Maternal exposure to dexamethasone or cortisol in early pregnancy differentially alters insulin secretion and glucose homeostasis in adult male sheep offspring

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De Blasio MJ, Dodic M, Jefferies AJ, Moritz KM, Wintour EM, Owens JA. Maternal exposure to dexamethasone or cortisol in early pregnancy differentially alters insulin secretion and glucose homeostasis in adult male sheep offspring. Am J Physiol Endocrinol Metab 293: E75–E82, 2007. First published March 13, 2007; doi:10.1152/ajpendo.00689.2006.—An adverse intrauterine environment increases the risk of developing various adult-onset diseases, whose nature varies with the timing of exposure. Maternal undernutrition in humans can increase adiposity, and the risk of coronary heart disease and impaired glucose tolerance in adult life, which may be partly mediated by maternal or fetal endocrine stress responses. In sheep, dexamethasone in early pregnancy impairs cardiovascular function, but not glucose homeostasis in adult female offspring. However, male offspring are often more susceptible to early life “programming”. Pregnant sheep were infused intravenously with saline (0.19 ml/h), dexamethasone (0.48 mg/h), or cortisol (5 mg/h), for 2 days from 26 to 28 days of gestation. In male offspring, size at birth and postnatal growth were measured, and glucose tolerance [intravenous glucose tolerance test (IVGTT)], insulin secretion, and insulin sensitivity of glucose, α-amino nitrogen, and free fatty acid metabolism were assessed at 4 yr of age. We show that cortisol, but not dexamethasone, treatment of mothers causes fasting hyperglycemia in adult male offspring. Maternal cortisol induced a second-phase hyperinsulinemia during IVGTT, whereas maternal dexamethasone induced a first-phase hyperinsulinemia. Dexamethasone improved glucose tolerance, while cortisol had no impact, and neither affected insulin sensitivity. This suggests that maternal glucocorticoid exposure in early pregnancy alters glucose homeostasis and induces hyperinsulinemia in adult male offspring, but in a glucocorticoid-specific manner. These consequences of glucocorticoid exposure in early pregnancy may lead to pancreatic exhaustion and diabetes longer term and are consistent with stress during early pregnancy contributing to such outcomes in humans.

The offspring of women exposed to famine at any stage during pregnancy develop hyperinsulinemia and impaired glucose tolerance, although this is most marked in those exposed late in gestation once in their fifties (14, 50, 52–55). Exposure in early pregnancy to the Dutch Winter Hunger Famine was additionally characterized by obesity, an atherogenic profile, and increased incidence of coronary heart disease in adult offspring (14, 50, 52–55, 68). Maternal undernutrition in early pregnancy remains a common challenge due to morning sickness or hyperemesis gravidarum and may similarly impact on the fetus. It has been proposed that endocrine responses of mothers and their offspring exposed to nutritional restriction may partly mediate the programming of later dysfunction (54). Women who experience hyperemesis gravidarum (severe morning sickness resulting in continual vomiting and hence undernutrition) exhibit increased circulating cortisol compared with nonhyperemesis women or nonpregnant women (34, 67). Such increases in circulating cortisol (1, 3, 39) may increase the exposure of the fetus to glucocorticoids. This may also occur when exogenous steroids are administered to the mother for conditions such as asthma and congenital adrenal hyperplasia (12, 37, 43), in association with reduced placental concentrations of 11β-hydroxysteroid dehydrogenase 2 (11β-HSD-2) (57). The developing fetal tissues are usually protected from high levels of maternal glucocorticoids by the actions of 11β-HSD-2 in the placenta or the fetal tissues themselves (7, 21, 57), which converts most (but not all) cortisol into an inactive form. Undernutrition in the rat and sheep at least downregulates placental 11β-HSD-2, however (9, 38, 42, 70), which, if it occurs in women, may further increase fetal exposure to maternal glucocorticoids. The aim of this study was to mimic a short-term stressful experience, such as morning sickness or a period of short-term undernutrition occurring during early gestation, by briefly exposing the mother to exogenous glucocorticoids at that stage and determining the outcomes for glucose homeostasis and insulin action in offspring. Certainly, maternal dexamethasone exposure in late gestation produces fasting hyperglycemia and/or glucose intolerance and an increased glucose-stimulated insulin secretion in adult offspring in the rat and sheep (20, 44, 46). In the rat, administration throughout pregnancy with carbonoxolone (an inhibitor of 11β-HSD) allows an increase in the passage of the natural glucocorticoid cortisol, reduces birth weight, and also impairs glucose tolerance in adult offspring (40, 41). But
whether maternal exposure to glucocorticoids in early gestation at different developmental stages in the fetus induces similar outcomes is largely unknown. Maternal dexamethasone induces the former, in part by increased hepatic levels of gluconeogenic enzymes, such as the rate-limiting enzyme phosphoenolpyruvate carboxykinase (PEPCK) (20, 46).

An additional aim was to assess to what extent any outcomes result directly from fetal exposure to glucocorticoids that cross the placenta or are indirect consequences of maternal and placental glucocorticoid exposure (28, 44). The effects of the synthetic glucocorticoid dexamethasone (which can cross the placental barrier to the fetal tissues without inactivation and acts on the glucocorticoid but not the mineralocorticoid receptor) and the natural glucocorticoid cortisol (which is partly inactivated by the placental barrier and acts on both receptors in fetal tissues) were, therefore, compared. Differential effects of maternal exposure to dexamethasone and cortisol have been demonstrated in sheep, where exposure to dexamethasone, but not cortisol or saline, in early pregnancy (27–29 days of gestation) increased responsiveness of central nervous system angiostensinergic mechanisms in response to angiotensin II and decreased heart rate and cardiac output (19). We, therefore, propose that maternal dexamethasone exposure will alter glucose homeostasis and insulin action and related parameters to a greater extent than cortisol in offspring.

There is also evidence from a range of species and experimental prenatal challenges to suggest that males are more sensitive to early life programming of insulin action than are females (23, 36). Males appear more susceptible to perinatal programming of metabolic and cardiovascular homeostasis than females in humans and in experimental models of fetal growth restriction (23, 64). At 20 yr of age following intrauterine growth restriction in humans, insulin sensitivity was reduced in men but not women who were light or short at birth (23). Early life influences also accounted for a greater proportion of variance in metabolic syndrome score at 50 yr of age in men than women, and birth weight and catch-up growth in childhood were related to metabolic score and to fasting insulin, respectively, in men, but not women, at 50 yr of age, suggesting that males are more susceptible to early life influences (51). We have previously shown that female offspring of pregnant ewes exposed to dexamethasone treatment in early pregnancy (27–29 days of gestation) also had increased insulin sensitivity of lipolysis, but unaltered glucose homeostasis or insulin action at 5 yr of age (25). We propose that, in contrast, maternal exposure to dexamethasone in early pregnancy will affect glucose control and insulin action in male offspring.

We, therefore, assessed insulin and glucose homeostasis in adult male offspring following short-term maternal exposure to dexamethasone, cortisol, or vehicle in early pregnancy from 26 to 28 days of gestation (~0.2 gestation). This is equivalent to ~50 days of gestation in the human and corresponds to the end of the human embryonic period. We hypothesized that maternal exposure to dexamethasone in early pregnancy would reduce size at birth; program fasting hyperglycemia, hyperinsulinemia, and hypoaminoacidemia, the latter partly due to increased gluconeogenesis; and impair glucose tolerance in male offspring compared with exposure to saline. We further hypothesized that maternal dexamethasone exposure in early pregnancy would impair insulin secretion in response to glucose and insulin sensitivity of glucose metabolism, but enhance that of lipolysis in male offspring. We further hypothesized that maternal cortisol exposure would have similar consequences, but of a lesser magnitude compared with dexamethasone for glucose homeostasis, insulin action, and related parameters in male offspring.

METHODS

All experiments were approved by the Animal Ethics Committee of Monash University before commencement of experimental protocols.

Pregnant ewes were treated with dexamethasone (Decadron; Merck, Sharp and Dohme) (0.48 mg/h), cortisol (5 mg/h), or saline (0.19 ml/h), given as an intravenous infusion for 48 h from 26 to 28 days of gestation. Ewes were allowed to lamb naturally. Adult male singleton offspring were castrated and tail docked at 2 mo of age and had carotid artery loops constructed under general anesthesia at ~1 yr of age (18). Male offspring were housed in metabolic crates and provided 0.8-kg lucerne chaff per day and water ad libitum, but were fasted overnight before the hyperinsulinemic euglycemic clamp (HEC) (4.08 ± 0.89 yr of age) and intravenous glucose tolerance tests (IVGTTs) (4.26 ± 0.89 yr of age).

Growth measures. Size at birth in terms of weight, crown-rump length, abdominal circumference, and placental weight was measured. Weight was measured in adult male offspring the day before the HEC.

HEC and insulin sensitivity of glucose metabolism. At 4.08 ± 0.89 yr of age, a HEC was performed. Sheep were weighed and then fasted overnight and allowed water ad libitum throughout. Arterial blood was sampled (2 ml) at −10, −5, and 0 min; human insulin (Actrapid, Novo Nordisk) was then continuously infused intravenously (4 mU·kg⁻¹·min⁻¹); and blood was sampled (0.2 ml) every 5 min for 120 min. Blood glucose at each time point was measured using a HemoCue glucometer (HemoCue). At 15 min after the start of the insulin infusion, glucose (25%) was infused intravenously, initially at 2 mg·kg⁻¹·min⁻¹. The glucose infusion rate (GIR) was then adjusted each 5 min after the measurement of blood glucose, to maintain euglycemia at the fasting blood glucose concentration, using a modification of a previously published algorithm (15, 25). The mean coefficient of variation (CV) in blood glucose during the second hour of the clamp was 10.1%. Plasma insulin was measured before the start of HEC (~10, ~5, 0 min) and during the second hour of the HEC (60, 75, 90, 105, 120 min), to determine the fasting plasma insulin concentration and the plateau plasma insulin concentration achieved, respectively. The insulin sensitivity of whole body glucose metabolism was determined as the mean GIR from 60 to 120 min during the HEC, divided by the mean plasma insulin concentration in the second hour of infusion measured every 15 min. The mean CV for GIR from 60 to 120 min in each lamb during the HEC was 38.2%.

Insulin sensitivity of circulating amino acids. Plasma α-amino nitrogen concentration was measured before and during the second hour of the HEC (~10, ~5, 0, 60, 75, 90, 105, 120 min) by colorimetric assay (22). The insulin sensitivity of circulating amino acids was calculated as the percentage change from fasting plasma α-amino nitrogen concentrations to those during the second hour of the HEC, corrected for the plateau plasma insulin concentration.

Insulin sensitivity of circulating free fatty acids. Plasma free fatty acid (FFA) concentration was measured before and during the second hour of the HEC (~10, ~5, 0, 60, 75, 90, 105, 120 min) by enzymatic analysis. The insulin sensitivity of circulating plasma FFA was calculated as the percentage change from fasting FFA concentrations to those during the second hour of the HEC, corrected for the plateau plasma insulin concentration.

Insulin clearance and posthepatic insulin secretory rate. Insulin clearance was calculated as the rate of insulin infusion during the HEC divided by the plateau plasma insulin concentration achieved in the second hour of the clamp. Posthepatic insulin secretory rate in the basal state was calculated as the fasting plasma insulin concentration before the IVGTT multiplied by the insulin clearance rate.
Disposition index. The disposition index is a measure of insulin action, reflecting both insulin abundance and sensitivity, and was calculated for glucose, amino acid, and FFA metabolism as the posthepatic insulin delivery rate in the fasting state multiplied by the insulin sensitivity of glucose, amino acids, or FFA metabolism (24, 49).

Insulin secretion in response to IVGTT. At 4.26 ± 0.89 yr of age, an IVGTT was performed. Sheep were fasted overnight before the experiment, but were allowed water ad libitum. Arterial blood samples (5 ml) were taken at −5, −3, 0, 2, 5, 10, 15, 20, 25, 30, 40, 60, 70, 80, 90, 100, 120, 140, 160, 180, and 210 min. At 0 min, 0.25 g glucose/kg live weight was rapidly infused intravenously over 30 s. Blood glucose concentration at each time was measured using a HemoCue glucometer. The remaining blood was centrifuged, and plasma was collected for measurement of plasma glucose and insulin concentrations. Fasting plasma glucose and insulin concentrations are the mean of the concentrations of the first three time points before glucose administration. Insulin secretion during the IVGTT was calculated as the area under the insulin concentration curve vs. time. Areas under the glucose and insulin curves, with the mean of the fasting concentrations as the baseline, were calculated using the Sigma Scan Pro v4 image analysis software package (Jandel Scientific Software).

Analysis of plasma metabolites and hormones. Plasma glucose, triglycerides, and cholesterol concentrations were measured by enzymatic analysis with a COBAS MIRA automated sample system, using the Glucose HK assay kit, Triglyceride kit, and Cholesterol kit, with the calibrator for automated systems, and quality controls: Precinorm U and Precipath U (Roche Diagnostics, Peakhurst, NSW, Australia). The mean CV for each individual assay was <3.3%. The quantitative determination of plasma FFAs was performed with a COBAS MIRA automated sample system using the NEFA-C FFA assay kit (Wako Pure Chemical Industries, Japan, intra-assay CV, 4%) and quality controls: QCS 1 and 2 (Bio-Rad, Australia). The mean CV for the assay of plasma FFAs was <4.6%. Plasma α-amino nitrogen concentrations were measured by deproteinizing 100 µl of plasma with 800 µl of 0.04 M H2SO4 and 100 µl of 0.3 M sodium tungstate. These were centrifuged for 15 min at 3,000 g, and 100 µl of supernatant were placed into a 96-well plate. To this, 24 µl sodium tetraborate (saturated, pH 9.6 with 5 M NaOH) and 20 µl 0.02 M β-naphtholquinone sulfonate were added and incubated for 20 min at 80°C, then cooled in ice water for 5 min. Water (120 µl), 36 µl of acid formaldehyde (0.6 M HCl, 0.045 M HCHO), and 20 µl of 0.05 M sodium thiosulphate were added and incubated at room temperature for 30 min. The absorbance was read at 490 and 690 nm on an ELISA plate reader. Plasma insulin was measured using a commercially available radioimmunoassay kit (Linco, Australian Laboratory Services).

Statistical analysis. Data are expressed as means ± SE. Pearson correlation analysis, multiple linear regression analysis, and between-factor ANOVA (3 levels) with Tukey’s post hoc test (SPSS 13.0 software package for Windows; SPSS, Chicago, IL) were used to test the overall effects of maternal exposure to glucocorticoids and specific a priori hypotheses that effects of cortisol would be of less magnitude than those of dexamethasone, as stated in the Introduction. The %CV was calculated as the standard deviation divided by the mean multiplied by 100. Statistical significance was assumed at P < 0.05.

RESULTS

Size at birth and postnatal growth rate. Prenatal treatment of glucocorticoids did not alter birth weight or placental weight in either group (Table 1). Prenatal treatment with dexamethasone reduced abdominal circumference at birth (dexamethasone: 35.9 ± 0.7 cm, saline: 39.8 ± 1.2 cm, P = 0.02) compared with saline (Table 1). Prenatal treatment of glucocorticoids also did not alter live weight at 4.5 yr of age (Table 1).

Birth weight and plasma glucose and insulin. Plasma glucose [saline: nonsignificant (NS); dexamethasone: NS; cortisol: r = 0.67, P < 0.05] and insulin (saline: NS; dexamethasone: NS; cortisol: r = 0.65, P < 0.05) correlated negatively with birth weight in cortisol-treated offspring only (Fig. 1).

Glycemia and insulin action on glucose metabolism. Maternal treatment with cortisol, but not dexamethasone, increased fasting plasma glucose concentration compared with saline (P < 0.05) (Fig. 2). Maternal dexamethasone reduced the area under the plasma glucose concentration curve during the IVGTT (P = 0.03) and reduced plasma glucose concentrations early (45–80 min) and late (120–140 min) during the IVGTT compared with saline (P < 0.05) (Fig. 3).

Maternal treatment with cortisol, but not dexamethasone, increased fasting plasma insulin concentrations, the fasting plasma insulin-to-glucose ratio (P < 0.05) (Fig. 1), and the basal posthepatic insulin delivery rate (P = 0.02) compared with saline (Table 2). Maternal dexamethasone increased plasma insulin concentrations from baseline until 30 min after glucose load, compared with saline (P < 0.05), indicative of a pronounced first-phase hyperinsulinemia (Fig. 3). Maternal treatment with cortisol did not alter plasma insulin concentrations during first-phase insulin secretion (from 2 to 30 min), but increased that from 40 to 140 min, compared with saline (P < 0.05), indicating a pronounced second-phase hyperinsulinemia (Fig. 3). Maternal dexamethasone also increased the insulin-stimulated glucose disposition index compared with saline (P = 0.04) (Table 2).

Other metabolites and insulin action. Maternal glucocorticoid treatment in early pregnancy did not alter fasting plasma FFAs, triglycerides, or cholesterol in adult male offspring (Table 3). Overall, fasting plasma triglycerides correlated positively with plasma insulin (r = 0.41, n = 21, P = 0.033), the basal insulin-to-glucose ratio (r = 0.38, n = 21, P = 0.045), the fasting posthepatic insulin delivery rate (r = 0.44, n = 21, P = 0.023), and the fasting glucose disposition index (r =

Table 1. Effect of maternal dexamethasone and cortisol on size at birth and growth of male adult offspring

<table>
<thead>
<tr>
<th>Size at Birth and Postnatal Growth Parameters</th>
<th>Saline</th>
<th>Dexamethasone</th>
<th>Cortisol</th>
<th>Overall ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>P &lt; 0.1</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>4.68±0.28</td>
<td>4.07±0.18</td>
<td>4.81±0.22</td>
<td>P &lt; 0.1</td>
</tr>
<tr>
<td>Birth crown-rump length, cm</td>
<td>50.9±1.2</td>
<td>48.7±1.2</td>
<td>51.3±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Birth abdominal circumference, cm</td>
<td>39.8±1.2</td>
<td>35.9±0.7*</td>
<td>38.7±1.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Placental weight, kg</td>
<td>0.52±0.04</td>
<td>0.43±0.03</td>
<td>0.46±0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Live weight at experiment, kg</td>
<td>63.2±1.8</td>
<td>57.9±2.9</td>
<td>63.4±2.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n, no. of animals. Treatment with glucocorticoids (3 levels: saline, dexamethasone, cortisol) shows specific contrasts. NS, nonsignificant. *Saline and dexamethasone, P < 0.05.
When treatment groups were analyzed separately, these correlations were present only in the offspring of saline-treated mothers (data not shown).

Glucocorticoids did not alter insulin sensitivity of glucose, FFA, or amino acid metabolism of adult male offspring (Table 2). Maternal glucocorticoid treatment reduced fasting plasma \(\alpha\)-amino nitrogen concentrations compared with saline (\(P < 0.05\)) (Table 3), while the fasting and stimulated \(\alpha\)-amino nitrogen disposition indexes were unaltered (data not shown).

**DISCUSSION**

This study has demonstrated that maternal exposure to glucocorticoids for a short period in early pregnancy alters metabolic homeostasis and insulin action in male adult sheep offspring. Specifically, the short 2-day infusion of cortisol into the pregnant sheep caused fasting hyperglycemia and hyperinsulinemia, while the infusion of dexamethasone caused a hyperinsulinemic response to glucose in adult male offspring. Maternal treatment with dexamethasone induced a first-phase hyperinsulinemia and improved glucose tolerance. In contrast, maternal cortisol treatment induced a second-phase hyperinsulinemia with no impact on glucose tolerance, while neither glucocorticoid affected insulin sensitivity. These outcomes are similar to those of offspring of mothers exposed to the Dutch Famine in early gestation, who did not develop impaired glucose tolerance as adults, but were hyperinsulinemic compared with unexposed people (14, 52). Therefore, glucocorticoid exposure in early pregnancy in the sheep is able to alter insulin abundance and secretion in adult male offspring, and in a manner that suggests it could lead to diabetes and other related disorders later on. Altered glucose homeostasis and insulin secretion in offspring following maternal glucocorticoid exposure in late gestation has been shown in several species such as the rat and also sheep (33, 44, 46), and here we have shown that similar consequences arise in adult male sheep from maternal glucocorticoid exposure in early gestation.

The hyperinsulinemia induced in adult male offspring by maternal glucocorticoid exposure in early gestation reflects altered insulin secretion, independent of insulin sensitivity, as the latter was unchanged and insulin action on glucose metabolism was increased, following dexamethasone at least. This suggests persistent effects on insulin secretion and the developing pancreas of glucocorticoids administered to the pregnant sheep from 26–28 days of gestation (\(\sim 0.2\) gestation). Little is known of ovine pancreatic development at this time, however, and whether direct effects of glucocorticoids on the endocrine pancreas or indirect consequences of maternal exposure are involved requires further investigation.

The differential effects of early gestation exposure of the mother to cortisol or dexamethasone on insulin secretion in offspring in adult life may arise from targeting of determinants of different phases of insulin secretion. Here we have shown that maternal dexamethasone treatment in early gestation increases the first-phase insulin secretion in response to a glucose load for a given insulin sensitivity in offspring, suggesting there is clear over-secretion of insulin, whereas cortisol induces similar changes in second-phase insulin secretion. The release of insulin from the pancreas is biphasic in nature, such that the first-phase insulin release in response to glucose is the result of the exocytosis of a small pool of granules from the \(\beta\)-cell within the pancreas (2, 13, 32, 71). This initial spike of insulin release is triggered by the glucose-induced closure of the KATP channel.

![Fig. 1. Effect of maternal glucocorticoid exposure in early pregnancy on the association of fasting plasma glucose (top) and insulin (bottom) with birth weight in offspring. Fasting plasma glucose [saline: nonsignificant (NS); dexamethasone: NS; cortisol: \(r = 0.67, P < 0.05\)] and insulin [saline: NS; dexamethasone: NS; cortisol: \(r = 0.65, P < 0.05\)] correlated negatively with birth weight in offspring exposed to cortisol only. Saline (●, solid line), dexamethasone (○, long dashed line), and cortisol (△, short dashed line) infused ewes are shown.](image1)

![Fig. 2. Maternal glucocorticoid exposure in early pregnancy and the fasting plasma glucose, insulin, and plasma insulin-to-glucose ratio in adult male offspring. Saline is represented by solid bars, dexamethasone is represented by light gray bars, and cortisol is represented by dark gray bars. Data are presented as means ± SE. *\(P < 0.05\).](image2)
ATP-sensitive potassium channels and activation of their signaling pathway, which depolarizes the β-cell to cause entry of calcium via voltage-dependent calcium channels (2, 13, 32, 71). Whether glucocorticoid exposure can affect any of these processes directly in the adult or earlier during development is unknown. The second-phase insulin secretion is incompletely understood, but requires a continuously elevated calcium and amplification of calcium action on exocytosis by ATP-sensitive potassium channel-independent mechanisms, as does the first phase to recruit vesicles from the reserve pool (47). The mechanism responsible for this is still unknown, but hypotheses include time-dependent changes in the concentration of signaling molecules (45), the time taken for the physical recruitment of vesicles to a ready reserve pool (48), or the recruitment of vesicles by other mechanisms (4). Recently, it has been suggested that alterations in either first- or second-phase insulin secretion may arise from changes in the size of the insulin-containing vesicle reserve pool in β-cells (30); however, the differential effects of each glucocorticoid suggest other processes are targeted. There is little information as to glucocorticoid effects on these various determinants of the dynamics of insulin secretion in any species to date, however. Glucocorticoid exposure of pregnant rats in late gestation also produces hyperglycemia and hyperinsulinemia in offspring, and the former appears due to alterations in hepatic glucose production by the liver via increased PEPCK (a key enzyme in gluconeogenesis) gene expression (46). Notably, we have also shown that exposure to maternal cortisol in early pregnancy induced hyperglycemia in adult male offspring, which may act via a similar pathway. Here, maternal exposure in early pregnancy to either glucocorticoid consistently decreased plasma α-amino nitrogen concentrations in adult male offspring compared with saline. This may be related to the fasting hyperglycemia observed in offspring of the cortisol-treated ewes, through increased utilization of gluconeogenic amino acids for increased glucose production. Previous studies have shown that treating pregnant rats in late gestation with dexamethasone results in offspring with reduced birth weight and hyperinsulinemia and hyperglycemia, due to elevated levels of gluconeogenic enzymes, such as the rate-limiting enzyme PEPCK (20, 46). De novo synthesis of glucose utilizes amino acids (all amino acids are available except leucine) as major substrates, and inhibition of PEPCK and gluconeogenesis in the rat results in hypoglycemia and increased plasma amino acid concentration (69).

The origins of glucocorticoid-specific differences in these outcomes of maternal exposure in early pregnancy are unclear. Both dexamethasone and cortisol are able to cross the placenta in rats (8, 40, 46, 58) and sheep (16, 17, 35) and lead to adverse outcomes for adult offspring, but the extent to which each glucocorticoid actually crosses the placenta into the fetal circulation and accesses fetal tissues in early gestation is unknown. Dexamethasone is poorly metabolized by 11β-HSD-2 and, therefore, most readily crosses the placenta (11, 59).

Therefore, it seemed likely that the placenta and fetal tissues would have a greater glucocorticoid exposure following dexamethasone compared with cortisol in the present study. We found instead that maternal cortisol exposure increased fasting plasma glucose and insulin concentrations in adult male offspring to a greater extent than did dexamethasone, suggesting that these barriers were not very protective in this cohort. In humans, it has been shown that ~10–20% of maternal cortisol can pass directly to the fetus, possibly due to bypassing the placental enzyme 11β-HSD-2 (27). Therefore, it is possible that both of the glucocorticoids were able to cross the placental barrier, but cortisol may have been able to exert a greater effect on the fetus than dexamethasone. This outcome is unexpected, given that competitive binding analysis studies in the rat pancreas have shown that the glucocorticoid receptor binds synthetic dexamethasone with a higher affinity than corticosterone (65), and the former would not have been in competition for the mineralocorticoid receptor.

Recently, the multidrug resistance phosphoglycoprotein transporter has been shown to be highly expressed in the placenta, especially in early pregnancy as well as in other tissues (63). This transporter is able to transport glucocorticoids, including dexamethasone, back into maternal blood, thus reducing placental transfer to the fetus (66). If this transporter is present and active in the sheep placenta, it could reduce access of maternal dexamethasone to the fetal circula-
tion and tissues, but presumably would act on cortisol as well. Alternatively, cortisol may be exerting some effects via the mineralocorticoid receptor. Finally, maternal betamethasone treatment restricts placental growth and labyrinthinationally, maternal glucocorticoids may also affect placental albeit of a short-term nature, however, which are known by 10.220.32.247 on October 14, 2017 http://ajpendo.physiology.org/ Downloaded from by 10.220.32.247 on October 14, 2017 http://ajpendo.physiology.org/ Downloaded from

Table 2. Effect of maternal dexamethasone and cortisol on insulin sensitivity and secretion in male adult offspring

<table>
<thead>
<tr>
<th></th>
<th>Insulin Action</th>
<th>Saline</th>
<th>Dexamethasone</th>
<th>Cortisol</th>
<th>Overall ANOVA</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td></td>
<td></td>
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<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Glucose, mg·ml⁻¹·U⁻¹·min⁻¹·kg⁻¹ (×100)</td>
<td>0.71±0.15</td>
<td>0.88±0.14</td>
<td>0.80±0.10</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Free fatty acids, meq·I⁻¹·U⁻¹·ml⁻¹</td>
<td>0.21±0.02</td>
<td>0.24±0.02</td>
<td>0.21±0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Amino acids, mg·min⁻¹·μU⁻¹</td>
<td>0.033±0.008</td>
<td>0.018±0.008</td>
<td>0.025±0.005</td>
<td>NS</td>
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<tr>
<td>Glucose tolerance (IVGTT), mmol·min⁻¹·I⁻¹</td>
<td>502±64</td>
<td>343±32*</td>
<td>480±39</td>
<td>P &lt; 0.1</td>
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<tr>
<td>Total insulin secretion (IVGTT), μU·min⁻¹·I⁻¹</td>
<td>1.10±122</td>
<td>1.051±251</td>
<td>1.319±528</td>
<td>NS</td>
<td></td>
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<tr>
<td>Insulin secretion corrected for glucose, μU/ml</td>
<td>2.45±0.38</td>
<td>3.22±0.78</td>
<td>2.69±0.50</td>
<td>NS</td>
<td></td>
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<tr>
<td>First-phase insulin secretion (AUC IVGTT), μU·min⁻¹·I⁻¹</td>
<td>400±71</td>
<td>658±177</td>
<td>569±99</td>
<td>NS</td>
<td></td>
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<tr>
<td>Second-phase insulin secretion (AUC IVGTT), μU·min⁻¹·I⁻¹</td>
<td>713±134</td>
<td>391±83</td>
<td>953±265</td>
<td>NS</td>
<td></td>
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<tr>
<td>Insulin clearance, ml·kg⁻¹·min⁻¹</td>
<td>10.41±0.97</td>
<td>10.89±0.77</td>
<td>10.26±0.44</td>
<td>NS</td>
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Postheaptic insulin delivery rate (IVGTT)

<table>
<thead>
<tr>
<th></th>
<th>Basal, μU·kg⁻¹·min⁻¹</th>
<th>Maximal, μU·kg⁻¹·min⁻¹</th>
<th>Basal disposition index</th>
<th>Glucose, mg·ml⁻¹·kg⁻¹·min⁻²</th>
<th>Free fatty acids, meq·ml⁻²·kg⁻¹·min⁻¹</th>
<th>Stimulated disposition index</th>
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<tbody>
<tr>
<td></td>
<td>51.3±4.8</td>
<td>74.3±10.3</td>
<td>235±49</td>
<td>0.37±0.10</td>
<td>10.9±1.6</td>
<td>1.63±0.44</td>
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<td></td>
<td>74.3±10.3</td>
<td>84.0±9.3#</td>
<td>420±101</td>
<td>0.71±0.18</td>
<td>18.5±3.5</td>
<td>3.83±1.07</td>
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<tr>
<td></td>
<td>274±57</td>
<td>NS</td>
<td>274±57</td>
<td>0.67±0.40</td>
<td>17.9±2.1</td>
<td>2.13±0.31</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Treatment with glucocorticoids (3 levels: saline, dexamethasone, cortisol) shows specific contrasts. IVGTT, intravenous glucose tolerance test; AUC, area under the curve. *Saline and dexamethasone, #saline and cortisol: P < 0.05.

Table 3. Effect of maternal dexamethasone and cortisol on fasting plasma metabolites in male adult offspring

<table>
<thead>
<tr>
<th></th>
<th>Plasma Metabolite Concentration</th>
<th>Saline</th>
<th>Dexamethasone</th>
<th>Cortisol</th>
<th>Overall ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids, mmol/l</td>
<td>1.02±0.25</td>
<td>1.18±0.16</td>
<td>1.09±0.27</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.22±0.01</td>
<td>0.22±0.01</td>
<td>0.26±0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>1.48±0.10</td>
<td>1.67±0.11</td>
<td>1.51±0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>α-Amino nitrogen, mM</td>
<td>2.96±0.17</td>
<td>2.53±0.08*</td>
<td>2.56±0.14#</td>
<td>P &lt; 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Treatment with glucocorticoids (3 levels: saline, dexamethasone, cortisol) show specific contrasts. *Saline and dexamethasone, #saline and cortisol: P < 0.05.
hyperinsulinemic and hyperglycemic male offspring. Increased exposure to steroids in early gestation may, therefore, predispose offspring to the development of metabolic and cardiovascular disease with aging. Furthermore, males may be more susceptible than females to the effects of prenatal exposure to glucocorticoids (25) and, accordingly, have a greater risk in developing later adult disease.

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REFERENCES


