Intrauterine growth restriction in rats is associated with hypertension and renal dysfunction in adulthood

MARIÈE-CLAUDIE BATTISTA,1,3 LUC L. OLIGNY,1,2 JEAN ST-LOUIS,1,3 AND MICHELE BROCHU1,3

1Research Center and 2Department of Pathology, Hôpital Sainte-Justine, and 3Department of Obstetrics and Gynecology, Université de Montréal, Montreal, Quebec, Canada H3T 1C5

Received 4 January 2001; accepted in final form 4 March 2002

Battista, Marie-Claude, Luc L. Oligny, Jean St-Louis, and Micheline Brochu. Intrauterine growth restriction in rats is associated with hypertension and renal dysfunction in adulthood. Am J Physiol Endocrinol Metab 283: E124–E131, 2002; 10.1152/ajpendo.00004.2001.—Epidemiological studies have produced evidence that unfavorable intrauterine environments during fetal life may lead to adverse outcomes in adulthood. We have previously shown that a low-sodium diet, given to pregnant rats over the last week of gestation, results in intrauterine growth restriction (IUGR). We hypothesize that pups born with IUGR are more susceptible to the development of hypertension in adulthood. IUGR fetuses and rats aged 1 wk were characterized for organ growth and renal morphogenesis. The adults (12 wk) were evaluated for weight, systolic blood pressure, activity of the renin-angiotensin-aldosterone system (RAAS), and renal function; hearts and kidneys underwent a histological examination. Brain and cardiac ventricle-to-body ratios were increased in IUGR fetuses compared with age-matched controls, whereas the kidney-to-body ratio was unchanged. Systolic blood pressure was elevated in both IUGR male and female adults. Plasma aldosterone levels were not correlated with increased plasma renin activity. Moreover, urinary sodium was decreased, whereas plasma urea was elevated in both males and females, and creatinine levels were augmented only in females, suggesting a glomerular filtration impairment in IUGR. In our model of IUGR induced by a low-sodium diet given to pregnant rats, high blood pressure, alteration of the RAAS, and renal dysfunction are observed in adult life. Differences observed between male and female adults suggest the importance of gender in outcomes in adulthood after IUGR.

fetal programming; renin-angiotensin-aldosterone system

Many epidemiological studies have linked diseases in adulthood, such as type 2 diabetes and hypertension, to adverse intrauterine environments during fetal life (for review see Ref. 3). The hypothesis of fetal programming of Barker et al. (4) proposes that adverse intrauterine environments can alter fetal growth, leading to malfunction of organ systems later in life. The underlying mechanisms of programming are not easy to study in humans because of longevity and many uncontrollable factors, such as stress, genetics, diet, and smoking. Development of different animal models of intrauterine growth restriction (IUGR) has supported a link between adverse intrauterine environment and adult disease. In these models, the mothers are manipulated or treated to impair fetal growth or produce fetal stress. For example, reduced calorie and protein intake by pregnant rats leads to elevated blood pressure in their offspring (29, 51). Reduced uterine blood flow in pregnant guinea pigs (40) induces high blood pressure in their adult offspring. Although the link is established, the underlying mechanisms are not clearly demonstrated.

It was reported that, in a group of IUGR human infants, cord venous angiotensin II concentration was elevated compared with an uncomplicated term pregnancy group (24). It was suggested that the fetal renin-angiotensin system (RAS) is activated during IUGR. In normally grown and IUGR fetal sheep, Edwards et al. (18) measured the arterial blood pressure between 115 and 145 days of gestation. No significant difference in the mean arterial blood pressure was noted between the IUGR and the control groups. However, during captopril infusion, after 135 days of gestation, there was a significant decrease in fetal arterial blood pressure in the IUGR group but not in the control group. These authors also observed that increasing intravenous doses of angiotensin II elevated diastolic blood pressure in the two groups but that the effects were different, with higher responses in the IUGR group. Therefore, they suggested that the fetal RAS is important in the maintenance of arterial blood pressure in growth-restricted sheep fetuses but not in the control group (18). These authors suggested that this system could be implicated in fetal programming of adult disease. IUGR could affect renal development. In fact, fewer nephrons were observed in kidneys from human IUGR stillbirths compared with live-born IUGR infants who died within the first year (23). This observation was also reported in IUGR rats (35).

We (43) reported that giving a low-sodium diet to dams in the last week of gestation induced birth of...
IUGR pups. This model is characterized, in the mother, by a decreased circulating volume and a greater activation of the renin-angiotensin-aldosterone system (RAAS). We have also observed reduced uterine artery diameters (26%) and decreased placental weights (12%), suggesting a decreased placental perfusion. Weight and length of the pups were consequently decreased without affecting the litter size. In humans, pregnancy-induced hypertension is known to be accompanied by a decreased circulatory volume (19) and is often treated with a low-sodium diet (34). Hence, we wanted to characterize the outcome of rat offspring born from dams on low-sodium diet. However, many studies have shown that dietary sodium restriction in humans does not reduce gestational hypertension (14, 47). Our findings in the rat would support the latter statement. Our model provides additional data on the detrimental effects of a gestational hyponatremic diet on the offspring.

The aim of the present study is to characterize the pups born from our IUGR model and to point out possible implications of RAAS and renal function in development of higher blood pressure.

MATERIALS AND METHODS

Animals and study design. Female Sprague-Dawley rats (Charles River Canada, St-Constant, QC, Canada) weighing 225–250 g were mated with male rats. Day 1 of pregnancy was determined by the presence of spermatozoa in vaginal smear. The animals were housed under controlled light (12:12 h light-dark cycle) and temperature (21 ± 3°C). The dams were randomly assigned to one of two diets for the last 7 days of pregnancy. One group was fed a normal diet containing 0.2% sodium (basal diet 5755; PMI Feed, Ren’s Feed and Supplies, Oakville, ON, Canada) and tap water. They gave birth to the control offspring group. The second group received a 0.03% sodium diet (low-sodium diet 5881; PMI Feed) and demineralized water. Their offspring composed the IUGR group (43).

At the end of the 7-day regimen (day 22 of gestation, term = day 23), some of the dams were killed by decapitation (0900–0930) to obtain fetuses. Two other groups of offspring were obtained after term delivery. At parturition, all dams received regular rat chow (0.3% sodium) and tap water. They gave birth to the control offspring group. The second group received a 0.03% sodium diet (low-sodium diet 5881; PMI Feed) and demineralized water. Their offspring composed the IUGR group (43).

At the end of the 7-day regimen (day 22 of gestation, term = day 23), some of the dams were killed by decapitation (0900–0930) to obtain fetuses. Two other groups of offspring were obtained after term delivery. At parturition, all dams received regular rat chow (0.3% sodium) and tap water. One-half of the litter was killed at 7 days (1-wk group), the remaining pups were killed at 12 wk of age (adult group). These animals were weaned at 4 wk of age and were separated into male and female subgroups. All offspring received a regular diet and tap water. This study received approval from the local animal care committee, which is accredited by the Canadian Council on Animal Care. Physiological measurements. Systolic blood pressure was measured in unanesthetized male and female offspring from 12 litters (6 controls and 6 IUGR) by the indirect tail cuff method (50-001 rat tail blood pressure system; Harvard Apparatus, St-Laurent, QC, Canada). Training in the indirect tail cuff method began when the rats in the adult groups were weaned at 4 wk of age, and blood pressure measurements were recorded at 2-day intervals between weeks 5 and 12.

After decapitation of male and female 12-wk adults, trunk blood was collected for hormone and electrolyte analysis. Brain and kidneys were taken and weighed in the fetal, 1-wk, and 12-wk groups. Both cardiac ventricles were obtained in fetuses and 1-wk offspring. Because hypertension is highly correlated with left ventricular hypertrophy, only the left ventricles were taken and weighed in the 12-wk adults.

Sample collection and analysis. Three days before they were killed, the 12-wk offspring were housed in individual metabolic cages to collect 24-h urine samples. On the day the animals were killed, an aliquot of urine was used to measure sodium, potassium, total calcium, and urea. At death, two blood samples were taken. One was rapidly centrifuged to determine hematocrit; the other sample was drawn into plain Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) and centrifuged at 3,000 rpm for 20 min at 4°C. An aliquot of plasma was analyzed to quantitate the concentration of the following substances: sodium and potassium with specific electrodes, total calcium by colorimetric reaction, urea by electrode conductibility, and creatinine by an enzymatic colorimetric test.

Plasma renin activity was measured indirectly by radioimmunoassay of angiotensin I generated during a 2-h incubation period, as described by Gutkowski et al. (21). The antibody used for this radioimmunoassay was purchased from Peninsula Laboratories (Belmont, CA). Plasma aldosterone measurements were obtained after plasma extraction by a solid-phase procedure with C18 Sep-Pak cartridges (Millipore; Waters, Montreal, QC, Canada) and then quantitated by radioimmunoassay, as previously described (8). Plasma renin activity and aldosterone levels were not measured in fetuses and 1-wk offspring because of the small amount of plasma available. Corticosterone was measured directly in plasma with a commercial radioimmunoassay kit (Medicorp, Montreal, QC, Canada) in fetuses, 1-wk offspring, and adults (males and females). For fetal and 1-wk offspring, plasma from individuals of the same litter was pooled.

Renal and left cardiac ventricle morphometric studies. Whole kidneys from all groups and left cardiac ventricles from adult groups were fixed in 10% buffered formalin and paraffin embedded. Four-micrometer-thick sections were stained with the hematoxylin-phloxine-saffron technique. Morphometry was performed with a Zeiss AxioHOME (Oberkochen, Germany) computerized system on histological sections from kidneys of animals at three different developmental stages: fetal, 1-wk offspring, and 12-wk adults. The total area of the kidney in fetuses and 1-wk offspring was determined by delimitation of the entire kidney section. For adults, the total area of the kidney excluded the pyelic urinary space. Every glomerulus present on each section was counted, and the total area of each glomerulus was measured. In adults, glomeruli were counted and stratified as being subcapsular, central, or juxtamedullary. Embryologically, the juxtamedullary glomeruli are the first to develop, the subcapsular glomeruli appearing last, thus giving a stratified architecture. The number of strata present in each section was counted as an indication of the progression of glomerulogenesis. Histological examination of the adults’ left cardiac ventricles was performed by an experienced anatomic pathologist to evaluate the endocardium, myocardium, and epicardium including its vessels. Particular attention was paid to subtle changes of early hypertrophy at the level of the myocardiocytes: nucleomegaly with hyperchromasia and cytomegaly with increased intracytoplasmic myofibrils. Endocardial fibroelastosis and the presence of myocardial collagen were also assessed subjectively, with the observer blinded with respect to the origin of the cases.

Northern hybridization. Total RNA was extracted from left cardiac ventricles of 12-wk male and female adults using TRIzol reagent. Atrium and liver were used as positive and negative controls, respectively. A 0.7-kb fragment of rat pre-pro-atrial natriuretic peptide (ANP; kindly provided by Dr. A.
Calderone) was labeled with Redivue [α-3H]dCTP (Amersham Pharmacia Biotech, Baie d’Urfé, QC, Canada) to a specific activity of 1 × 10^6 counts·min·μg·μCi by the Rediprime II random prime labeling system (Amersham Pharmacia Biotech) and hybridized to nylon membranes (Boehringer Mannheim, Laval, QC, Canada) for 24 h at 42°C. Membranes exposed to the cDNA probes were washed twice (15 min at room temperature) with 300 mmol/l NaCl-30 mmol/l trisodium citrate and 0.1% SDS and once (15 min at 60°C) with the same solution (9). Nylon membranes were subsequently exposed to Kodak XAR film with an intensifying screen at −80°C, and films were scanned with Scion Image computer software (Scion, National Institutes of Health, Bethesda, MD). Steady-state mRNA levels were expressed as arbitrary densitometric units and standardized by comparison with hybridization results obtained with random prime-labeled 18S ribosomal RNA. The experiment was performed on five animals per group. These animals were from different litters.

Statistical analysis. The IUGR group was compared with age-matched controls. Adults were also matched for gender. Systolic blood pressure measurements were compared between IUGR and control offspring by use of a two-way ANOVA with repeated measures. The impact of age was also assessed. Statistical analysis of organ weight, morphometric studies, and plasma and urinary parameters as well as ANP mRNA were performed by Student’s t-test. All results are expressed as means ± SE. P < 0.05 was considered to be significant.

RESULTS

Physiological characterization of IUGR. As described in Table 1, fetal (22 days of gestation) body weight of the IUGR group was lower than that of controls. Kidney weight of the IUGR fetuses was also decreased compared with controls, leading to similar kidney-to-body weight ratios between the two groups (IUGR: 9.65 × 10⁻³ vs. control: 9.44 × 10⁻³). Weights of brains and cardiac ventricles were not different between IUGR and control fetuses, resulting in increases of these organs’ mass-to-body weight ratio in IUGR fetuses (brain: 4.73 × 10⁻² vs. 3.93 × 10⁻²; cardiac ventricles: 6.04 × 10⁻³ vs. 5.41 × 10⁻³; IUGR vs. controls, respectively). In 1-wk offspring, IUGR rats were still smaller than their counterparts (Table 1). Brain and cardiac ventricle weights were significantly lower in the IUGR group.

The adults never recovered from their growth restriction. In IUGR males, total body, brain, left cardiac ventricle, and kidney weights remained significantly lower than in controls (Table 1). In IUGR females, however, even though total body, brain, and kidney weights were lower, left cardiac ventricle weight was not different from those of controls. This particularity is also reflected by an increased ratio of left cardiac ventricle to total body weight in IUGR females (3.21 × 10⁻³ vs. 2.93 × 10⁻³; IUGR vs. controls respectively).

Systolic blood pressure during development. Systolic blood pressure increased with age in all four groups of rats, but at approximately the 8th wk of age, the pressure tended to stabilize (Fig. 1). Subsequently, higher systolic blood pressures were observed in animals born with IUGR, reaching 143 ± 2.11 mmHg at 12 wk old in IUGR males compared with 135 ± 1.29 mmHg in matched controls and 144 ± 1.38 mmHg in IUGR females compared with 135 ± 1.12 mmHg in their controls. Systolic blood pressure increased with age (P = 4.36 × 10⁻¹¹) and IUGR status (P = 2.44 × 10⁻⁵).

RAAS and corticosterone levels. Corticosterone levels were significantly elevated in IUGR fetuses (n = 3 litters; 1.07 ± 0.07 nmol/ml) compared with the controls (n = 6 litters; 0.82 ± 0.07 nmol/ml). This difference was not observed between 1-wk IUGR and control offspring. Adult IUGR males showed increased plasma renin activity (Table 2). Plasma aldosterone and corticosterone levels were not different between the two groups. In females, despite increased plasma renin activity, plasma aldosterone levels were decreased in the IUGR group. Plasma corticosterone levels were augmented in IUGR females compared with the controls.

Functional and structural evaluation of the kidney. In IUGR males, plasma measurements did not reveal any changes except for an increased urea, whereas plasma potassium, urea, and creatinine concentrations were elevated in IUGR females (Table 3).

As shown in Table 4, urinary sodium and potassium were decreased in IUGR males. IUGR females showed decreased urinary sodium and increased urea concentrations compared with controls.

### Table 1. Total body, brain, cardiac ventricle, and renal weights of fetuses, 1-wk-old offspring, and 12-wk male and female adult offspring with mothers on normal (controls) or low-sodium (IUGR) diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Total, g</th>
<th>Brain, g</th>
<th>Cardiac Ventricle, mg</th>
<th>Left Cardiac Ventricle, g</th>
<th>Kidney, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (8)</td>
<td>5.0 ± 0.2</td>
<td>0.196 ± 0.004</td>
<td>27 ± 1</td>
<td>0.047 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>IUGR (8)</td>
<td>4.1 ± 0.1‡</td>
<td>0.186 ± 0.005</td>
<td>25 ± 1</td>
<td>0.040 ± 0.002*</td>
<td></td>
</tr>
<tr>
<td>Offspring 1 wk</td>
<td>Controls (18)</td>
<td>14.5 ± 1.4</td>
<td>0.618 ± 0.025</td>
<td>97 ± 8</td>
<td>0.202 ± 0.029</td>
</tr>
<tr>
<td>IUGR (38)</td>
<td>10.5 ± 1.3‡</td>
<td>0.478 ± 0.038§</td>
<td>80 ± 62</td>
<td>0.144 ± 0.015‡</td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (16)</td>
<td>451 ± 9</td>
<td>2.07 ± 0.04</td>
<td>1.17 ± 0.04</td>
<td>3.33 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>IUGR (15)</td>
<td>383 ± 8‡</td>
<td>1.90 ± 0.04*</td>
<td>1.02 ± 0.03†</td>
<td>2.79 ± 0.11‡</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (16)</td>
<td>258 ± 6</td>
<td>1.83 ± 0.03</td>
<td>0.73 ± 0.03</td>
<td>1.99 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>IUGR (15)</td>
<td>233 ± 5‡</td>
<td>1.70 ± 0.04*</td>
<td>0.74 ± 0.02</td>
<td>0.78 ± 0.05†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. IUGR, intrauterine growth restricted. For fetuses, the values represent the mean fetal weight in a given litter for the number of litter indicated. In 1-wk offspring, individual rats were studied (sample size in parentheses). In adults, the numbers of rats used are shown in parentheses. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. age-matched controls.
Plasma renin activity, pmol Ang I-1 ml-1 h-1
Aldosterone, pmol/ml
Corticosterone, nmol/ml

Table 2. Renin-angiotensin-aldosterone system and plasma corticosterone levels of 12-wk male and female offspring with mothers on control or IUGR diets

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (14)</td>
<td>IUGR (13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma renin activity</td>
<td>1.37 ± 0.20</td>
<td>2.05 ± 0.26*</td>
</tr>
<tr>
<td>Aldosterone, pmol/ml</td>
<td>1.53 ± 0.15</td>
<td>1.81 ± 0.18</td>
</tr>
<tr>
<td>Corticosterone, nmol/ml</td>
<td>0.77 ± 0.07</td>
<td>0.95 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. The numbers of rats used are shown in parentheses. *P < 0.05 vs. gender-matched controls.

Fig. 1. Time course of systolic blood pressure in intrauterine growth-restricted (IUGR) male (A) and female (B) rat offspring during development between the ages of 5 and 12 wk. Statistical analyses were performed using a two-way ANOVA with repeated measures. Results are expressed as means ± SE.

Histological morphometric measurements were performed on the renal glomeruli. The ratio of the number of glomeruli to total area of the kidney as well as the mean area of glomeruli were not different between the two groups of animals in the three stages of development (Table 5). The number of strata was not altered (data not shown).

Structural evaluation of the left cardiac ventricle. Histological assessment of the left cardiac ventricle in 12-wk offspring showed no difference between the male and female offspring with mothers on control or IUGR diets.

Table 3. Plasma glucose, electrolytes, hematocrit, urea, and creatinine of 12-wk male and female offspring with mothers on control or IUGR diets

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (9)</td>
<td>IUGR (10)</td>
</tr>
<tr>
<td>glucose, mmol/l</td>
<td>8.4 ± 0.2</td>
<td>8.1 ± 0.3</td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>141 ± 1</td>
<td>137 ± 4</td>
</tr>
<tr>
<td>K⁺, mmol/l</td>
<td>7.4 ± 0.2</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>Total Ca²⁺, mmol/l</td>
<td>2.61 ± 0.03</td>
<td>2.54 ± 0.07</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>47 ± 1</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>4.9 ± 0.2</td>
<td>6.6 ± 0.3*</td>
</tr>
<tr>
<td>Creatinine, µmol/l</td>
<td>29 ± 2</td>
<td>31 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. The numbers of rats used are shown in parentheses. *P < 0.05, †P < 0.001 vs. gender-matched controls.

IUGR and control animals for both males and females. Neither the cytoplasm nor the nuclei of cardiomyocytes were hypertrophic, and interstitial collagen was not increased. There were no histopathological changes in the IUGR groups, and their cardiac histology was essentially identical to that of the control group (data not shown).

ANP mRNA expression in left cardiac ventricles. As shown in Fig. 2, the steady-state mRNA levels of prepro-ANP in left cardiac ventricles were significantly increased in 12-wk female adults born with IUGR compared with their controls (0.97 ± 0.05 vs. 0.72 ± 0.06 densitometric arbitrary units, respectively; P < 0.02). This increase was not observed between IUGR and control males (0.87 ± 0.02 vs. 0.84 ± 0.07 densitometric arbitrary units, respectively).

DISCUSSION

In this study, we have further characterized our IUGR model (43) by linking adverse fetal conditions and potential hypertension later in life. Moreover, we have shown that, in IUGR rats, 1) fetal brain and heart masses are increased relative to body weight, whereas relative kidney mass is unchanged; 2) by 12 wk of age, growth restriction is not compensated; 3) females have left ventricular hypertrophy; 4) elevated blood pressure is observed after the 8th wk of age; 5) the RAAS is regulated differently in males and females; and 6) diminution of the glomerular filtration rate (creatinine filtration rate (creatinine

Table 4. Urinary electrolytes and urea of 12-wk male and female offspring with mothers on control or IUGR diets

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (9)</td>
<td>IUGR (9)</td>
</tr>
<tr>
<td>Na⁺</td>
<td>223 ± 31</td>
<td>130 ± 26*</td>
</tr>
<tr>
<td>K⁺</td>
<td>309 ± 5</td>
<td>262 ± 19*</td>
</tr>
<tr>
<td>Total Ca²⁺</td>
<td>1.6 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Urea</td>
<td>968 ± 124</td>
<td>1,002 ± 60</td>
</tr>
</tbody>
</table>

Values are means ± SE in mmol/l. The numbers of rats used are shown in parentheses. *P < 0.05, †P < 0.01 vs. gender-matched controls.
lambs that oxygen deficiency generates a hypoxic state, affecting the developing fetus. It has been shown in uterine weight suggests reduced maternofetal exchanges, uteroplacental blood flow. Moreover, decreased placental weight suggests reduced maternofetal exchanges, affecting the developing fetus. It has been shown in lambs that oxygen deficiency generates a hypoxic state, causing a redistribution of cardiac output to the heart and brain at the expense of renal blood flow (13). It has also been demonstrated that the brain-to-body weight ratio in IUGR rabbits is higher than in controls (22). In humans, Veille et al. (48) observed that IUGR infants have larger hearts. Similar results were obtained in our model, as shown by an increase in the brain-to-body weight and ventricle-to-body weight ratios in the IUGR group.

At 1 wk of age, IUGR offspring are still smaller than controls. At that time, contrary to the fetal stage, organ weights (brain, cardiac ventricles, and kidney) of the young IUGR rats are decreased compared with their controls. In humans with IUGR, a lack of catch-up growth in postnatal life was also observed (49), as it was in another rat model of IUGR induced by maternal undernutrition (50). Contrary to our present longitudinal experiments lasting 12 wk, our previous study (43) showed that IUGR animals appeared to recover by the 2nd wk. However, in that first study, pups were evaluated on days 1 and 14 only. This 2nd wk may be a transient growth rate stage. A similar observation was made by Woodall et al. (50), who compared growth curves of growth-restricted and control rats. Indeed, the former had a lower weight at birth, almost recovered by the 3rd wk of life, and then stayed smaller than the controls throughout the length of the study.

We have shown that the weight of the left cardiac ventricle in IUGR adult female rats is similar to that of controls. The increased ratio of the left cardiac ventricle to total body weight in IUGR females suggests a left ventricular hypertrophy in these animals, although cardiac histology did not show the hallmark signs of mycardiocyte hypertrophy. It is possible that myocytes may be enlarged to a certain level that cannot be detected using the histological methods employed in our study. In addition, the greater mass of the left ventricle was not caused by an increase in collagen. Measuring of heart or ventricle relative to body weight has been reported to be a reliable method to evaluate hypertrophy of the heart (5, 32). It has been shown that activation of some cardiac genes, including the ANP gene, occurs during cardiac hypertrophy (12). Using female rats, Calderone et al. (9) have shown that left cardiac ventricle ANP mRNA levels are increased in a pathological model of cardiac hypertrophy (norepinephrine induced) but not in a physiological model (exercise training). Transgenic mice overexpressing angiotensin II type 1 receptor in cardiomyocytes develop cardiac hypertrophy (37). In this model, expression of ventricular ANP was also increased. To further characterize cardiac hypertrophy in our female model of IUGR, we have analyzed expression of ANP. We have demonstrated that ANP mRNA is overexpressed (33%) in female IUGR compared with their controls, whereas no changes are observed in males. In a model of cardiac hypertrophy induced by selective deletion of GLUT4 associated with an increased ventricular ANP expression, no gross morphological abnormalities of myocyte architecture and no increase in interstitial collagen were observed (1). For these reasons, we believe that cardiac hypertrophy in our adult female rats is pathological. This increased ANP expression could

Table 5. Number and area of glomeruli present in the kidney of fetuses, 1-wk offspring, and adult rats with mothers on control or IUGR diets

<table>
<thead>
<tr>
<th>Age</th>
<th>Group</th>
<th>No. of Glomeruli/</th>
<th>Area of Total Kidney,</th>
<th>Mean Area of Glomeruli, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>Controls</td>
<td>21.1 ± 2.7</td>
<td>1.78 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IUGR</td>
<td>15.7 ± 1.9</td>
<td>2.21 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>Offspring, 1 wk</td>
<td>Controls</td>
<td>25.1 ± 1.5</td>
<td>2.22 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IUGR</td>
<td>22.2 ± 0.8</td>
<td>2.14 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Adult, 12 wk</td>
<td>Controls</td>
<td>4.0 ± 0.6</td>
<td>8.75 ± 0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IUGR</td>
<td>4.0 ± 0.4</td>
<td>9.32 ± 0.77</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 animals per group. Note that there is no statistical difference observed between the IUGR and the control groups.

and urea) is not related to a decline in the number or size of glomeruli.

In the mothers of our IUGR rats, it is believed that lowered plasma volume (43) and decreased diameter of the uterine arteries (J. St-Louis, B. Sicotte, and M. Brochu, unpublished observations) would decrease uteroplacental blood flow. Moreover, decreased placental weight suggests reduced maternofetal exchanges, affecting the developing fetus. It has been shown in lambs that oxygen deficiency generates a hypoxic state, causing a redistribution of cardiac output to the heart and brain at the expense of renal blood flow (13). It has also been demonstrated that the brain-to-body weight ratio in IUGR rabbits is higher than in controls (22). In humans, Veille et al. (48) observed that IUGR infants have larger hearts. Similar results were obtained in our model, as shown by an increase in the brain-to-body weight and ventricle-to-body weight ratios in the IUGR group.

At 1 wk of age, IUGR offspring are still smaller than controls. At that time, contrary to the fetal stage, organ weights (brain, cardiac ventricles, and kidney) of the young IUGR rats are decreased compared with their controls. In humans with IUGR, a lack of catch-up growth in postnatal life was also observed (49), as it was in another rat model of IUGR induced by maternal undernutrition (50). Contrary to our present longitudinal experiments lasting 12 wk, our previous study (43) showed that IUGR animals appeared to recover by the 2nd wk. However, in that first study, pups were evaluated on days 1 and 14 only. This 2nd wk may be a transient growth rate stage. A similar observation was made by Woodall et al. (50), who compared growth curves of growth-restricted and control rats. Indeed, the former had a lower weight at birth, almost recovered by the 3rd wk of life, and then stayed smaller than the controls throughout the length of the study.

We have shown that the weight of the left cardiac ventricle in IUGR adult female rats is similar to that of controls. The increased ratio of the left cardiac ventricle to total body weight in IUGR females suggests a left ventricular hypertrophy in these animals, although cardiac histology did not show the hallmark signs of mycardiocyte hypertrophy. It is possible that myocytes may be enlarged to a certain level that cannot be detected using the histological methods employed in our study. In addition, the greater mass of the left ventricle was not caused by an increase in collagen. Measuring of heart or ventricle relative to body weight has been reported to be a reliable method to evaluate hypertrophy of the heart (5, 32). It has been shown that activation of some cardiac genes, including the ANP gene, occurs during cardiac hypertrophy (12). Using female rats, Calderone et al. (9) have shown that left cardiac ventricle ANP mRNA levels are increased in a pathological model of cardiac hypertrophy (norepinephrine induced) but not in a physiological model (exercise training). Transgenic mice overexpressing angiotensin II type 1 receptor in cardiomyocytes develop cardiac hypertrophy (37). In this model, expression of ventricular ANP was also increased. To further characterize cardiac hypertrophy in our female model of IUGR, we have analyzed expression of ANP. We have demonstrated that ANP mRNA is overexpressed (33%) in female IUGR compared with their controls, whereas no changes are observed in males. In a model of cardiac hypertrophy induced by selective deletion of GLUT4 associated with an increased ventricular ANP expression, no gross morphological abnormalities of myocyte architecture and no increase in interstitial collagen were observed (1). For these reasons, we believe that cardiac hypertrophy in our adult female rats is pathological. This increased ANP expression could

Fig. 2. Effect of IUGR on atrial natriuretic peptide (ANP) mRNA in the left cardiac ventricles of 12-wk-old female and male adults. Northern blot analyses were performed on 20 µg of total RNA. Top: autoradiogram of a representative experiment. Bottom: means ± SE of 5 separate experiments after standardization with the 18S ribosomal probe. *P < 0.05 vs. controls.
explain the decreased plasma aldosterone levels in female rats.

The observation that systolic blood pressure is elevated in IUGR animals is of prime importance. This is consistent with the hypothesis of Barker et al. (4) of fetal programming and confirms observations in other models of IUGR induced by either maternal undernutrition (50) or a low-protein diet (29). In the present study, this elevation of blood pressure was recorded from the 8th wk of age. However, we have observed that at week 7, the blood pressure curve of the IUGR groups (males and females) tends to meet that of the controls. This alteration in the systolic blood pressure curve could reflect hormonal changes during the postnatal period.

Elevation of systolic blood pressure is present in both genders, left cardiac hypertrophy is observed only in females, and alterations of RAAS and renal function are less marked in males. These data are surprising, because it is well known that men are at greater risk for cardiovascular and renal disease compared with women of similar ages (46). This has also been shown in rats (20, 41). However, two studies conducted on humans showed that gender has an important influence on the left ventricle adaptation pattern to pressure overload due to aortic stenosis. In fact, women developed a greater degree of left ventricular hypertrophy, even though their cardiac performance is better for cardiovascular and renal disease compared with men (10, 26). It was suggested that the smaller heart size of females allows a greater degree of hypertension that compensates for the systolic overload seen in aortic valve stenosis (17). In rats, it was described that the hypertrophic response to renovascular hypertension is less marked in males than in females (33). It was also shown that male rats develop cardiac insufficiency symptoms earlier than females, the latter tending to respond with compensatory myocardial hypertrophy (39). Our findings are in agreement with the results published by these authors. In our study, males and females both developed high systolic blood pressure. Furthermore, only females developed left ventricular hypertrophy. This could be explained by a decreased sodium excretion that induced a volume expansion as shown by a tendency to have lower hematocrits. It could be that IUGR females develop compensatory mechanisms (increased volume, sodium retention, cardiac hypertrophy, etc.) in response to their higher blood pressure. We could not find literature comparing male and female urinary data. We observed that urinary sodium is reduced in females compared with males. It is probably well explained by the difference in food consumption: 38 g/day for males vs. 20 g for females. Moreover, our plasma values of electrolytes are closely related to those obtained by Lillie et al. (30).

In humans, Konje et al. (25) observed that renin activity was significantly elevated in cord plasma from growth-restricted babies. In rats born from mothers receiving low-protein diets during pregnancy and who developed hypertension in adulthood, it was shown that, if they received angiotensin I-converting enzyme inhibitors (captopril), they underwent a significant reduction of blood pressure to levels similar to those measured in control animals (44). These studies suggest a role for the RAS in the development of hypertension programmed by IUGR. To further investigate the role of angiotensin II in the programming of hypertension, low-protein-exposed offspring were treated with losartan between 2 and 4 wk of age. By the 4th wk, the animals had not developed hypertension, and blood pressure was still normal by the 12th wk (45). We have shown that the RAAS system was altered in our adult rats. In IUGR males, increased plasma renin activity was observed without change in aldosterone levels. However, in IUGR females, plasma renin activity was heightened with a decrease in plasma aldosterone. To our knowledge, the only gender difference in regard to the RAAS was reported in spontaneously hypertensive rats (SHR) (42). It was concluded from that study that the development of hypertension in SHR, regardless of sex steroids, is mediated by the RAAS. Those authors also suggested that androgens promote the exacerbation of hypertension in male SHR via a mechanism involving the RAAS (42). Our group is the first to show that the RAAS and corticosterone levels are modified differently in males and females.

The elevated corticosterone levels in IUGR females could be associated with hypertension. We observed that corticosterone concentrations were higher in IUGR fetuses than in the controls. Increased fetal corticosterone secretion is known to induce early parturition in UGR, a condition often seen in our model. Indeed, in IUGR offspring, electrolyte balance in adulthood, possibly leading to subsequent postnatal diseases such as hypertension. In fact, excess of glucocorticoids in fetal life is known to exert adverse effects on the structural development of the kidney and, hence, further influence blood pressure control, maybe through altered renin synthesis (11). The effects of excess glucocorticoids in fetal life, leading to hypertension, have also been observed in sheep (16).

Development, growth, and functional capacity of the kidney have been shown to be impaired by IUGR in humans (15, 23, 27) and rats (35). Because IUGR is a predisposing condition to subsequent development of hypertension, it was proposed that this predisposition might be conferred by the kidney (6). In humans, a prospective study concluded that blood pressure and creatinine levels are linked (38). Lucas et al. (31) demonstrated that, in IUGR rats induced by 50% food restriction of dams during pregnancy, there is true impairment of glomerular hemodynamics in the IUGR progeny. We investigated whether hypertension could be caused by major impairment of renal function in our model. Indeed, in IUGR offspring, electrolyte balance was modified, whereas plasma urea and creatinine levels were both increased. Because hematocrit values did not change between groups, we speculated that decreased glomerular filtration could be implicated in the development of hypertension. To document such
glomerular impairment, we looked for a reduction in the number of glomeruli or a reduction in glomerular surface. We could not document any such alteration, since both parameters were comparable in IUGR animals and controls. Langley-Evans et al. (28) postulated that, even though IUGR adult offspring of dams fed a low-protein diet during pregnancy had a lowered nephron complement from birth, they maintained a normal glomerular filtration rate. Moreover, it has been reported that, in SHR, the number of renal glomeruli was not significantly smaller than in normotensive Wistar-Kyoto rats (2). This implies that other renal excretory defects might be involved in the pathogenesis of hypertension (7). These findings support our observation that the number of glomeruli and glomerular filtration do not necessarily conspire in the pathogenesis of hypertension. We observed that the number of strata was not altered, indicating a normal development of nephrons. Kidney damage in our IUGR animals may be due to more subtle modifications of renal homeostasis, impairments of the fine structure of the kidney, or even by pathophysiological alteration of the tubular portion (36).

In summary, this study, using IUGR rats born from mothers of our original model of low-sodium diet, provides evidence that fetal programming in these rats leads to the development of hypertension, alterations of renal function, and modifications of the RAAS. Moreover, our results indicate gender differences in the expression of these alterations in adult rats. It is expected that the results provided by investigations with our model will provide new and important findings that will help understanding of the link between fetal programming in IUGR and the pathogenesis of some forms of hypertension.

We thank Dr. Patrick Vinay for valuable comments on this model, and Dr. Angelo Calderone for the gift of rat prepro-ANP cDNA. Our studies were supported by grants from La Fondation des Maladies du Coeur du Québec and the Canadian Institutes of Health Research (MOP-37902). M.-C. Battista received a studentship from La Société Québécoise d’Hypertension Artérielle and La Fondation de l’Hôpital Sainte-Justine. M. Brochu is a scholar from Fonds de la Recherche en Santé du Québec.

REFERENCES
27. Kostkiewicz M, Tracz W, Olszowska M, Podolec P, and Drop D. Left ventricular geometry and function in patients with...


