Uninephrectomy in Young Age or Chronic Salt Loading Causes Salt-Sensitive Hypertension in Adult Rats

Mattias Carlström, Johan Sällström, Ole Skøtt, Erik Larsson, A. Erik G. Persson

Abstract—The importance of nephron endowment and salt intake for the development of hypertension is under debate. The present study was designed to investigate whether reduced nephron number, after completion of nephrogenesis, or chronic salt loading causes renal injury and salt-sensitive hypertension in adulthood. Rats were operated at 3 weeks of age (after completed nephrogenesis) and then subjected to either normal or high-salt diets for 6 to 8 weeks. Four different experimental groups were used: sham-operated animals raised with normal-salt diet (controls) or high-salt diet (HS) and uninephrectomized animals raised with normal-salt diet (UNX) or high-salt diet (UNX+HS). In the adult animals, renal and cardiovascular functions were evaluated and blood pressure recorded telemetrically under different sodium conditions (normal, high, and low). Hypertension was present in UNX+HS (122±9 mm Hg), UNX (101±3 mm Hg), and HS (96±1 mm Hg) groups on normal-salt diets compared with the controls (84±2 mm Hg), and the blood pressure was salt sensitive (high- versus normal-salt diet; 23±3, 9±2, 7±2, and 1±1 mm Hg, respectively). The hypertensive groups (UNX+HS, UNX, and HS) had increased diuresis and reduced ability to concentrate urine. The glomerular filtration rate (milliliters per minute) in anesthetized rats was reduced in the UNX+HS (2.36±0.30) and UNX animals (2.00±0.31) compared with both HS animals (3.55±0.45) and controls (3.01±0.35). Hypertensive groups displayed reduced plasma renin concentrations during high sodium conditions and hypertrophic kidneys and hearts with various degrees of histopathologic changes. In conclusion, at a young age after completed nephrogenesis, uninephrectomy or chronic salt loading causes renal and cardiovascular injury with salt-sensitive hypertension. (Hypertension. 2007;49:1342-1350.)

Key Words: blood pressure ■ cardiovascular diseases ■ fibrosis ■ glomerular filtration rate ■ hypertension renal ■ nephrectomy ■ sodium dietary

The kidneys play a key role in the homeostatic regulation of body fluid volume and electrolyte balance and consequently possess a dominant role in long-term blood pressure control. Hypertension is the most common chronic disorder worldwide, and secondary forms of hypertension are found in approximately 10% of the hypertensive population, of which all can be linked to renal disease.1

The hypothesis that a reduced number of nephrons at birth causes renal injury and hypertension in adult age was proposed ≈2 decades ago.2 However, it has been argued that if nephron underdosing causes hypertension, as some studies have indicated, that nephrectomy for live kidney donation in adults does not increase the prevalence of hypertension,3–5 whereas others have found an increased blood pressure.6–8

Nephrogenesis in humans occurs during embryonic/fetal development and is complete by week 36 of gestation. In rats, however, the period for nephron development is extended until approximately postnatal day 8.9 For this reason, the age at which nephron reduction occurs is an important factor for the outcome of the nephrectomy. The compensatory increase in kidney weight and function after nephrectomy appears more pronounced in immature than in adult kidneys, as determined by experimental studies.9,10

Furthermore, uninephrectomy, before completion of nephrogenesis, causes salt-sensitive hypertension and compromised renal function.11–13 Therefore, it is considered that the immature kidney is more susceptible to the development of renal damage and that neonatal and adult nephrectomy differs with respect to effects on blood pressure.

The debate on salt intake and blood pressure control is long standing. The kidneys appear to play a central role in the functional disturbances linking salt intake to arterial blood pressure. Clinical and experimental studies have provided critical insights into the relation among salt intake, renal salt handling, and arterial blood pressure,14–16 but, still, the effects on blood pressure, after acute and prolonged high salt loading, are controversial. Although the available data from experimental studies in other mammalian species indicate

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that blood pressure appears to increase with the magnitude of the salt intake, the results are difficult to interpret, because data were scattered and overlapped between groups. The present study was designed to examine the effects of unilateral nephrectomy at a young age when nephrogenesis was complete, on blood pressure and renal and cardiovascular function, and to examine the consequences of chronic salt loading during adolescence in rats.

Methods

The studies were performed on male Sprague-Dawley rats (Møllgaard, Copenhagen, Denmark). The animals were uninephrectomized or sham operated at 3 weeks of age and then subjected to either normal-salt diet (0.7% NaCl, SD389-R36, Lactamin) or high-salt diet (4% NaCl, SD312-R36, Lactamin) for 6 to 8 weeks. In this way, 4 different experimental groups were created: sham-operated animals raised on a normal-salt diet (controls) or a high-salt diet (HS) and uninephrectomized animals raised on a normal-salt diet (UNX) or a high-salt diet (UNX+HS). The experimental periods were carried out in adult animals during different sodium conditions.

Young Animals

Uninephrectomy

Unilateral nephrectomy or sham operations were performed on 3-week-old animals in the same way as described previously (details are available in an online supplement at http://hyper.ahajournals.org). After surgery, all of the animals were left to grow with free access to either normal- or high-salt diets for 6 to 8 weeks.

Adult Animals

The experiments were undertaken, after uninephrectomy or sham operations, in 2 separate series. In series 1, blood pressure and heart rate were measured telemetrically, followed by renal excretion analysis of volume and electrolytes in metabolism cages. Finally, plasma samples were obtained for renin analysis. In series 2, whole-kidney glomerular filtration rate (GFR) was measured. The kidneys and hearts from animals in both series were subject to histological and stereological examination.

In series 1, adult animals were subjected to different salt diets consecutively: normal-, high-, and low-salt diet (0.02% NaCl, SD441-R36, Lactamin). The experiments in series 2 were all conducted during normal sodium conditions. All of the animals were allowed to equilibrate for 10 days on each diet before measurements commenced.

Series 1

Telemetric Measurements

The telemetric device (PA-C40, DSL, Transoma Medical) was implanted, and the telemetric measurements were commenced in adult animals as described previously (see the data supplement).

Renal Excretion Measurements In Metabolism Cages

For urine collection, the animals were housed individually for 24 hours in metabolism cages with different salt diets and water ad libitum. Urine production was determined gravimetrically, sodium and potassium concentrations were determined by flame photometry (FLM3, Radiometer), and osmolality was determined by depression of the freezing point (Fiske Micro-Osmometer, Model 210, Fiske Associates).

In a separate series of experiments, the ability to concentrate the urine was further investigated. In their normal cages, the animals were deprived of food and water for 12 hours before an additional 12-hour collection period (nighttime) with water deprivation in the metabolism cages.

Renin Sampling and Assay

Blood samples, taken from the tail tip, were obtained for renin analysis at the end of each dietary period. The plasma renin concentration (PRC) was measured by radioimmunoassay of angiotensin I by the antibody-trapping technique as described previously.

Figure 1. MAP and heart rates (24-hour means) in adult animals treated with different salt diets given in the following order: normal-, high-, and low-salt diet. All of the values are expressed as mean±SEM. *P<0.05 vs controls on the same diet. #P<0.05 vs both normal- and low-salt diets within the same group.
Mild; 2 the severity of change (0 changes, and the hearts were investigated for fibrosis and hypertrophy. Samples were taken in the middle of each period. Values obtained for the 3 clearance periods were averaged to give a single value for each animal (see the data supplement).

### Series 2

#### Whole-Kidney GFR Measurements

The GFR was calculated in anesthetized animals as renal clearance of [3H]-methoxy-inulin. All of the animals had been given a normal-salt diet for 10 days before the experiment. Catheters were placed to monitor blood pressure and for continuous infusion. Urine was collected during 3 consecutive 20-minute periods, and blood samples were taken in the middle of each period. Values obtained for the 3 clearance periods were averaged to give a single value for each animal (see the data supplement).

#### Histological Examination

The animals, which had been given a normal-salt diet for 10 days before the examination, were euthanized, and the kidneys and hearts were explanted and weighed. Hearts and sagittal slices of the renal tissue were fixed in formalin (4% in PBS) and embedded in paraffin. Embedded tissue blocks were cut into 5-μm-thick sections and stained with hematoxylin/eosin, periodic acid-Schiff, and Picrosirius red for a blinded histopathologic evaluation.

In the renal tissues, the cortex, medulla, and papilla were investigated for fibrosis, inflammation, and glomerular and tubular changes, and the hearts were investigated for fibrosis and hypertrophy. The tissues evaluated were given a score of 0 to 3 depending on the severity of change (0=no observable changes; 1=mild; 2=moderate; and 3=severe changes).

#### Stereological Examination

A light microscope (Leitz DMRB, Leica Microsystems) with a charge-coupled device camera (AxioCam Color, Carl Zeiss) was used to take photomicrographs of all of the tissues investigated. The renal cortical area of 1 section, from each kidney and animal, was photographed, and the mean glomerular area was determined with a computer program (Scion Image, Scion Corporation). From the area measured, an approximation of the glomerular volume was calculated with the equation $V=4/3\pi D^3$, where $D$ is the real diameter, and $d$ is the measured mean glomerular diameter.

For the hearts, the mean lengths of both left and right ventricular walls and the thickness of the septum were measured. Furthermore, the thickness of the cardiac myocyte fibers and nuclei in the left ventricle were measured with the Scion Image program.

### Calculations and Statistics

Values were presented as mean±SEM. Single comparisons between normally distributed parameters were tested for significance with Student’s paired or unpaired $t$ test. Multiple comparisons were analyzed with 2-way ANOVA followed by the Fisher’s posttest. Scored data for the histological evaluation were analyzed by the Kruskal–Wallis test followed by the Mann–Whitney $U$ test. Differences were considered to be statistically different if $P<0.05$.

### Ethics

The experiments were approved by the Uppsala Ethical Committee for Animal Experiments.

### Results

All of the animals used in this study were in good condition. At the beginning of the experiments (ie, 6 to 8 weeks postsurgery) the UNX+HS (318±6 g) but not the UNX (340±4 g) and HS animals (330±14 g) had a slightly lower
body weight compared with the controls (365±16 g). However, no differences were found during the experimental periods of series 1 or 2.

Series 1

**Telemetric Measurements**

Mean arterial blood pressures (MAPs) and heart rates (24-hour mean) are presented in Figure 1. Both nephrectomized groups ([UNX+HS; n=10] and [UNX; n=8]) and the high sodium–loaded animals (HS; n=6) had higher blood pressure, on all of the diets, than the controls did (n=12). All of the groups displayed a salt-sensitive blood pressure with significant difference in MAP between the low- and high-salt diets for the UNX+HS (33±6 mm Hg), UNX (12±2 mm Hg), and HS groups (12±4 mm Hg), but this was not the case for the control group (3±1 mm Hg). The UNX+HS animals had higher blood pressure than the UNX and the HS animals on all of the diets; however, no differences were determined between the UNX and the HS groups.

Heart rates were increased in the UNX+HS, UNX, and HS groups compared with the controls on all of the diets, but the differences were only significant on the high-salt diet (Figure 1). The UNX+HS, UNX, and HS groups had similar heart rates with no changes during the different sodium conditions. When the control group was subjected to a higher salt intake, heart rates decreased compared with both normal- and low-salt diets.

**Renal Excretion Measurements**

Renal excretion data for the different groups are summarized in Table 1. Diuresis was increased in the UNX+HS, UNX, and HS groups on all of the diets compared with the controls; however, this had borderline significance during the high-salt diet for the HS group (P=0.08). As expected, both water intake and diuresis increased in all of the groups during the high-salt period. The osmolality was reduced in the UNX+HS, UNX, and HS groups compared with the controls on both low- and high-salt diets but was reduced only in the UNX+HS group during normal sodium conditions. Furthermore, the absolute electrolyte excretion rate was increased under all of the diets in the UNX+HS and HS groups compared with controls but only during normal- and low-sodium conditions in the HS group. Sodium and potassium excretion were increased in all of the experimental groups, compared with the controls, both during normal- and low-sodium conditions.

Figure 2 shows an analysis of the relationship between the MAP and the steady-state sodium excretion in control (n=12), HS (n=6), UNX (n=8), and UNX+HS (n=10) animals under the different sodium conditions. All of the groups displayed abnormal renal function curves with pressure natriuresis compared with the controls. In the 24-hour food- and water-deprived period, both UNX+HS and UNX groups displayed reduced osmolar concentration compared with the controls, and the UNX+HS had an increased diuresis (Table 2).

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**Table 2. Renal Excretion Data in 24-Hour Fasted and Water-Deprived Animals**

<table>
<thead>
<tr>
<th></th>
<th>Normal Salt Diet</th>
<th>UNX+HS</th>
<th>UNX</th>
<th>HS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water, mL/24 h</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>UV, ml/12 h</td>
<td>8.0±0.2*</td>
<td>6.4±0.9</td>
<td>6.0±0.2</td>
<td>5.8±0.2</td>
<td></td>
</tr>
<tr>
<td>Na⁺ excretion, μmol/12 h</td>
<td>290±37</td>
<td>287±93</td>
<td>305±65</td>
<td>335±36</td>
<td></td>
</tr>
<tr>
<td>K⁺ excretion, μmol/12 h</td>
<td>1320±59</td>
<td>1239±133</td>
<td>1333±68</td>
<td>1191±114</td>
<td></td>
</tr>
<tr>
<td>Osm, mM</td>
<td>922±10*</td>
<td>1043±62*</td>
<td>1148±76</td>
<td>1181±42</td>
<td></td>
</tr>
<tr>
<td>Osmexcretion, mmol/12 h</td>
<td>7±0</td>
<td>7±1</td>
<td>7±0</td>
<td>7±0</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; UV indicates urine flow rate; n, number of animals; Osm, osmolality; Osmexcretion, osmolar excretion. *P<0.05 vs controls.
PRC
The results from the plasma renin analysis are displayed in Figure 3. In all of the groups, a significant difference in PRC was found between the low- and high-salt diets. There were no differences in PRC among the UNX/H11001 (n=10), UNX (n=8), and the HS (n=6) groups. The controls (n=12) had higher PRC than all of the other groups on all diets did, but the difference was only significant during high-sodium conditions.

Series 2
Whole-Kidney GFR Measurements
Data from the clearance measurements are shown in Table 3. MAP was higher in UNX+HS and HS groups than in the controls, but not for the UNX animals (P=0.09). The total kidney weight was decreased in the nephrectomized groups compared with the HS and the control groups. Furthermore, the hematocrit was lower in the UNX+HS group than in the controls. Absolute GFR was reduced in UNX+HS and UNX groups compared with the controls (Figure 4). In the HS group, there was a tendency for an increased GFR compared with the controls (P=0.10). When GFR was normalized to the total kidney weight, no differences were determined between the groups.

Series 1 to 2
Histology and Stereology
All of the experimental groups displayed increased kidney and heart weights compared with the controls. The results from the histology and stereology are summarized in Table 4. For the kidneys, the UNX+HS group displayed the most severe changes among the hypertensive groups (Figure 5); however, there were large individual variations in all of the tissues ranging from mild to severe injuries. Two of the UNX-HS animals displayed severe destructive changes in all of the compartments, including glomerular matrix increase, extracapillary changes with crescents and tubular atrophy, dilatation, and interstitial inflammation (plasma cells and lymphocytes). Moreover, vascular changes with hypertrophy of the media were also observed.

The UNX and the HS groups displayed similar fibrotic and tubular changes (hyaline material in the lumen, atrophy, and also thickening of the basal membrane) and glomerular changes (ie, sclerosis, mesangial matrix increase, and

### Table 3. Kidney and Heart Weight, Blood Pressure, and Renal Excretion Data for Whole-Kidney GFR Measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UNX+HS</th>
<th>UNX</th>
<th>HS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kw (total), g</td>
<td>2.61±0.13*</td>
<td>2.21±0.06*</td>
<td>3.05±0.13</td>
<td>2.91±0.04</td>
</tr>
<tr>
<td>Hw, g</td>
<td>1.55±0.05*</td>
<td>1.50±0.09*</td>
<td>1.44±0.08</td>
<td>1.36±0.03</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>150±7*</td>
<td>129±8</td>
<td>135±5*</td>
<td>116±4</td>
</tr>
<tr>
<td>Hct, %</td>
<td>44±1*</td>
<td>48±1</td>
<td>47±1</td>
<td>48±1</td>
</tr>
<tr>
<td>GFR, µL/min</td>
<td>2363±304*</td>
<td>2003±315*</td>
<td>3548±453</td>
<td>3011±351</td>
</tr>
<tr>
<td>GFR, % of controls</td>
<td>78.5</td>
<td>66.5</td>
<td>117.8</td>
<td>100</td>
</tr>
<tr>
<td>GFR/Kw, µL/min/g</td>
<td>909±112</td>
<td>926±160</td>
<td>1148±119</td>
<td>1038±121</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; Kw indicates kidney weight; Hw, heart weight; Hct, hematocrit; n, number of animals.

*P<0.05 vs controls.
shrunk glomeruli). In contrast to the UNX + HS group, the observed changes were milder, and neither UNX nor HS groups displayed infiltration of inflammatory cells. The control animals displayed normal renal histoarchitecture with no changes in any of the tissues investigated, with the exception of very mild fibrosis in 2 kidneys.

The hearts were hypertrophic in the UNX + HS (1.64±0.08 g), UNX (1.57±0.07 g), and HS (1.49±0.05 g) groups compared with the controls (1.37±0.03 g). This hypertrophy was associated with an increased thickness of the left ventricular wall in all of the uninephrectomized groups. All of the hypertensive groups displayed various degrees of fibrosis (Figure 6) and an increased thickness of the cardiac myocyte fibers and nuclei compared with the controls (Table 4). Furthermore, the glomerular volume was increased in all of the hypertensive groups (Table 4).

**Discussion**
This study clearly demonstrated that nephrectomy after completed nephrogenesis or chronic salt loading separately caused salt-sensitive hypertension in adulthood. Furthermore, the consequence of the combination of young nephrectomy and long-term treatment with a high-salt diet gave rise to more pronounced hypertension and salt sensitivity.

**TABLE 4. Histology and Stereology of the Kidney and Heart**

<table>
<thead>
<tr>
<th>Variable</th>
<th>UNX + HS</th>
<th>UNX</th>
<th>HS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney Kw, mean, g</td>
<td>3.00±0.23*</td>
<td>2.57±0.14*</td>
<td>1.59±0.08*</td>
<td>1.43±0.02</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1.4±0.2*</td>
<td>1.0±0.0*</td>
<td>1.0±0.0*</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.9±0.3*</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Tubular changes</td>
<td>1.1±0.3*</td>
<td>0.8±0.2*</td>
<td>0.5±0.2*</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Glomerular changes</td>
<td>1.1±0.4*</td>
<td>0.3±0.2*</td>
<td>0.3±0.2*</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Mean glomerular volume, μm³×10⁶</td>
<td>2.38±0.21*</td>
<td>1.71±0.13*</td>
<td>1.17±0.09*</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Heart Hw, g</td>
<td>1.69±0.15*</td>
<td>1.69±0.08*</td>
<td>1.57±0.06*</td>
<td>1.37±0.02</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1.2±0.2*</td>
<td>1.0±0.0*</td>
<td>1.00±0.35*</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Cardiac myocyte fiber thickness, μm</td>
<td>2.32±0.07*</td>
<td>2.08±0.04*</td>
<td>2.03±0.09*</td>
<td>1.79±0.06</td>
</tr>
<tr>
<td>Cardiac myocyte nuclei thickness, μm</td>
<td>0.57±0.01*</td>
<td>0.54±0.01*</td>
<td>0.51±0.02</td>
<td>0.47±0.02</td>
</tr>
<tr>
<td>Left ventricular wall thickness, mm</td>
<td>2.97±0.04*</td>
<td>2.68±0.09*</td>
<td>2.45±0.08</td>
<td>2.27±0.09</td>
</tr>
<tr>
<td>Septal wall thickness, mm</td>
<td>2.26±0.17</td>
<td>2.43±0.06*</td>
<td>2.01±0.25</td>
<td>1.95±0.11</td>
</tr>
<tr>
<td>Right ventricular wall thickness, mm</td>
<td>1.30±0.09</td>
<td>1.13±0.04</td>
<td>1.11±0.06</td>
<td>1.11±0.05</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Evaluation of fibrosis of the kidney and the left ventricle wall, inflammation (ie, infiltration of plasma cells and lymphocytes), tubular changes (ie, hyaline material and atrophy), and glomerular changes (ie, sclerosis, mesangial matrix increase, and shrunken glomeruli). All of the evaluated sections were given a score of 0 to 3 (0=no observable changes; 1=mild; 2=moderate; and 3=severe changes). Values are expressed as mean±SEM. Kw indicates kidney weight; Hw, heart weight; n, number of animals.

*P<0.05 vs controls.
The kidneys have a key role in the long-term blood pressure control. It has been proposed that there is an inverse relationship between nephron endowment and the risk of developing cardiovascular disease and hypertension later in life. In humans, all nephrons are developed 4 to 5 weeks before birth. In rats, however, a large part of nephrogenesis takes place during the first week after birth.

A reduction of 50% of the nephrons by uninephrectomy in neonatal rats causes hypertension and progressive renal disease. However, uninephrectomy in adult rats does not cause hypertension or renal injuries and, therefore, neonatal and adult nephrectomy appear to have different effects on blood pressure regulation. In the present study, nephrectomy was performed in postnatal animals, which all developed various degrees of hypertension in adulthood. This occurred despite all of the nephrons having been formed at the time of nephrectomy, and, therefore, no compensatory nephrogenesis could take place. This demonstrates that animals are not only susceptible to develop renal injury and hypertension if nephron deficiency is induced during nephrogenesis but also during a sensitive period after the neonatal period.

Figure 5. Representative photomicrographs of kidney sections, stained with Picrosirius for fibrosis in the different groups: control animal with a normal renal tissue (inner cortex and outer medulla), with no fibrotic changes; HS animal with increased fibrosis in the cortical interstitial tissue, without prominent tubular atrophy; UNX animal with increased periglomerular and interstitial fibrosis; and UNX + HS animal with pronounced fibrosis in the inner cortex and the outer medulla.

Figure 6. Representative photomicrographs of heart sections (left ventricle wall), stained with Picrosirius for fibrosis in the different groups: Control animal with a normal myocardium, without any fibrotic changes; HS animal with fibrotic changes in the myocardium, especially subendocardially and around the papillary muscles; UNX animal with prominent fibrotic changes; and UNX + HS animal with areas of pronounced fibrosis in the myocardium.
Because normal functioning kidneys have the ability to excrete large amounts of excessive salt, it is generally considered that an increased salt intake for a shorter period of time (ie, days or even a few weeks) does not cause any significant changes in arterial blood pressure. However, the long-term effects of high sodium intake are much more controversial.

In the present study, adult animals, subjected to chronic high sodium loading from a young age, developed permanent hypertension. Uninephrectomized animals and those subjected to chronic salt treatment displayed changed renal function and renal hypertrophy. In uninephrectomized animals, this compensation in kidney growth has been described as somewhat more pronounced in immature kidneys than in fully developed kidneys.\(^9,10\)

Compared with the controls, all of the hypertensive groups (ie, UNX+HS, UNX, and HS groups) displayed reduced urine concentrating ability. This was associated with an increased diuresis on all of the diets and elevated osmolar excretion. Increased diuresis, as well as the reduced capacity of concentrating the urine, was also found in water-deprived animals with a reduced kidney mass (ie, UNX+HS and UNX groups). Pressure natriuresis and diuresis have been used to describe the regulation of arterial blood pressure on both the functional and pathological states of the kidneys.\(^25–28\) In all forms of chronic hypertension, the renal pressure natriuresis mechanism is abnormal; however, it is still unclear whether this phenomenon is a cause or a consequence of hypertension.\(^29\) In theory, pressure natriuresis resetting requires increased blood pressure to maintain a constant salt and water balance, but it could also occur secondarily to hypertension, because renal pathological changes occur as a consequence of chronic hypertension and must, therefore, be considered as an alternative explanation for pressure natriuresis in hypertension. In our study, it was unclear whether the changed renal excretion pattern was a cause or a consequence of hypertension.\(^30,31\) In theory, pressure natriuresis resetting requires increased blood pressure to maintain a constant salt and water balance, but it could also occur secondarily to hypertension, because renal pathological changes occur as a consequence of chronic hypertension and must, therefore, be considered as an alternative explanation for pressure natriuresis in hypertension. In our study, it was unclear whether the changed renal excretion pattern was a cause or a consequence of hypertension. However, it has been shown that hypertension after neonatal uninephrectomy in rats precedes glomerular damage, because the increased blood pressure was present earlier in life than were the signs of glomerular disease. Therefore, hypertension, associated with neonatal nephrectomy is a contributor rather than a result of the onset of renal disease.\(^12\)

The effects of chronic salt load on renal function and long-term blood pressure control have been discussed. Studies have suggested that excessive salt intake for longer periods can cause renal injury and abnormal kidney function.\(^17,30–32\) Furthermore, in the present study, the pressure natriuresis and diuresis were more pronounced in animals subject to chronic salt loading, which could very well be explained by renal pathological changes.

All of the groups displayed increased blood pressure and heart rate compared with the controls, which was associated with both renal and cardiovascular pathohistological changes. Both nephrectomized and chronic high salt–treated animals exhibited various degrees of fibrosis, as well as glomerular and tubular changes, whereas inflammation was only found in the kidneys of the UNX+HS group. The degree of pathological changes appeared to correlate with the degree of hypertension (ie, with higher blood pressure, the changes were the more severe).

Furthermore, it has been demonstrated that hypertension, after neonatal uninephrectomy, progresses slowly over time and correlates with a decrease in GFR.\(^12\) The present study supported the finding that uninephrectomy caused a reduced total GFR in adult animals and that chronic sodium loading (alone or in combination with nephrectomy) appeared to increase the GFR, even after the animals have returned to a normal-salt diet. However, no significant differences were determined among the groups when GFR was adjusted for total kidney weight, indicating hypertrophy of the remaining kidney. Furthermore, all of the hypertensive groups displayed an increased glomerular volume. Taken together, this suggested that UNX+HS, UNX, and HS animals had a changed renal function with hyperfiltration of the remaining nephrons that could lead to the observed pathohistological changes and consequently contribute to hypertension in adulthood.

The effects of excessive salt intake have not only been discussed in terms of blood pressure. Dietary salt also appears to have detrimental effects on the cardiovascular system, such as increased left ventricle mass, thickened and stiffened conduit arteries, narrowed resistance arteries, increased sensitivity of platelet aggregation, and deposition of collagen and fibrotic tissue.\(^33,34\) In the present study, cardiac fibrosis was evident in all of the hypertensive groups, and both heart weight and left ventricle wall thickness were increased compared with the controls. It has been shown\(^35\) that there is a relationship, independent of the blood pressure, between the left ventricle mass and cardiovascular mortality and morbidity. In the present study, the ventricular hypertrophy could possibly be a consequence of a raised preload after chronic salt intake, hypertension and increased heart rate, or, more likely, a combination of these factors, because the UNX+HS animals were the ones with the most pronounced pathohistological changes. Other possible explanations are dysregulation of the renin–angiotensin system or that the sympathetic nervous system acts as a mediator.\(^35\)

The mechanisms behind the salt-sensitive hypertension, as illustrated in Figure 1, and by the slopes and the rightward shift of the renal function curves in Figure 2 could partly be explained by reduced renal mass in the nephrectomized animals. This would also be consistent with an inappropriate regulation of the renin–angiotensin system,\(^25,29\) which is known to have an important role in long-term blood pressure regulation. In the present study, PRCs were only reduced during high sodium load. That no suppression was found under either normal- or low-sodium conditions, despite an elevated blood pressure, indicated that the regulation of the renin–angiotensin system was not entirely normal. It has been suggested\(^28\) that both pressure natriuresis and decreased angiotensin II formation are important mechanisms in maintaining sodium balance and in minimizing hypertension during high salt intake in animals with a reduced number of nephrons. Although PRC was suppressed in all of the groups on the high-salt diet in the presence of hypertension, it is unclear whether the suppression was sufficient.

In conclusion, these results demonstrate that not only a reduced nephron number at birth, but also during a sensitive
period at young age, may increase the risk of developing hypertension and renal injury. In addition, the present data indicate a relationship between chronic dietary salt intake, from a young age, and blood pressure level in adulthood. The mechanisms behind the hypertension are unclear, but it is possible that both reduction of the renal mass at a young age, as well as chronic salt intake, could lead to glomerular hyperfiltration, which, in the long term, will lead to renal injury with subsequent hypertension.

**Perspectives**

This study clearly demonstrated that both reduction in nephron number, after completed nephrogenesis, and chronic salt loading during young age cause salt-sensitive hypertension in adulthood. The current study gives substantial support to the hypothesis that a reduced number of nephrons and salt intake are related to hypertension and renal injury in the adulthood. From a clinical point of view, a reduction in salt intake should be advised, particularly at a young age and, if nephrectomy has been performed, salt restriction should be imposed to prevent the development of renal injury and hypertension.

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**Disclosures**

None.

**References**

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Methods

Uninephrectomy

A unilateral nephrectomy was performed on three-week-old animals. Anesthesia with spontaneous inhalation of 2-2.5% isoflurane (Forene®, Abbot Scandinavia AB, Kista, Sweden) was maintained throughout the surgery and the body temperature was kept stable at 37.5°C with a servo regulated heating pad. The abdomen was opened sterile through a midline incision and the left kidney was exposed. The renal vessels and the ureter were carefully isolated and a single ligature placed around them, which was tied tightly. The distal portions were then cut and the kidney removed. Sham operations in control animals were performed in the same way, but without removal of the kidney and the ureter. Finally, the abdominal wall was closed and the animals were allowed to wake up under a heating lamp, and were not returned to their cages until fully awake. All animals were then left to grow with free access to either normal or high salt diets.

Series I

Telemetric measurements

The telemetric device (PA-C40) (DSITM, Transoma Medical, St Paul, MN, USA) was implanted 6-8 weeks following uninephrectomy or sham-operation in 3-weeks old animals. Inhalation anesthesia was used as described above, the skin was sterilized and an abdominal midline incision made. A 20 mm long segment of the abdominal aorta was dissected free and the catheter of the telemetric probe was inserted into the aortic lumen. The entry site was sealed by application of n-butyl-cyanoacrylate tissue adhesive (Vetbond™, 3M Animal Care Products, St Paul, MN, USA). The transmitter was placed in the peritoneal cavity and sutured to the inside of the abdominal wall, and the abdomen was closed.
For measurements of blood pressure and heart rates, the telemetric device was activated and the cage placed on a receiver plate that transferred the signals to a computer, where calibrated blood pressure values were measured. Data were collected for five seconds every second minute for at least 48-hours at a time. The recorded data were continuously analyzed by a computer program (PC-Lab 5.0, AstraZeneca, Mölndal, Sweden).

**Whole kidney GFR measurements**

The rats were anaesthetized with an intraperitoneal injection of thiobutabarbitral sodium (120 mg/kg Bw) (Inactin®, Sigma, St Louis, MO, USA). All animals had been given a normal salt diet for 10 days prior to the experiment. The body temperature was kept stable at 37.5°C with a servo regulated heating pad. A tracheotomy was placed to allow spontaneous breathing. Polyethylene catheters were placed in the femoral artery to monitor blood pressure and in the femoral vein for infusing Ringer solution (5 mL/h/kg Bw). The bladder was cannulated for urine collection. After completion of surgery, an initial bolus of 5 µCi [³H]-methoxy-inulin (American Radiolabeled Chemicals, St Louis, MO, USA) was followed by a continuous dosage of 5µCi/h. All animals were allowed to equilibrate for 45 min before measurements started. Urine was collected during three consecutive 20-min periods and blood samples (20µL) were taken in heparinized glass capillaries from the femoral artery in the middle of each period. Values obtained for the three clearance periods were averaged to give a single value for each animal. The urine volume and osmolality, as well as the concentrations of sodium and potassium, were assayed in the same way as for Series I. Samples were centrifuged and the [³H]-activity in aliquots of plasma and urine analyzed according to standard laboratory procedures. Inulin clearance was then calculated as a measure of GFR.