Fetal Programming

Prenatal Dexamethasone Exposure Does Not Alter Blood Pressure and Nephron Number in the Young Adult Marmoset Monkey

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Abstract—The influence of prenatal factors on the development of arterial hypertension has gained considerable interest in recent years. Prenatal dexamethasone exposure was found to induce hypertension and to alter nephron number and size in rodents and sheep. However, it is not clear whether these findings are applicable to nonhuman primates. Thus, we examined the effects of prenatal dexamethasone treatment on blood pressure (BP) and nephron number in marmoset monkeys. Fifty-two marmosets were allotted to 3 groups according to the gestational stage during which their mothers were exposed to oral 5-mg/kg dexamethasone for 7 days (gestation period: 20 weeks): (1) the early dexamethasone group at week 7; (2) the late dexamethasone group at week 13; and (3) the control group. BP was determined by telemetric (n=12) or cuff measurements (n=30), along with cystatin C, proteinuria, and body weight. All of the animals were euthanized at the age of 24 months, and glomerular number and volume were determined. Prenatal exposure to dexamethasone did not lead to a significant difference between the groups with regard to BP, kidney morphology and function, or body weight. BP correlated significantly with body weight, relative kidney weight, and mean glomerular volume and the body weight with the glomerular volume regardless of dexamethasone treatment. In conclusion, prenatal exposure to dexamethasone in marmosets does not, in contrast to other mammals studied, result in hypertension or changes in kidney morphology. Our data support the role of body weight as a predictor of elevated glomerular volume and BP development rather than prenatal dexamethasone exposure. (Hypertension. 2009;54:1115-1122.)

Key Words: hypertension • dexamethasone • prenatal programming • marmoset monkeys • glomeruli number

Synthetic glucocorticoids such as dexamethasone (DEX) are often given to pregnant women with expected preterm birth to accelerate fetal pulmonary maturation and to prevent respiratory distress syndrome. However, prenatal administration of DEX may induce hypertension in combination with a lower number and increased volume of glomeruli in the later life of the offspring, as has been demonstrated for rodents and sheep.1

The fetal origin of chronic diseases in adulthood was first proposed by Barker et al (“Barker hypothesis”).2 The original epidemiological studies linked low birth weight with the risk of fatal ischemic heart disease2 and with an increased risk for metabolic syndrome, including hypertension, dyslipidemia, and type 2 diabetes mellitus.3 Therefore, the prevalence of metabolic syndrome, including hypertension, increased progressively, in both men and women, from those who had the highest birth weights.3 Several subsequent experimental studies in rats and sheep inducing intrauterine growth restriction by maternal glucocorticoid exposure confirmed these epidemiological data.1 A decreased number of glomeruli in the kidney were, therefore, proposed as one possible pathogenetic mechanism for the induction of hypertension.5,4 The presumed mechanism is an “underdosing” of nephron number relative to metabolic and excretory needs. Thus, the reduced number is proposed to lead to glomerular hyperfiltration and hypertension in the existing nephrons with subsequent glomerular injury leading finally to systemic hypertension (“hyperfiltration hypothesis”).1 However, these changes were also seen without alterations in birth weight, particularly in those with short-term prenatal glucocorticoid exposure.5 One of the most distinct studies regarding prenatal programming of hypertension was con-

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ducted by Celsi et al in rats. Daily treatment with DEX throughout gestation led to a significantly higher systolic blood pressure (BP; 130±4 versus 107±1 mm Hg) at the age of 60 days in combination with a 50% reduction in nephron number, relative to controls. Even short-term administration with DEX during pregnancy programmed an increase in BP and renal injury in rats. For example, injection of DEX on gestational days 15 and 16 resulted in a significant 20% reduction in glomerular number and a significant increase of BP in male rats at month 6.

Conversely, other studies in rats and sheep have failed to demonstrate any direct effects of prenatal DEX treatment on BP and/or glomeruli number. Studies reported elevated BP but without differences in glomerular number, reduced nephron number but without BP increases, or a lack of association between reduced nephron number and hypertension. Thus, the influence of prenatal DEX exposure on subsequent development of hypertension and reduction in glomerular number is unclear.

We evaluated the effects of prenatal DEX exposure on the development of arterial hypertension and glomeruli number in the common marmoset monkey. The common marmoset (Callithrix jacchus) is a small New World monkey (350 to 450 g) that exhibits dizygotic twinning, relatively high genomic homology to humans (eg, corticosteroid receptor genes), primate typical placentation, and prenatal maturation with a gestation period of 144 days (20 weeks). In our study, 52 female marmoset monkeys were treated daily either with DEX over a period of 7 days during week 7 or 20 of gestation or with vehicle only.

The study was performed within the Glucocorticoid Hormone Programming in Early Life and Its Impact on Adult Health Consortium (www.eupeah.org). Results of this project could already demonstrate that fetal DEX overexposure leads to abnormal development of motor, affective, and/or cognitive behaviors, dependent on the timing of glucocorticoid overexposure, and also to a significant decrease in the proliferation of dentate gyrus cells. Here we report on the effects of DEX on BP in combination with nephron number in the offspring.

Methods

Animals and Maintenance

The study was conducted with common marmosets (C. jacchus) at 2 different laboratory colonies: a cohort of 22 animals (14 males and 8 females) was bred and maintained at the Laboratory of Behavioral Neurobiology of the Swiss Federal Institute of Technology (Zürich, Switzerland), and a cohort of 30 animals (all male) was bred and maintained at the German Primate Center.

The main results of this study, including the telemetric BP measurement in combination with the determination of the glomeruli number and mean glomerular volume, were obtained from the Zürich cohort. To confirm these data and to enlarge the impact of the study, additional BP measurements were obtained with the Gottingen cohort (n=30). However, because these subjects were also studied in a different project using MRL, and animals could not be fitted with radiotelemetry devices, BP measurements were obtained by cuff measurement in these animals. In both groups (n=52), additional data were evaluated, including body weight at birth and at the age of 24 months, cystatin C serum concentrations, and urinary protein concentrations.

Breeding and experimental animals were housed in family groups in Zürich and in pairs in Gottingen, as described previously. Animal experiments were conducted in accordance with the European Communities’ Council Directive of November 24, 1986, and were approved by the Lower Saxony State Food Office for Consumer Protection and Food Safety and the Cantonal Veterinary Office Zürich.

Prenatal DEX Treatment

Dose-finding experiments were performed by the administration of 0.05, 1.00, 2.50, 5.00, and 10.00 mg of DEX per kilogram of body weight per day for 7 consecutive days in week 7 (days 42 to 48) or week 13 (days 90 to 96) of gestation, in ≥2 animals for each dose. Contrary to the dosage of 10 mg/kg per day of DEX, 5 mg/kg per day did not inhibit fetal growth or induce abortion but did markedly reduce plasma (early DEX [E-DEX]: ~50%; late DEX [L-DEX]: ~100% compared with vehicle [VEH]) and urinary cortisol titers (~80% to 90%) of pregnant females and, thus, was selected as the study dose. This high dose reflects the relative glucocorticoid resistance of the common marmoset compared with other species, including humans.

Two time windows were selected for the treatment of the pregnant animals during the 21-week pregnancy. The first window (week 7) is comparable with weeks 5 to 12 in humans and is probably a critical time point for nephron development. The second window (week 13) was chosen because of the clinical relevance in the prevention of respiratory distress syndrome in preterm birth at the end of the second trimester.

Fifty-two common marmosets were divided into 3 different treatment groups according to their estimated gestational age at which their mothers were administered DEX (gestation period: 144 days): (1) the E-DEX group, with administration of 5 mg/kg per day of DEX from day 42 to 48 postconception and VEH from day 90 to 96 (Zürich: n=8; Gottingen: n=10); (2) the L-DEX group, with administration of VEH from day 42 to 48 and 5 mg/kg per day of DEX from day 90 to 96 postconception (Zürich: n=7; Gottingen: n=10); and (3) the VEH group, receiving VEH only at both estimated gestational periods (Zürich: n=7; Gottingen: n=10). DEX tablets (Jenapharm) were crushed and suspended in 3 mL of palatable fruit syrup to yield an oral dose of 5 mg/kg per day of DEX, which was given at 9:00 AM.

BP Evaluation

In the Zürich group, 6 of the 12 cages containing the study families were fitted with telemetry receivers, so that 2 pairs of VEH twins (2 males and 2 females), 2 pairs of E-DEX twins (2 males and 2 females), and 2 pairs of L-DEX twins (3 males and 1 female) could be measured. In most cases, the twins were brother-sister pairs. BP was measured over a 72-hour period in the home cage using a radiotelemetry system, with a ≥1-week interval between recordings from the first twin and the second twin in each twin pair. Subjects were aged 16 to 19 months and were, therefore, young adults (sexual maturation occurs at months 15 to 18). The radiotelemetry system (Transoma Medical) allowed for the continuous recording of systolic and diastolic BPs in freely moving animals. At the age of 12 months, a pressure-sensitive radiotransmitter (model TAI11PA-C20; weight: 4 g) was implanted in the peritoneal cavity with the pressure-sensitive fluid-filled catheter inserted into the descending aorta pointing upstream. Details of the surgical procedure and the system applied have been described previously. Briefly, at 2 hours before surgery, 0.05 mg/mL of carprofen/midamyl was injected IM, and immediately before surgery, 0.3 mL of saffan (10 mg/mL) was applied IM. During the whole procedure, 600 mL/min of isoflurane/O2 (2%) were provided continuously. To prevent general bacterial infection, 0.1 mL of the antibiotic erythromycin was given SC directly after surgery. For detection of the radio signal in the home cage, 6 flat biotelemetry receivers (model RMA2000) were located on the inside surface of the ceiling of the home cage, and a single cylindrical receiver (model RL-A3000) was suspended underneath the perforated floor of the sleeping box. The transmitters operated over a distance of 25 to 30 cm, such that it was possible to...
Kidney Morphology Including Glomerular Number and Volume

Nephron number and volume were determined in all of the animals of the Zürich cohort (n=22). Kidneys were removed after killing the animals at the age of 24 months by administration of an intrahepatic overdose of pentobarbital (0.2 mL/50 mg per milliliter; Vetalancol; Veterinaria AG), and kidneys were frozen at −70°C.

Number and volume of glomeruli were determined using established stereological techniques. In brief, the right kidney was weighed and cut into 2-mm sections in the cranial to caudal direction. After fixation in HOPE solution (Innovative Diagnostic Systems) the kidney blocks were embedded in paraffin. Afterward, blocks were serially sectioned (3 μm) and stained by periodic acid-Schiff reaction. To estimate the number and volume of glomeruli per kidney, light microscopy was performed with a system of coordinates dividing the field into 100 squares. Subsequently, the number of points on the grid that coincided with the whole kidney, the cortex, the medulla, and the glomeruli was determined together with kidney weight. These data served to calculate the sampling volume (corrected for shrinkage by multiplication with a factor of 1.4), as well as the relative and absolute volumes of the cortex and medulla. In addition, the number of glomeruli per unit of sectional area (millimeters squared) and then per cortex was determined. The absolute number of glomeruli was estimated by the multiplication of numeric density of the cortex and the absolute volume of the cortex. The total glomerular volume per kidney was estimated by multiplying the number of glomeruli by the mean glomerular volume.

Determination of Cystatin C, Proteinuria, and Concomitant Kidney Diseases

Serum and urine samples from marmoset monkeys were taken at the age of 24 months and were frozen immediately at −70°C until examination. Cystatin C was determined in serum samples from both cohorts (n=52). ELISA test kits were used to determine levels of cystatin in the serum (Alexa Biochemicals). The tests were performed according to the manufacturer’s instructions. Concentrations of cystatin C were measured spectrophotometrically at 450 nm.

Proteinuria was determined in spot urine of the total group (n=52). Urinary creatinine concentrations were determined using an enzymatic colorimetric assay on automated clinical chemistry analyzers (Roche/Hitachi Modular P) to determine the protein:creatinine ratio.

Because of the assumed higher incidence of IgA nephritis in marmoset monkeys, kidneys were explored by light microscopy for mesangial cell proliferation and matrix expansion. In addition, immunohistochemistry using antibodies against IgA and C3c (DAKO) was conducted to exclude IgA nephritis.

Statistics

Data were analyzed using SAS 9.1 software (SAS Institute). To investigate the effect of prenatal DEX on BP, we used a linear mixed-regression model with repeated measures. We checked the distribution of the different parameters with the Kolmogorov-Smirnov test and optically with box plots. For BP we could assume a normal distribution, but for the other parameters we could not, so we used the ranks of the values for the regression (ANOVA). A P<0.05 was considered to be statistically significant. Results are expressed as mean±SD (BP) and as median with quartiles for the other parameters.

Results

Blood Pressure

In the 12 Zürich marmosets fitted with an intra-arterial BP transmitter and sampled for 72 hours at the ages of 16 to 19 months, systolic and diastolic BP values decreased at nighttime by ≈10 mm Hg (Figure 1). BP values were not significantly different among the VEH, E-DEX, and L-DEX groups, neither over the total 72 hours (VEH: 93.0/73.0±5.5/2.9 mm Hg; E-DEX: 88.6/68.2±10.2/6.7 mm Hg; L-DEX: 91.0/72.0±8.2/6.9 mm Hg), nor during the day (VEH: 98.4/77.6±6.3/3.4 mm Hg; E-DEX: 94.8/72.8±11.9/7.2 mm Hg; L-DEX: 97.8/77.8±9.5/8.5 mm Hg) or night (VEH: 88.8/69.6±5.8/3.6 mm Hg; E-DEX: 82.6/63.8±11.3/8.1 mm Hg; L-DEX: 86.8/69.0±7.5/6.2 mm Hg; Figure 1).

These findings without significant differences were supported in the 30 Gottingen marmosets using cuff measurements at the age of 24 months (VEH: 153.5/71.1±19.4/13.3 mm Hg; E-DEX: 157.3/67.6±17.1/13.8 mm Hg; L-DEX: 153.6/75.1±23.7/13.2 mm Hg). Moreover, no significant differences were seen in BP values at earlier time...
Figure 2. Correlation between telemetric and cuff blood pressure measurements in handheld marmosets. Parallel sampling using both methods in 6 animals on a total of 27 occasions showed comparable systolic values for the 2 methods. The 2 methods of BP measurement yielded significantly correlated values (systolic BP: \(r^2=0.5; P<0.001\)).

Kidney Morphology Including Glomerular Number and Volume

Kidney weight and number and volume of glomeruli were determined at the age of 24 months in the Zürich cohort (n=22). Although there was no significant effect of prenatal DEX on these parameters, absolute and relative kidney weights were lowest in the L-DEX group (absolute kidney weight: 0.85 g [0.73 to 1.15 g]; relative kidney weight: 2.41 g/kg [2.15 to 2.66 g/kg], followed by the VEH group (absolute kidney weight: 1.05 g [0.96 to 1.27 g]; relative kidney weight: 2.45 g/kg [2.37 to 2.78 g/kg]) and the E-DEX group (absolute kidney weight: 1.29 g [0.89 to 1.47 g]; relative kidney weight: 2.93 g/kg [2.13 to 3.31 g/kg]). The absolute and relative kidney weights showed a high interindividual variability (absolute kidney weight: minimum: 0.7 g; maximum: 1.9 g; relative kidney weight: minimum: 1.78 g/kg; maximum: 3.85 g/kg; Table 1).

Both glomerular number and volume showed a high interindividual variability. Glomerular number ranged from 10 820 to 28 884 per kidney, and the mean glomerular volume ranged between 2.15 and 3.49×10⁻³ mm³. Neither glomerular number \(P=0.257\) nor volume \(P=0.058\) was significantly different among the 3 treatment groups (Figure 3).

Table 1. Glomeruli Number, Mean Glomerular Volume, and Kidney Weight After Prenatal DEX Administration

<table>
<thead>
<tr>
<th>Kidney Morphology</th>
<th>VEH</th>
<th>E-DEX</th>
<th>L-DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli No. per kidney</td>
<td>15 293</td>
<td>15 341</td>
<td>15 638</td>
</tr>
<tr>
<td>25th to 75th percentile</td>
<td>12 494 to 17 422</td>
<td>12 478 to 17 712</td>
<td>14 566 to 19 433</td>
</tr>
<tr>
<td>Mean glomeruli volume, (\times10^{-3}) mm³</td>
<td>3.131</td>
<td>3.113</td>
<td>2.791</td>
</tr>
<tr>
<td>25th to 75th percentile</td>
<td>2.898 to 3.323</td>
<td>2.582 to 3.192</td>
<td>2.267 to 3.018</td>
</tr>
<tr>
<td>Absolute kidney weight, g</td>
<td>1.05</td>
<td>1.29</td>
<td>0.85</td>
</tr>
<tr>
<td>25th to 75th percentile</td>
<td>0.96 to 1.27</td>
<td>0.89 to 1.47</td>
<td>0.73 to 1.15</td>
</tr>
<tr>
<td>Body weight, 24 mo</td>
<td>423.5</td>
<td>441</td>
<td>384</td>
</tr>
<tr>
<td>25th to 75th percentile</td>
<td>414 to 459</td>
<td>394 to 484</td>
<td>336 to 415</td>
</tr>
<tr>
<td>Relative kidney weight, g/kg</td>
<td>2.45</td>
<td>2.93</td>
<td>2.41</td>
</tr>
<tr>
<td>25th to 75th percentile</td>
<td>2.37 to 2.78</td>
<td>2.13 to 3.31</td>
<td>2.15 to 2.66</td>
</tr>
</tbody>
</table>

Data were obtained in 22 animals (VEH: n=8; E-DEX: n=7; L-DEX: n=7) and are presented as medians with quartiles.
Cystatin C, Proteinuria, and Concomitant Kidney Diseases

No significant effect of prenatal DEX administration was detected for cystatin C (VEH: 0.19 μg/L [0.15 to 0.41 μg/L]; E-DEX: 0.20 μg/L [0.14 to 0.32 μg/L]; L-DEX: 0.31 μg/L [0.09 to 0.38 μg/L]). Proteinuria was highest in the L-DEX group (313 mg/g of creatinine [192 to 1092 mg/g of creatinine]), followed by the E-DEX group (242 mg/g of creatinine [186 to 410 mg/g of creatinine]) and the VEH group (240 mg/g of creatinine [171 to 797 mg/g of creatinine]). However, the differences were not significantly different.

Despite the assumed higher incidence of IgA nephritis in marmoset monkeys, IgA nephropathy, as indicated by mesangial cell hyperplasia, mesangial matrix expansion, and mesangial deposition of IgA and C3c, was not detected. In addition, other obvious kidney diseases and focal or global glomerulosclerosis were excluded by extensive light microscopic investigations in all of the examined animals (n=52).

Maternal Body Weight, Intrauterine Growth, and Body Weight of the Offspring

Maternal body weights were not significantly different before pregnancy in the Göttingen cohort (VEH: 450.0 g [416.8 to 505.0 g]; E-DEX: 398.5 g [390.0 to 440.0 g]; L-DEX: 409.5 g [361.3 to 457.5 g]) and the Zürich cohort, as described by Hauser et al.11 In the Göttingen cohort, mothers of the L-DEX group had a significantly decreased body weight compared with VEH at day 80 of pregnancy (414.0 g [375.8 to 479.8 g] versus 477.0 g [460.0 to 569.0 g]; P=0.027) and 1 day after parturition (392.5 g [373.5 to 449.5 g] versus 469.0 g [416.5 to 516.5 g]; P=0.0147), as well as a trend toward a decreased body weight at day 144 of pregnancy (474.0 g [463.5 to 585.3 g] versus 593.0 g [517.0 to 612.0 g]; P=0.0528). No further significant differences were seen among VEH, E-DEX, and L-DEX, including the Zürich animals.11

Despite the decreased maternal weight in the L-DEX group, intrauterine growth was not different, as assessed by the biparietal diameters of the fetus at day 80 until day 130 of pregnancy by ultrasound.11 Moreover, DEX administration was not leading to a different length of the gestation period, and all of the offspring were born in a species-typical gestation period.11 Prenatal glucocorticoid treatments did not lead to a significant effect on birth weight (VEH: 29.9 g [28.8 to 32.0 g]; E-DEX: 28.8 g [27.2 to 30.4 g]; L-DEX: 27.0 g [26.8 to 33.1 g]; Figure 4) or on body weight at 24 months (VEH: 417.0 g [401.2 to 480.8 g]; E-DEX: 415.0 g [376.5 to 459.5 g]; L-DEX: 400.0 g [341.8 to 485.5 g]; Figure 4) in the total group of all of the examined animals (n=52). Both body weight at birth and at the age of 24 months showed high interindividual variability, particularly in VEH subjects at birth (minimum to maximum ranges: 25.5 to 38.2 g) and in L-DEX subjects at the age of 24 months (minimum to maximum ranges: 203 to 582 g). In contrast to the body weight at birth, the age at which body weight is first assessed.
Table 2. Significant Correlations Among BP, Body Weight, and Kidney Parameters Independent of the Prenatal Administration

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Estimator of Regression</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>Birth weight</td>
<td>0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Body weight (24 mo)</td>
<td>0.116</td>
<td>0.0340</td>
</tr>
<tr>
<td></td>
<td>Relative kidney weight*</td>
<td>0.223</td>
<td>0.0310</td>
</tr>
<tr>
<td></td>
<td>Mean glomerular volume*</td>
<td>0.237</td>
<td>0.0003</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>Birth weight</td>
<td>0.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Body weight (24 mo)</td>
<td>0.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Mean glomerular volume*</td>
<td>0.32</td>
<td>0.0040</td>
</tr>
</tbody>
</table>

*Data show the determination only in the Zürich cohort (n=22).

weight, crown-heel lengths showed only very small variations between 29 and 31 cm in our animals.

**Correlation Analysis and Characterization of Animals With Elevated BP**

Independent of the prenatal treatment, systolic and diastolic BPs were significantly positively correlated with the mean glomerular volume, birth weight, and body weight at age 24 months (Table 2). In addition, systolic but not diastolic BP was significantly positively correlated with the relative kidney weight (Table 2), and the body weights at 24 month were significantly positively correlated with the mean glomerular volume ($r^2=0.4911$; $P=0.0274$).

There was no significant correlation of the glomerular number with the systolic ($r^2=0.0251$; $P=0.731$) or diastolic ($r^2=0.1933$; $P=0.2375$) BP. The litter size of the offspring varied from 2 to 4 and was significantly positively correlated with the birth weight ($r=0.0838$; $P=0.0083$) but not the BP (diastolic: $r^2=0.1892$; $P=0.5421$; systolic: $r^2=0.00838$, $P=0.7833$), glomeruli number ($r^2=0.1717$; $P=0.4444$), or volume ($r^2=0.0709$; $P=0.7544$).

As noted above, the 12 animals (8 in the Göttingen cohort and 4 in the Zürich cohort) with BP values above the 75th percentile of the total group (telemetric measurement: >97/74 mm Hg; cuff measurement >162/79 mm Hg) were composed of 2 VEH subjects, 5 E-DEX subjects, and 5 L-DEX subjects. However, these animals were only partially associated with high birth weight (n=5), low birth weight (n=1), elevated body weight at the age of 24 months (n=6), elevated cystatin C (n=1), and proteinuria (n=4), with “high” defined as values above the 75th percentile and “low” as values below the 25th percentile of the total group. Of the 4 animals of the Zürich cohort with BP values above the 75th percentile, only 1 subject displayed a high glomeruli number, and 2 subjects displayed a high glomerular volume.

**Discussion**

The association among prenatal DEX exposure, increased BP, and reduced nephron number is an area of biological and clinical controversy. Most studies on this subject have been performed in rodents and sheep. Our study is the first to examine the effects of prenatal DEX exposure on BP in combination with kidney morphology and function in a nonhuman primate. Comparing 2 different time points for prenatal DEX administration against untreated animals, no significant effects on BP and kidney morphology were observed. In fact, our data confirmed the dominant influence of the adult body weight as a predictor of the mean glomerular volume and BP development rather than prenatal DEX exposure.

The rationale for this study was based on the numerous experiments in different animal species, mainly rodents and sheep, demonstrating a link between prenatal exposure to glucocorticoids and altered BP and renal function in adulthood. However, because of the different length of gestation, maturity state of the newborn, kidney development, and adult characteristics, the results are not necessarily applicable to higher species.

This consideration may be confirmed by the negative findings of our study in marmoset monkeys and by human studies demonstrating a lack of BP changes after prenatal glucocorticoid administration at the age of 19 and 30 years. However, human studies may be confounded by several factors influencing BP. Thus, a number of human studies showed increased BP after prenatal glucocorticoid administration. For example, Doyle et al reported that BP values of 14-year-old children who received antenatal corticosteroid therapy were moderately increased relative to peers without antenatal corticosteroid treatment: mean systolic BP was 4 mm Hg higher (118 versus 114 mm Hg) and diastolic BP 3 mm Hg higher (68 versus 65 mm Hg). Moreover, in contrast to our negative findings in marmoset monkeys, de Vries et al demonstrated a remarkable increase of systolic (17 mm Hg) and diastolic (17 mm Hg) BPs at the age of 12 to 14 months in vervet monkeys after daily administration of 200 μg/kg per day of DEX from midgestation onward. However, BP measurement in vervet monkeys was carried out under general anesthesia, but ours were done in conscious animals. Unfortunately, none of these studies analyzed glomeruli number or mean glomerular volume of the kidney.

There are several points that will need to be clarified before the contradictory results in this field can be rationalized. In our view, there are 4 main points to discuss. First, the length of prenatal DEX administration seems to be important regarding the long-term effects of prenatal DEX on BP and kidney morphology. In contrast to our study with short-term glucocorticoid administration at week 7 or 13 of gestation, the increased BP values of the vervet monkeys were attributed to a daily dose of DEX from midgestation onward. Moreover, glucocorticoid administration in rats throughout the whole gestation period showed the most impressive results, with an elevated BP of 23 mm Hg (17.7%) in combination with a 50% reduction in nephron number at the age of 60 days relative to the control group. In contrast, short-term DEX exposure (0.2 mg/kg of body weight) on days 15 and 16 of gestation resulted only in a 20% reduction in nephron number and a male-specific elevation in tail-cuff BP in offspring at the age of 6 to 9 months. Thereby, the effects on BP and nephron number were less pronounced after glucocorticoid administration at days 17 and 18 and absent after treatment in very early (days 11 and 12) and later (days 19 and 20) pregnancy. These findings led to the hypothesis that glucocorticoids must be administered during a critical period,
which would probably be the early, preglomerular stage of
metanephric development during which excess glucocorti-
coids may affect both final nephron number and predispose to
hypertension. At what stage of gestation this occurs in
marmoset monkeys is currently unknown. Compared with
humans, in whom it is thought to take place between weeks 5
and 12, the time point of DEX exposure in marmosets at
weeks 7 and 13 may have been too late in a gestation period
of ≈20 weeks. On the other hand, DEX exposure analogous
to our L-DEX study protocol is comparable with the clinical
regimes used to accelerate fetal pulmonary maturation and
may therewith indicate the harmlessness of short-term DEX
exposure in later pregnancy. These results concur with the
missing BP changes in 30-year–old humans in a double-
blind, placebo controlled, randomized trial of antenatal beta-
methasone exposure for 2 days between weeks 24 and 26 of
gestation. The second point, which may be of importance for the
development of hypertension by prenatal glucocorticoids, is
the stress responsiveness of the treated animals. This hypo-
thesis is based on the observation that antenatal DEX admin-
istration leads to an impaired resilience to stressors in
adults. Interestingly, O’Regan et al demonstrated that
antenatal DEX treatment in rats in the last week of pregnancy
results in lower basal BP values of ≈4 to 8 mm Hg, whereas
even mild disturbances or a more severe stressor lead to
increased BP values ≥30 mm Hg higher than controls.
Thereby, they used a radiotelemetry method to monitor BP
that is unaffected by stress artifacts of measurement. In fact,
our telemetric BP values reflect the basal BP, and cuff
measurement itself may be a too-low stressor in trained
marmosets to cause DEX exposure–induced BP changes.
Third, although subjects were at the age of young adults
when measurement and sampling were performed, BP mea-
surement may still have been performed too early, and
treatment effects could have developed later.
Fourth, other factors, including genetic background, sex,
ethnic group, body weight, maternal deficiencies, and extra-
uterine growth, may influence the effect of prenatal DEX
exposure on BP and/or nephron number. For example, DEX
exposure in rats on days 15 and 16 of gestation led to BP
elevation in males but not in females at the age of 6 months,
which may reveal a predisposition of individual animals.
Potential evidence that DEX exposure–induced hypertension
occurs only in predisposed animals could be the higher
prevalence of marmosets with elevated BP values (>75th
percentile of the total group) in the E-DEX (n=5) and L-DEX
(n=5) groups compared with the VEH group (n=2). This
may be because of an interaction of DEX and predisposition
factors. The study cohort was certainly heterogeneous, as
demonstrated by the wide range of values for proteinuria,
serum cystatin C concentrations, and birth and adult body
weights within each treatment group. However, given that
body weights correlated significantly with the BP and the
mean glomerular volume, we would propose that this is the
dominant risk factor for hypertension in marmosets rather
than prenatal DEX exposure. These findings are in line with
observations of Rea et al. They found a positive correlation
among the glomerular size, the body mass index, and the BP
in living kidney donors. However, it remains unclear
whether the elevated BP in obese patients is because of or
independent of the increased glomerular volume. The positive
association between birth weight and adult BP is of course
contrary to the original hypothesis of Barker et al. However, a
study of close to 30 000 7-year–old white children observed an
inverse correlation between BP and birth weight, whereas in a
black sample, birth weight and BP were positively correlated.
Perspectives
Prenatal administration of DEX during week 7 or 13 of
gestation did not lead to basal hypertension or changes in
glomerular number or size in young adult common marmoset
monkeys relative to VEH controls. Independent of the pre-
natal treatment, BP was significantly correlated with body
weight at birth and at the age of 24 months. These data
provide support from a nonhuman primate for the importance
of the adult body weight on the development of hypertension
and mean glomerular volume and do not provide support for
an effect of prenatal DEX administration, although we note
several provisos for why this last conclusion needs to be
treated cautiously.

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