels. All the measures were done in individual fetuses. In E21 fetuses, plasma ACTH levels were measured in unextracted plasma using a commercial kit (ACTHK-PR; Cis Bio International). The sensitivity of the assay was 10 pg/ml. Plasma corticosterone levels were measured using a commercial kit (Corticosterone DA; MP Biomedicals, Strasbourg, France). The minimum level of detection of the assay was 7.7 ng/ml. Plasma corticosteroid-binding globulin (CBG) binding capacity was determined as previously described (34). The apparent maximum binding capacity and dissociation constant of CBG for corticosterone were individually evaluated from Scatchard plots. Plasma insulin levels were measured using a previously described RIA (16). The sensitivity of the assay was 0.25 ng/ml. IGF-I plasma levels in E21 fetal blood were measured with a commercial kit IGF-R20 from Mediagnost (D-72770, Reutlingen). The assay sensitivity was 0.16 ng/ml. Growth hormone (GH) plasma levels analysis was made using the assay kit RPA 551 (Amersham Biosciences). The sensitivity of the assay was 0.16 ng/ml.

**RESULTS**

Effects of restraint stress on the dam physiology. Restraint stress altered maternal pregnancy. Stressed dams showed a decrease in body weight gain [F1,4–88] = 5.25, P < 0.001; Fig. 1A]. Although both stressed and control females progressively increased food intake during gestation (data not shown), stressed females had lower overall food intake than controls [F1,12] = 10.19, P < 0.05; Fig. 1B]. At term, stressed dams had increased adrenal weight (stressed: 93.1 ± 7.4 mg; CTL: 68.2 ± 2.7 mg; t14 = 3.17, P < 0.05), whereas their thymus weight was reduced (stressed: 223 ± 11 mg; control: 295 ± 12 mg; t14 = 4.44, P < 0.05).

Effects of PS on placental functions. PS did not affect the weight of the placenta [PS rats: 95.9 ± 3.59 mg/g body wt; CTL rats: 93.6 ± 3.65 mg/g body wt; t14 = 0.67, not significant (NS)]. PS significantly modified glucose transporter protein levels in the placenta (Fig. 2). GLUT1 was strongly reduced in PS placentas (t18 = 8.37, P < 0.001; Fig. 2). In
contrast, levels of GLUT3 and GLUT4 were augmented by PS (GLUT 3: t18 = 2.13; P < 0.05; GLUT4: t18 = 7.64, P < 0.001).

PS strikingly decreased placental 11β-HSD2 mRNA levels (t8 = 8.06, P < 0.001; Fig. 3A) and 11β-HSD2 activity (t11 = 4.14, P < 0.05; Fig. 3B). PS was also associated with a small decrease in placental 11β-HSD1 mRNA (t5 = 2.26, P < 0.05), but this was not linked to any change in NADP-driven activity (Fig. 3B).

Effects of PS on the E21 fetuses physiology. In Table 1, the weight of male PS fetuses (n = 7–9) at term (E21) was significantly lower than CTL (n = 7, t13 = 2.98, P < 0.01). E21 PS fetuses had reductions in adrenal (t14 = 2.98, P < 0.01), pancreatic (t8 = 2.37, P < 0.05), and testis (t14 = 2.83, P < 0.05) weights, whereas liver (t13 = 0.42, NS) and thymus (t13 = 0.80, NS) weights were similar to CTL. Moreover, the ano-genital distance of male PS fetuses (n = 16) was shorter (t22 = 5.72, P < 0.01) than CTL (n = 22).

Associated with the decreased weight of the pancreas, PS induced a significant decrease in pancreatic β-cell mass (t8 = 4.05, P < 0.01; Fig. 4A).

However, in Table 2 (CTL: n = 7; PS: n = 5–11), plasma insulin levels were not changed in PS fetuses (t18 = 0.26, NS), although they had lower plasma glucose levels (t20 = 2.32, P < 0.05). In terms of growth and counterregulatory hormones, E21 PS fetuses had lower plasma levels of GH, although without change in circulating IGF-I (t17 = 0.36, NS). Plasma ACTH was decreased (t12 = 4.22, P < 0.01), whereas corticosterone and CBG were unchanged (t17 = 0.35, NS, and t8 = 0.85, NS, respectively).

Although PS tended to increase hepatic 11β-HSD1 mRNA levels (t8 = 2.11, P = 0.06; Fig. 4B), no changes in liver GR mRNA levels were observed (t8 = 0.49, NS). previous observations (35). Furthermore, we reported here that maternal stress affected mothers’ physiology during pregnancy, extending our previous observations (10) showing that gestational stress has long-lasting effects on emotional reactivity of dams. The mechanisms by which PS affects pup growth remain largely unknown. However, it has been postulated (1, 13, 53) that overexposure to the catabolic effects of maternal glucocorticoids in utero could underlie such alteration. Indeed, maternal administration of dexamethasone, a synthetic glucocorticoid that readily crosses the placenta, reduces fetal growth in rats and in other mammals (7, 43, 53). This hypothesis is in accord with our previous data (1) showing that elevation of plasma corticosterone levels in stressed pregnant female rats decreased HPA axis feedback mechanism in their litters. Reduced adrenal, pancreatic, and testis weights were noted in PS rats. Reduced adrenal, pancreatic, and testis weights were noted in PS fetuses, whereas their liver and thymus weights were similar to controls. The ano-genital length was measured on 22 CTL and 16 PS fetuses. ∗P < 0.05; †P < 0.01.

Table 1. Mean characteristics of the E21 male fetuses

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus body weight, g</td>
<td>5.76±0.03</td>
<td>5.48±0.08†</td>
</tr>
<tr>
<td>Adrenals, mg/g body wt</td>
<td>0.72±0.07</td>
<td>0.51±0.04†</td>
</tr>
<tr>
<td>Pancreas, mg/g body wt</td>
<td>5.19±0.22</td>
<td>4.58±0.27∗</td>
</tr>
<tr>
<td>Liver, mg/g body wt</td>
<td>57.82±2.63</td>
<td>55.94±3.52</td>
</tr>
<tr>
<td>Thymus, mg/g body wt</td>
<td>2.28±0.15</td>
<td>2.05±0.23</td>
</tr>
<tr>
<td>Testis, mg/g body wt</td>
<td>1.21±0.08</td>
<td>0.91±0.04∗</td>
</tr>
<tr>
<td>Ano-genital length, mm</td>
<td>3.90±0.15</td>
<td>2.92±0.15†</td>
</tr>
</tbody>
</table>

Values are means ± SE [control rats (CTL): n = 7; prenatal stress (PS) rats: n = 7–9]. The weight of PS fetuses was significantly lower than CTL rats. Reduced adrenal, pancreatic, and testis weights were noted in PS fetuses, whereas their liver and thymus weights were similar to controls. The ano-genital length was measured on 22 CTL and 16 PS fetuses. ∗P < 0.05; †P < 0.01.

DISCUSSION

We demonstrate that chronic maternal stress affects the growth and organ development of the fetus. Specifically, PS reduces fetal growth, targeting some organs more than others, strikingly attenuates placental 11β-HSD2 and GLUT1 expression, and diminishes fetal plasma glucose, GH, and ACTH levels. We suggest that PS induces early feto-placental unit dysfunction that may contribute to the development of the long-term behavioral and metabolic alterations seen previously in the adult offspring (39).

The present study shows that PS alters fetal growth, resulting in a decrease of body weight at term, in accord with our previous observations (35). Furthermore, we reported here that maternal stress affected mothers’ physiology during pregnancy, extending our previous observations (10) showing that gestational stress has long-lasting effects on emotional reactivity of dams. The mechanisms by which PS affects pup growth remain largely unknown. However, it has been postulated (1, 13, 53) that overexposure to the catabolic effects of maternal glucocorticoids in utero could underlie such alteration. Indeed, maternal administration of dexamethasone, a synthetic glucocorticoid that readily crosses the placenta, reduces fetal growth in rats and in other mammals (7, 43, 53). This hypothesis is in accord with our previous data (1) showing that elevation of plasma corticosterone levels in stressed pregnant female rats decreased HPA axis feedback mechanism in their litters. Reduced adrenal, pancreatic, and testis weights were noted in PS rats. Reduced adrenal, pancreatic, and testis weights were noted in PS fetuses, whereas their liver and thymus weights were similar to controls. The ano-genital length was measured on 22 CTL and 16 PS fetuses. ∗P < 0.05; †P < 0.01.

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levels, as we have previously shown (1), and reduced placental 11β-HSD2 activity per se and/or to reduced maternal food intake and/or other mediators of stress. However, recent crosses of mice heterozygous for a null allele of the 11β-HSD2 gene show that within the same mother, birth weight correlates closely with feto-placental 11β-HSD2 genotype (lowest in 11β-HSD2−/− offspring) (25). Thus, the reduced placental 11β-HSD2 in PS rats is compatible with increased transfer of corticosterone from the maternal to the fetal compartment and may be sufficient to reduce birth weight and program offspring physiology. Indeed, in mice, rats, and humans (6, 52, 54), reduced body birth weight is associated with deficient placental 11β-HSD2 (52) allowing more corticosterone to cross the placenta barrier. Perhaps as a consequence of the increased fetal glucocorticoid exposure, PS fetal HPA axis function is attenuated since we found atrophied adrenals and reduced plasma ACTH levels without change in the plasma corticosterone in E21 PS fetuses. The maintained corticosterone levels in PS fetuses are also compatible with the increased transplacental glucocorticoid transfer (from mother to fetus), as their adrenals are atrophied. In late gestation, the development and activity of the fetal adrenals depends mainly on the secretion of pituitary ACTH, which in turn is controlled by hypothalamic CRH (21). It was shown that maternal hypersecretion of pituitary ACTH, which in turn is controlled by hypothalamic glucocorticoids (34) as well as exogenous administration of CRH (21). It was shown that maternal hypersecretion of glucocorticoids (34) as well as exogenous administration of dexamethasone (12) reduce both fetal adrenal growth and activity in correlation with drastic reduction of hypothalamic CRH and suppression of plasma ACTH. Thus, the present results suggest that the HPA axis alterations observed in PS fetuses can be related to the negative feedback control exerted by high levels of maternal glucocorticoids on the fetal HPA axis.

Present data show that maternal stress induced a decrease in testis weight as well as a reduction of the ano-genital length. These alterations are in accord with the observations made by Ward (64) in 1983. Ward et al. (63) have suggested that these early gonadal disturbances could be responsible for the reduction of the adult sexual behavior of male PS rats and could be the result of a depression in early gonadal steroidogenesis, the origin of which remains to be found.

During late gestation, fetal growth is also mainly dependent on the utero-placental unit to deliver oxygen and nutrients as well as the activity of the fetal GH-IGF endocrine system (26). A primary nutrient for the developing fetus is glucose, which crosses the placental barrier through GLUTs that mediate the transport of glucose across plasma membranes by facilitated diffusion (30). We report here that chronic maternal restraint stress reduces placental GLUT1 protein levels but slightly increases GLUT3 and GLUT4 protein levels at term. GLUT1 is the highest-expressed GLUT in rodent placentas (32) and is considered rate limiting for glucose transport from mother to fetus (5). Decreased placental GLUT1 therefore suggests reduced glucose transfer across the placenta, a notion in accord with the observed hypoglycemia in PS fetuses. The small increases in the minor placental transporters GLUT3 and GLUT4 might reflect an adaptation to increase placental and/or fetal glucose supply in response to the downregulation of GLUT1, although proof of this notion requires direct studies to measure glucose flux in these animals. How PS alters placental GLUT protein levels remains unknown. However, because glucocorticoids regulate expression of GLUT transporters in rat placenta (22), the increased maternal corticosterone secretion induced by chronic stress may play a role. Additionally, as maternal undernutrition alters placental GLUT transporters (34), the reduced food intake in PS dams might be involved. In accord with our data, intrauterine growth retardation in humans is also associated with alterations in placental nutrient transport (29). Systemic levels of glucose production arepowerfully regulated in the liver by glucocorticoids, whose action is determined by intracellular regeneration of active corticosterone by 11β-HSD1 and the density of GRs. In our study, since hepatic GR and 11β-HSD1 gene expression remain unaffected in the PS fetuses, the fetal hypoglycemia is not due to deficient glucocorticoid action on the fetal liver gluconeogenesis. The
observed hypoglycemia in PS fetuses is likely to be due to placental failure and/or GLUT1 deficiency.

Maternal restraint stress provoked a decrease in fetal plasma GH levels without circulating plasma IGF-I levels being altered. Abnormalities in the GH-IGF axis are commonly described (24, 52, 59) in growth-retarded fetuses and neonates as well as in many adult diseases associated with low birth weight. Before birth, GH has been thought to have only a minor role in regulating fetal growth, contrary to the IGFs (both IGF-I and IGF-II) that are the principle regulators of fetal growth. Interestingly, it has been described (15, 45) that IGF-I levels are highly regulated in fetuses by nutritional factors. In late gestation, even if fetal levels of both IGF-I and liver GH receptor are low, GH-inducible genes are expressed in fetal liver, suggesting that GH may be physiologically active before term (47). The development of the fetal somatotropic axis during late gestation is dependent on the plasma glucocorticoid levels, since the surge of fetal corticosterone (or cortisol) is essential to initiate the switch from GH-independent local production of IGFS in utero to GH-dependent hepatic production of endocrine IGF-I (15). Thus, fetal overexposure to glucocorticoid may prematurely activate the fetal growth axis, inducing an altered somatic development. Here, although the fetal plasma GH levels were diminished, the IGF-I levels remained surprisingly unaffected. One can speculate that PS reduces pituitary GH secretion and/or its hypothalamic control through glucocorticoid effects or undernutrition (48). Interestingly, in E21 fetuses, Fowden et al. (14) reported that IGF-I production is controlled by paracrine factors rather than by GH alone. Consequently, both nutrient restriction and glucocorticoid overexposure of PS fetuses could have disturbed hepatic sensitivity to GH and/or the local control of IGF-I production. Future experiments will be needed to clarify this point as well as to investigate the effects of PS on IGF-II, which is an important factor for early fetoplacental unit development.

Associated with the low birth weight of PS fetuses, gestational stress reduced fetal pancreatic weight and impaired β-cell development at term. A similar decrease of pancreatic β-cell mass is observed in rat model of maternal food restriction in late pregnancy and implies a rise of both maternal and fetal plasma corticosterone levels (7). Moreover, treatment of pregnant rats with dexamethasone or with an 11β-HSD2 inhibitor, carbonoxolone, also produced a reduction in β-cell number in fetuses at term (7). Since glucocorticoids play a pivotal role in pancreatic endocrine cell lineages at specific developmental windows (17), the increased glucocorticoid levels induced by PS can give rise to impairment of β-cell development and differentiation. Such early effects of PS on pancreatic β-cells could contribute to long-term alterations, as we showed (35) that PS programs glucose intolerance in aged rats. Collectively, these data emphasize how some of the late alterations observed in humans born with intrauterine growth retardation may result from fetal disturbances such as altered β-cell development, as initially suggested by Hales and Barker (23).

In conclusion, our data emphasize the impact of PS on fetal endocrine functions as well as on placental physiology. Besides HPA axis alteration, PS fetuses also exhibit altered somatotropic and endocrine activities. Mechanisms involved in this altered fetal phenotype could be identified, at least in part, on 1) maternal food intake that is diminished, 2) GLUT system that is disrupted, and 3) maternal corticosterone levels that are increased during restraint stress and by the decreased fetoplacental 11β-HSD2, which usually protects the fetus from maternal glucocorticoid excess. Since the action of glucocorticoids on homeostasis is widespread, affecting most of the body compartments as well as the brain, one can speculate that the chronic stress applied to mothers can generate a deleterious environment for the fetus development. The early-altered phenotype obtained in this study may subsequently be at the origin of the adult pathophysiology programmed in PS offspring.

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REFERENCES

Prenatal Stress and Fetal Programming

Hales CN, Barker DJ.


Gluckman PD, Hanson MA, Spencer HG, Bateson P.

Holt RI.

Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR.


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