Electrolyte and Water Balance in Young Spontaneously Hypertensive Rats

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SUMMARY In metabolic balance studies the intake and excretion of sodium, potassium, and water were measured in spontaneously hypertensive rats (SHRs) of the Okamoto-Aoki strain and age-matched Wistar Kyoto rats (WKYs) that were 3 through 13 weeks of age. While fed their usual chow, young SHRs exhibited differences in excretion as compared to WKYs consuming essentially equivalent amounts of food and water. Fractional sodium and water excretion (percent of amount ingested) by SHRs were significantly less during Weeks 4-6 and 6-7, respectively, due to lower rates of urinary excretion. Potassium excretion was less in SHRs at 4-5 weeks. These observations indicate that SHRs retain more urinary sodium, potassium, and water during an early phase of hypertension than normotensive, age-matched WKYs. After 8 weeks of age, fractional excretion of electrolytes and water did not differ appreciably between strains.

In another group of rats, sodium intake was restricted and observations were made from 3 through 13 weeks of age. Although SHRs excreted slightly less sodium, cumulative sodium balance was similar in SHR and WKY. Sodium restriction slowed the increase in arterial pressure in SHRs younger than 9 weeks of age and reduced the magnitude of the hypertension in 10- through 13-week-old SHRs. At the latter age, arterial pressure was not as high in sodium-restricted SHR as in SHR on the standard sodium diet, but it was elevated above that in either WKY groups. Thus dietary sodium restriction retards the development, but does not prevent the hypertension in SHR.

Key Words • hypertension • blood pressure • kidney • sodium excretion • sodium restriction

ALTHOUGH animals with genetic or spontaneous hypertension have been studied extensively to ascertain the mechanisms initiating and maintaining hypertension in humans, our understanding of the etiology of this complex, polygenic or multifactoral disorder is incomplete. Of the several basic mechanisms that may be involved, one proposal centers on a reduced capacity of the kidneys to excrete salt and water in proper relation to intake. Some function of sodium metabolism appears to be important in the pathogenic process in the Okamoto-Aoki strain of spontaneously hypertensive rats (SHR) since chronic consumption of excess sodium chloride augments, whereas sodium restriction generally attenuates the hypertension. In addition, cross-transplantation studies indicate that a defect in renal function plays an important role in determining the level of arterial pressure.

Previous balance studies examining urinary excretion over a 1- or 3-week period provide evidence of renal dysfunction in young genetically hypertensive rats of the Milan strain (MHS) and stroke-prone sub-strain of SHR (SHRSP). Similar results were recently reported by Herlitz et al. for 7-week-old SHR as compared with normotensive Wistar rats; however, no data were presented for the appropriate genetic control, WKY.

In the present balance study, we assessed renal excretion by SHR early during the development of hypertension between the ages of 3 through 13 weeks as compared with age-matched WKY. An important aspect of our investigation was the measurement of cumulative electrolyte and water balance for 10 consecutive weeks after weaning. This minimized the possibility that subtle weekly differences between strains would escape detection. Another group of SHR and WKY was fed a low sodium diet to assess excretion in response to prolonged sodium restriction and to deter-
mine if the development of hypertension in SHR could be dissociated from enhanced retention of salt and water.

Methods

Two long-term metabolic balance studies were conducted on age-matched, male offspring of sibling-mated SHR and randomly outbred WKY.²⁰ Our local colonies originated from breeding stock supplied by Dr. Carl Hansen of the National Institutes of Health. Immediately after weaning at 3 weeks of age, two groups of SHR (generation F-41) and WKY were fed diets of differing sodium content. Fifteen SHR and 13 WKY were given a standard rat chow (Purina rat chow: sodium content, 138 μEq/g; potassium, 290 μEq/g) and were studied from 3 through 13 weeks of age. The second group consisted of six SHR and six WKY that were fed a low sodium diet (Teklad test diet: sodium, 0.5 μEq/g; potassium, 290 μEq/g) and studied from 3 through 13 weeks of age. Both chows were of similar vitamin and nutrient content. Observations were made on rats individually housed in metabolic cages located in a room with controlled temperature (22 ± 1°C), humidity (40–60%, RH), photoperiod (7 a.m.–7 p.m.), and air turnover (15 changes/hour). Rats in each dietary group were fed a uniform amount of granular chow on a given day. To assure complete consumption with minimal spillage, the amount offered was slightly less than they would have consumed ad libitum. The food ration was increased periodically to accommodate growth but still assure complete consumption.¹¹ The animals had free access to distilled water throughout the 10-week observation period. Food and water consumption were determined daily and twice weekly, respectively. Body weight was recorded twice a week.

Urine from each cage drained into a volumetric cylinder containing mineral oil. After recording urine volume, the cage baffle was rinsed with a known volume of distilled water. Feces were collected twice weekly and pooled for each strain of rats within a dietary group. Following weighing and mixing with distilled water, the homogenized samples were digested for 72 hours in 0.05 M nitric acid. Sodium and potassium concentrations of urine, feces, and chow were determined by flame photometry (Instrumentation Laboratory, Inc., Lexington, Massachusetts).

Systolic arterial pressure was estimated each week by tail-cuff plethysmography using a programmed electrophysymomanometer (Narco-Biosystems, Houston, Texas) connected to a Hewlett-Packard recorder. The system was standardized with a mercury manometer. An unanesthetized rat was restrained in a plastic or wire-mesh cage on a warmed (35°–38°C) heating table and allowed to acclimate for approximately 5 minutes. Recording values represent the mean of three consecutive readings agreeing within 10 mm Hg of each other. The accuracy of this technique was verified by comparing tail-cuff values with simultaneous direct measurements of systolic pressure recorded in a femoral artery by a Statham P23 Db pressure transducer; the ratio of tail-cuff/direct values was 1.00 ± 0.03 (sp) for pressures ranging from 80 to 200 mm Hg in 12 conscious SHR and WKY.

Presented values of electrolyte and water balance were based on weekly averages of individual animal means ± se. Sodium intake (mEq/wk) was determined from the daily consumption of chow and sodium content. Total excretion of sodium (mEq/wk) represents the combined urinary and fecal excretion. Fractional sodium excretion (%) was computed as the percentage of dietary sodium excreted in the urine and feces each week.¹¹,¹² Total cumulative sodium balance (mEq/wk · g body wt) was calculated by summing the weekly differences between intake and total excretion and then normalizing for small differences in body weight. Potassium balance was analyzed in terms of intake, absolute excretion in urine and feces, and fractional excretion. Water balance was evaluated from determinations of intake and absolute and fraction urinary excretion; these estimates do not include fecal and insensible water losses.

Student's t test for unpaired variates was performed for analysis of significance. Results are reported as being statistically significant when p values were less than 0.05.

Results

Standard Diet

Balance data for the SHR and WKY fed the standard diet are summarized in figures 1–4. As shown in figure 1, systolic arterial pressure in SHR was significantly higher than in WKY from 4.5 through 13 weeks of age.
age. During the study SHR's pressure increased from $118 \pm 11$ (se) to $191 \pm 9$ mm Hg (6.9 mm Hg/wk) as compared with a slower rise from $84 \pm 12$ to $115 \pm 7$ mm Hg (3.4 mm Hg/wk) in WKY. The arterial pressure in SHR appeared to plateau after 10 weeks of age. Between 10 and 13 weeks, arterial pressure averaged $181 \pm 4$ mm Hg in SHR and $110 \pm 3$ mm Hg in WKY ($p < 0.001$). Initial body weight was slightly but significantly less in SHR. Although SHR grew more rapidly (16 vs 13 g/wk, $p < 0.001$) over the observation period, no differences were observed after 5 weeks of age and the weight gain was uniform for each strain.

Data for sodium balance are presented in figure 2. In general, food and therefore sodium intakes were similar in SHR and WKY; small but significant differences were noted during the 5th and 6th weeks. An important finding was that SHR excreted less sodium than WKY during the initial 3 weeks of observation. This was attributed to differences in urinary sodium excretion ($2.9 \text{ vs } 3.9 \text{ mEq/wk, } p < 0.005$) as fecal losses were similar ($1.4 \text{ vs } 1.7 \text{ mEq/wk, } p > 0.4$). The tendency for SHR to excrete less sodium was reversed when SHR were 10, 11, and 13 weeks old. However, fractional excretion in the older animals differed during Week 11 only. Fecal sodium excretion never differed between SHR and WKY. It was 30% of total sodium excretion initially, thereafter progressively declining to 12% at 13 weeks. Of particular interest is the observation that the reduced excretion by SHR during the initial 3 weeks of study occurred while sodium ingestion did not differ appreciably between strains. This is further highlighted by the significant differences in fractional sodium excretion during the same time period (fig. 2). As a result of the relative retention of
sodium, SHR exhibited a greater cumulative sodium balance through 8 weeks of age. These differences were statistically significant whether expressed in absolute terms or per gram of body weight. The cumulative retention of sodium in SHR appeared to plateau after 7 weeks, whereas in WKY continued to increase at a relatively constant rate. Cumulative sodium balance did not differ statistically between strains after 9 weeks of age.

Data for potassium balance are summarized in figure 3. Overall, potassium intake was similar in SHR and WKY. Small but significant differences were observed during Weeks 5, 11, and 12. Potassium excretion by SHR was significantly lower when the animals were 4 and 5 weeks old. As was the case for sodium, this was a consequence of reduced urinary excretion (5.1 vs 7.2 mEq/wk, p < 0.001) as fecal losses (2.6 vs 2.4 mEq/wk, p > 0.2) were almost identical. The differences noted during Weeks 10, 11, and 13 reflect greater potassium excretion of SHR in both urine and feces. However, fractional potassium excretion was significantly different only at 5 weeks of age.

The pattern of urinary water excretion (fig. 4) generally paralleled that of sodium. Although SHR tended to excrete 10-15 ml/wk less from 4 through 9 weeks of age, the means for absolute water excretion were statistically different only during the 6th and 7th weeks. Likewise, fractional water excretion by SHR was significantly less by about 10% at 6 and 7 weeks of age. Analysis of cumulative water excretion also revealed that SHR retained more water than WKY during this two week period, whereas balance did not differ after the 8th week. Since cumulative balance mirrored fractional excretion and the latter is considered a more sensitive index, cumulative data are not presented.

Low Sodium Diet

Summarized in figures 5-8 are the results for SHR and WKY maintained on a low sodium diet after weaning at 3 weeks of age. As shown in figure 5, SHRs at 3.5 weeks of age were hypertensive relative to WKY as systolic arterial pressures were 121 ± 7 mm Hg and 87 ± 9 mm Hg, respectively (p < 0.05). Thereafter, continual sodium restriction retarded the development of hypertension; no significant pressure differences were detected between groups from 4.5 to 9 weeks of age. After 10 weeks of age, SHRs' pressure (135 ± 2 mm Hg) was less variable and significantly higher than that in WKY (99 ± 4 mm Hg, p < 0.001). Although the low-sodium SHR were hypertensive as compared to either age-matched, low-sodium WKY or standard diet WKY (p < 0.005 for both), their mean systolic pressure after 9 weeks of age was 25% lower (p < 0.001) than age-matched SHR consuming the standard chow. In contrast, sodium restriction had little, if any, effect on arterial pressure in WKY.

SHR and WKY on the low sodium diet had practically identical body weights each week of the study. As shown in the bottom panel of figure 5, the rate of growth was approximately 4 g/wk, markedly attenuated as compared with the 15-20 g/wk observed for age-

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**Figure 4.** Water intake, urinary water excretion, and fractional urinary water excretion as a function of age in SHR and WKY fed a standard diet. Values are means plus or minus SE. * = p < 0.05. ** = p < 0.005.

**Figure 5.** Systolic arterial pressure and body weight as a function of age in SHR and WKY fed a low sodium diet. Values are means plus or minus SE. * = p < 0.05. ** = p < 0.005.
matched rats fed the standard diet (fig. 1). The animals on the severely reduced sodium intake gradually became lethargic and poorly groomed. During the 9th week, two SHR and one WKY died; another WKY died during the 13th week.

Figure 6 presents the results for sodium balance. In general, both groups consumed similar amounts throughout the study. Very small but statistically significant differences in sodium intake were observed at 4 and 5 weeks of age. After a fall during the initial weeks of observation, total sodium excretion appeared to level off between 20–40 μEq/wk. Sodium excretion by SHR was significantly less than that by WKY from 6 to 13 weeks of age. The differences appeared to result from less fecal excretion by SHR as urinary excretion was similar in both strains at all ages studied. At 4 weeks of age, fecal excretion was 21%–22% of total sodium excretion. During the 10 weeks of sodium restriction, this percentage averaged to 44% ± 16% in WKY and 26% ± 12% in SHR. Since intakes were similar, fractional sodium excretion was also significantly lower in SHR beginning with the 6th week. However, these relatively small quantitative differences in fractional excretion were not apparent when cumulative sodium balance was analyzed. After an initial sodium loss, the magnitude of sodium retention never differed between SHR and WKY.

Data for potassium balance are shown in figure 7. Potassium intake, and absolute and fractional excretion were remarkably similar in both groups. Fractional excretion was relatively constant at 60% from 4 through 13 weeks of age, not markedly different from the 60%–80% observed in rats fed the standard diet (fig. 3).

Water consumption by SHR and WKY on the sodium-restricted diet did not differ during any of the weeks (fig. 8). Although generally comparable, absolute urinary excretion by SHR was greater during the

**Figure 6.** Sodium intake, total sodium excretion, fractional sodium excretion, and cumulative sodium balance as a function of age in SHR and WKY fed a low sodium diet. Values are means plus or minus se. * = p < 0.05. ** = p < 0.005.

**Figure 7.** Potassium intake, total potassium excretion, and fractional potassium excretion as a function of age in SHR and WKY fed a low sodium diet. Values are means plus or minus se. * = p < 0.05. ** = p < 0.005.
7th and 8th weeks. SHR also exhibited a higher fractional water excretion during weeks 6 through 8, after which it declined to WKY levels at 40%-50%, as compared with 30%-40% in rats on standard sodium intake (fig. 4).

Discussion

Our findings document that young SHR developing hypertension while maintained on their standard diet retain more sodium and water than age-matched WKY consuming similar amounts of sodium and water. The significant differences in absolute and fractional excretion observed while the animals were between 4 and 7 weeks of age can be attributed to renal mechanisms. During this period, urinary excretion was less in SHR while fecal losses were similar in both strains. Consequently, SHR retained more sodium and water than WKY from 4 to 8 weeks of age. Of course, this does not preclude the possibility that younger animals exhibit patterns of excretion similar to those found immediately after the pups were weaned. In contrast, as the animals matured and systolic arterial pressure rose above 150 mm Hg, fractional total and renal sodium excretion normalized in 8- through 13-week-old SHR.

Analysis of initial measurements from both dietary groups revealed that systolic arterial pressure was significantly higher in our SHR than in WKY from 3 to 3.5 weeks of age (117 ± 7 mm Hg (n = 21) vs 87 ± 6 mm Hg (n = 19), p < 0.005). Similar results have been reported for SHR derived from other colonies, indicating that current generations manifest a relative hypertension as early as 3 to 4 weeks of age. Thus, our observations were made early during the developmental phase rather than in a so-called prehypertensive period.

In the balance study of Bianchi et al., young MHS exhibited a period of greater positive sodium balance early during the development of hypertension than normotensive controls. As in our SHR, after the MHS were 8.5 weeks old, no differences in sodium balance were evident. Their findings concerning water balance differ from ours in that the MHS drank and excreted more water the first week after weaning and that water retention did not accompany the observed sodium retention.

Young SHRSP also have a reduced fractional urinary sodium excretion. While 6-week-old SHRSP ingested less sodium and water than WKY, they appeared to retain more sodium; fecal excretion was not measured. Recently Herlitz et al. reported that 7-week-old SHR excrete less sodium in the urine (28% vs 54% of intake) and more in the feces (32% vs 12%) than normotensive Wistar rats. Although the authors state in another communication that WKY, the appropriate genetic control, behaved as normotensive Wistar rats, no data were presented for WKY. In the present study, fecal excretion rates for sodium and potassium were consistently low, similar in SHR and WKY throughout the observation period, and comparable to those published for other rats. The reason for the discrepancy in results for fecal sodium excretion by SHR is not apparent. Herlitz et al. did not report on water balance.

Interpretation of studies on renal excretion in adult rats with genetic hypertension is complicated by the interdependency of renal salt and water excretion and arterial pressure. Several reports indicate that basal rates of excretion are similar in 12- to 18-week-old WKY and SHR with established hypertension. However, when renal perfusion pressure was reduced to the range observed in normotensive control rats, excretion by SHR was reduced. These acute studies indicate that SHR kidneys require a higher arterial pressure than kidneys of normotensive rats to excrete a given amount of salt and water under basal conditions. Our present balance findings are consistent with this view. When young SHR had a relatively low systolic arterial pressure, they excreted less sodium and water than age-matched WKY that were essentially pair-fed. After systolic pressure rose above 150 mm Hg in older SHR, sodium and water balance did not differ between strains. Clearly an in-depth understanding of the kidneys' involvement in hypertension requires a better definition of the relation between urinary excretion of salt and water and arterial pressure.

Sodium retention could initiate or contribute to the development of hypertension by interacting with a variety of mechanisms, e.g., vascular smooth muscle,

![Figure 8](https://example.com/figure8.png)
sympathetic nervous system.\textsuperscript{20} It could also produce hypertension by an initial expansion of effective extracellular fluid or plasma volume.\textsuperscript{1} There is an impressive similarity in the time course of the changes we observed and differences in body fluids and cardiac output reported by other investigators. Trippodo et al.\textsuperscript{14} found that extracellular fluid volume and total body water but not plasma volume tended to be greater in very young SHR than in weight-matched WKY. Also evident was an inverse relationship of extracellular fluid volume and total body water with body weight (arterial pressure) in SHR; WKY did not display such correlations. Perhaps even larger strain differences are present in younger rats. Until more is known about vascular and tissue compliance in young rats with genetic hypertension, absolute volumes should be interpreted with caution. Smith and Hutchins\textsuperscript{21} reported that a transient increase in cardiac index in 5- to 6-week-old SHR preceded a rise in total peripheral vascular resistance. By the time the animals were 11 and 17 weeks old, this relation had reversed.

The mechanism by which sodium retention occurs in young genetically hypertensive rats is not known. It could result from an inherent renal defect or extrarenal stimuli. Evidence supporting the hypothesis that a functional impairment of the pregglomerular and/or glomerular vasculature is involved\textsuperscript{12} is provided by recent studies. Relative to values in WKY, 6-week-old SHR have an elevated renal vascular resistance with a reduced glomerular filtration rate (GFR) and renal blood flow.\textsuperscript{22} Similarly, GFR has been reported to be lower in 6-week-old MHS.\textsuperscript{24} These important differences in renal hemodynamics may reflect an enhanced vascular reactivity to circulating vasoconstrictors in vivo such as that displayed by isolated-perfused kidneys from 4-week-old SHRSP.\textsuperscript{25} If there is enhanced tubular reabsorption, it is probably not mediated solely by aldosterone. The plasma concentration of aldosterone is reported to be lower in 7- to 8-week-old SHR\textsuperscript{26} and 12-week-old SHR\textsuperscript{27} than in WKY, and adrenalectomy does not prevent the hypertension in SHR.\textsuperscript{4} We did not observe potassium wasting associated with sodium retention. To the contrary, the lower fractional urinary excretion of sodium and potassium observed in the present study during the 4th and 5th weeks suggests a more general effect such as a low GFR and/or enhanced tubular reabsorption in segments proximal to a sodium-potassium exchange site.

Results of several studies suggest that the involvement of the sympathetic nervous system in the pathogenesis of genetic hypertension may be mediated in part by its influence on renal function. An elevated effenter renal adrenergic tone could promote urinary retention of salt and water by eliciting arteriolar constriction and/or enhancing tubular reabsorption. Electrical stimulation of the renal nerves in acute experiments enhances sodium reabsorption,\textsuperscript{24} in particular in the proximal convoluted tubule,\textsuperscript{29} and effenter renal nerve traffic as assessed by multifiber\textsuperscript{30,22} and single unit activity\textsuperscript{33} is elevated in 8- to 24-week-old SHR. Unfortunately, such recordings have not been made on younger SHR. Chronic stimulation of the renal nerves or intrarenal infusion of norepinephrine is capable of producing hypertension.\textsuperscript{34,35} Conversely, bilateral renal denervation in 5- to 8-week-old SHR delays the development of hypertension for approximately 3 weeks.\textsuperscript{36-38} The elevated fractional sodium excretion during this 3-week period when the pressure rise is attenuated provides evidence of a functional relation between the renal nerves and sodium excretion in SHR developing hypertension. Further, the subsequent rise in arterial pressure was associated with increases in renal norepinephrine content, an index of reinnervation.\textsuperscript{39} In SHRSP, bilateral renal denervation at 3 weeks of age is reported to prevent an increase in pressure for 6 weeks.\textsuperscript{5}

Restriction of dietary sodium produced an initial negative sodium balance in both SHR and WKY. It also had a dramatic effect on arterial pressure in SHR, attenuating the rate of increase markedly in animals between 3 and 8 weeks of age and also the magnitude of stable hypertension from 8 through 13 weeks. The failure of arterial pressure in sodium-restricted SHR to rise to the level observed in SHR fed their standard diet was probably not a consequence of the animals' ill health or poor general condition caused by sodium restriction. Such a nonspecific response cannot explain the observation that pressure was affected selectively in SHR while WKY pressure was essentially the same in sodium-restricted and normal sodium groups. Growth was retarded to a similar extent in SHR and WKY.

Throughout most of the study, sodium-restricted SHR tended to excrete slightly less sodium than WKY, a difference largely due to greater fecal losses in WKY. Considering the very small quantities of sodium consumed, it is not surprising that cumulative sodium balance did not differ appreciably between strains. Thus the delayed development of hypertension in SHR between 4 and 7 weeks of age was associated with a period during which SHR did not retain excess sodium because of the severe restriction of dietary sodium. In another study in which a low sodium diet was introduced to SHR at 6 weeks of age, the development of hypertension was slowed but pressure eventually reached the same level of stable hypertension as that in 16-week-old SHR fed their normal diet (unpublished observations). These findings suggest that an early period of salt retention is critical for the early development and full expression of the hypertension. However, it is clear from the present results and those reported previously\textsuperscript{24} that sodium restriction does not prevent an elevated arterial pressure in this model of genetic hypertension. This may reflect the polygenic nature of the hypertension in SHR and the involvement of several components, at least one of which is sodium-dependent.\textsuperscript{5,39}

Our observations suggest that several interacting mechanisms participate in the initiation and maintenance of hypertension in SHR. Genetically determined differences in the pattern of renal excretion may be responsible for or at least contribute to the various
levels of arterial pressure. While sodium restriction can modify the evolution of the disease process, mechanisms other than renal retention of sodium and water are also involved. The sodium or volume dependency of genetic hypertension and the relative importance of the various factors may vary according to the different phases of hypertension and environmental factors.

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