CALL FOR PAPERS | Fetal Physiological Programming

Prenatal programming of adult blood pressure: role of maternal corticosteroids

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Woods, Lori L., and Douglas A. Weeks. Prenatal programming of adult blood pressure: role of maternal corticosteroids. Am J Physiol Regul Integr Comp Physiol 289: R955–R962, 2005. First published June 2, 2005; doi:10.1152/ajpregu.00455.2004.—Both maternal glucocorticoid administration and maternal dietary protein or food restriction in pregnancy cause fewer nephrons and hypertension in the adult offspring. The purpose of these studies was to determine the extent to which nutritional factors contribute to programming of offspring hypertension by maternal glucocorticoids. Pregnant rats were treated with dexamethasone (100 μg·kg⁻¹·d⁻¹ sc) on days 1–10 (ED) or days 15–20 (LD) of pregnancy. Additional groups of pregnant animals were pair fed to the early (EDPF) and late (LDPF) dexamethasone-treated groups, and another group was untreated or given vehicle (C). The dams treated with dexamethasone reduced their food intake and lost or failed to gain a normal amount of weight during treatment; body weights of ED dams caught up to normal after the treatment period, whereas those of LD dams did not. In adulthood (~21 wks), chronically instrumented male offspring of ED had normal blood pressures (125 ± 2 mmHg vs. 126 ± 1 mmHg in C), whereas LD offspring were hypertensive (136 ± 3 mmHg). However, LDPF offspring were equally hypertensive (134 ± 2 mmHg). Glomerular filtration rates normalized to body weight were not significantly different among groups. Qualitatively similar results were found in female offspring. Thus the long-term effects of maternal glucocorticoid administration at this dose on offspring’s blood pressure may, in large part, be accounted for by the reduction in maternal food intake. These data suggest that maternal glucocorticoids and maternal food or protein restriction may, at least in part, share a common mechanism in programming offspring for hypertension. The fetal theory is that increased fetal exposure to maternal glucocorticoids may play a key role in programming the offspring for hypertension (2). The fetus is normally thought to be protected from maternal glucocorticoids, which are deactivated by the placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD). However, under conditions in which placental 11β-HSD activity is diminished and/or maternal glucocorticoid levels are increased, fetal exposure would occur. In support of this idea, administration of dexamethasone, a synthetic corticosteroid not metabolized by this enzyme, or carbenoxolone, an inhibitor of 11β-HSD, to pregnant rats leads to offspring of low birth weight that become hypertensive later in life (2, 20, 21, 27).

A second theory regarding fetal programming for adult hypertension invokes a key role for nutritional factors. Generalized undernutrition or inadequate supply of specific nutrients during development could lead to impaired development of specific organ systems, such as the kidney, thereby altering long-term control of blood pressure. In support of this hypothesis, we and others (15, 32, 34) have shown that maternal dietary protein restriction or global food restriction during pregnancy results in hypertension and reduced glomerular number in adult offspring, indicating a link between fetal undernutrition, changes in renal structure, and adult hypertension. Dexamethasone and carbenoxolone at the doses used previously may impair maternal food intake and weight gain, suggesting that these two postulated mechanisms of fetal programming may interact or even share a common pathway. However, it has been reported that maternal and fetal corticosterone levels during modest protein restriction are not different from those in controls (11). Thus the precise relationship between maternal glucocorticoids and fetal undernutrition in programming for adult hypertension is unknown. The purpose of the present study was to determine whether maternal nutritional factors may contribute to programming for offspring hypertension by maternal glucocorticoid administration.

METHODS

All procedures were approved by the Institutional Animal Care and Use Committee. Female Sprague-Dawley rats (Simonsen) weighing ~250–300 g were bred at Oregon Health & Science University, Portland, OR, and maintained on a normal protein (19% protein, Purina basal diet 5755) diet throughout pregnancy and lactation. The Na+ content of the diet was 0.20%. Each female was housed with a male, and the day sperm were seen in a vaginal smear was designated as day 1 of pregnancy. One group of dams (n = 5) were untreated (control group). A second group of dams (n = 3) were injected with...
dexamethasone (100 µg·kg⁻¹·d⁻¹ in 4% ethanol/0.9% saline, 100 µg/ml) subcutaneously for days 1–10 of pregnancy (ED). This dose was chosen because it is the dose used by other investigators in previous studies (2, 7, 24). A third group of dams (n = 3) were weight matched at breeding and subsequently pair fed to the animals receiving dexamethasone in early pregnancy (EDDP). A fourth group of dams (n = 5) were injected with dexamethasone (100 µg·kg⁻¹·d⁻¹) on days 15–20 of pregnancy (LD). A fifth group (n = 8) was fed ad libitum until day 15 of gestation, individually paired with dexamethasone-treated dams showing similar weight gains in early pregnancy, and subsequently pair fed with this group until delivery (LDPF). The large number of dams in this latter group was necessary because of an imbalance in the gender distribution of the pups in several of the litters. Finally, three dams were injected with vehicle in late pregnancy. The data from vehicle-injected dams and their offspring were not different from controls, so the two groups were combined in further analyses (control). Maternal food intake was measured daily, and all pregnant animals were weighed daily, except for those that received dexamethasone during the last half of pregnancy. This group was weighed hourly through the last day of treatment and then weighed only once after that to minimize stress to the animal near the time of delivery. Litters were counted and weighed within 15 h of birth (0 days of age) and were subsequently counted and weighed at 1, 5, 10, 15, and 21 days. All pups were weaned to the normal diet at 22 days of age and maintained on that diet until adulthood. The animals were housed in a room with a controlled temperature and a 12:12-h light-dark cycle. Immediately before surgery, each animal was housed overnight in a metabolic cage for collection of a 24-h urine sample.

**Surgical preparation of adult animals.** At ~20 wks of age, adult male and female offspring were chronically instrumented as described previously (34). Briefly, the rats were anesthetized with a mixture of 55% ketamine (100 mg/ml), 28% xylazine (20 mg/ml), 11% acepromazine (10 mg/ml), and 6% sterile water, administered at 1.0 ml/kg ip. A stainless steel Silastic-covered catheter was implanted in the bladder, flushed with chloramphenicol sodium succinate (30 mg/ml), and plugged. Sterile catheters made of Tygon microbore tubing were implanted into the left femoral artery and vein and tunneled under the skin to exit on top of the head. The catheters were filled with heparin (500 U/ml) following surgery, and plugged with stainless steel wire pins. For the first 24 h after surgery, a mixture of rat chow and 5% dextrose was provided in a bowl to encourage eating. Animals were allowed to recover in individual cages for a minimum of 6 days before any experiments were conducted and were maintained on the normal protein-normal sodium diet. Vascular catheters were flushed every 2 or 3 days with 5% dextrose to maintain patency. During the recovery period, the animals were placed in a wire restrainer in the study room for at least 2 h on at least three occasions to allow them to become acclimatized to the study conditions.

**Experimental protocol.** At 21 wks of age, mean arterial pressure and renal function were measured in conscious animals (34). The rat was placed in a wire restrainer in the study room, the bladder plug was removed, and urine was allowed to drain continuously into a tube throughout the experiment. Mean arterial pressure was measured through the arterial catheter using a pressure transducer (Statham, Oxnard, CA) connected to a polygraph (Grass Instruments, Quincy MA), and a reading was taken after at least 30 min once the pressure had stabilized. Arterial pressures were always measured between 6:00 and 9:00 AM. After the pressure measurement, a small blood sample was taken from the arterial catheter for measurement of microhematocrit and plasma protein.

**Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF).** GFR were measured using standard clearance techniques. Inulin (Sigma, St. Louis, MO) and para-aminohippuric acid (PAH) (Sigma) in 5% dextrose were given intravenously as a bolus (0.45 ml containing 56 mg inulin and 5.6 mg PAH) followed by a continuous infusion (0.024 ml/min of 74 mg/ml inulin and 7.4 mg/ml PAH) throughout the rest of the experiment. To encourage urine flow, an additional infusion (0.036 ml/min) of 0.9% saline and 5% dextrose in a 2:1 ratio was begun 40 min after beginning the inulin infusion and continued for the rest of the experiment. One hour after beginning the inulin/PAH infusion, urine was collected during four successive 20-min clearance periods. Urine volume was determined gravimetrically. A blood sample was taken in a sterile heparinized syringe at the midpoint of each clearance period. After centrifugation of the blood and removal of the plasma, red blood cells were resuspended in an equivalent volume of saline and returned to the animal. The plasma was frozen at −20°C for later analysis. When all experiments were completed or when the instrumentation was no longer functional, the rats were killed with a commercial euthanasia solution. The right kidney was fixed in 10% phosphate-buffered formalin and embedded in paraffin.

**Analytical measurements.** Inulin in plasma and urine was measured by a modification of the method of Waugh (31) after deproteinization with zinc sulfate. PAH was assayed on the same samples using the method of Brun (6). GFR was calculated as the renal clearance of inulin [GFR = (Uin/Pin) × V], where Uin and Pin are the urine and arterial plasma inulin concentrations, respectively, and V is the urine flow rate. ERPF was calculated as the renal clearance of PAH. Values obtained for the four clearance periods were averaged to give a single value for each animal. Plasma protein was measured by refractometry (National Instrument, Baltimore MD). Urine protein was measured by precipitation with sulfosalicylic acid.

**Histopathology evaluation.** Approximately three sections (5 μm) of the kidney were cut from each block and stained with hematoxylin and eosin. Sections were scored for renal pathology as follows: 0 = normal kidney; 1+ = minimal focal tubulointerstitial injury (including tubular atrophy, dilation, and fibrosis); 2+ = moderate tubulointerstitial injury, involving multiple microscopic fields at ×100; 3+ = extensive tubulointerstitial injury, involving more than half of microscopic fields at ×100. The observer was blinded to the codes indicating treatment.

**Statistical analysis.** The data are expressed as means ± SE. For the groups were compared using two-way (treatment x gender) ANOVA, followed by a post hoc test (Bonferroni). Histopathology scores were analyzed using a Kruskal-Wallis nonparametric ANOVA. Statistical significance was assumed with a value of P < 0.05 or better.

**RESULTS**

**Effects of dexamethasone on maternal food consumption and weight gain in pregnancy.** Dexamethasone had a profound effect on food intake and weight gain in pregnant animals, as shown in Fig. 1. Dams receiving dexamethasone in early pregnancy (ED) reduced their food intake by an average of 20% compared with controls and lost weight during the period of dexamethasone treatment. After dexamethasone administration was stopped, food intake exceeded that of controls and weight gain was more rapid than in controls such that the net gestational weight gain was normal in these dams. In animals receiving dexamethasone during late pregnancy (LD), food intake was reduced ~30% during and after the period of administration, and normal weight gain was markedly impaired. Indeed, the total gestational weight gain in these animals was only about 40% of that in the controls. Animals pair fed to those receiving dexamethasone in early or late pregnancy showed weight gain patterns that were intermediate to those of dexamethasone-treated and control animals.

**Effect of dexamethasone on offspring growth.** Birth weights also were significantly lower in offspring of dams that received dexamethasone in late pregnancy (Table 1). These offspring were also significantly smaller than normal or pair-fed controls throughout life. Interestingly, although the maternal weight gain and birth weight in pair-fed (LDPF) dams was intermediate between control and dexamethasone-treated (LD) animals, by the

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time of weaning, the pair-fed group had caught up to controls in body weight. This was not the case for pups of dams pair fed to the mothers treated with dexamethasone in early pregnancy. Thus although pair feeding during early or late pregnancy resulted in pups of similar average birth weight, those pair fed during late gestation caught up in weight by the age of weaning, whereas those pair fed during early gestation had not yet caught up by this age. This difference cannot be explained by differences in the size of the litters being nursed because they were similar in the two groups. Kidney weights were not different among the groups, although the kidney-to-body weight ratio was significantly increased in male LD offspring and tended to be increased in their female littermates as well. Kidney and heart weights were greater in males than in females, reflecting their greater body weights. However, there was no gender difference in absolute adrenal weight. The adrenal-to-body weight ratio was higher in females than in males but was generally not different across treatment groups except that it was higher in ED females than in female controls. Heart-to-body weight ratio was also higher in females than males and was higher in LD males than controls. Out of all variables analyzed, only the adrenal-to-body weight ratio showed a significant interaction between gender and treatment.

Effects of dexamethasone or pair feeding on physiological variables in the offspring. Physiological variables in offspring of dexamethasone-treated, pair-fed, and control animals are shown in Fig. 2 and Table 2. Mean arterial pressure in the
The histopathology scores for tubulointerstitial injury were any of the groups in the degree of glomerulosclerosis noted.

**DISCUSSION**

There were no obvious differences between any of the groups in the degree of glomerulosclerosis noted. The histopathology scores for tubulointerstitial injury were significantly greater in males than in females, but in no group were they significantly greater than in controls.

**Effects of maternal protein restriction on renal histopathology in offspring.** There were no obvious differences between any of the groups in the degree of glomerulosclerosis noted. The histopathology scores for tubulointerstitial injury were significantly greater in males than in females, but in no group were they significantly greater than in controls.

**DISCUSSION**

The most important findings of the present study are that 1) treatment with dexamethasone at this dose during early pregnancy (before the window of nephrogenesis) has no long-term effect on offspring’s blood pressure in either males or females; and 2) although maternal dexamethasone treatment at this dose in late pregnancy programs both male and female offspring for hypertension in adult life, pair feeding of pregnant dams, without administration of dexamethasone, has equivalent effects on offspring’s blood pressure. These findings support the idea that exposure of the fetus to excess glucocorticoids from the mother (by maternal dexamethasone treatment at this dose) programs offspring hypertension indirectly, by reducing maternal nutrition. Previous studies suggest that this involves impaired renal development and permanent changes in renal structure and function in the offspring (34).

Although an inverse relationship between early growth and adult hypertension in humans has now been well established (1, 8, 9, 19, 29), the precise physiological mechanisms by which this occurs are still not well understood. At least two theories have been proposed. The first theory involves fetal undernutrition (due to maternal undernutrition or other causes) and consequent impairment of renal development, and the second theory involves exposure of the fetus to maternal glucocorticoids. We and others (18, 34) have provided evidence of an important role for maternal nutritional factors, in particular protein intake, and impaired renal development in this programming for adult hypertension. In the rat, modest maternal protein restriction throughout pregnancy leads to hypertension in the adult offspring, at least in males (34). In our hands, female gender appears to be relatively protective against this programming by maternal undernutrition; modest protein restriction fails to cause hypertension in female offspring.
Fig. 2. Arterial pressure and renal hemodynamics in adult (22 wks) offspring of control mothers (Con), mothers treated with dexamethasone during early (ED) or late (LD) pregnancy, or mothers pair fed to the dexamethasone-treated mothers (EDPF, LDPF). Data are means ± SE; number of animals is in parentheses. *P < 0.05 compared with controls of the same gender.
plasma protein, g/dL

Excess exposure of the fetus to maternal glucocorticoids (2) holds that fetal programming for hypertension occurs through suppression of the intrarenal renin-angiotensin system in the fetus/newborn and consequent impairment of renal function, including both the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) (3). This programming appears to occur early in gestation, whereas a more severe restriction programs females as well as males (35, 38). This programming appears to occur as a response to maternal weight gain during pregnancy generally leads to offspring of lower birth weight (2, 20), although this may be related to the normal pregnancies that become hypertensive (16, 21). However, an important concern with many of these previous studies is that the doses of dexamethasone and carbenoxolone used may have been higher than those necessary to produce physiologic/pathophysiological levels of glucocorticoids in the fetus. Maternal weight gain during administration of these compounds was frequently impaired (2, 20), and maternal health may have been significantly compromised (12).

We addressed this issue in the present studies. We found that injection of dexamethasone at 100 μg·kg⁻¹·d⁻¹ (the most common dose used in previous studies) (2, 7) in late gestation reduced maternal food intake by ~30%, and the pregnant mothers either failed to gain their normal amount of weight or even lost weight during treatment without ever completely recovering. Although these mothers indeed produced offspring of low birth weight that became hypertensive, pair feeding of noninjected mothers led to offspring that were equally hypertensive. Thus it appears that the reduced food intake in mothers treated with dexamethasone at this dose may account for the majority of hypertension in their offspring. Although it remains to be determined whether a lower dose of dexamethasone, one that does not reduce maternal food intake, can still program for offspring hypertension, our present data do not support a direct role for fetal exposure to maternal glucocorticoids in programming for hypertension.

It is likely that changes in renal structure and function play an important role in programming for offspring hypertension in the present study. Other studies (7) have reported that maternal treatment with dexamethasone (100 μg·kg⁻¹·d⁻¹) reduces the nephron number by 60% in the offspring. Global food restriction and protein restriction also reduce the nephron number by about 15–45% in the offspring (18, 22, 25, 30, 34). Thus, although we did not measure glomerular number in the present study, it is likely that the number of nephrons was reduced in offspring of mothers treated with dexamethasone in late pregnancy, as well as in the LDPF group. This suggests that even if total GFR was unchanged in these groups, the filtration rate per nephron was likely increased. In the case of LD animals, we found that absolute GFR was reduced by only 14% (males) and 21% (females). Coupled with the previously reported reduction in nephron number on the order of 60% in offspring of dexamethasone-treated mothers (7), it is likely that the remaining nephrons in these animals were also hyperfiltering. Hyperfiltration in the remaining nephrons is a well-known mechanism by which maternal hypertension is transmitted to offspring (39). Therefore, it appears that the changes in renal functional and structural parameters we observed, including the increased ERPF and reduced ERPF, may be important in programming for offspring hypertension.

**Table 2. Physiological variables and histopathology scores**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ED</th>
<th>EDPF</th>
<th>LD</th>
<th>LDPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR/kidney wt, ml/min g⁻¹</td>
<td>M 1.11±0.03</td>
<td>1.12±0.05</td>
<td>1.10±0.03</td>
<td>1.00±0.06</td>
<td>1.05±0.03</td>
</tr>
<tr>
<td></td>
<td>F 1.17±0.02</td>
<td>1.19±0.18</td>
<td>1.34±0.05</td>
<td>1.00±0.09</td>
<td>1.19±0.04</td>
</tr>
<tr>
<td>ERPF/kidney wt, ml/min g⁻¹</td>
<td>M 3.54±0.13</td>
<td>3.97±0.29</td>
<td>3.22±0.13</td>
<td>3.41±0.34</td>
<td>3.40±0.11</td>
</tr>
<tr>
<td></td>
<td>F 3.77±0.08</td>
<td>4.05±0.67</td>
<td>3.96±0.13</td>
<td>3.26±0.32</td>
<td>3.85±0.13</td>
</tr>
<tr>
<td>GFR/body wt, ml/min·100 g⁻¹</td>
<td>M 0.770±0.018</td>
<td>0.724±0.036</td>
<td>0.782±0.014</td>
<td>0.750±0.042</td>
<td>0.735±0.017</td>
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<tr>
<td></td>
<td>F 0.874±0.039</td>
<td>0.825±0.087</td>
<td>0.925±0.036</td>
<td>0.807±0.055</td>
<td>0.884±0.021</td>
</tr>
<tr>
<td>ERPF/body wt, ml/min·100 g⁻¹</td>
<td>M 2.47±0.09</td>
<td>2.57±0.18</td>
<td>2.28±0.07</td>
<td>2.66±0.17</td>
<td>2.41±0.07</td>
</tr>
<tr>
<td></td>
<td>F 2.83±0.15</td>
<td>2.81±0.33</td>
<td>2.73±0.09</td>
<td>2.64±0.19</td>
<td>2.86±0.10</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>M 0.49±0.01</td>
<td>0.45±0.01</td>
<td>0.45±0.01</td>
<td>0.44±0.01</td>
<td>0.46±0.01</td>
</tr>
<tr>
<td></td>
<td>F 0.41±0.01</td>
<td>0.43±0.01</td>
<td>0.44±0.02</td>
<td>0.41±0.01</td>
<td>0.42±0.01</td>
</tr>
<tr>
<td>Plasma protein, g/dL</td>
<td>M 6.7±0.1</td>
<td>6.9±0.1</td>
<td>7.1±0.1</td>
<td>6.6±0.1</td>
<td>6.7±0.1</td>
</tr>
<tr>
<td></td>
<td>F 6.5±0.1</td>
<td>6.6±0.1</td>
<td>6.9±0.1</td>
<td>6.2±0.1</td>
<td>6.6±0.1</td>
</tr>
<tr>
<td>Urine protein excretion, mg/d</td>
<td>M 16±2</td>
<td>15±4</td>
<td>38±9</td>
<td>30±8</td>
<td>25±6</td>
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<tr>
<td></td>
<td>F 2±1</td>
<td>6±1</td>
<td>5±1</td>
<td>22±12</td>
<td>8±2</td>
</tr>
<tr>
<td>Histopathology score</td>
<td>M 2.5±0.2 (10)</td>
<td>2.3±0.2 (6)</td>
<td>2.7±0.2 (10)</td>
<td>2.5±0.3 (4)</td>
<td>2.1±0.3 (9)</td>
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<tr>
<td></td>
<td>F 1.4±0.2 (11)</td>
<td>1.5±0.5 (4)</td>
<td>1.6±0.2 (10)</td>
<td>2.1±0.4 (7)</td>
<td>1.4±0.3 (7)</td>
</tr>
</tbody>
</table>

Values are means ± SE; (n) = animals for histopathology scores. There were no significant differences between ED, EDPF, or LDPF and control or LD groups. Numbers of animals are the same as indicated in Table 1. GFR, glomerular filtration rate; ERPF, effective renal plasma flow.
response to nephron loss and has important implications for progression of renal disease. If present, it would be expected to contribute to progressive glomerulosclerosis and hypertension, thus creating a vicious cycle of worsening renal damage and increasing blood pressure (3, 4). That we did not yet see renal histopathological damage in these animals is not surprising because we have shown in another model of hypertension due to early nephron loss that the hypertension precedes signs of renal damage (37). Presumably, this would eventually become apparent if the animals in the present study were allowed to grow older.

Another finding of this study relates to the importance of timing of fetal exposure to a maternal insult in programming for hypertension. We found that exposure to dexamethasone for 6 days during the latter half of pregnancy or to reduced maternal food intake from day 15 until term led to reduced birth weight and hypertension in the offspring, whereas exposure to dexamethasone only early in pregnancy did not. Because nephrogenesis in the rat occurs from about midgestation until ~10 days after birth, this finding is consistent with a role for impairment of renal development in programming for hypertension. These findings are also consistent with another study from our laboratory in which we found that the window of sensitivity of offspring blood pressure to maternal dietary protein restriction also coincides with nephrogenesis (38).

Other investigators have shown that blockade of 11β-HSD with carbenoxolone also fails to program for hypertension if it is only given during very early pregnancy (16, 21). Finally, recent reports (26) suggest that even a short (2-day) exposure to maternal dexamethasone treatment during late pregnancy can program for hypertension. Thus, in the rat, it appears that the insult must occur during late pregnancy to program for hypertension. In contrast, recent reports (10) suggest that short-term administration of betamethasone to the pregnant sheep very early in pregnancy programs the offspring for hypertension in adulthood. However, as the window of nephrogenesis occurs earlier in gestation in the sheep compared with the rat, this finding is also consistent with the hypothesis that nephrogenesis is impaired by glucocorticoid administration in both species.

Although pair feeding and dexamethasone administration had identical effects on offspring’s blood pressure, their effects on several other variables were at least quantitatively if not qualitatively different. For example, maternal weight gain was more severely impaired in LD than in LDPF animals, despite identical food intake, suggesting less efficient use of nutritional energy for growth in LD mothers. Likewise, the birth weights of the offspring were more severely reduced in LD animals, and unlike the LDPF animals, they remained smaller than normal throughout life. Thus dexamethasone in late pregnancy permanently altered the offspring growth trajectory, independent of its effects on maternal food intake. Absolute kidney function was also reduced in LD compared with LDPF animals. Because the kidney is recognized to be the major long-term controller of arterial pressure (13), this suggests that changes in renal function could have contributed more to the hypertension in LD offspring than in LDPF offspring. However, the kidney-to-body weight ratios and GFRs normalized to either kidney or body weight were not different among the groups. Thus renal function was reduced proportionally to the reduced body size in LD offspring, making a differential role for renal mechanisms in causing the hypertension in the two groups less likely. It remains possible, however, that programming of factors we did not measure, such as the hypothalamic-pituitary-adrenal axis or the renin-angiotensin system, may have contributed to hypertension in one group but not in the other.

Results of this study do not totally rule out a possible role for glucocorticoids in programming offspring hypertension. Placental 11β-HSD activity has been reported to be reduced in protein-restricted rat dams (17). Also, despite one report to the contrary (11), maternal corticosterone levels could be increased by global food or protein restriction. Thus fetuses of food- or protein-restricted mothers may indeed be exposed to increased levels of maternally derived corticosteroids. However, the present study does not support a direct role for maternal glucocorticoids in fetal programming of blood pressure, but rather suggests that dexamethasone programs for offspring hypertension at least in part through an indirect effect, mediated by a reduced maternal food intake.

One surprising finding in this study was that although early dexamethasone treatment did not lead to hypertension, maternal food restriction (pair feeding) during early pregnancy did cause hypertension in male but not in female offspring. The reasons for this are not clear. It seems unlikely to be due to random chance, because the males in that group came from three different litters and all but one of the animals was hypertensive. It may be that the physiological effects of reduced food intake in early pregnancy can carry over into the later part of gestation. Although the birth weights of these offspring were not significantly different from controls, they did tend to be lower, which would be consistent with a programming effect. In any case, the fact that the males were affected, whereas their female littermates were not, is consistent with other findings from our laboratory and others (24, 33, 35, 37) suggesting that females are relatively protected from more subtle maternal insults.

**Perspectives**

Human babies that are born smaller have an increased risk for developing hypertension and other diseases in adulthood than do larger babies. This is true across the normal range of birth weights and for full-term infants (i.e., it is unrelated to prematurity). The “normal range” of nephron number in humans is quite broad, and Brenner and colleagues (5, 23) have proposed that essential hypertension may be due in part to a reduced nephron endowment at birth. Indeed, a recent report indicates that patients with primary hypertension have fewer nephrons than normotensive patients (14). Although the mechanisms that lead to a reduced nephron endowment are still not well understood, our previous studies (34) in rats support as key factors fetal undernutrition and a suppressed intrarenal renin-angiotensin system during development. Other studies (2, 16, 20, 21) have suggested that fetal exposure to excess maternal glucocorticoids programs the offspring for hypertension; however, the present study does not support such a direct role for maternal corticosteroids. From this and other studies (15, 28, 30, 32, 34), it appears that fetal undernutrition is one critical factor that can lead to hypertension later in life. However, it must be recognized that fetal undernutrition may be caused not only by maternal undernutrition, but also by im-
paired uteroplacental blood flow or impaired nutrient exchange at the maternal-fetal interface in an otherwise well-nourished mother.

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GRANTS

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REFERENCES