Antenatal Dexamethasone Treatment in Midgestation Reduces System A-Mediated Transport in the Late-Gestation Murine Placenta

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Clinically, approximately 30% of women who receive synthetic glucocorticoids (sGC) for risk of preterm labor carry to term. In vitro studies have shown that sGC acutely regulate the placental system A amino acid transporter, but there are no comparable data in vivo. Hence, the objective of our study was to examine the acute [embryonic day (E)15.5] and longer-term (E17.5 and E18.5) consequences of midgestation antenatal sGC [dexamethasone (DEX); 0.1 mg/kg on E13.5 and E14.5] on placental system A-mediated transfer in the mouse (measured in vivo as maternal-fetal unidirectional 14C-methylaminoisobutyric acid transfer per gram of placenta). System A transfer and Slc38a mRNA expression significantly increased from E12.5 to E18.5 (P = 0.05), corresponding to increased fetal growth. DEX treatment had no acute effect at E15.5 or longer-term effect at E17.5 but significantly decreased system A-mediated transfer before term (E18.5; P < 0.05) in placentae of male and female fetuses. There was no effect of DEX on Slc38a gene expression. Administration of DEX in this regime had no effect on birth weight. We conclude that sGC treatment in midgestation leads to a substantial decrease in placental system A-mediated transport in late gestation, suggesting that prenatal sGC therapy may lead to a reduction in availability of neutral amino acids to the fetus if gestation persists to term. (Endocrinology 152: 3561–3570, 2011)

A dministration of synthetic glucocorticoids (sGC) for threatened preterm labor has become standard obstetrical practice (1–3). Treatment with sGC promotes fetal lung maturation and decreases the incidence of respiratory distress syndrome in the neonate (1). Initially, it was proposed that sGC therapy was maximally beneficial between 24 h and 7 d after treatment (4). This observation led to the administration of repeat courses of sGC if the risk of preterm delivery persisted after initial treatment. Although sGC treatment has clear neonatal benefit, exogenous GC exposure may also adversely affect the growth of the fetus (5–7). Controlled clinical trials have shown that multiple courses of sGC treatment are associated with decreased fetal weight, height, and head circumference (8). Birth weight within a healthy range is critical, because deviations from the normal growth trajectory, such as those that occur in fetal growth restriction and/or macrosomia, predict a predisposition to hypertension, glucose intolerance, and the metabolic syndrome in later life (9). Furthermore, evidence from animal models indicates that exposure to excess GC in utero can contribute to the programming of disease in adulthood (3, 10–13).

Although the mechanisms of fetal growth restriction remains to be elucidated, current evidence suggests that changes in fetal development are mediated by alterations in placental structure and/or function. In pregnant sheep,
maternal, but not fetal, administration of sGC resulted in fetal growth restriction (14). In sheep and mice, elevations in maternal GC during pregnancy reduced fetal and placental growth, which was associated with altered placental nutrient transfer, gene expression, and endocrine signaling (15–16).

One critical determinant of fetal growth is placental amino acid transfer. The placental system A amino acid transporter is composed of three functionally independent protein/gene isoforms sodium-coupled neutral amino acid transporter (SNAT)1/Slc38a1, SNAT2/Slc38a2, and SNAT4/Slc38a4 (17–18), that transport small zwitterionic neutral unbranched amino acids (19). System A activity is reduced in conditions of fetal growth restriction and in pregnancies of small for gestational aged infants (20–22). In vitro experiments have shown that human placental system A activity is increased by insulin-like growth factors, leptin, and GC treatment (endogenous and synthetic) and down-regulated by hypoxemia and nutrient deficiency (23–27). In vitro, sGC administration (48-h treatment) resulted in a stimulatory effect on system A activity in term placental villous explants in culture (27). This increase in system A transfer was also associated with a promotion of syncytial regeneration and differentiation. This may represent an artifact of the culture model used and thus may not reflect the effects of sGC on placental function in vivo. There are no data concerning the acute effects of sGC in vivo or the potential longer-term effects of treatment. However, in 11β-hydroxysteroid dehydrogenase type 2 knockout mice, placental system A activity is differentially regulated at embryonic day (E)15 compared with E18, suggesting a gestational-specific response (16). In these mice, placental metabolism of maternal corticosterone is substantially reduced, which elevates the level of endogenous GC across gestation. Because approximately 70% of pregnant women who receive sGC for threatened preterm labor do not deliver within 7 d and greater than 30% carry to term (8), it is important to investigate the longitudinal effects of sGC treatment on placental system A transfer in vivo.

Male fetuses grow faster from the first trimester, tend to weigh more, are longer, and have larger placentae compared with female fetuses. Male fetuses also have increased mortality rates and worse outcomes reported in preterm labor (28, 29). Whether a sexual dimorphic function of placental nutrient transporters contributes to the differences in male and female growth across gestation and/or in cases of maternal sGC treatment has yet to be determined. Hence, the objectives of the present study were to 1) delineate changes in system A activity and isoform gene expression during the second half of murine gestation, 2) determine the acute and longer-term effects of exogenous sGC on system A, and 3) examine whether these effects depend upon the sex of the fetus, because sex differences are clearly emerging as being of importance in the expression of key placental regulatory activities (30–32).

**Materials and Methods**

**Animal breeding and treatment**

C57BL/6 mice (6–8 wk; Charles River, Germantown, NY) were bred overnight, and the morning in which a vaginal plug was found was designated E0.5. Litters with fewer than five were excluded to avoid any small litter effects. As such, no significant differences in litter size occurred in any experiment. All protocols were approved by the Animal Care Committee at the University of Toronto and in accordance with the Canadian Council for Animal Care. Animals were fed ad libitum with standard rodent chow during a 0700 h lights on and 1900 h lights off cycle. A subset of mice was used to examine system A transport across gestation and received no handling before tissue collection and transplacental transfer assay. To determine any longitudinal effects of exogenous GC, pregnant dams were injected sc with either saline (control) or 0.1 mg/kg of dexamethasone (DEX) (Vétoquinol N.-A., Inc., Lavaltrie, Québec, Canada) at midgestation on E13.5 and E14.5. This dose has been commonly used in rodent models of antenatal sGC therapy (33, 34). Administration at midgestation allows for the examination of acute and chronic effects of sGC treatment on placental system A.

**Tissue collection and system A-mediated transplacental transfer assay**

To characterize the developmental profile of system A-mediated transplacental transfer in the murine placenta during the second half of gestation, pregnant dams were examined at E12.5, E15.5, and E18.5 (term = E19–E20; n = 6–8 dams per gestational age). To examine any potential timing effects of sGC treatment on system A-mediated transfer, pregnant dams (n = 6–11 dams per treatment and gestational age) were examined 24 h (E15.5; acute), 72 h (E17.5; longer term), and 96 h (E18.5; longer term) after treatment.

System A-mediated transfer was determined using established protocols (24, 35, 36). At each gestational time point, pregnant dams were iv administered the specific system A substrate 14C-N-methylated aminoisobutyric acid (MeAIB) (37) [3.5 μCi (24), NEC671; PerkinElmer, Inc., Boston, MA] in saline (100 μl). A subset of mice (n = 4–6 dams per treatment and gestational age) was used to examine placental permeability using the paracellular diffusion marker 14C-mannitol [1.75 μCi (35), NEC377050UC; PerkinElmer, Inc.] in saline (100 μl). Animals were anesthetized and euthanized by cardiac puncture, 4 min after injection. Maternal blood was collected, and plasma was separated by centrifugation at 10,000 rpm for 2 min at 4 C. Whole concepti were dissected out from the maternal uterus. Afterwards, fetal units (consisting of fetus, yolk sac, amniotic sac, and amniotic fluid) were collected by removal of the placental from the whole conceptus. Fetal units for system A-mediated activity measurements were arbitrarily removed from various positions throughout the uterine horn to avoid any positional effects on growth (38). In any experiment, half the
litter (three to five fetal units) was weighed, and tissues were collected for translaplacental transfer assay. The remaining fetuses (approximately three to five per litter) were removed from the amniotic sac and collected to measure fetal weight. Placental tissue was collected, weighed, frozen, and stored at −80 C. Fetal tails were collected for DNA extraction needed for sex determination. A subset of dams (n = 4–5) was allowed to carry to term (−E19.5). Mice were monitored from E19 to E20. Immediately after delivery, neonates were removed and weighed.

Fetal units were homogenized in PBS. Homogenate (200 µl) was added to SOLVABLE (1 ml; PerkinElmer, Inc.) along with H2O2 (30%; 100 µl) and scintillation fluid (10 ml, Ultima-Gold; PerkinElmer, Inc.). Radioactivity was measured using a Tri-Carb β-Counter (PerkinElmer, Inc.) in both homogenates of fetal unit and maternal plasma, with a counting efficiency of more than or equal to 94%. System A-mediated translaplacental transfer was calculated as a ratio of radioactivity present in the fetal unit (disintegrations per minute) relative to maternal plasma (disintegrations per minute) standardized per gram of placenta (36).

Sex determination

DNA was extracted and amplified using Sigma REExtract-N-AMP Tissue PCR kit (XNAT; Sigma Chemical Co., St. Louis, MO) according to manufacturers’ instructions. PCR primer sequences for the male sex determining region Y were forward, TCA TGA GAC TGC CAA CCA CAG and reverse, CAT GAC and maternal plasma, with a counting efficiency of more than or equal to 94%. System A-mediated translaplacental transfer was calculated as a ratio of radioactivity present in the fetal unit (disintegrations per minute) relative to maternal plasma (disintegrations per minute) standardized per gram of placenta (36).

Quantitative real-time PCR

Placentae from male and female fetuses (one to two arbitrarily chosen per litter) were homogenized in TRIzol reagent (1 ml; Invitrogen, Burlington, Ontario, Canada) and total RNA extracted as described in the manufacturer’s protocol. Contaminating genomic DNA was removed by treating RNA samples with DNA-free deoxyribonuclease treatment (Ambion, Austin, TX). RNA purity and concentration were assessed using spectrophotometric analysis, and RNA integrity was verified using gel electrophoresis. RNA samples were combined to give a mean male and female sample per litter. RNA was stored at −80 C until further use.

DNA was converted to cDNA using Multiscribe Reverse Transcriptase (50 U/µl), deoxynucleotide triphosphate mix, and random (hexamer) primers (Applied Biosystems, Foster City, CA). Samples were incubated at 25 C for 10 min, 37 C for 120 min, and 85 C for 5 min using the C1000 Thermal Cycler (Bio-Rad, Hercules, CA).

Real-time PCR was performed using the C1000 Thermal Cycler and quantified using the CFX96 Real-Time System (Bio-Rad). Samples were prepared using TaqMan Universal PCR Master Mix (Applied Biosystems, Hammonton, NJ), primer-probes sets for the following genes: GAPDH (lot no. 4332932E), Slc38a1 (Mm00506391_m1), Slc38a2 (Mm00628416_m1), Slc38a4 (Mm00459056_m1), TATA box binding protein (TBP) (Mm00446971_m1) (Applied Biosystems), and cDNA template (50 ng) using ratios according to manufacturers instructions. Data analysis was undertaken using CFX Manager Software (Bio-Rad). For each primer probe set, a standard curve was generated by serial dilution of a pooled reference sample with a minimum efficiency more than or equal to 90.0%. Samples were run in triplicate. Relative mRNA expression was calculated as gene of interest expression normalized [ΔΔc(t)] to reference gene expression (GAPDH and TBP) (34, 40). GAPDH and TBP were not differentially regulated across gestation or altered by DEX treatment. For each plate, a nontemplate control (containing H2O in place of template cDNA) and nonamplification control (containing H2O in place of template RNA) was run to verify amplification and RT specificity.

Morphometric analyses

An additional group of pregnant dams (n = 7–9) was treated on E13.5 and E14.5 with either vehicle or DEX as described above. On E18.5, placentae were collected for morphometric analyses. One male and one female placenta closest to the mean litter placental weight were used as a representative sample. Placentae were hemisected along the midline using the umbilical cord insertion as the central reference. Tissue was fixed paraformaldehyde (4%, 24 h), then dehydrated and processed into paraffin. Sections were prepared from the full sagittal central plane of the placenta and stained with hematoxylin and eosin. Analysis was undertaken using NDP.view NanoZoomer (Hamamatsu Photonics K.K., Bridgewater Township, NJ), by an operator blinded to treatment. The total area of both the labyrinth and junctional zone was measured. Final comparisons were made as a regional percentage of the total placental area per section.

Corticosterone measurements

Maternal plasma was collected from pregnant dams treated with either saline or...
DEX (E13.5 and E14.5) as stated above at E18.5 via cardiac puncture (n = 10 vehicle and n = 8 DEX). A small sample (<15 μl) of fetal blood was collected from each fetus and blood from each litter pooled. Corticosterone was measured using a RIA (MP Biomedicals, Solon, OH) according to the manufacturers instructions. The samples were analyzed in a single assay (intraassay variability ≤8%), where the lower limit of detection was 25 ng/ml.

**Statistical analysis**

Statistical analysis was conducted using SPSS/WIN version 18.0 (SPSS, Inc., Chicago, IL). No significant intralitter variation occurred; therefore, sex-matched siblings were averaged per litter. Two-way ANOVA was used to examine differences in transport due to sex across gestation (ontogeny) or due to sGC treatment across gestation on datasets that were normally distributed with equal variances with Bonferroni post hoc analyses. Corticosterone and morphometric comparisons on E18.5 were conducted using unpaired t test. Statistical outliers greater than two SD from the mean were excluded from the analyses. Datasets that failed to meet the required criteria were log transformed before analyses. Significance was determined at P < 0.05, and values are presented as mean ± SEM.

**Results**

**Ontogeny of system A**

System A activity increased from E12.5 to E18.5 (P < 0.01), with no difference between placentae from male and female fetuses (Fig. 1A). Fetal weight increased significantly from E12.5 to E18.5 (P < 0.05), with no significant differences between male and female fetuses at any gestational age (Fig. 1B). Placental weight increased significantly from E12.5 to E15.5 (P < 0.01), with no further significant difference between E15.5 and E18.5. There was a strong trend toward an effect of sex on placental weight irrespective of gestational age, with reduced placental weight from pregnancies with a female fetus compared with a male fetus (P = 0.0532) (Fig. 1C). Maternal weight increased significantly across gestation (P < 0.05) from E12.5 (26.88 ± 0.35 g) to E15.5 (32.40 ± 0.67 g) and E18.5 (37.17 ± 0.86 g).

Consistent with increased activity across mid to late gestation, mRNA expression of system A genes Slc38a1, Slc38a2, and Slc38a4 increased from E12.5 to E18.5 (P < 0.05) relative to GAPDH and TBP reference gene expression (Fig. 2, A–C). No sex-specific difference in mRNA expression occurred for any one of the system A isoforms.

**The effects of antenatal DEX treatment on system A activity and mRNA expression**

DEX treatment did not significantly affect system A-mediated transfer 24 or 72 h after treatment at E15.5 and E17.5 in placentae from either male or female fetuses. However, DEX significantly reduced system A-mediated placental transfer transport just before term on E18.5 (Fig. 3, A and B). To determine the specificity of sGC treatment on system A transfer, we examined the paracellular transfer of 14C-mannitol per gram of placenta. 14C-mannitol transfer increased from E15.5 to E18.5 in placentae from male and female fetuses (P < 0.05). However, DEX did not affect passive transfer in placentae from either sex at E15.5 or E18.5 (Fig. 3, C and D).

Antenatal DEX treatment had no effect on the levels of mRNA for Slc38a1, Slc38a2, or Slc38a4 at E15.5, E17.5 (data not shown), or E18.5 (Fig. 4, A–C) in placentae of male or female fetuses despite the significant decrease in system A-mediated transfer at E18.5.

![Graph](https://example.com/graph.png)
The effects of antenatal DEX treatment on fetal and placental growth

DEX treatment did not affect fetal weight in utero for either male or female fetuses between E15.5 and E18.5, nor did it affect fetal birth weight at term (Fig. 5, A and B). Antenatal DEX treatment did not significantly affect placental weight at E15.5 or E17.5. On E18.5, DEX did not significantly affect placental weight with a male fetus (Fig. 5C), but it reduced placental weight with a female fetus at E18.5 (P < 0.05) (Fig. 5D). Placental efficiency, measured as the ratio of fetal weight to placental weight, was not significantly affected by DEX treatment in males (Fig. 5E). Female placental efficiency was not affected by DEX at E15.5 or E17.5; however, the ratio of fetal to placental weight was significantly increased before term at E18.5 after DEX treatment (Fig. 5F). DEX did not alter the relative proportion of either the labyrinth or junctional zone in placentae giving rise to male or female fetuses on E18.5 (Table 1).

The effects of antenatal DEX treatment on maternal and fetal plasma corticosterone

There was no difference in maternal or fetal plasma corticosterone concentrations between the vehicle and sGC-treated animals by E18.5 (Table 2). System A-mediated transfer and mRNA expression increased between mid- and late gestation in placentae from murine pregnancies with either male or female fetuses, consistent with increased fetal growth during this period. Placental system A-mediated transfer was differentially affected by the timing of exposure to maternal sGC treatment. Although DEX given at midgestation did not have any acute effects on system A-mediated transfer, system A mRNA expression, fetal weight, or placental weight, it significantly reduced system A transport before term (E18.5) in placentae giving rise to both male and female fetuses. DEX did not affect passive permeability or regional fractions of the placenta, thus demonstrating the specificity of effect on system A. Changes in system A-mediated transfer due to DEX treatment were not mediated via alterations in system A Slc38a1, Slc38a2, or Slc38a4 gene expression. Despite the effect of DEX on system A, there was no effect on male or female fetal weight across gestation or weight at term. DEX treatment significantly reduced placental weight in females before term (E18.5) but did not significantly affect placental weight in males. Alterations in system A-mediated transfer cannot be attributed to chronic alterations in endogenous GC levels, because DEX treatment (E13.5 and E14.5) did not result in altered circulating levels of fetal or maternal corticosterone at E18.5.

This is the first study to investigate the effects of antenatal sGC treatment on the placental system A transporter, in vivo. Recently, it has been demonstrated that initial rate system A activity is comparable in microvillous membrane vesicles isolated from both human and mouse placenta (41). We have demonstrated that system A-mediated transfer increases across gestation in the murine placenta and follows a similar trend to the change in system A activity in the human placenta across gestation: system A activity measured in microvillous membrane at term is significantly increased compared with first trimester (42). Therefore, both murine and human placental system A activities increase across gestation, most likely to meet the growing demands of the fetus.

In the present study, we have used a murine model to examine the regulatory effects of sGC in vivo, without any confounding effects of preterm labor or other pregnancy
complications on placental nutrient transport as would occur in human pregnancies. In contrast to previous studies in human term placental explants, in which we have shown that sGC stimulate the system A transporter after 48 h treatment in vitro (27), the present data demonstrate a longer-term inhibition on system A activity levels in the mouse. A possible reason for the contrast may reflect the difference between model systems used. Investigation of human placental explants in vitro requires a 7-d culture period, during which the outer syncytiotrophoblast layer is shed and regenerated before exogenous sGC can be administered (43). Treatment with the sGC DEX not only stimulated system A activity but also heightened syncytialization of explants in a dose-dependent manner during the culture period (27). Thus, the potent differentiating actions of DEX also increased microvillous membrane formation of the newly generated syncytial layer, which may contribute to increased system A activity and transport capacity within explants.

By examining sGC treatment in vivo using a murine model, Baisden et al. (44) demonstrated that antenatal DEX treatment (0.5 mg/kg) on E14.5–E16.5 down-regulates 1212 genes and up-regulates 1382 genes in the murine placenta at term. Therefore, DEX treatment given in an in vivo animal model may alter placental gene expression and indirectly contribute to longer-term changes in nutrient transport. However, we were unable to detect direct changes in any of the system A transporter isoforms at the level of gene expression in either the in vitro studies (human placental explants) (27) or the current in vivo studies (mouse). It is not uncommon to report disconnect between placental transporter activity, protein, and mRNA expression for system A and other placental transport systems (18, 34). System A may therefore be subject to posttranscriptional/translational regulation. Because sGC treatment did not chronically alter maternal hypothalamic-pituitary-adrenal function, the mechanism of decreased system A activity 96 h after exposure may be mediated by long-term reductions in SNAT protein expression and/or protein recruitment to the microvillous membrane. This aspect underlying sGC-induced changes in placental transport warrants further investigation.

We have demonstrated that approximately 40–50% reduction of system A transport did not affect fetal weight outcome. Transport activity rates are reduced by 15–40% in human microvillous membrane vesicles isolated from both small for gestational aged infants as well as infants that are pathologically growth restricted (20, 21). However, studies in rodent models demonstrate that reductions in system A-mediated transfer in vivo precede fetal growth restriction (26, 45). Thus, it is perhaps not surprising that in our model, where system A-mediated transfer is only decreased just before term, the fetus escapes an effect on growth. Across gestation, system A activity follows a similar trend to fetal weight, because both parameters significantly increase across the second half of gestation. The largest increase in fetal growth occurs from E15.5 to E18.5 (∼267% increase in fetal weight) compared with E18.5 to term (∼18% increase in fetal weight). Therefore, if reductions in system A-mediated transfer had occurred earlier in gestation, it is quite possible that this would translate to reductions in fetal growth. This study examines changes in placental function after sGC treatment due to the importance of antenatal sGC therapy given to women for risk of preterm labor. However, we also acknowledge that there are limitations of using a mouse model. Gestation in a mouse is relatively short (∼19.5 d), and the definitive placenta is only established at E12.5 (∼64% of total gestational length) (46). As such, the developmental profile of murine placental and fetal development cannot be mapped directly to that of humans. We cannot examine sGC treat-
ment at an earlier time point to determine whether changes in transport precede reductions in fetal weight, because the administration of sGC earlier than E13.5 would target early placental development rather than placental function. Currently, the National Institutes of Health recommends a single course of antenatal corticosteroid treatment as the standard of care for the management of the patient presenting with apparent preterm labor (2). This practice has been shown to decrease the rate of respiratory distress syndrome immediately after treatment, and clinical trials have demonstrated no effect of a single course of sGC administration on fetal growth (47, 48). However, a recent smaller study by Davis et al. (7) was the first to examine longer-term exposure of sGC. In this study, a single course of sGC treatment caused a reduction in fetal length, weight, and head circumference in sGC-exposed infants who carried to term. These reductions were not related to fetal size before antenatal midgestation treatment (7). A mouse model has allowed for the investigation of differences in placental system A nutrient transport across gestation based on duration since sGC exposure. Clearly, additional studies are required to further map the effects of sGC exposure over time after treatment.

In the present study, it is possible that a single course sGC treatment may cause a compensatory up-regulation of other nutrient transport systems, which could maintain fetal weight. Dose-dependent increases in glucose transporter (GLUT)1 and GLUT3 protein expression occur before term in rats given DEX (0.1–0.2 mg/kg) starting at E15 (49). In 11β-hydroxysteroid dehydrogenase type 2 knockout mice, system A activity was initially increased as E15, and fetal weight was protected despite reduced placental weight. It was only later in gestation on E18 that a fall in fetal weight occurred coincident with a decrease in placental glucose transport and GLUT3 mRNA expression and a return of system A activity to control (16). Therefore, it is possible that the placenta up-regulates glucose transport in an attempt to maintain fetal weight after exposure to sGC.

Evidence from human populations, as well as various animal models, has consistently demonstrated that in utero adaptations associated with altered growth rates can result in adverse health outcomes that persist into adult life (9, 11, 15, 50). Smaller neonates are at greater risk of adult hypertension, ischemic heart disease, and insulin resistance, whereas being born large for gestational age increases the incidence of obesity and metabolic syndrome in adulthood (9). Although fetal weight can be used as one parameter indicating increased risk of disease in adulthood, programming effects can occur despite a lack of change in fetal weight with sGC treatment (51, 52). Differential adaptations in placental transfer mechanisms may alter fetal body composition that may persist long-term and alter adult phenotype (40, 53). Whether the mechanism of sGC-induced long-term programming, with/without changes to total fetal body weight, is mediated by in utero adaptations caused by altered placental nutrient delivery warrants further investigation.

![Graph](https://example.com/graph.png)

**FIG. 5.** The effects of midgestation DEX (0.1 mg/kg administered on E13.5 and E14.5) treatment on fetal weight (A and B) and placental weight (C and D). E and F, Placental efficiency is determined as the fetal to placental weight ratio (F:P). Vehicle treatment is designated in white, and DEX treatment is designated in black (males: A, C, and E) and gray (females: B, D, and F). Data presented as mean ± SEM; n values are denoted in bars. An asterisk denotes significant difference between vehicle and DEX treatment (P < 0.05). Letters that are different represent significant differences between E (P < 0.05).
When assessing placental function, most studies pool data from placentae of both male and female fetuses. Although this provides a representative measure, analyzing data by this method may mask any opposing sex-dependent effects that may be present. The present study is novel in that it is one of the first to examine sex-specific differences in murine placental system A transport. Placentae from both male and female fetuses exhibited similar rates of transport during normal gestation and after antenatal sGC treatment. Clifton and Murphy (31) suggest that male and female fetuses exhibit differential mechanisms to cope with adverse intrauterine environments. In adverse uterine conditions, female fetuses reduce growth rate, which confers a protective mechanism for any secondary adversities (31). In contrast, male fetuses grow normally in utero despite adversities, and the lack of compensation in growth rates increases their susceptibility to altered environmental factors (29, 32). Our data suggest that female placentae may be more susceptible to reduced growth when treated with a single course of sGC. As such, female placentae exposed to midgestation sGC treatment have significantly increased fetal to placental weight ratio, which may predict an altered placental efficiency in the female when exposed to sGC therapy.

Repeat courses of sGC reduce human and rodent placental growth in a dose-dependent manner (54, 55). In rodent models, various dosing regimes have targeted placental growth with specific reductions in both the labyrinth and junctional zones (36). We have demonstrated that although DEX (0.1 mg/kg on E13.5 and E14.5) reduced female placental weight at E18.5, these reductions were proportionally distributed to both the labyrinth (transport region) and junctional zone (region of hormonal secretion). Because DEX did not affect mannitol transfer (marker of flow-limited diffusion) (22, 35), we can eliminate any profound alteration on vascularity contributing to reduced 14C-N-methylated aminoisobutyric acid transport. Overall, this demonstrates specificity for the DEX-induced reduction of system A-mediated transfer per gram of placental tissue before term at E18.5.

Currently, there is a significant increase in the number of women who are exposed to sGC therapy for risk of preterm labor who carry to term. In 2004, Polyakov et al. (57) report approximately 11.2% of all pregnant women received sGC, which was increased from 8.4% in 1998. This increase in sGC exposure also includes a larger proportion of women who received sGC yet delivered after 34 wk of gestation (57). If our data can be extrapolated to the clinical situation, they would suggest that the system A transporter is differentially regulated based on timing from sGC exposure. There are no short-term regulatory effects of midgestation sGC treatment. However, a substantial reduction in system A-mediated transport may occur if pregnancy continues to term. Whether this is the clinical case with antenatal therapy requires further investigation. The benefits of reduced neonatal morbidity and mortality due to sGC therapy clearly outweigh the risks of use. Our study does not suggest that antenatal sGC treatment should not be administered but reinforces the need for accurate diagnosis of preterm labor. Further understanding the molecular effects of antenatal corticosteroid therapy at the level of the placenta will help maximize fetal maturational benefit and minimize potential adverse outcome.

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References

17. Mackenzie B, Erickson JD 2004 Sodium-coupled neutral amino acid (system N/A) transporters of the SLC38 family gene. Pflugers Arch 447:784–795
specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. Proc Natl Acad Sci USA 101:8204–8208
52. Ortiz LA, Quan A, Zarzar F, Weinberg A, Baum M 2003 Prenatal dexamethasone programs hypertension and renal injury in the rat. Hypertension 41:328–334