Dexamethasone during Late Gestation Exacerbates Peripheral Insulin Resistance and Selectively Targets Glucose-Sensitive Functions in β Cell and Liver

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We examined whether low-dose dexamethasone administration during late pregnancy modifies hepatic and/or peripheral insulin action or glucose-stimulated insulin secretion. Dexamethasone (100 μg/kg maternal body weight/d) was administered via an osmotic minipump from d 14–19 of gestation. Maternal glucose-insulin homeostasis was assessed on d 19 of pregnancy in the postabsorptive state. Insulin secretion and glucose tolerance was assessed after iv glucose, and insulin action examined during insulin infusion at euglycemia. Dexamethasone treatment during late pregnancy elicited fasting hyperinsulinaemia (by 88%; P < 0.001) and hyperglycaemia (by 20%; P < 0.05), and enhanced endogenous glucose production (by 29%; P < 0.001). Insulin secretion and rates of glucose disappearance after iv glucose were greatly impaired (by 44% and 39% respectively; P < 0.05). Suppression of endogenous glucose production by insulin was enhanced by dexamethasone treatment, but insulin’s ability to promote glucose clearance was diminished. We demonstrate that excess maternal glucocorticoids during late pregnancy impairs glucose-stimulated insulin secretion and insulin-simulated glucose clearance but enhances insulin’s ability to suppress endogenous glucose production. The data also indicate that elevated maternal glucocorticoids impair adaptations of the endocrine pancreas to pregnancy in vivo in that insulin hypersecretion in response to deteriorating peripheral insulin action is no longer apparent, leading to impaired glucose tolerance. (Endocrinology 142: 3742–3748, 2001)

PREGNANCY IS ASSOCIATED with the progressive development of maternal insulin resistance that suppresses maternal glucose utilization (1–4). Because uterine uptake and the placental transport of glucose is relatively unaffected by the maternal insulin status, this adaptation facilitates glucose utilization by the developing fetus (5, 6). Maternal insulin resistance during late pregnancy is also associated with increased pancreatic islet number, enhanced pancreatic β-cell insulin secretion and a lowered threshold for stimulation of insulin secretion by glucose (reviewed in Ref. 7). The placent al lactogens (PL-I and PL-II) and PRL are thought to be important mediators of the adaptations of the endocrine pancreas to pregnancy (see e.g. Refs. 8–11), in particular islet proliferation and islet cell hypertrophy. Elevated levels of stress hormones, including glucocorticoids, can induce insulin resistance (12) that can lead to glucose intolerance. Glucocorticoids also counteract the effect of lactogens on insulin secretion and β-cell proliferation in vitro (13). Dexamethasone also suppresses insulin secretion by islets previously cultured with PRL to mimic the islet adaptive response to pregnancy (13), inhibits β-cell proliferation induced by PRL, and promotes β-cell apoptosis (13). Dexamethasone stimulates leptin synthesis and secretion in rat adipocytes (14), and leptin has been shown to markedly impair glucose-stimulated insulin secretion (GSIS) (15–17). In addition, leptin has been proposed as a potential factor regulating fetal growth (reviewed in Ref. 18). The present study sought to establish whether low-dose dexamethasone administration leads to altered characteristics of GSIS or modified hepatic or peripheral insulin action during late pregnancy, and the extent to which modulation of plasma leptin levels may contribute to such effects.

Materials and Methods

Materials

General Laboratory reagents were from Roche Diagnostics (Lewes, East Sussex, UK) or from Sigma (Poole, Dorset, UK), with the following exceptions. Organic solvents were of analytical grade and obtained from BDH (Poole, Dorset, UK). Plasma insulin and leptin were measured using commercial kits from Phadeseph Pharmacia (Uppsala, Sweden) and Linco Research, Inc. (St. Louis, MO) respectively. Human Actrapid insulin was from Novo Nordisk (Bagsvaerd, Denmark). Radiochemicals were purchased from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK). Dexamethasone (sodium phosphate) was obtained from David Bull Laboratories, Inc. (Warwick, UK). Mini-osmotic pumps were purchased from Charles River Laboratories, Inc. (Margate, Kent, UK).

Animals

All studies were conducted in adherence to the regulations of the United Kingdom Animal Scientific Procedures Act (1986). Female Wistar rats (200–250 g) were purchased from Charles River Laboratories (Margate, Kent, UK). Rats were subjected to a standard light-dark cycle (0800–2000 h dark, 2000–0800 h light) cycle in a temperature-controlled room (21 ± 2 °C). The rats were housed in individual cages and were given free access to food and water. Rats were maintained on standard, pelleted rodent diet purchased from Special Diet Services (Witham, UK).
Essex, UK). This diet consisted of 52% carbohydrate, 15% protein, 3% lipid, and 30% non-digestible residue (by weight), and contained 2.61 kcal metabolizable energy/g. Rats were time-mated by the appearance of sperm plugs (d 0 of pregnancy) and randomly assigned to two groups. Dexamethasone was administered sc at a low dose (100 µg/kg maternal body weight per d) via a chronically implanted osmotic minipump to rats from d 14–19 of pregnancy (term = 22–23 d) or, for an equivalent period, to age matched unmated female rats. An initial priming dose (0.1 mg) of dexamethasone was given by sc injection before minipump implantation. This procedure led to almost total suppression of endogenous corticosterone levels (results not shown). Pregnant rats with less than eight fetuses were not included in the study. Sham operations involving incision and manipulation under anesthesia identical to the procedure for implantation of the osmotic minipump were undertaken on control rats.

In vivo studies of glucose kinetics

Maternal glucose-insulin homeostasis was assessed on d 19 of pregnancy in the postabsorptive state and during insulin infusion at euglycemia. On the d of the experiment, food was withdrawn at the end of the dark (feeding) phase and studies undertaken in the postabsorptive state. For euglycemic-hyperinsulinemic clamp studies, rats were fitted with chronic indwelling jugular cannulae for infusion and sampling. Euglycemic-hyperinsulinemic clamp studies were conducted at 5 d after cannulation to permit full recovery from surgery in unstressed conscious rats as described in Refs. 19, 20. A constant infusion of human Actrapid insulin was given for up to 2 h. The insulin dose was standardized to 4 mU/kg per min. The infusion of exogenous glucose was initiated at 1 min after the start of insulin infusion. Blood was sampled from the right jugular vein at 5-min intervals. Adjustments in the exogenous glucose infusion rate were made to maintain glycemia at approximately 4.2 mm. Blood glucose concentrations during the clamp were determined using a glucose analyzer (YSI, Inc., Yellow Springs, OH). A plateau for the exogenous glucose infusion rate was reached after 60–90 min. The glucose infusion rate required to maintain euglycemia during the plateau phase of the clamp is denoted as glucose infusion rate (GIR).

Rates of endogenous glucose production (Ra) and peripheral glucose disposal (Rd) were estimated in the basal state and during a euglycemic-hyperinsulinemic clamp using primed (0.5 µCi)-continuous (0.2 µCi/min per rat) iv infusion of [3-3H]glucose (19, 20). A steady-state of plasma was maintained sample was immediately centrifuged (10,000 × g/11003 H) and the supernatant retained for subsequent assay of blood glucose. The remaining sample was immediately centrifuged (10,000 × g) at 4 °C, and the supernatant retained for subsequent assay of blood glucose. The remaining sample was immediately centrifuged (10,000 × g) at 4 °C, and plasma was stored at −20 °C until assayed for insulin.

Biochemical and physiological determinations

Plasma insulin concentrations were measured by RIA using rat insulin standards (Phadepharm Pharmacia). Plasma leptin concentrations were determined by a commercially available RIA using rat leptin standards (Linco Research, Inc.).

Statistical analyses

Statistical comparisons were made with StatView (Abacus Concepts, Berkeley, CA). Multiple comparisons were made by ANOVA and in individual comparisons by Fisher post hoc tests. Comparisons between just two sets of data were performed with the unpaired t test. All data are presented as the means ± SEM.

Results

Maternal dexamethasone treatment during pregnancy in the rat decreases maternal body weight and food intake

Food intakes and body weight gain of control and dexamethasone-treated dams from d 0–19 of pregnancy are shown in Fig. 1. The body weight of control dams increased steadily from d 0–19 of gestation (Fig. 1). This was accompanied by an increase in food intake in control dams from d 1–17 of gestation compared with d 0 of gestation, but food intake subsequently declined thereafter before parturition (Fig. 1). Dexamethasone treatment led to a modest, but statistically significant decline in food intake compared with that of control dams on d 15 (16%; P < 0.01), 16 (19%, P < 0.001), and 17 (12%; P < 0.05) of pregnancy. This was associated with a significant decline in body weight in dexamethasone-treated dams compared with control dams on d 17 (9%; P < 0.05), 18 (9%; P < 0.001), and 19 (11%; P < 0.001) of pregnancy (Fig. 1). Glucocorticoid administration did not compromise pregnancy to term and fetal numbers per dam were unchanged (control, 12.3 ± 0.5; dexamethasone-treated, 12.0 ± 0.7 [8–13 litters]; N.S.).

Maternal dexamethasone treatment during pregnancy leads to hyperinsulinaemia and hyperglycaemia in the postabsorptive state

Plasma insulin concentrations were significantly higher (by 88%; P < 0.001) in dexamethasone-treated pregnant rats compared with controls on d 15 (91%; P < 0.001), 16 (87%; P < 0.001), and 17 (88%; P < 0.001) of pregnancy. Plasma glucose concentrations were unchanged (control, 12.3 ± 0.5; dexamethasone-treated, 12.0 ± 0.7 [8–13 litters]; N.S.).
compared with the control pregnant group in the postabsorptive state (Table 1). This relative hyperinsulinaemia was accompanied by higher postabsorptive blood glucose concentrations (20%; \( P < 0.05 \)) in the dexamethasone-treated pregnant group (Table 1). The insulin: glucose ratio, which is an index of the relative response of insulin to fasting glycaemia, was significantly higher (43%, \( P < 0.05 \)) in the dexamethasone-treated pregnant group compared with controls. We assessed to what extent these responses to dexamethasone were specifically related to the pregnant state by treating age-matched nonpregnant female rats with dexamethasone for 5 d. Dexamethasone treatment of nonpregnant rats also resulted in elevated postabsorptive insulin concentrations compared with those found in nonpregnant control rats (control, \( 10 \pm 1 \mu U/ml \); dexamethasone-treated, \( 29 \pm 5 \mu U/ml \); \( P < 0.01 \)), but did not alter postabsorptive glycaemia.

Maternal dexamethasone treatment during pregnancy increases endogenous glucose production and glucose metabolic clearance rates in the postabsorptive state

The groups of pregnant rats were infused with [3-3H] glucose for measurement of glucose kinetics in the postabsorptive state. Despite their having significantly higher insulin concentrations, \( R_a \) calculated from [3-3H] glucose specific activity and expressed relative to body weight was significantly higher (by 29%; \( P < 0.001 \)) in the dexamethasone-treated pregnant rats under basal conditions (Fig. 2). Basal blood GCR, an index of glucose disposal taking into account differences in prevailing glucose concentrations, was also significantly increased (by 17%; \( P < 0.01 \)) in the dexamethasone-treated pregnant group (Fig. 2).

Maternal dexamethasone treatment during pregnancy is associated with impaired glucose clearance during the first 15 min after iv glucose challenge

Administration of iv glucose (500 mg/kg) elevated blood glucose levels to approximately 10 mM in both control and the dexamethasone-treated pregnant rats after 2 min, and blood glucose levels at 2, 5, and 10 min after iv glucose challenge did not differ significantly between the control and dexamethasone-treated pregnant groups (Fig. 3). A trend toward higher glucose levels was evident in the dexamethasone-treated pregnant group after 15 min and the rate of glucose disappearance (K value) calculated over the first 15 min was significantly lower (by 39%; \( P < 0.05 \)) for the dexamethasone-treated pregnant group than for the controls (control pregnant, \( 3.1 \pm 0.4\%/min \); dexamethasone-treated pregnant, \( 1.9 \pm 0.2\%/min \)). Dexamethasone treatment of nonpregnant rats resulted in a trend toward a lower K value (a 21% decrease, but this did not achieve statistical significance) compared with control nonpregnant rats (control nonpregnant, \( 2.9 \pm 0.2\%/min \); dexamethasone-treated nonpregnant, \( 2.3 \pm 0.2\%/min \)). Thus, the impact of dexamethasone treatment on rates of glucose clearance after an iv glucose challenge is more pronounced in pregnant than in nonpregnant rats.

Maternal dexamethasone treatment during pregnancy leads to impaired glucose-stimulated insulin secretion after an iv glucose challenge

To evaluate insulin secretory responses, plasma insulin concentrations were measured at intervals after the iv administration of the glucose bolus. The relative response of insulin to increased glycaemia was suppressed by dexamethasone administration in late pregnancy as reflected by a substantial decrease (by 44%; \( P < 0.05 \)) in the incremental area under the curve for insulin (IAUC-insulin) (control, \( 215 \pm 27 \mu U/min per liter \); dexamethasone-treated, \( 121 \pm 20 \mu U/min per liter \)). Plasma insulin concentrations at 2 min after iv administration of glucose were significantly lower (by 38%, \( P < 0.05 \)) in the dexamethasone-treated pregnant group than in the control pregnant rats (\( 78 \pm 7 \mu U/ml \) and \( 48 \pm 9 \mu U/ml \), respectively) (Fig. 3). Insulin concentrations in rats of the dexamethasone-treated pregnant group remained significantly lower (by 36%; \( P < 0.05 \)) than those of the control pregnant group at 5 min after iv glucose administration (\( 82 \pm 11 \mu U/ml \) and \( 52 \pm 8 \mu U/ml \), respectively) (Fig. 3). The incremental area under the curve for insulin (IAUC-insulin) was, as expected, significantly higher in late preg- nant control rats than in age-matched unmated control rats (control nonpregnant, 149 \( \pm 8 \mu U/min per liter \); control pregnant, \( 215 \pm 27 \mu U/min per liter \); a 44% increase, \( P < 0.05 \)). Dexamethasone treatment did not significantly affect IAUC-insulin values for nonpregnant rats (dexamethasone-treated nonpregnant, \( 121 \pm 34 \mu U/min per liter \)). Thus, dexamethasone treatment specifically impairs the enhancement of insulin secretion that is normally observed in response to pregnancy and IAUC-insulin values for nonpregnant and late-pregnant rats after dexamethasone treatment do not differ significantly (dexamethasone-treated nonpregnant, \( 121 \pm 34 \mu U/min per liter \); dexamethasone-treated pregnant, \( 121 \pm 20 \mu U/min per liter \); NS).

Effect of maternal dexamethasone treatment during pregnancy on whole body insulin action and glucose disposal during hyperinsulinaemia

Previous studies have demonstrated that an insulin infusion rate of approximately 4 mU/kg per min leads to plasma insulin concentrations in the upper physiological range (ap-

### TABLE 1

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<tr>
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<th>Control</th>
<th>Dexamethasone-treated</th>
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<tr>
<td>Blood glucose (mM)</td>
<td>3.0 ± 0.2 (18)</td>
<td>3.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt; (7)</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>8 ± 1 (18)</td>
<td>15 ± 1&lt;sup&gt;b&lt;/sup&gt; (7)</td>
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<tr>
<td>Insulin/glucose ratio (µU/µmol)</td>
<td>2.8 ± 0.5 (18)</td>
<td>4.0 ± 0.2&lt;sup&gt;b&lt;/sup&gt; (7)</td>
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Results are means ± sem for the numbers of rats indicated in parentheses. Statistically significant effects of dexamethasone treatment are indicated by: <sup>a</sup> \( P < 0.05 \); <sup>b</sup> \( P < 0.001 \).
proximately 75 μU/ml) during clamp (19, 21). In the present study, steady-state plasma insulin concentrations attained during clamp were similar in the control and dexamethasone-treated pregnant groups (control, 69 ± 7 μU/ml [n = 10]; dexamethasone-treated, 74 ± 5 μU/ml [n = 4]). The standardization of blood glucose concentrations during insulin infusion enables direct comparison of whole-body insulin action between the two pregnant groups. Steady-state blood glucose concentrations during hyperinsulinemia did not differ significantly between the two groups of pregnant rats (control, 4.1 ± 0.2 mM [n = 10]; dexamethasone-treated, 4.2 ± 0.1 mM [n = 4]). The coefficients of variance for blood glucose concentrations were <15% for both groups.

Maternal plasma leptin levels at d 19 of gestation, measured in the postabsorptive state, were significantly higher (by 2.2-fold; P < 0.05) in the pregnant group treated with dexamethasone compared with the control group. Insulin infusion (2 h) significantly increased (2-fold; P < 0.001) plasma leptin levels in the control pregnant group, but increases in plasma leptin levels did not achieve significance in the dexamethasone-treated pregnant group. Basal and clamp leptin levels are plotted against prevailing steady-state insulin levels in Fig. 4. The plasma insulin–plasma leptin relationships (slopes of the lines) provide indices of the response of plasma leptin levels to 2-h hyperinsulinaemia at euglycemic-hyperinsulinaemia in control pregnant rats (open symbols) or pregnant rats treated with dexamethasone at a dose of 100 μg/kg maternal body wt per d from d 14 of pregnancy (closed symbols). Results are presented as means ± SEM for 5–10 rats. Statistically significant effects of dexamethasone treatment are indicated by: †, P < 0.05; ‡, P < 0.01; *, P < 0.001.

Effect of maternal dexamethasone treatment during pregnancy on endogenous glucose production during hyperinsulinaemia

Although Ra was suppressed by hyperinsulinaemia in both groups, suppression of Ra was greater in the dexamethasone-treated pregnant group (P < 0.05) under hyperinsulinaemic conditions (Fig. 2). Furthermore, whereas GIR was greater than Rd for each member of the dexamethasone-treated pregnant group, which is typically interpreted to indicate that endogenous glucose production has been suppressed completely, GIR was less than Rd for the control pregnant group, indicating that incomplete suppression of endogenous production of glucose had been obtained.

Maternal leptin levels are increased by dexamethasone administration

Maternal plasma leptin levels at d 19 of gestation, measured in the postabsorptive state, were significantly higher (by 2.2-fold; P < 0.05) in the pregnant group treated with dexamethasone compared with the control group. Insulin infusion (2 h) significantly increased (2-fold; P < 0.001) plasma leptin levels in the control pregnant group, but increases in plasma leptin levels did not achieve significance in the dexamethasone-treated pregnant group. Basal and clamp leptin levels are plotted against prevailing steady-state insulin levels in Fig. 4. The plasma insulin–plasma leptin relationships (slopes of the lines) provide indices of the response of plasma leptin levels to 2-h hyperinsulinaemia at euglycemic-hyperinsulinaemia in control pregnant rats (open symbols) or pregnant rats treated with dexamethasone at a dose of 100 μg/kg maternal body wt per d from d 14 of pregnancy (closed symbols). Results are presented as means ± SEM for 5–10 rats. Statistically significant effects of dexamethasone treatment are indicated by: †, P < 0.05; ‡, P < 0.01; *, P < 0.001.

FIG. 2. Relationship between plasma insulin concentrations and rates of endogenous glucose production (Ra; A), glucose disposal (Rd; B) and glucose clearance rates (GCR; C) in the basal state and after 2 h euglycaemic-hyperinsulinaemia in control pregnant rats (open symbols) or pregnant rats treated with dexamethasone at a dose of 100 μg/kg maternal body wt per d from d 14 of pregnancy (closed symbols). Results are presented as means ± SEM for 5–10 rats. Statistically significant effects of dexamethasone treatment are indicated by: †, P < 0.05; ‡, P < 0.01; *, P < 0.001.

FIG. 3. Blood glucose (A) and plasma insulin (B) concentrations before and at intervals after the iv administration of a glucose bolus to control pregnant rats (open symbols) or pregnant rats treated with dexamethasone at a dose of 100 μg/kg maternal body wt per d from d 14 of pregnancy (closed symbols). Results are presented as means ± SEM for 5–7 rats. Statistically significant effects of dexamethasone treatment are indicated by: †, P < 0.05.

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Fig. 4. Maternal circulating leptin concentrations in relation to steady-state plasma insulin concentrations in 19-d-old control pregnant rats (open symbols) and 19-d-old pregnant rats administered dexamethasone at a dose of 100 μg/kg maternal body weight per d from d 14 of pregnancy (closed symbols). Results are presented as means ± SEM for 6–12 rat dams. Statistically significant effects of maternal dexamethasone administration are indicated by: †P < 0.05.

cæma. There was no indication that the elevated postabsorptive leptin levels observed in the dexamethasone-treated pregnant group could be solely attributed to relative hyperinsulinaemia, and it appeared that the leptin response to a rise in insulin was unimpaired by dexamethasone treatment in late pregnancy (Fig. 4).

Discussion

The present study sought to establish whether low-dose dexamethasone administration leads to altered characteristics of GSIS or modified hepatic or peripheral insulin action during late pregnancy, and the extent to which modulation of plasma leptin levels may contribute to such effects.

The liver is a major target tissue for the glucocorticoids (reviewed in Ref. 22). In the nonpregnant state, glucocorticoids increase hepatic glucose production by stimulating gluconeogenesis (23). The expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) has been identified as a key target. Hepatic PEPCK expression is also regulated by insulin, and the effect of insulin is dominant over that of the glucocorticoids (24). Ra in the postabsorptive state was increased in late-pregnant dams treated with dexamethasone, compared with the controls. This effect was not associated with changes in liver weight as a consequence of dexamethasone treatment (control, 14.5 ± 0.3 g; dexamethasone-treated, 13.9 ± 0.4 g). Postabsorptive GCR was also increased by dexamethasone administration in late pregnancy, and thus the modest increase in postabsorptive glycæma observed in this group can be entirely attributed to increased glucose production. The observation of increased Ra in the postabsorptive state in late pregnant dams treated with dexamethasone compared with the controls pregnant dams suggests that hepatic sensitivity to glucocorticoids with respect to glucose production is retained in late pregnancy.

It is established that gestation is characterized by adaptations of carbohydrate metabolism, including a progressive state of maternal insulin resistance, that impede maternal glucose utilization (1–4). The development of maternal insulin resistance confers a competitive advantage to the developing fetus because uterine uptake and the placental transport of glucose appear to be relatively unaffected by changes in the maternal insulin status (5, 6). It is also well known that stress hormones, such as the glucocorticoids, induce insulin resistance (12, 22, 25), and can precipitate glucose intolerance when they are in excess. However, since late pregnancy is already a state of insulin resistance, a key question addressed in the present study was whether the administration of dexamethasone during late pregnancy would further depress insulin action. Whole-body insulin action, as assessed by the rate of glucose infusion required to maintain euglycaemia during steady-state hyperinsulinaemia (GIR), was unaffected by maternal dexamethasone treatment during late pregnancy. However, kinetic analysis revealed a shift in insulin sensitivity, with augmented insulin resistance of peripheral tissues but enhanced hepatic insulin sensitivity. The identification of peripheral insulin resistance as a component of the maternal response to excess glucocorticoids during late pregnancy is supported by the finding of a lower rate of glucose disappearance (K value) during the iv glucose tolerance test in the dexamethasone-treated pregnant group. Importantly, the impact of dexamethasone treatment on rates of glucose disappearance was more pronounced in pregnant than in nonpregnant rats. The question remains as to why the increased Ra in the postabsorptive state is not associated with an impaired response of Ra to hyperinsulinaemia. It appears that hyperinsulinaemia can effectively counter the effect of dexamethasone treatment to increase Ra. Overall, our findings suggest that increased postabsorptive Ra in the dexamethasone-treated pregnant group reflects a direct effect of dexamethasone to increase hepatic glucose production which is not due to an action to impair hepatic insulin sensitivity. Thus, our results are consistent with the previously demonstrated dominance of insulin over dexamethasone with respect to regulation of PEPCK expression (24).

During normal pregnancy, pancreatic islets undergo a number of adaptive changes to meet the increased demand for insulin secretion imposed by maternal insulin resistance (reviewed in Ref. 7). These include increased GSIS, a reduction in the glucose-stimulated threshold, and β cell proliferation. The lowering of the threshold for GSIS is thought to be the primary mechanism by which the β cells can release significantly more insulin under normal blood glucose concentrations (7). In the present experiments, in which the administration of dexamethasone exaggerated the physiological peripheral insulin resistance of late pregnancy, the higher insulin:glucose concentration ratio observed in the dexamethasone-treated pregnant group in the postabsorptive state suggests that insulin secretion may occur at a lower level of glycaemia. It therefore appears that the leftward shift in the insulin secretory response curve typical of late pregnancy is not only retained, but augmented. The adaptation in β cell function observed in dexamethasone-treated pregnant rats in the present study parallels the apparent enhancement of insulin sensitivity with respect to suppression.
of Ra observed in this group, suggesting that there may be a common underlying signal/mechanism. Enhanced basal insulin secretion was, however, accompanied by a marked impairment in GSIS as a consequence of dexamethasone administration, suggesting a dissociation between enhanced glucose sensing and augmented glucose responsiveness in vivo.

It has emerged that lactogenic hormones—the placental lactogens (PL-I and PL-II) and PRL—may be important for mediating some of the adaptations of the endocrine pancreas to pregnancy (see e.g. Refs. 8–11), in particular the increase in islet proliferation and islet cell hypertrophy. In rodents, PL-I is normally made in the trophoblast giant cells of the placenta (26–28) and the PLs interact on islet cells with the PRL receptor, a member of the cytokine family of receptors (29, 30). In the present study, dexamethasone treatment during pregnancy led to decreased placental weights (results not shown) and thus placental PL-I production may be impaired. Perhaps even more importantly, recent work has shown that glucocorticoids, whose concentrations normally increase in late pregnancy to assist maturation of fetal tissue function (31), counteract the effect of lactogens on insulin secretion and β cell proliferation (13), presumably to curtail insulin hypersecretion postpartum. In vitro, dexamethasone at a concentration equivalent to the plasma glucocorticoid concentration found during late pregnancy was shown to exert a significant inhibitory effect on insulin secretion by islets previously cultured with PRL to mimic the islet adaptive response to pregnancy (13). Of major importance was the finding that dexamethasone inhibited β-cell proliferation induced by PRL and promoted β-cell apoptosis (13). Studies of the effects of long-term high-dose dexamethasone treatment (2 mg/kg daily for 12 d) on GSIS in isolated islets from male rats demonstrated a marked leftward shift in the glucose dose-response relationship after dexamethasone treatment, but no difference in insulin secretion at 20 mM glucose (32). Our data therefore provide direct in vivo support for the concept that compensatory insulin hypersecretion in pregnancy is opposed by excessive exposure of the β cell to glucocorticoids, probably by an effect to impair β cell proliferation and islet mass augmentation in response to PL-I, but that the lowered GSIS threshold occurs via a different mechanism that is not adversely affected (and may even be enhanced) by glucocorticoids. It is possible that high basal insulin secretion but substantially reduced GSIS in the dexamethasone-treated dams may reflect sustained exposure of the β cells to a relatively elevated glucose concentration as rats made hyperglycaemic by chronic (48 h) glucose infusions develop β-cell glucose unresponsiveness despite high basal insulin secretion (33, 34).

Leptin has been proposed as a potential factor in fetal growth. In the nonpregnant state, leptin expression in adipose tissue of lean rodents changes in parallel with the changes in insulin concentrations associated with feeding and fasting (reviewed in Ref. 18). In starved rats, circulating leptin levels are increased with insulin infusion at euglycemia (35–37). In the present experiments with late pregnant rats, insulin infusion at euglycemia significantly elevated leptin levels. Leptin levels were already elevated in the postabsorptive state in the dexamethasone-treated dams and further elevated by hyperinsulinaemia in the treated group. Given that leptin has been reported to influence hepatic and peripheral actions of insulin (38–41) and to impair GSIS (15–17), we cannot exclude a role for leptin in modulating or mediating altered maternal insulin-glucose interactions invoked by inappropriately high glucocorticoid concentrations during late pregnancy.

Intrauterine growth retardation (IUGR) (assessed as low birth weight) in humans has been identified as a risk factor for the development of disorders in adult life, including glucose intolerance and insulin resistance (reviewed in Ref. 42). However, the mechanisms underlying IUGR remains to fully elucidated. The fetus is normally protected from maternal glucocorticoids by the placental enzyme 11β-hydroxysteroid dehydrogenase type-2 (11β-HSD2), which catalyzes the rapid conversion of active glucocorticoids to inert 11-keto derivatives (43). The synthetic glucocorticoid dexamethasone is a poor substrate for 11β-HSD2 (43) and, in the rat, dexamethasone administration during the last third of pregnancy leads to fetal growth retardation (44, 45). Early growth retardation induced by maternal dexamethasone treatment in the rat produces fasting and postglucose hyperinsulinaemia (46) in the adult offspring. The administration of benoxolone, an inhibitor of placental 11β-HSD2, to pregnant rats prevents the normal degradation of maternal glucocorticoids exposing the fetus to elevated levels of maternal glucocorticoids. This treatment also leads to a significant reduction in birth weight (47). Although these data provided strong evidence that excessive exposure to glucocorticoids affects fetal growth directly, the possibility nevertheless existed that an adverse impact of dexamethasone treatment on maternal glucose handling or insulin sensitivity might additionally impair nutrient provision to the developing fetus and thereby contribute to IUGR. We observed significant (15% P < 0.001) fetal growth retardation at d 19 of gestation in response to maternal dexamethasone treatment in the present study (mean fetal weight (g): control, 2.62 ± 0.07 [6 litters]; dexamethasone-treated, 2.22 ± 0.03 [13 litters]). However, the data obtained in the present study indicate that maternal peripheral insulin-dependent glucose utilization is impaired by dexamethasone treatment, and therefore it would be predicted that even more glucose would be made available for fetal use. Thus, fetal IUGR in response to dexamethasone treatment is unlikely to reflect a maternal metabolic defect with respect to inadequate suppression of insulin-dependent glucose disposal. Leptin has been proposed as a potential factor in fetal growth and, therefore, dexamethasone-induced IUGR could reflect, in part, an inappropriately low plasma leptin level. However, the present study demonstrates an effect of dexamethasone treatment to enhance leptin levels during pregnancy, indicating that an inappropriately low plasma leptin level is unlikely to contribute to IUGR.

In summary, dexamethasone treatment during late pregnancy elicited fasting hyperinsulinaemia and hyperglycaemia and significantly enhanced endogenous glucose production. Insulin secretion after iv glucose challenge and insulin secretion after iv glucose challenge and thereby contribute to IUGR. We observed significant (15% P < 0.001) fetal growth retardation at d 19 of gestation in response to maternal dexamethasone treatment in the present study (mean fetal weight (g): control, 2.62 ± 0.07 [6 litters]; dexamethasone-treated, 2.22 ± 0.03 [13 litters]). However, the data obtained in the present study indicate that maternal peripheral insulin-dependent glucose utilization is impaired by dexamethasone treatment, and therefore it would be predicted that even more glucose would be made available for fetal use. Thus, fetal IUGR in response to dexamethasone treatment is unlikely to reflect a maternal metabolic defect with respect to inadequate suppression of insulin-dependent glucose disposal. Leptin has been proposed as a potential factor in fetal growth and, therefore, dexamethasone-induced IUGR could reflect, in part, an inappropriately low plasma leptin level. However, the present study demonstrates an effect of dexamethasone treatment to enhance leptin levels during pregnancy, indicating that an inappropriately low plasma leptin level is unlikely to contribute to IUGR.

In summary, dexamethasone treatment during late pregnancy elicited fasting hyperinsulinaemia and hyperglycaemia and significantly enhanced endogenous glucose production. Insulin secretion after iv glucose challenge and insulin’s ability to promote glucose clearance were impaired, whereas suppression of endogenous glucose production by hyperinsulinaemia was enhanced by dexamethasone treatment. The data indicate that elevated maternal glucocorti-
coids impair adaptations of the endocrine pancreas to pregnancy in that hypersecretion of insulin in response to deteriorating peripheral insulin action is no longer apparent leading to impaired glucose tolerance. Increased glucose production in the basal state is likely to underlie lasting hyperglycaemia; however, the response of 1α to insulin is sensitized supporting the concept that impaired glucose tolerance is due to impaired peripheral glucose disposal.

Acknowledgments

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