High Sodium Intake Increases Blood Pressure and Alters Renal Function in Intrauterine Growth–Retarded Rats

Marijke W. Sanders, Gregorio E. Fazzi, Ger M.J. Janssen, Carlos E. Blanco, Jo G.R. De Mey

Abstract—A suboptimal fetal environment increases the risk to develop cardiovascular disease in the adult. We reported previously that intrauterine stress in response to reduced uteroplacental blood flow in the pregnant rat limits fetal growth and compromises renal development, leading to an altered renal function in the adult offspring. Here we tested the hypothesis that high dietary sodium intake in rats with impaired renal development attributable to intrauterine stress, results in increased blood pressure, altered renal function, and organ damage. In rats, intrauterine stress was induced by bilateral ligation of the uterine arteries at day 17 of pregnancy. At the age of 12 weeks, the offspring was given high-sodium drinking water (2% sodium chloride). At the age of 16 weeks, rats were instrumented for monitoring of blood pressure and renal function. After intrauterine stress, litter size and birth weight were reduced, whereas hematocrit at birth was increased. Renal blood flow, glomerular filtration rate, and the glomerular filtration fraction were increased significantly after intrauterine stress. High sodium intake did not change renal function and blood pressure in control animals. However, during high sodium intake in intrauterine stress offspring, renal blood flow, glomerular filtration rate, and the filtration fraction were decreased, and blood pressure was increased. In addition, these animals developed severe albuminuria, an important sign of renal dysfunction. Thus, a suboptimal fetal microenvironment, which impairs renal development, results in sodium-dependent hypertension and albuminuria. (Hypertension. 2005;46:71-75.)

Key Words: hypertension, sodium-dependent

A healthy intrauterine environment is prerequisite for normal development. A suboptimal intrauterine environment may permanently alter some tissues and organs that enable the fetus to survive in utero but cause a predisposition to (cardiovascular) disease later in life.1 Particularly, the development of the kidney can be affected by this process of fetal programming.2 A possible mechanism might be an impaired nephrogenesis as a result of a suboptimal intrauterine environment. This may lead to reduced nephron endowment during development, which is associated with high blood pressure3 and renal dysfunction4 in the adult. Previous studies in our laboratory have shown that a suboptimal intrauterine environment, induced by bilateral ligation of the uterine arteries of pregnant rats, resulted in a decreased glomerular number, an increased glomerular size, and an altered renal function in the adult offspring. In young adults, we could not demonstrate a change in blood pressure as a result of a suboptimal fetal environment, but we have shown that the renal compensatory capacity after unilateral nephrectomy is reduced.5

Not only fetal programming, but also dietary influences such as high sodium intake, increase the risk of cardiovascular and renal diseases, independently of other cardiovascular risk factors, including blood pressure.6 High sodium intake may have detrimental effects on glomerular hemodynamics by inducing glomerular hyperfiltration and increasing the filtration fraction and possibly intraglomerular pressure.7 Furthermore, high dietary sodium can induce left ventricular hypertrophy and is associated with albuminuria.6,8,9 This led us to the hypothesis that impaired renal development by intrauterine stress (IUS) results in an increased blood pressure, altered renal function, and possibly organ damage during high dietary sodium intake.

To investigate this hypothesis, IUS was induced by bilateral distal ligation of the uterine arteries at day 17 of pregnancy in Wistar rats. At the age of 12 weeks, offspring were given high-sodium drinking water (2% NaCl) for 4 weeks. At the age of 16 weeks, rats were instrumented for measuring glomerular filtration rate, renal blood flow, and blood pressure. After experimentation, animals were euthanized to determine cardiac and renal structural properties.

Methods

Experiments were approved by the local ethical committee for animal research of the University of Maastricht. Male and female Wistar rats (Charles River; Maastricht, The Netherlands) had free access to pelleted food and tap water and were maintained on a 12-hour light/dark cycle at 21°C.
Induction of IUS
Female rats were anesthetized with ketamine (40 mg/kg IP) and xylazine (3 mg/kg SC). IUS was induced at day 17 of pregnancy as described previously. The offspring of mothers that underwent bilateral distal ligation of the uterine arteries during pregnancy are referred to as IUS group, and the offspring of mothers that underwent a sham operation are referred to as control group. Rats delivered spontaneously at 23 days, and litter size and birth weight of the offspring were determined within 1 hour after birth. For identification purposes, a toe was amputated from the offspring at birth, and blood was collected with micro-hematocrit tubes (9 μL; Modulohm) to measure hematocrit values (7 minutes in hematocrit centrifuge). Immediately after this, litter size of the control group was reduced to match that of the IUS group. Because long-lasting consequences of a suboptimal fetal environment can be influenced by gender, the present study was restricted to male offspring.

High-Sodium Diet
At the age of 12 weeks, both groups of animals were given drinking water containing 2% NaCl for 4 weeks. During this period, the water intake was measured daily. Before and after 4 weeks of treatment with high sodium, animals were kept in metabolic cages for 24 hours to determine their urinary output.

Instrumentation and Renal Hemodynamics
At the age of 16 weeks, rats were anesthetized with ketamine (40 mg/kg IP) and xylazine (3 mg/kg SC). The right femoral artery and vein were cannulated for blood pressure monitoring and the measurement of inulin (inutest; Laevosan Gesellschaft) and parahippurate (PAH; MSD) clearances to evaluate renal hemodynamic function. The procedure of instrumentation and the clearance techniques were as described by Fischer et al. Measurements were performed 2 days after instrumentation in conscious unrestrained animals.

Morphometric Analysis of the Left Ventricles
Hearts of 16-week-old rats were dissected and fixed overnight in phosphate-buffered (pH 7.4) formaldehyde (4%). Tissues were transferred to 70% ethanol until further processing and eventually embedded in paraffin. Parallel transversal sections were stained with hematoxylin and eosin. Cross-sectional area of the left ventricular wall was measured by subtracting the lumen area from the area enclosed by the outer border of the left ventricle. Left ventricular wall thickness was calculated by dividing the cross-sectional area of the left ventricle by the medial area of the cross-sectional area using video images generated by a Zeiss Axioscope, a Leica DFC 280 camera, and commercially available software (Leica Qwin Pro).

Renal Histology
Kidneys of 16-week-old rats were isolated, fixed overnight in phosphate-buffered (pH 7.4) formaldehyde (4%), and embedded in paraffin. Parallel transversal sections were stained using Jones methenamine silver or periodic acid Schiffs’s solution to determine whether there was any development of sclerosis or other signs of renal damage.

Albumin and Glucose Assays
Before the experiment and at the end of the treatment with high-sodium drinking water, rats were kept in metabolic cages for 24 hours. Urine was collected and kept at −20°C until further processing. Urine samples were centrifuged (1500 rpm; 10 minutes) and diluted in distilled water. Albumin concentrations were measured with the rat albumin enzyme immunoassay obtained from SPI-BIO. Blood glucose concentrations were determined in the fasted state using the Euroflash blood glucose analyzer from Lifescan Benelux.

Data Analysis
Differences between findings in both groups of rats were tested with a 1-way ANOVA followed by Bonferroni post hoc test. A value of P<0.05 was considered statistically significant. Data are presented as mean±SEM.

Results

At Birth
Bilateral distal ligation of the uterine arteries at day 17 of pregnancy resulted in significantly reduced birth weights (5.03±0.05 g versus 5.35±0.04 g; n=137 and n=204; P<0.001) and litter size (5±1 versus 11±1; n=22 and n=16; P<0.001) in the offspring. Hematocrit at birth was increased compared with control animals (0.44±0.01 versus 0.39±0.01; n=77 and n=41; P<0.001).

Adult
Before treatment with the high-sodium diet, body weights of the control and IUS animals were not significantly different. After 4 weeks of treatment, both groups showed a similar increase in body weight (+15%), and there were no significant differences in the sodium-induced increases in water intake and urine volume between the 2 groups (Table). Blood glucose levels did not differ significantly between control and IUS animals and were not altered by high sodium intake (Table).

Renal function and hemodynamics in IUS versus control animals are presented in Figure 1. The clearances of inulin (Figure 1a) and PAH (Figure 1b) and the filtration fraction (Figure 1c, filtration/perfusion ratio) were significantly increased in IUS animals on normal drinking water. However, blood pressure was not affected (Figure 1d).

Figure 1a shows that IUS animals did not reach the same level of glomerular filtration rate during high sodium intake than on normal drinking water, whereas in control animals, the glomerular filtration rate was maintained during high

<table>
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<th>With Treatment</th>
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<tr>
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<td>IUS (n=5)</td>
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<td>Body weight (g)</td>
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<td>Water intake (g/24 hours)</td>
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<td>29±2</td>
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<td>Urine volume (mL/24 hours)</td>
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<tr>
<td>Blood glucose (mmol/L)</td>
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<tr>
<th></th>
<th>CON-HS (n=8)</th>
<th>IUS-HS (n=10)</th>
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<td>Water intake (g/24 hours)</td>
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<td>Urine volume (mL/24 hours)</td>
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<tr>
<td>Blood glucose (mmol/L)</td>
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Control rats (CON) and rats that had been exposed to IUS before and after 4 weeks of treatment with 2% NaCl in drinking water (CON– high sodium [HS] and IUS-HS). Values are mean±SEM; *P<0.05 CON vs CON-HS; †P<0.05 IUS vs IUS-HS.
sodium intake. High sodium intake also reduced renal blood flow (Figure 1b) and filtration fraction (Figure 1c) in IUS animals, whereas control animals did not show sodium-induced changes in these parameters. The decrease in glomerular filtration rate and renal blood flow in IUS animals during high sodium intake was accompanied by an increased blood pressure (Figure 1d). In addition, high sodium treatment led to a significantly increased excretion of albumin in the urine of IUS animals compared with control animals and IUS animals on normal drinking water (Figure 2).

After euthanizing the animals, we measured left ventricular wall thickness to investigate whether IUS animals are more susceptible to develop left ventricular hypertrophy during high sodium intake. However, we could not demonstrate any differences in left ventricular wall thickness between the experimental groups during high sodium treatment (CON-HS versus IUS-HS; 2359 ± 53 versus 2467 ± 43 μm).

Although we previously demonstrated a decreased glomerular number and an increased size of the glomeruli after IUS, we did not observe any additional histological signs of glomerular disease or glomerulosclerosis 4 weeks after treatment with high sodium (data not shown).

**Discussion**

IUS as a consequence of reduced uteroplacental blood flow limits fetal growth and compromises renal development, which results in an altered renal function in the adult offspring. The present study demonstrates that in IUS animals, high sodium intake leads to increased blood pressure and albuminuria. Although IUS resulted in reduced birth weight, body weights were not significantly different between control and IUS animals at 12 weeks of age. The intervention thus resulted in fetal growth retardation and postnatal catch-up growth, which have been associated with an increased risk for cardiovascular disease in the adult human. During the 4 weeks of treatment with high sodium, the body weights increased to the same extent in both groups. The weight gain

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**Figure 1.** Inulin (A) and PAH (B) clearances, filtration fraction (C), and mean arterial pressure (MAP; D) in control (CON) rats without and with 4 weeks of high sodium intake (CON-HS) and in rats exposed to IUS without (IUS) and with 4 weeks of high sodium intake (IUS-HS). GFR indicates glomerular filtration rate; RBF, renal blood flow; FF, filtration fraction. Data are mean ± SEM. *P*<0.05 IUS vs CON; †*P*<0.05 IUS vs IUS-HS; §*P*<0.05 CON-HS vs IUS-HS.

**Figure 2.** Urinary albumin excretion in control (CON) rats without (CON), and with 4 weeks of high sodium intake (CON-HS) and in rats exposed to IUS without and with 4 weeks of high sodium intake (IUS-HS). Data are mean ± SEM. ‡*P*<0.05 IUS vs CON; †*P*<0.05 IUS vs IUS-HS; §*P*<0.05 CON-HS vs IUS-HS.
between 12 and 16 weeks was to be expected because of normal growth of the animals. In both groups of animals, high sodium intake resulted in comparable increases in water intake and urinary output.

IUS alone resulted in reduced nephron endowment during renal maturation, followed by an increased glomerular filtration rate, renal blood flow, and filtration fraction in the adult.5 High sodium intake resulted in a significant decrease of these renal hemodynamic parameters. The pathophysiological mechanisms of the altered glomerular hemodynamics in response to sodium loading are not fully elucidated. Glomerular filtration is linked to the extracellular fluid volume by feedback loops that include elements such as renal nerves, the renin-angiotensin system, and natriuretic peptides or the concentration of sodium in the tubular fluid that reaches the macula densa by tubuloglomerular feedback. These feedback systems compete for the control of glomerular filtration. In the onset of diabetes, the kidneys grow and start to hyperfiltrate. It has been proposed that in diabetes, tubular hypertrophy increases sodium reabsorption in the proximal tubules and less sodium reaches the macula densa. In the normal rat, the macula densa mediates renin release by sensing decreases in luminal sodium chloride and thereby regulates afferent arteriolar tone. However, reduced salt intake increased renal blood flow in diabetic rats and caused renal vasodilatation in diabetic patients. Thomson and Vallon demonstrated that the increase in proximal reabsorption leads to a paradoxical effect of dietary sodium on glomerular filtration rate; a high sodium intake resulted in a decreased glomerular filtration rate and renal vasoconstriction in diabetic rats. They found a negative impact of dietary sodium on reabsorption upstream from the macula densa and an important decrease in proximal reabsorption. The increased sensitivity of the proximal tubules in diabetes results in a strong influence of dietary sodium on the tubuloglomerular feedback signal, implying that a higher sodium intake leads to enhanced activation of the tubuloglomerular feedback system and vice versa. In our study, we also observed that the glomerular filtration rate and renal blood flow were increased as a result of IUS, and that these parameters were reduced in response to high sodium intake. IUS impairs nephrogenesis and promotes glomerular and tubular hypertrophy and might make the tubules more sensitive to dietary sodium intake as in diabetes. Diabetes is relevant for intrauterine growth retardation and catch-up growth because Simmons et al reported that uterine artery ligation in the pregnant rat (using a protocol that differs slightly from ours) results in offspring that exhibit glucose tolerance, insulin resistance, hyperglycemia, and obesity at later stages of life. In our 16-week-old rats, the loss of pancreatic β-cell mass might not have evolved yet to result in hyperglycemia under baseline conditions. Moreover, high sodium might not only restore glomerular filtration rate to control levels via the tubuloglomerular feedback system, but it might also affect other feedback loops. They may include vasoconstriction of glomerular arterioles attributable to activation (or inadequate suppression) of neuroendocrine systems such as the renin-angiotensin system or the sympathetic nervous system. Possibly, these elements play an important role in returning renal blood flow to control levels in IUS animals. Although high sodium intake, via an increased response of renal nerves and the renin-angiotensin system locally, restores renal hemodynamic function to control levels, systemically, it might be responsible for the increased blood pressure in IUS animals.

An additional manifestation of renal dysfunction is the elevated urinary albumin concentration in IUS animals compared with control animals before and after the intake of high sodium. High sodium might be harmful to the selective permeability of the glomerular basement membrane and worsen the urinary excretion of albumin in IUS animals. In the glomerulus, filtration takes place across the capillary wall into the Bowman’s space. The wall includes 3 layers: an inner layer of glomerular endothelial cells, an outer (urinary side) layer of glomerular epithelial cells or podocytes, and, lying between the 2 cellular layers, the glomerular basement membrane. It is generally accepted that the glomerular podocyte is the cell primarily responsible for the prevention of albuminuria in health, and that podocyte damage/dysfunction underlies albuminuria in disease.26 Albumin destined for excretion appears obligated to pass through the degradation pathway within lysosomes. The majority of albumin is excreted as low—molecular weight peptide fragments, and there is evidence that albuminuria in cardiovascular disease (including diabetic kidney disease, preeclampsia, and hypertension) may be the result of factors affecting the degradation pathway of filtered albumin, rather than a primary effect on glomerular permeability. The major underlying factors associated with tissue damage and fibrosis in cardiovascular and kidney disease are the upregulation and action of growth factors such as transforming growth factor-β (TGF-β) and cytokines produced in response to changes in systemic factors, particularly blood pressure. TGF-β is linked to increased levels of intact albumin in the urine by affecting the albumin uptake and the lysosomal breakdown of filtered albumin by proximal tubular cells before excretion. Additionally, there is an interaction between TGF-β and the renin-angiotensin system. Inhibition of the renin-angiotensin system corresponds to suppression of TGF-β production but also activation of lysosomal activity. Perhaps the increase in blood pressure in IUS animals during high sodium intake raised the activity of TGF-β and increased the urinary albumin excretion. Again, more research is needed to provide evidence for this hypothesis.

It has been shown that high dietary sodium leads to the development of target organ damage such as left ventricular hypertrophy and renal fibrosis. Yet, in this study, we could not demonstrate any high sodium-induced increase of left ventricular wall mass or of renal glomerular or peritubular fibrosis. It is well possible that the duration of the treatment with sodium was not extensive enough to cause visible signs of left ventricular hypertrophy or collagen deposition in the kidneys. On the other hand, studies in sodium-sensitive hypertensive patients have described impaired left ventricular diastolic function without any differences in left ventricular wall mass compared with salt-resistant patients and irrespective of blood pressure. Thus, although these animals did not develop left ventricular
hypertrophy, this does not mean that they do not have early signs of left ventricular dysfunction.

Perspectives

Together, our results indicate that IUS and additional high sodium intake result in an altered renal function, albuminuria, and an increased blood pressure. This suggests that although IUS alone does not result in hypertension, it predisposes these animals to be more sensitive to high dietary sodium intake. An unfavorable intrauterine environment might be a factor that interferes with the response to sodium, and this could explain why some persons are more susceptible to sodium-induced effects on blood pressure than others.

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References

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