Perinatal Salt Intake Alters Blood Pressure and Salt Balance in Hypertensive Rats

Robert Di Nicolantonio, Kerryn Hoy, Sue Spargo, and Trefor O. Morgan

Blood pressure and the rate of excretion of an oral salt load were examined in spontaneously hypertensive rats of the Okamoto strain after exposure in utero and during suckling to a high salt (3% NaCl, wt/wt), low salt (0.1%), control salt (0.8%), or high potassium (1% KCl) maternal diet. After weaning, all offspring were given a diet containing 0.8% NaCl. There were small but significant differences in growth rate among offspring groups over the 60 weeks of observation, with rats exposed to perinatal low salt and high salt diet being lighter than those given control or high potassium diet. There were positive, significant correlations between body weight and blood pressure in all dietary groups at 8 weeks of age but not 16 or 24 weeks. Rats exposed to perinatal low salt diet had significantly lower blood pressures than the other three groups, which had similar blood pressures. Low salt rats also exhibited an exaggerated natriuresis after a single, oral salt load (0.15 M saline, 1% body weight) compared with the other three diet groups, which were not different from each other. High potassium rats had a reduced kaliuresis and diuresis after the salt load when compared with the other three groups.

At 60 weeks of age, rats that received perinatal low salt diet had significantly heavier adrenal glands when compared with the other groups, and the high potassium group had significantly elevated plasma renin concentrations. Thus, maternal electrolyte intake during the perinatal phase may alter body fluid homeostasis in genetically susceptible individuals at maturity.

Epidemiological studies in human infants present logistical difficulties; therefore researchers have examined the possible mechanism by which perinatal salt intake alters blood pressure and fluid balance in experimental animal models under more controlled conditions. An early study found that perinatal exposure of normotensive rats to a variety of dietary and pharmacological regimes resulted in hypertension in offspring at 12 months of age. However, we have not been able to replicate these findings in several normotensive rat strains. A more recent study found that a high perinatal salt diet exacerbated the hypertension of spontaneously hypertensive rats (SHR) if the diet was continued after weaning until maturity. In view of these findings, we examined the effect of variable salt diets confined to the in utero and perinatal phase on the blood pressure of SHR at maturity. Further, given the evidence that salt balance and renal hemodynamics may be altered in hypertension, we also examined whether such diets result in alteration in the ability to concomitantly salt load at maturity.

Methods
Male and female SHR (200–250 g) were obtained from the Biological Research Laboratories (Austin Hospital, Melbourne, Australia), maintained under
conditions of a 12-hour light/dark cycle, and received standard laboratory food (Barastoc GR2+, Melbourne, Australia) and tap water ad libitum. After tail-cuff blood pressure measurement by a method previously described,9 rats were assigned to one of four dietary groups such that the average starting blood pressures of these groups were not different. The experimental diet consisted of a corn flour/casein base with the addition of vegetable oil, essential amino acids, minerals, trace elements, and a multivitamin preparation (Pentavite, Nicholas, Melbourne, Australia). The four experimental diets consisted of this base diet with the addition of either 0.8% NaCl (control salt diet [CSD]), 3% NaCl (high salt diet [HSD]), 0.1% NaCl (low salt diet [LSD]), or 1% KCl (high potassium diet [HKD]). These dietary electrolyte levels correspond to dietary sodium/potassium ratios of 40, 141, 7, and 0.02 in the CSD, HSD, LSD, and HKD, respectively.

Rats were maintained individually on one of these diets for 2 weeks and then the blood pressure was remeasured. On the basis of these measurements, male and female rats on the same diet were paired and housed individually such that there was no difference in average parental blood pressure among dietary groups.

When pregnancy was detected, the male was removed. Litter number and pup weight were recorded 3, 10, and 28 days postpartum. The mother was continued on the same diet until the pups were weaned at 4 weeks of age. All offspring were maintained thereafter on standard laboratory fodder (Barastoc GR2+; 0.8% NaCl wt/wt). At 8 weeks of age, offspring had their blood pressure measured and were segregated by sex into boxes with three to four rats per cage. Except for litter statistics, only the data from male offspring are discussed below.

Body weight was measured weekly throughout the study, and blood pressure was measured at 12, 16, 24, and 48 weeks of age. The procedure for measurement of blood pressure was considered a significant risk past this age. At 12 weeks of age, eight randomly selected rats from each dietary group were housed in individual metabolic cages with neither food nor water. After a 2-hour control period, a urinary collection was made after the rats were given a whiff of ether and pressure was applied to the bladder.

This urine sample was discarded, and the rats were given a single oral salt load by gavage (0.15 M NaCl, 1% body weight). Cumulative urinary volume and Na+ and K+ excretion were measured 2, 4, and 6 hours after the oral saline bolus. Electrolyte concentration was measured by flame photometry (model 943, Instrumentation Laboratories, Milan, Italy).

At 16 months of age, rats were decapitated, organ weights were recorded, and trunk blood was collected for estimation of plasma Na+ concentration and plasma renin concentration (PRC).

Results presented are mean±SEM. Differences in growth rates, blood pressure, and urinary excretion among groups were assessed by analysis of variance (ANOVA) and subsequent contrasts if the F value was significant. Differences in organ weights and PRC were assessed with Student's t test and corrections for multiple comparisons were made with the Student-Newman-Keuls method.10

### Results

Parental body weights and blood pressure before and after 2 weeks of exposure to variable salt diets are shown in Table 1. There was no significant difference in parental body weight among dietary groups either before (F3,47=2.4, p>0.08) or after (F3,47=1.4, p>0.2) the 2-week period on these diets. Similarly, there was no significant difference in maternal body weight either before (F3,39=0.2, p>0.9) or after (F3,39=0.5, p>0.7) 2 weeks of exposure to the variable salt diets (Table 1). There was no significant difference in maternal blood pressure among dietary groups either before (F3,39=0.3, p>0.8) or after (F3,39=1.7, p>0.2) the 2-week period on the experimental diets. Although maternal blood pressure 10 days postpartum was highest in the HSD group and lowest in the HKD group, this difference was only of borderline significance (F3,39=2.7, p=0.06) (Table 1).

There was no significant difference in litter size (F3,45=1.5, p>0.2) or pup male/female ratio (F3,39=1.4, p=0.2) among the dietary groups (Table 2). Three-day-old male and female pups whose mothers received the HSD were significantly heavier than

### Table 1. Parental Body Weight and Blood Pressure Before and 2 Weeks After Exposure to High Salt, Low Salt, High Potassium, or Control Salt Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Male Before</th>
<th>Female Before</th>
<th>Male After</th>
<th>Female After</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSD</td>
<td>161±9</td>
<td>148±9</td>
<td>267±8</td>
<td>195±9</td>
<td>176±4</td>
<td>187±6</td>
<td>191±7</td>
<td>173±4</td>
</tr>
<tr>
<td>HSD</td>
<td>200±14</td>
<td>182±4</td>
<td>284±15</td>
<td>200±5</td>
<td>178±3</td>
<td>182±5</td>
<td>194±7</td>
<td>185±6</td>
</tr>
<tr>
<td>LSD</td>
<td>182±3</td>
<td>153±6</td>
<td>281±6</td>
<td>205±5</td>
<td>177±4</td>
<td>184±5</td>
<td>188±6</td>
<td>169±5</td>
</tr>
<tr>
<td>HKD</td>
<td>180±11</td>
<td>149±2</td>
<td>260±8</td>
<td>203±6</td>
<td>176±4</td>
<td>188±6</td>
<td>185±7</td>
<td>173±6</td>
</tr>
</tbody>
</table>

Results are mean±SEM with n=7 for both male and female breeders in each dietary group. BP, blood pressure; CSD, control salt diet; HSD, high salt diet; LSD, low salt diet; HKD, high potassium diet.
TABLE 2. Litter Size, Sex Ratios, and Pup Weights at Various Ages During Weaning

<table>
<thead>
<tr>
<th>Maternal diet</th>
<th>n</th>
<th>Litter size</th>
<th>Male/female ratio</th>
<th>Pup weight (g) 3 days</th>
<th>Pup weight (g) 10 days</th>
<th>Pup weight (g) 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSD</td>
<td>9</td>
<td>6.9±0.8</td>
<td>1.8±0.5</td>
<td>6.3±0.5</td>
<td>13.0±1.3</td>
<td>82±6</td>
</tr>
<tr>
<td>HSD</td>
<td>10</td>
<td>8.3±0.7</td>
<td>2.7±1.2</td>
<td>8.1±0.4*</td>
<td>16.8±0.8*</td>
<td>80±9</td>
</tr>
<tr>
<td>LSD</td>
<td>10</td>
<td>8.9±0.6</td>
<td>0.8±0.2</td>
<td>6.5±0.3</td>
<td>12.9±0.8</td>
<td>69±6</td>
</tr>
<tr>
<td>HKD</td>
<td>10</td>
<td>8.5±0.7</td>
<td>1.9±0.5</td>
<td>7.2±0.3</td>
<td>15.3±1.0</td>
<td>86±6</td>
</tr>
</tbody>
</table>

Results are mean±SEM. Some females in each dietary group were allowed to litter more than once with n equal to the number of litters per dietary group. CSD, control salt diet; HSD, high salt diet; LSD, low salt diet; HKD, high potassium diet.

*p<0.05 when compared with CSD and LSD.

Those receiving the LSD or CSD (Student-Newman-Keuls, p<0.05) but not rats whose mothers received the HKD (Table 2). Although 10-day-old male pups receiving the HSD were significantly heavier than those on the CSD and LSD (Student-Newman-Keuls, p<0.05), there were no significant differences among dietary groups in 10-day-old female pups. There were no significant differences in either male or female pup weights among the dietary groups at 28 days of age (Table 2). Cannibalism resulted in fewer rats being weaned than predicted from litter numbers and size.

There were small but significant (F2,92=5.0, p<0.005) differences in body weights among the different dietary groups when compared over the 60-week observation period (Figure 1). The HKD group had significantly greater body weights than the HSD (F2,92=7.1, p<0.01) and LSD (F2,92=13.1, p<0.001) groups, but rats exposed to the LSD were also significantly lighter than CSD rats (F2,92=4.6, p<0.05). More specifically, at 12 weeks of age, there were significant differences in body weight among the four dietary groups (F1,95=7.0, p<0.001). Although there were no significant differences at this age in body weight between rats exposed prenatally to the LSD or HSD (255±6 vs. 264±4 g, respectively; Student-Newman-Keuls, p>0.05) or between rats given the CSD or HSD (283±5 vs. 282±4 g, respectively; Student-Newman-Keuls, p>0.05), the LSD and HSD rats were significantly lighter than those exposed to perinatal CSD or HKD (Student-Newman-Keuls, p<0.05).

There was a significant (F1,111=8.9, p<0.001) difference in tail-cuff blood pressure among the dietary groups over the 8-48-week period of measurement (Figure 2). Subsequent ANOVA contrasts found that the LSD group had significantly lower blood pressure than the three other dietary groups (F1,111=22.2, p<0.01). There was also a significant difference (F1,111=6.05, p<0.02) in blood pressure between rats on HSD and LSD at 16 and 48 weeks of age (Figure 2).

Rats exposed to perinatal LSD had a significantly greater (F1,36=8.6, p<0.01) rate of Na+ excretion after an oral salt load when compared with the other three dietary groups, which were not significantly different (F1,36=0.2, p>0.7) to each other (Figure 3). There was also a significant difference (F1,36=4.1, p<0.02) in K+ excretion among dietary groups over the 6-hour observation period (Figure 4). The LSD group had a significantly greater rate of K+ excretion after the oral salt load than the three other dietary groups (F1,36=2.4, p<0.1) to each other (Figure 4). The HKD group had a significantly (F1,36=8.5, p<0.01) reduced diuresis after the oral salt load, when compared with the other three groups, which were not significantly different (F1,36=0.8, p>0.3) to each other (Figure 5).
At 16 months of age SHR exposed to perinatal LSD had significantly \((F_{3,54}=4.0, p<0.02)\) heavier adrenal glands when compared with the other dietary groups (Table 3). Rats that had received perinatal HKD had significantly \((F_{3,60}=4.6, p<0.01)\) elevated PRC at 16 months of age when compared with the other groups.

The relation between body weight and blood pressure in the four dietary groups at 8, 24, and 48 weeks of age is shown in Table 4. There were significant, positive correlations \((p<0.05)\) between body weight and blood pressure in rats exposed to perinatal HSD, LSD, and HKD at 8 weeks but not at 24 weeks of age (Table 4). There were no significant correlations \((p>0.05)\) between blood pressure and body weight in the CSD, LSD, or HKD groups at 48 weeks of age.

**Discussion**

The majority of studies examining the effect of dietary salt on the hypertension of the SHR have focused on the period after weaning (for review, see Reference 11). An early study by Grollman and Grollman\(^5\) claimed that perinatal treatment with a wide variety of electrolyte or drug treatments resulted in hypertension in genetically normotensive rats at 1 year of age. However, we have been unable to replicate these findings.\(^6\) A more recent study examining the effect of prenatal dietary salt in normotensive rats did not report alterations in blood pressure but found that LSD-fed rats had a significantly increased water intake at maturity.\(^12\) Conversely Contreras and Kosten\(^13\) found that perinatal high salt diet reduced the water intake of genetically normotensive rats given a choice of water and saline as drinking fluid. Although blood pressure was not reported in this study, Contreras has more recently shown that perinatal high salt diet (3% NaCl) increased blood pressure in Sprague-Dawley rats when compared with control rats given 1% NaCl.\(^14\)

The pathophysiological significance of this 9.7 mm Hg difference in blood pressure remains to be determined. Karr-Dullien and Bloomquist\(^7\) found that perinatal high salt diet in the SHR resulted in fulminating hypertension at maturity if offspring were maintained on such a diet after weaning. A comparable elevation in the perinatal salt intake of baboons resulted at maturity in significant hypertension that was exacerbated by uninephrectomy.\(^15\) Although the authors of this study suggested that this salt-induced elevation in blood pressure might be a mechanism by which excess sodium is excreted, in the present study the converse was found in that the significantly reduced blood pressure of SHR given low perinatal salt diet was associated with an increased ability to excrete sodium. Interestingly, in the Dahl salt-sensitive strain of rat, a model inbred specifically for sensitivity to dietary salt, perinatal salt intake fails to modify blood pressure at maturity.\(^16\)

In the present study, low maternal salt intake during pregnancy and lactation resulted in a significant attenuation of blood pressure in offspring throughout life, even though salt intakes after wean-
ing were not different among groups. Perinatal high salt diet failed to exacerbate the hypertension of the SHR. This is in contrast with the period after weaning where numerous studies have shown that HSD exacerbates the elevated blood pressure of this strain (for review, see Reference 11). Similarly, although a HKD has been shown to ameliorate the hypertension of the SHR\textsuperscript{17} and Dahl strain\textsuperscript{18} and reduce the incidence of stroke in the stroke-prone substrate of the SHR,\textsuperscript{19} exposure to a high maternal potassium intake in utero and during suckling failed to alter the blood pressure of offspring throughout life.

It has been reported that food restriction in the SHR, from weaning until 90 days of age, results in not only an expected reduction (40\%) in body weight but also a significant fall (14\%) in blood pressure (see Figure 1 from Wright et al\textsuperscript{20}). No change in blood pressure was observed in normotensive Sprague-Dawley rats on the same dietary regime. In the present study, the body weights of SHR given perinatal high or low salt diet were significantly reduced (7 and 10\%, respectively) when compared with control rats and these body weight differences may have contributed to the lower blood pressure in these groups. This possibility is supported by our finding of significant, positive correlations between blood pressure and body weight in the four dietary groups at 8 weeks of age. If the same relation is assumed between blood pressure and body weight reduction (0.23 mm Hg/g) as that indicated by the data in the previous study by Wright and colleagues,\textsuperscript{20} then the reduced body weights of HSD and LSD groups in the present study would result in reductions in blood pressure of 4 and 6 mm Hg, respectively, or approximately half that observed. It should be stressed, however, that the rats used in the present study were exposed to variable salt diet only in the perinatal period and were given standard laboratory fodder ad libitum thereafter. Therefore, given the possible metabolic and endocrinological changes associated with semistarvation, it is possible that the relation between blood pressure and body weight in food-restricted rats is different to that in the replete SHR used in the present study. It is of interest that significant, positive correlations between body weight and blood pressure were found at 8 but not 24 or 48 weeks of age. At early ages, the body weight increase presumably is largely due to increasing lean body mass and an associated positive electrolyte and fluid balance that may contribute to the rising blood pressure in these rats at this stage.\textsuperscript{11,21} At older ages, increases in body weight are mainly due to accumulation of body fat which may not, per se, influence blood pressure.

Low maternal salt intake during pregnancy and lactation also led to an exaggerated natriuretic response to an oral salt load in the offspring. This response was not a “pressure natriuresis” as the LSD rats had significantly lower blood pressure than the other three dietary groups. Thus, it is possible that the lower blood pressure in the rats receiving perinatal LSD may be secondary to an increased ability to excrete a dietary salt load. This possibility is strengthened by the finding that reduced renal blood flow and glomerular filtration rate are genetically linked to the elevated blood pressure of the SHR.\textsuperscript{8} It would be of interest to examine what effects a low perinatal salt intake has on renal blood flow and glomerular filtration rate in young SHR before hypertension develops. Alternatively, perinatal LSD may have induced a humoral substance altering both blood pressure and renal function.\textsuperscript{8} Or conversely perinatal LSD may have inhibited the production of a hypertensinogenic substance present in the other dietary groups. The possible role of some other “natriuretic hormone”\textsuperscript{22} or atrial natriuretic factor\textsuperscript{23} remains to be determined. Furthermore, alterations in sympathetic nerve activity,\textsuperscript{11} alterations in Ca\textsuperscript{2+} balance,\textsuperscript{24} or alterations in gonadal hormones\textsuperscript{25} cannot be ruled out.

After the oral salt load, the K\textsuperscript{+} excretion of SHR exposed to perinatal LSD was greater than that of

\begin{table}
\centering
\caption{Organ Weights, Plasma Sodium Concentration, and Plasma Renin Concentration of 16-Month-Old Spontaneously Hypertensive Rats Exposed to Perinatal Control, High Salt, Low Salt, or High Potassium Diet}\\
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Diet & \text{Heart} & \text{Kidney} & \text{Adrenal} & \text{Spleen} & \text{Plasma [Na\textsuperscript{+}] (mmol/l)} & \text{PRC (ng/ml/hr)} \\
& (g) & (g) & (mg) & (g) & & \\
\hline
CSD & 12 & 2.2±0.1 & 1.9±0.1 & 36±4 & 1.0±0.1 & 141±1 \\
LSD & 8 & 2.6±0.2 & 2.0±0.1 & 50±2* & 1.0±0.1 & 136±1 \\
HSD & 20 & 2.3±0.1 & 2.1±0.1 & 39±3 & 0.9±0.1 & 139±1 \\
HKD & 21 & 2.5±0.1 & 2.1±0.1 & 35±2 & 1.0±0.1 & 139±1 \\
\hline
\end{tabular}
\end{table}

Results are mean±SEM. [Na\textsuperscript{+}], sodium concentration; PRC, plasma renin concentration; CSD, control salt diet; LSD, low salt diet; HSD, high salt diet; HKD, high potassium diet.

\*p<0.05 when compared with CSD.

\begin{table}
\centering
\caption{Correlation Between Body Weight and Blood Pressure in Male Spontaneously Hypertensive Rats at Various Ages}\\
\begin{tabular}{|c|c|c|c|}
\hline
Diet & \text{Age (wk)} & 8 & 24 & 48 \\
\hline
CSD & 30 & 0.33 & 0.19 & 0.16 \\
HSD & 33 & 0.52* & -0.29 & 0.40\textsuperscript{+} \\
LSD & 19 & 0.49* & 0.37 & -0.13 \\
HKD & 37 & 0.45* & -0.17 & 0.08 \\
\hline
\end{tabular}
\end{table}

Rats were only exposed to the variable salt diets prenatally and up to 4 weeks of age. CSD, control salt diet; HSD, high salt diet; LSD, low salt diet; HKD, high potassium diet.

\*p<0.05.

\textit{fn}=27.
the CSD group as was the kaliuretic response to the oral salt load in rats that received the HKD. This latter difference may be related to the reduced diuretic response to the salt load in the HKD group when compared with the other three dietary groups that were not significantly different from each other.

Perinatal exposure to LSD resulted in a significantly heavier adrenal weight at 16 months of age. This is in agreement with a previous study in the normotensive rat demonstrating adrenal hypertrophy in mature rats exposed to perinatal low salt diet. It is possible that maternal low salt diet induces adrenal hyperplasia in utero, which persists throughout the lifetime of these rats. Also of interest is the lifelong elevation in plasma renin in the HKD rats, an effect seen with postnatal HKD in mature adults. Aldosterone levels and renal and adrenal histology will be required to further examine the mechanism of these changes and their possible role in the alterations in blood pressure and salt handling. These findings indicate that the mechanisms responsible for blood pressure and salt handling in the SHR are in some way related to maternal sodium intake during the prenatal and suckling period, a time before blood pressure begins to rise. The role of humoral substances or structural changes associated with the above findings, or whether the prenatal or postnatal phase is the more important remains to be determined. Furthermore, given that maternal behavior and feeding patterns may influence cardiovascular parameters in rat pups, the possible role of salt-induced changes in either maternal or pup behavior needs to be addressed. Whether these findings apply to genetically susceptible humans also remains to be determined.

Acknowledgments

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References

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