Altered Placental Function of 11β-Hydroxysteroid Dehydrogenase 2 Knockout Mice

Caitlin S. Wyrwoll, Jonathan R. Seckl, and Megan C. Holmes

Endocrinology Unit, Centre for Cardiovascular Science, Queen's Medical Research Institute, The University of Edinburgh, Edinburgh EH16 4TJ, United Kingdom

Fetal glucocorticoid exposure is a key mechanism proposed to underlie prenatal "programming" of adult cardiometabolic and neuropsychiatric disorders. Regulation of fetal glucocorticoid exposure is achieved by the placental glucocorticoid "barrier," which involves glucocorticoid inactivation within the labyrinth zone of the murine placenta by 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2). Thus, the absence of placental 11β-HSD2 may impact on fetal and placental development. The current study investigated transport of amino acids and glucose, key factors required for fetal growth, and vascular development in placentas from 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses derived from 11β-HSD2$^{+/-}$ matings. At embryonic d 15 (E15) (term = E19), 11β-HSD2$^{-/-}$ fetal weight was maintained in comparison to 11β-HSD2$^{+/+}$ fetuses. The maintenance of 11β-HSD2$^{-/-}$ fetal weight occurred despite a reduction in placental weight, suggesting that compensatory changes occur in the placenta to maintain function. However, by E18, 11β-HSD2$^{-/-}$ fetal and placental weights were both reduced. Transport studies revealed up-regulation of placental amino acid transport to 11β-HSD2$^{-/-}$ offspring at E15, coinciding with an increase in the expression of the amino acid transporters. Furthermore, at E18, placental glucose transport to 11β-HSD2$^{-/-}$ offspring was markedly reduced, correlating with lower fetal weight and a decrease in glucose transporter 3 expression. Stereological analyses of the labyrinth zone of the placenta revealed that the reduction in placental weight at E18 was associated with restriction of the normal increase in fetal vessel density over the final third of pregnancy. Our data suggest that restriction of fetal growth in 11β-HSD2$^{-/-}$ mice is mediated, at least in part, via altered placental transport of nutrients and reduction in placental vascularization. **(*Endocrinology* 150: 1287–1293, 2009)**

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Although glucocorticoids are essential for fetal maturation in late gestation, excessive glucocorticoid exposure reduces fetal growth, and associates with susceptibility to later hypertension, insulin resistance, and anxiety related disorders (1–4). Impairment of fetal growth has predominantly been attributed to direct effects of glucocorticoids on the fetus, prematurely shifting tissue development from a proliferative to a more functionally mature state (5). However, fetal growth is dependent on a complex interplay of maternal, placental, and fetal endocrine signals, and glucocorticoid-mediated fetal growth retardation is likely also to relate to disturbances in placental growth and function (6, 7).

Transfer of maternal glucocorticoids to the fetus is controlled by the placental glucocorticoid "barrier," 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which catalyzes rapid inactivation of physiological glucocorticoids (corticosterone to 11-dehydrocorticosterone in rodents) within the placenta. In the mouse, 11β-HSD2 is present in the labyrinthine trophoblast (8), the fetal portion of the placenta and the key site of maternal-fetal exchange (9). Labyrinthine 11β-HSD2 mRNA is highly expressed, peaking at embryonic d 15.5 (E15.5) and turning off by E16.5 (8). Crucially, activity of 11β-HSD2 within the placenta in rats and humans correlates with birth weight (1, 10, 11), suggesting that normal variation in fetal exposure to maternal glucocorticoids impacts on fetal growth. Indeed, maternal treatment during pregnancy with dexamethasone, a poor substrate for 11β-HSD2, reduces placental and birth weight with "adverse" outcomes for adult cardiometabolic, neuroendocrine, and

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For editorial see page 1073

Abbreviations: E15.5, Embryonic d 15.5; GLUT, glucose transporter; 11β-HSD2, 11β-hydroxysteroid dehydrogenase 2; PPAR, peroxisome proliferator-activated receptor; SNAT, system A amino acid transporter; VEGF, vascular endothelial growth factor.

behavioral function. Furthermore, 11β-HSD2 knockout mice (11β-HSD2$^{-/-}$) have a lower birth weight than congenic littermate controls (11β-HSD2$^{+/+}$) of 11β-HSD2$^{+/-}$ crosses (2). Of course, disruption of 11β-HSD2 in the fetally derived labyrinth zone of 11β-HSD2$^{-/-}$ fetuses exposes not only the fetus but also the placenta to increased local corticosterone levels, thus potentially altering placental function.

Treatment of rats with glucocorticoids such as dexamethasone, which are poor substrates for 11β-HSD2, restricts placental vascular development, via inhibition of the endothelial cell-specific mitogen, vascular endothelial growth factor (VEGF)-A, and peroxisome proliferator-activated receptor γ (PPARγ), which regulates VEGF-A expression (6, 12). Impaired vascular arborization within key areas of the placenta involved in nutrient exchange between the maternal and fetal circulations are likely to have effects on placental function. However, glucocorticoid effects on placental function have been discordant. Thus, chronic restraint stress during late gestation in rats reduces placental 11β-HSD2 expression and expression of glucose transporter (GLUT) 1, with an associated reduction in fetal plasma glucose (7), whereas late gestation dexamethasone increases placental GLUT1 and 3 expression (13), and another synthetic glucocorticoid, triamcinolone, down-regulates placental GLUT1 and 3 protein and mRNA (14). Any physiological relevance of these manipulations is unresolved. Furthermore, whereas system A amino acid transporter (SNAT) activity and expression are upregulated by cortisol exposure in BeWo cells (15), they are unaltered in human placental villous fragments exposed to cortisol (16, 17), and any effects of glucocorticoids on placental amino acid transport in rodent pregnancy are presently unexplored.

The purpose of this current study was to elucidate the importance of 11β-HSD2 in placental function. This was achieved via characterization of placental function and morphology of 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses derived from 11β-HSD2$^{+/-}$ matings, thus eliminating maternal pathophysiology as a contributing factor.

Materials and Methods

Animals

Male and female 11β-HSD2$^{+/-}$ mice congenic on the C57BL/6J background were mated overnight, and the morning on which a vaginal plug was present was designated d 1 (E1) pregnancy. Offspring consisting of 11β-HSD2$^{+/+}$, $^{+/-}$, $^{-/-}$ mice were compared within the same litter. Genotyping was performed by PCR as described previously (2). Animals were given standard chow and water *ad libitum*, lights were on between 0700 and 1900 h, and all studies were performed to the highest standards of humane animal care under the aegis of the United Kingdom Animals Scientific Procedures Act, 1986.

At E15 and 18 (term = E19), pregnant females were decapitated, and fetuses and placentas were removed and weighed. Fetal tails were collected for genotyping, and at E18, fetal trunk blood was collected for plasma glucose measurements. Placental zones were separated by blunt dissection and frozen for real-time quantitative RT-PCR or fixed in formaldehyde and processed for paraffin histology for subsequent stereological analyses. Placental dry weights were obtained by drying the placentas in an oven at 37 C until a constant weight was obtained. The water content of each placenta was calculated by subtracting the dry from the wet weights and expressing it as a percentage of wet weight.

Placental transport of radiolabeled glucose and amino acids

Placental transport of radiolabeled glucose and amino acids was established using modified methods (18). Briefly, pregnant mice at E15 and 18 were anesthetized. A total of 100 μl PBS containing 3.5 μCi ^{14}C-MeAIB (NEC671; PerkinElmer, Beaconsfield, UK) or 3.5 μCi ^{14}C-methyl-D-glucose (NEC377050UC; PerkinElmer) was injected iv, and 4 min after injection, animals were killed by cervical dislocation, and fetuses and placentas were removed and weighed. Fetal tails were collected for genotyping, and the remainder of the fetus was lysed overnight at 55 C in Biosol (National Diagnostics, Hessle Hull, UK). Fractions of fetal samples were then added to scintillation tubes for β-counting (Tri-Carb 2100TR; Packard, Pangbourne, UK). Radioactive counts in each fetus were then used to calculate the amount of radioisotope transferred per gram of placenta (to give a relative measure of placental transfer of the solute), or per gram of fetus (to give a relative measure of the amount of solute received by the fetus). Average values for wild-type, heterozygote, and knockout fetuses within a litter were then calculated. These values were then used to calculate a mean for all litters at E15 and 19.

Glucose assay

Plasma glucose was measured by hexokinase assay (Infinity Glucose Kit; ThermoScientific, Buckinghamshire, UK).

Immunohistochemistry

Placental sections were exhaustively sectioned at 7-μm thickness, deparaffinized, and rehydrated, then incubated for 20 min in 3% H_2O_2 to block endogenous peroxidases. Proteinase K solution (Dakocytomation, Ely, UK) was applied to assist epitope retrieval. Polyclonal rabbit primary antibody to von Willebrand factor (to distinguish fetal capillary endothelium; Dakocytomation) was used at a dilution of 1:500 for 1 h at room temperature, followed by a 30-min room temperature incubation in goat antirabbit secondary (Vector Laboratories, Ltd., Peterborough, UK). After washing, slides were incubated with ABC solution (Vector Laboratories), and specific binding was detected using the 3′3-diaminobenzidine HCl system (Vector Laboratories). Slides were counterstained with hematoxylin before dehydrating in graded alcohols and mounting in Di-N-Butyle Phthalate in Xylene (VWR International Ltd, Poole, UK).

Placental stereological assessment

Placental volume, placental component volume, and detailed labyrinth zone analyses were all assessed as detailed previously (19). Briefly, absolute placental volume was assessed by point counting at ×1.25 magnification to enable a complete view of the section. Volumes of placental components were then assessed at ×10 magnification by random sampling of 15 fields of view, and point counting was conducted to estimate labyrinth zone, spongiotrophoblast, and decidual volumes. The labyrinth zone was assessed at ×40 magnification, and volumes of fetal capillaries, maternal blood spaces, and trophoblasts were obtained by point counting. Surface area measurements for these labyrinth zone compartments were determined by overlaying a grid of cycloid arcs. Estimation of capillary length, diameter, and density was obtained by using a counting frame with two contiguous forbidden lines (20). Measures were adjusted for tissue shrinkage by measurement of average maternal erythrocyte diameter before and after tissue processing (19). All measurements were performed blind, and intraobserver error was less than 5%.

Measurement of mRNA expression by quantitative RT-PCR analysis

Total RNA was extracted from tissue samples using TRIZOL reagent (Life Technologies Inc., Paisley, UK), and extracted RNA was treated with deoxyribonuclease I (Invitrogen, Paisley, UK) to remove contaminating genomic DNA. RNA (1 μg) was then reverse transcribed at 55 C for 50 min using Moloney murine leukemia virus reverse transcriptase (Promega, Southampton, UK) according to the manufacturer's instruc-

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tions. Real-time PCR was performed using the TaqMan ABI Prism 7900 sequence detector (Applied Biosystems, Chester, UK). Expression levels were quantified using Lightcycler 480 Probes Master (Roche Diagnostics, Burgess Hill, UK) with primer probe sets (Applied Biosystems) for the following genes: *18S* (lot no. Hs99999901_s1), *Pparγ* (Mm00440945_m1), *Slc2a1* (Mm00441473_m1), *Slc2a3* (Mm00441483_m1), *Slc38a1* (Mm00506391_m1), *Slc38a2* (Mm00628416_m1), *Slc38a4* (Mm00459056_m1), and *Vegf-a* (Mm00437304_m1). Data acquisition used Sequence Detector 1.6.3. software (Applied Biosystems). A standard curve for each primer probe set was generated in triplicate by serial dilution of pooled cDNA. Each sample was run in triplicate, and the mean values of the triplicates were used to calculate transcript level from the standard curve. The results are expressed as a ratio to *18S* rRNA to normalize the transcript levels. Reverse transcriptase negative controls and intron spanning primers were used where possible to prevent genomic DNA amplification.

Statistical analysis

All data are expressed as mean ± SEM, with each litter representing n = 1. For fetal and placental weights, n = 35–43. For each experimental group for stereological and real-time RT-PCR and plasma glucose measures, n = 6–8, whereas for transport studies, n = 8–15. Two-way ANOVA was used to assess variation in placental and fetal weights, placental transport, stereology, and plasma glucose measures for gestational age and genotype. When significant interaction ($P < 0.05$) was found, subsets of data were analyzed by one-way ANOVA, followed by Tukey's *post hoc* test.

Results

Fetal and placental weights

All fetuses regardless of 11β-HSD2 genotype increased in weight between E15 and 18 (4-fold; $P < 0.001$; Table 1). Placental weight remained stable with time, but the placentas from 11β-HSD2$^{-/-}$ mice were consistently smaller than either 11β-HSD2$^{+/+}$ or $^{+/-}$ littermates. Therefore, at E15, 11β-HSD2$^{-/-}$ fetal weight was similar to control despite a reduction (11%; $P < 0.05$; Table 1) in placental weight. Consequently, fetal to placental weight ratios were higher in the 11β-HSD2$^{-/-}$ than wild-type fetuses. However, by E18, 11β-HSD2$^{-/-}$ fetal and placental weights were both reduced in comparison to 11β-HSD2$^{+/+}$ littermates (fetus: 12%, $P < 0.01$; placenta: 6%, $P < 0.05$; Table 1), and this was accompanied by a reduction in the fetal to placental ratio. The water content of 11β-HSD2$^{-/-}$ placentas did not differ from their wild-type or heterozygous littermates, showing that the reduction in weight is not due to altered fluid/water content (Table 1).

Placental transport of amino acids and glucose

To determine whether the impaired growth of the placentas from 11β-HSD2$^{-/-}$ fetuses had an impact on the transport of nutrients to the fetus, placental transport of amino acids and glucose was determined.

Amino acid transport

System A placental transport capacity of ^{14}C-MeAIB increased in all genotypes from E15-18 (Fig. 1, A and B). Interestingly, at E15 the transport capacity of placentas from 11β-HSD2$^{-/-}$ was significantly higher than $^{+/+}$ or $^{+/-}$ littermates (53 and 43%, respectively; $P < 0.01$; Fig. 1A), but this did not result in a significant change in the relative amount of ^{14}C-MeAIB received by the fetus (Fig. 1B). No effect of genotype was observed on amino acid transport at E18.

Glucose transport

Placental transport capacity of ^{14}C-glucose significantly increased at E18 in comparison to E15 in placentas from fetuses of all genotypes (Fig. 1C). At E15, levels of glucose transport across the placenta were independent of genotype, but at E18 the glucose transport capacity of the placenta was significantly reduced in both 11β-HSD2$^{+/-}$ and $^{-/-}$ fetuses (53 and 25%, respectively, in comparison to wild type; $P < 0.01$; Fig. 1C); in both $^{-/-}$ and $^{+/-}$ fetuses, this resulted in the fetus receiving less glucose per gram fetal weight than wild-type littermates (57 and 35% less, respectively; $P < 0.01$; Fig. 1D).

Fetal plasma glucose measurements

Levels of fetal plasma glucose at E18 reflected the placental transport findings, with 11β-HSD2$^{-/-}$ and $^{+/-}$ fetuses having lower plasma glucose than wild type (11β-HSD2$^{+/+}$: 83.6 ± 4.4 mg/dl; 11β-HSD2$^{+/-}$: 64.5 ± 9.1 mg/dl; 11β-HSD2$^{-/-}$: 68.9 ± 5.7 mg/dl; $P < 0.05$).

Placental stereological measures

Stereological measurements were performed to determine which regions of the placenta from 11β-HSD2$^{-/-}$ mice contribute to the reduced placental size. All placentas exhibited a significant expansion in the labyrinth zone between E15 and 18 (43% in the wild-type placenta; $P < 0.01$), however, the normal gestational increase in labyrinthine fraction was compromised in placentas from 11β-HSD2$^{-/-}$ fetuses, with an expansion of only

TABLE 1. Fetal and placental wet weights, fetal to placental ratio, and placental dry weight of 11β -HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses at E15 and 18

	E15			E18		
	+/+	+/−	−/−	+/+	+/−	−/−
Placental weight (mg)	101 ± 4	98 ± 3	90 ± 5[a]	98 ± 2	98 ± 2	92 ± 2[a]
Fetal weight (mg)	**203 ± 5**	**198 ± 3**	**193 ± 7**	**863 ± 21**	**820 ± 31**	**757 ± 13**[a]
Fetal to placental ratio	**2.06 ± 0.1**	**2.09 ± 0.06**	**2.52 ± 15**[a]	**8.89 ± 0.3**	**8.48 ± 0.3**	**8.41 ± 0.2**[a]
Placental water content (%)	84.2 ± 0.6	83.6 ± 1.1	84.9 ± 0.8	83.5 ± 0.9	83.6 ± 0.6	82.2 ± 1.1

Values are the mean ± SEM (n = 35–43 per group). *Bold text* denotes an overall effect of gestational age (two-way ANOVA, $P < 0.05$).

[a] Differences from 11β -HSD2$^{+/+}$ within the time point (one-way ANOVA, $P < 0.05$).

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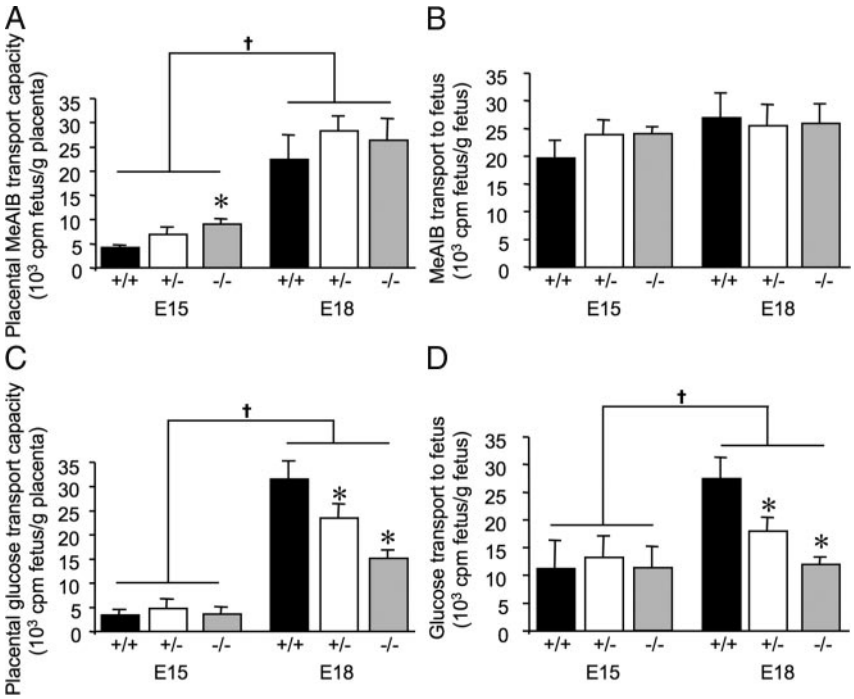

FIG. 1. Changes in placental transport of ^{14}C-MeAIB and ^{14}C-glucose in 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses at E15 and 18 expressed per gram of placenta (A and C, respectively) or per gram of fetus (B and D, respectively). Values are the mean ± SEM. †, An overall effect of gestational age (two-way ANOVA, $P < 0.05$); *, differences from 11β-HSD2$^{+/+}$ within the time point (one-way ANOVA, $P < 0.05$).

18% between E15 and 18 ($P < 0.05$; Table 2). In contrast, the spongiotrophoblast fraction decreased ($P < 0.05$) between E15 (48.5% of total placental volume) and 18 (29% of total placental volume), whereas there was no significant change in the decidual fraction between those two time points. Neither of the spongiotrophoblast nor the decidual fraction was modified by genotype.

Because the labyrinth zone is the site of 11β-HSD2 expression and is also crucial for nutrient transfer from mother to fetus, this zone was investigated in greater detail. The gestational increase in the labyrinth zone of all placentas was found to be attributable to increased volume and surface area of both maternal blood spaces and fetal capillaries, the latter reflecting a concomitant increase in fetal capillary length (Table 2). Although the maternal blood space and fetal capillary volume were not significantly altered in 11β-HSD2$^{-/-}$ placentas compared with wild-type littermates at E15, by E18 there was a dramatic decrease in fetal capillary volume (58% less than wild type at E18; $P < 0.01$), surface area density (34% less than wild type at E18; $P < 0.01$), length (12% less than wild type at E18; $P < 0.01$), and diameter ($P < 0.05$; Table 2). However, maternal blood space volume remained unaltered in placentas of 11β-HSD2$^{-/-}$ fetuses in comparison to wild-type littermates. Thus, the vascular neogenesis normally observed in placental fetal capillaries over the final third of gestation was impaired in 11β-HSD2$^{-/-}$ fetuses.

Quantification of placental gene expression

Because there were significant functional and structural changes in placentas from 11β-HSD2$^{-/-}$ mice, we wished to determine whether there is a concomitant change in expression of nutrient transporters and growth factors within the zones of the placenta that may impact on placental function. At E15, real-time quantitative PCR revealed that expression of *Slc38a2* and *Slc38a4* was up-regulated 4-fold ($P < 0.05$; Fig. 2, B and C) in placentas from 11β-HSD2$^{-/-}$ fetuses in comparison

TABLE 2. Stereological measures of placentas from 11β -HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses at E15 and 18

	E15			E18		
	+/+	+/−	−/−	+/+	+/−	−/−
Placental fractions (% of total placental volume) decidua basalis	25.4 ± 3.11	31.3 ± 2.14	19.6 ± 2.67	18.5 ± 1.50	17.7 ± 1.61	21.9 ± 1.66
Spongiotrophoblast	**38.5 ± 2.6**	**32.6 ± 3.08**	**40.85 ± 3.04**	**18.4 ± 3.07**	**18.7 ± 3.56**	**28.7 ± 2.95**
Labyrinth zone	**33.9 ± 3.16**	**33.7 ± 2.37**	**37.2 ± 1.29**	**59.5 ± 2.75**	**59.6 ± 3.05**	**45.6 ± 2.44**[a]
Chorion	**2.16 ± 0.03**	**2.37 ± 0.02**	**2.3 ± 0.03**	**3.6 ± 0.03**	**3.9 ± 0.03**	**3.7 ± 0.04**
Absolute MBS volume (10^{-3} cm^3)	**3.8 ± 0.3**	**3.3 ± 0.1**	**2.8 ± 0.6**	**9.1 ± 1**[a]	**9.4 ± 1**	**6.4 ± 2**
Absolute FC volume (10^{-3} cm^3)	**4.3 ± 0.1**	**4.1 ± 0.3**	**3.4 ± 0.2**	**14.8 ± 2**[a]	**14.5 ± 1**	**6.3 ± 1**[a]
MBS surface area density (cm^2/cm^3)	**270.56 ± 30.21**	**276.70 ± 40.26**	**194.42 ± 35.94**	**431.09 ± 46.34**	**398.13 ± 20.09**	**498.65 ± 66.49**
FC surface area density (cm^2/cm^3)	**370.29 ± 27.33**	**373.02 ± 28.55**	**285.96 ± 38.2**	**473.39 ± 24.93**	**450.26 ± 16.77**	**314.11 ± 45.87**[a]
FC length (m)	**25.34 ± 5.12**	**24.98 ± 3.45**	**25.21 ± 4.61**	**152.9 ± 9.33**	**153.2 ± 11.0**	**134.6 ± 8.48**[a]
FC diameter (μm)	**14.3 ± 1.4**	**14.0 ± 0.6**	**14.2 ± 0.8**	**10.7 ± 0. 1**	**10.6 ± 0.3**	**9.0 ± 0.4**[a]

Values are the mean ± SEM (n = 6–8 per group). *Bold text* denotes an overall effect of gestational age (two-way ANOVA, $P < 0.05$). FC, Fetal capillaries; MBS, maternal blood space.

[a] Differences from 11β -HSD2$^{+/+}$ within the time point (one-way ANOVA, $P < 0.05$).

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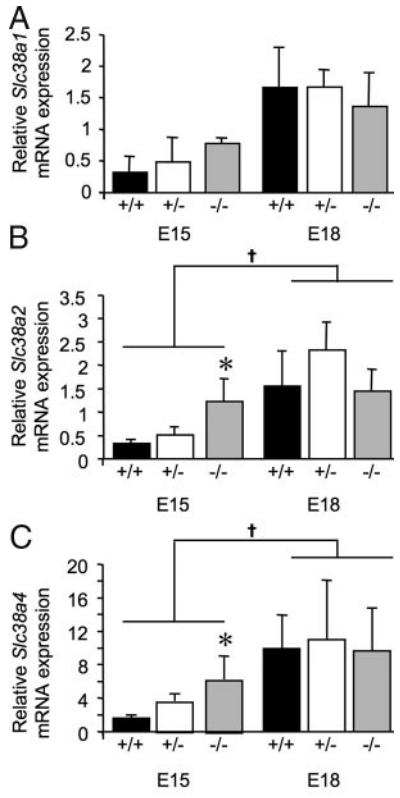

FIG. 2. Relative mRNA expression of the SNATs *Slc38a1* (A), *Slc38a2* (B), and *Slc38a4* (C) in the labyrinth zone of placentas from 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses at E15 and 18. Values are the mean ± SEM. †, An overall effect of gestational age (two-way ANOVA, $P < 0.05$); *, differences from 11β-HSD2$^{+/+}$ within the time point (one-way ANOVA, $P < 0.05$).

to wild-type littermates. The gestational increase in expression of SNATs *Slc38a2* (SNAT2) and *Slc38a4* (SNAT4) in wild-type and 11β-HSD2$^{+/-}$ placentas was absent in 11β-HSD2$^{-/-}$ placentas (Fig. 2), such that there were no differences in expression levels across the genotypes at E18. The expression of *Slc38a1* was not affected by genotype. Interestingly, the up-regulation of the amino acid transporters at E15 parallels the observed increased system A transporter function at this time point in 11β-HSD2$^{-/-}$ placentas (Fig. 1A).

The expression of GLUTs exhibited a different profile. There was no significant change in *Slc2a1* (GLUT1) attributable to fetal genotype, though there was a trend for developmental decline in expression from E15-18 (Fig. 3A). The expression of the GLUT *Slc2a3* (GLUT3) was increased by 52% ($P < 0.05$) between E15 and 18 in the wild-type placentas (Fig. 3B). Importantly, at E18, *Slc2a3* expression was reduced by 67% ($P < 0.05$) in 11β-HSD2$^{-/-}$ than in wild-type littermate placentas, abolishing the gestational increase observed in the wild-type placentas. These results are consistent with the impaired glucose transport observed across the placenta of E18 11β-HSD2$^{-/-}$ mice.

Vegf-a mRNA expression, a marker for angiogenesis, increased over E15-18 in the labyrinth zone of placentas from wild-type fetuses (8-fold; $P < 0.01$; Fig. 4A), but levels were decreased 3-fold ($P < 0.05$) in 11β-HSD2$^{-/-}$ littermates in comparison to wild-types at E18, abolishing the normal gestational increase in *Vegf-a* expression. A similar pattern of expression was revealed for mRNA encoding of a major regulator of *Vegf-a* expression, *Ppar*γ, with a halving of the normal gestational increase in expression ($P < 0.05$; Fig. 4B). Together, the low expression levels of these genes, which are key for angiogenesis, may be responsible, at least in part, for the inadequate fetal capillary growth observed in the 11β-HSD2$^{-/-}$ placentas.

Discussion

Here, we show that the absence of 11β-HSD2 compromises not only fetal but also placental growth. 11β-HSD2$^{-/-}$ placentas had elevated amino acid transport at E15 in conjunction with increased expression of *Slc38a2*, whereas expression of *Slc2a3* and glucose transport was diminished at E18. The altered placental function was associated with reduced capillary networks. Thus, 11β-HSD2$^{-/-}$ placentas had significantly reduced fetal capillary development within the labyrinth zone, the zone regulating nutrient exchange, accompanied by a decline in *Vegf-a* and *Ppar*γ mRNA expression, factors known to regulate angiogenesis. These changes in placental function and morphology are likely to have ramifications for fetal development with consequences for later adult health.

At E15, despite a reduction in placental size, fetal weight is maintained, generating an increase in fetal to placental ratio that is indicative of enhanced placental function. Indeed, placental amino acid transport of 11β-HSD2$^{-/-}$ fetuses was up-regulated at E15, alongside increased expression of *Slc38a2* and *Slc38a4*. These observations likely contribute to the maintenance of fetal weight because amino acids contribute a large proportion of the carbon and nitrogen required for fetal growth (21). Increase in amino acid system A transport activity after glucocorticoid exposure has been previously demonstrated *in vitro* (15), whereas the data here suggest that this also occurs *in vivo*. Further studies are required to characterize the specific mechanisms by which excess placental glucocorticoid exposure up-regulates amino acid transport.

Later in pregnancy, at E18, the smaller placenta of the 11β-HSD2$^{-/-}$ fetus appears unable to maintain normal fetal growth, and fetal weight falls behind control littermates. At this time the transplacental transfer of glucose and plasma glucose levels was re-

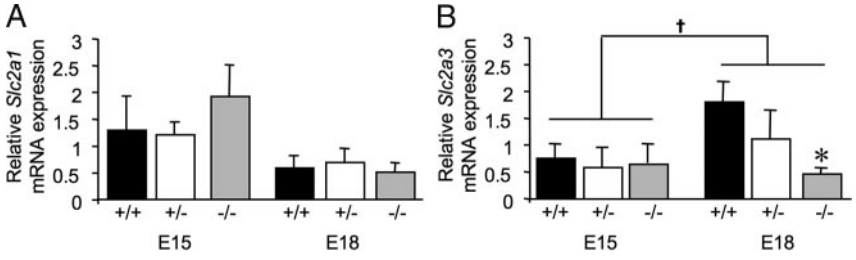

FIG. 3. Relative mRNA expression of the GLUTs *Slc2a1* (A) and *Slc2a3* (B) in the labyrinth zone of placentas from 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses at E15 and 18. Values are the mean ± SEM. †, An overall effect of gestational age (two-way ANOVA, $P < 0.05$); *, differences from 11β-HSD2$^{+/+}$ within the time point (one-way ANOVA, $P < 0.05$).

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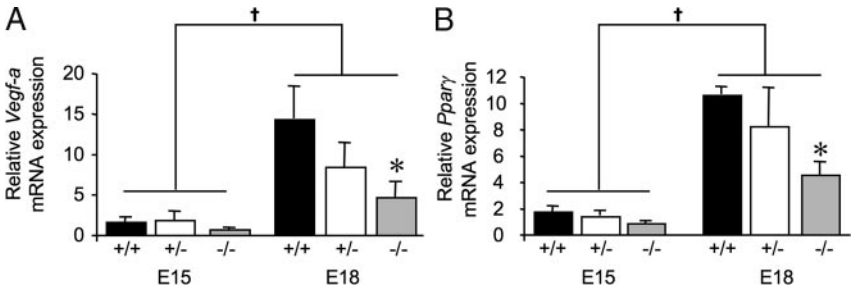

FIG. 4. Relative expression of *Vegf-a* (A) and *Pparγ* (B) mRNAs in the labyrinth zone of placentas from 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses at E15 and 18. Values are the mean ± SEM. †, An overall effect of gestational age (two-way ANOVA, $P < 0.05$); *, differences from 11β-HSD2$^{+/+}$ within the time point (one-way ANOVA, $P < 0.05$).

duced in 11β-HSD2$^{-/-}$ fetuses. Glucose is a primary nutrient required for fetal development, and is transported across the placenta by facilitated diffusion primarily via GLUT1 and GLUT3 (22). The expression of GLUT1 has been localized to basal placental membranes and blood vessels, suggestive of a role for glucose transport to placental tissue (23, 24). In contrast, GLUT3 is present on the maternal-facing side of the labyrinth trophoblasts and, therefore, may be responsible for fetal glucose delivery. Thus, our observations of reduced *Slc2a3* expression in the labyrinth zone of placentas from 11β-HSD2$^{-/-}$ fetuses most likely accounts for the reduction in the transplacental transfer of glucose. Regulation of placental GLUT expression by glucocorticoids has been previously demonstrated in rat pregnancy and human villous extracts (7, 13, 14). Thus, it appears that the supply of glucose to the fetus is a critical feature of models of glucocorticoid excess in pregnancy. This may have important ramifications for "setting" fetal metabolism and adult health in later life, but further research is required to establish the significance of decreased placental transfer of glucose.

Interestingly, the 11β-HSD2$^{+/-}$ fetuses, which exhibit approximately 50% of wild-type 11β-HSD2 activity, also exhibited increased transplacental transfer of MeAIB at E15 and decreased transplacental glucose transfer at E18 alongside corresponding changes in glucose and amino acid transporter expression. This suggests that 11β-HSD2$^{+/-}$ fetuses may also be "programed," and exhibit an intermediate phenotype between 11β-HSD2$^{+/+}$ and 11β-HSD2$^{-/-}$ fetuses. However, previous work conducted on 11β-HSD2$^{+/-}$ mice from heterozygous matings has not revealed any alteration in behavior (2) or metabolic phenotype (Abrahamsen, C. T., unpublished observations) to date, despite an intermediate birth weight (2).

At E15 there was no significant alteration in fetal capillary density in 11β-HSD2$^{-/-}$ placentas compared with wild-type littermates. This time point is just after the initial quiescent phase of labyrinthine fetal capillary development, which ceases at around E14.5 (19). Therefore, impairment of fetal capillary development in placentas from 11β-HSD2$^{-/-}$ fetuses as a consequence of excess placental glucocorticoid exposure must occur after E14.5. This is supported by our observations that reveal that at E15, whereas not significant, there is a trend for reduced fetal capillary volume, surface area, and length. However, by E18, fetal capillary development was markedly impaired in placentas from 11β-HSD2$^{-/-}$ fetuses, and this corresponded to a reduction in *Vegf-a* and *Pparγ* mRNAs in the labyrinth zone. These results are consistent with recent studies of rat placentas from dexamethasone-treated pregnancies (6, 12). Thus, it appears that glucocorticoid excess during rodent pregnancy retards fetal vessel growth within the labyrinth zone, at least in part, by direct effects of glucocorticoids on the expression of VEGF-A. Previous studies have implicated VEGF-A as a key factor for placental vascular remodeling (25, 26). Furthermore, glucocorticoids inhibit VEGF-A expression (27–30), perhaps via changes in PPARγ, which positively regulates VEGF-A expression (31, 32), and is suppressed by dexamethasone (12). Importantly, it is unknown whether the previous observations of altered placental function in dexamethasone-treated pregnant rats are a direct effect of glucocorticoids on the placenta or via indirect effects on the dam. In contrast, our model of 11β-HSD2 heterozygous matings, whereby 11β-HSD2$^{+/+}$, $^{+/-}$, and $^{-/-}$ fetuses are generated by the same mother, clearly demonstrates a direct effect of increased glucocorticoid exposure on placental function. Indeed, the placenta may be key to the effects of glucocorticoids on fetal growth because studies in sheep reveal that administration of glucocorticoids directly to the fetus does not retard fetal growth (33).

Although 11β-HSD2 is absent in the labyrinth zone of placenta from 11β-HSD2$^{-/-}$ fetuses, it is also absent in the fetal tissues, including kidney, gut, lung, and brain (8). Therefore, we cannot eliminate the possibility that our observed changes in placental function may be a result of altered fetoplacental cross talk. Until a placenta-specific knockout of 11β-HSD2 is developed, the differential significance of placental 11β-HSD2 for fetal and placental development cannot be comprehensively elucidated. Furthermore, there is evidence for a fetal gender-specific effect on placental glucocorticoid sensitivity (34), although it is uncertain if this is also the case in the present study. Further studies need to be conducted on a much larger scale to verify if the effects of placental 11β-HSD2 absence on placental function are sex specific.

In conclusion, deletion of 11β-HSD2 and, therefore, overexposure of the fetal-placental unit to high maternal glucocorticoids result in reduced fetal and placental growth. Placentas from 11β-HSD2$^{-/-}$ fetuses have impaired labyrinth zone capillary development and altered transport of nutrients. These alterations in placental phenotype are likely a consequence of increased placental glucocorticoid exposure. The initial consequence of placental 11β-HSD2 absence is an up-regulation of amino acid transport that coincides with maintained fetal weight. However, as gestation progresses, normal fetal capillary development is compromised, and placental glucose transport is diminished that may contribute to the observed reduction in fetal weight. These data suggest that placental 11β-HSD2 is crucial for optimal fetal development, and that the deleterious effect of excess glucocorticoids on fetal development and subsequent adult health is mediated, at least in part, by altered placental function.

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Address all correspondence and requests for reprints to: C. S. Wyrwoll, Endocrinology Unit, Queen's Medical Research Institute, Centre for Cardiovascular Science, 47 Little France Crescent, Edinburgh EH16 4TJ, United Kingdom. E-mail: cwyrwoll@staffmail.ed.ac.uk.

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