Longitudinal Changes During the Development of Hypertension in Rats Fed Excess Chloride and Sodium (43615)

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Abstract. **The effects of supplemental NaCl, KCl, and Na acetate on the blood pressure of weanling rats fed semipurified diets and diets based on naturally high salt products, like cottage cheese, were examined in two studies. Within 2 weeks of initiation of dietary treatments, rats fed supplemental chloride had elevated blood pressure and lowered plasma renin activity, which persisted throughout the 8-week study. The effect of supplemental sodium on blood pressure was not significant until after 6 weeks of dietary treatment. The initial increase in blood pressure preceded the slowed growth observed in rats fed excess chloride or sodium. Urinary volume and urinary excretion of calcium, magnesium, phosphorus, sodium, and chloride were increased when supplemental chloride or sodium was fed, but tissue electrolyte and plasma atrial natriuretic peptide concentrations remained constant. Two changes preceded the rise in blood pressure: rats fed supplemental chloride had enlarged kidneys, and those fed supplemental sodium had elevated hematocrits, suggesting a transient shift among fluid compartments, after only 6 days of treatment. These data suggest that the hypertension induced by ingestion of supplemental (14.6 mg Cl/g of diet) chloride is mediated by changes in renal function. Ingestion of excess sodium depressed bone magnesium concentrations in Study 1 and after 24 days in Study 2; the impact of this "relative" magnesium depletion on blood pressure deserves further study.** [P.S.E.B.M. 1993, Vol 203]

The association between dietary salt and blood pressure has been assumed to be related primarily to the sodium content of the salt (1, 2). However, a number of investigators have demonstrated that the ingestion of sodium chloride elevated blood pressure and depressed plasma renin activity more than the ingestion of equimolar quantities of other sodium salts in humans (3, 4) and animal models (5–9).

Despite the evidence that chloride as well as sodium may influence the etiology of hypertension, the food industry has focused efforts on reducing only the sodium content of foods. Many low salt products contain high levels of chloride, because potassium chloride is used as a salt substitute (10).

Thus, one objective of these studies was to compare the effects of supplemental sodium chloride, potassium chloride, and sodium acetate on blood pressure of rats fed semipurified diets and diets based on a natural food product. We used cottage cheese in these studies as an example of a product that contains relatively high levels of salt (1162 mg of NaCl in a half-cup serving) (11). Moreover, the sodium and chloride contents of this product could be easily adjusted. We hypothesized that the ingestion of chloride salts would have a greater effect on blood pressure than the ingestion of sodium salts, regardless of the protein source.

A second objective of these studies was to assess longitudinally the early changes in rats fed supplemental chloride in terms of plasma hormones (renin and atrial naturetic peptide), kidney function, and mineral metabolism. Previously, we demonstrated that Sprague-Dawley rats fed supplemental chloride for 7 weeks not only had elevated blood pressure, but also had increased urinary calcium excretion and enlarged kidneys (5, 6, 12, 13). Other investigators have demonstrated that plasma renin activity (14) and plasma atrial natriuretic peptide concentrations (15) responded rapidly to the infusion of sodium chloride. We hypothesized that those changes in plasma renin or atrial

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natriuretic peptide, mineral metabolism, and/or growth or kidney size and function that preceded the development of the hypertension were apt to be part of the etiology of the chloride-induced hypertension.

Materials and Methods

Experimental Design. Study 1 was a 2 × 2 × 2 factorial design in which rats were fed cottage cheese- or lactalbumin-based diets with 1.64 or 11.3 mg of Na/g diet (by analysis) and 1.87 or 14.5 mg of Cl/g diet (by analysis). The basal cottage cheese-based (Diet Basal-CC) and lactalbumin-based (Diet Basal-L) diets were supplemented with potassium chloride (Diets KCl-CC and KCl-L), sodium acetate (Diets NaA-CC and NaA-L), or sodium chloride (Diets NaCl-CC and NaCl-L) to achieve the eight dietary treatments. Rats (n = 8/treatment) were fed for 58 days.

In Study 2, rats were fed lactalbumin-based diets containing 1.63 or 12.5 mg of Na/g diet (by analysis) and 1.88 or 14.7 mg of Cl/g diet (by analysis) for 24 days. The basal diet (Diet Basal-L) was supplemented with potassium chloride (Diet KCl-L), sodium acetate (Diet NaA-L), or sodium chloride (Diet NaCl-L) to achieve the desired sodium and chloride levels. Six rats in each treatment group were sacrificed on days 6, 12, and 24.

Animals. Male weanling Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) were used in both studies. Rats were housed individually in stainless steel, wire-bottom cages in rooms maintained at 23–24°C with a 12-hr light, 12-hr dark cycle. The facilities and protocols met the requirements of an institutional animal care and use committee.

Deionized water was provided *ad libitum* throughout the study. Feed consumption was monitored daily.

Diets. One large batch of cottage cheese curd was prepared at the University of Wisconsin-Madison dairy plant according to a commercial formula, with 39.75% dressing and 60.24% curd by weight. It was split into four batches. Variations in the levels of sodium and chloride in each of the four batches were obtained by adding reagent grade sodium chloride, potassium chloride, or sodium acetate (Mallinkrodt, Inc., Paris, KY) directly into the cottage cheese dressing before addition to the curd. All four batches of cottage cheese were freeze-dried (Freeze Mobile 12 Consol with Quick Defrost, VirTis Co., Inc., Gardiner, NY) and ground to a fine powder.

All diets provided 24% protein as either cottage cheese or lactalbumin (Table I). The same batches of sodium chloride, sodium acetate, and potassium chloride that were added to the cottage cheese were added to the lactalbumin-based diets. The concentrations of sodium and chloride were equimolar in the equivalent cottage cheese- and lactalbumin-based diets. To make the lactalbumin-based diets equivalent to the cottage cheese-based diets in terms of disaccharides and fat as well as protein content, the basal AIN-76 formula (16) was altered to provide additional sucrose and corn oil, with equivalent reductions in cornstarch. Based on analysis of the freeze-dried cottage cheese and lactalbumin, the composition of the AIN-76 mineral mixture was altered so that all diets provided similar levels of calcium (Study 1, 4.24 mg of Ca/g of diet; Study 2: 4.54 mg of Ca/g of diet), phosphorus (Study 1, 4.35 mg of P/g diet; Study 2, 4.09 mg of P/g of diet), and magnesium (Study 1, 0.45 mg of Mg/g of diet; Study 2, 0.48 mg of Mg/g of diet). Diets supplemented with potassium chloride in Studies 1 and 2 contained 21.23 mg of K/g of diet. Basal diets and diets supplemented with sodium acetate or NaCl contained 4.16 mg of K/g of diet in Study 1 and 3.46 mg of K/g of diet in Study 2. The diets provided 42 mg of Zn/kg of diet and 7 mg of Cu/kg of diet.

Table I. Composition of Diets in Studies 1 and 2

Treatments	Lactalbumin basal diet[a] (%)	Cottage cheese basal diet[a] (%)
Lactalbumin[b]	27.9	—
Freeze-dried cottage cheese	—	40.4
Sucrose	35.1	30.0
Cellulose[b]	5.0	5.0
Corn oil[c]	13.6	5.0
Mineral mixture	3.5[d]	3.5[e]
AIN-76 vitamin mixture[b]	1.0	1.0
DL-Methionine[b]	0.3	0.3
Choline dihydrogen citrate[b]	0.2	0.2
Cornstarch[f]	7.5–13.4	14.6

[a] Lactalbumin-based diets were fed in Studies 1 and 2; cottage cheese-based diets were fed in Study 1 only.
[b] Teklad Test Diets (Madison, WI).
[c] ADM Packaged Oils (Decatur, IL).
[d] Modified AIN-76 mineral mixture for lactalbumin-based diets (grams/kg mineral mixture): $CaHPO_4$, 437; NaCl, 80; $K_3C_6H_5O_7 \cdot H_2O$, 220; K_2SO_4, 52; MgO, 22.3; $MnCO_3$, 3.5; ferric citrate (16.7% Fe), 6.0; $ZnCO_3$ (52% Zn), 1.10; $CuSO_4 \cdot 5H_2O$ (53–55% Cu), 0.28; KIO_3, 0.01; $Na_2SeO_3 \cdot 5H_2O$, 0.01; $CrK(SO_4)_2 \cdot 12H_2O$, 0.55.
[e] Modified AIN-76 mineral mixture for cottage cheese-based diets (grams/kg mineral mixture): $CaHPO_4$, 257; NaCl, 25; $K_3C_6H_5O_7 \cdot H_2O$, 220; K_2SO_4, 52; MgO, 12.6; $MnCO_3$, 3.5; ferric citrate (16.7% Fe), 6.0; $ZnCO_3$ (52% Zn), 0.41; $CuSO_4 \cdot 5H_2O$ (53–55% Cu), 0.24; KIO_3, 0.01; $Na_2SeO_3 \cdot 5H_2O$, 0.01; $CrK(SO_4)_2 \cdot 12H_2O$, 0.55.
[f] In Diet NaCl-L, 2.5 g NaCl; in Diet NaA-L, 5.9 g Na acetate; and in Diet KCl-L, 3.3 g KCl were substituted for an equivalent amount of cornstarch.

Systolic Blood Pressure. Systolic blood pressure measurements were made in Study 1 during weeks 1, 2, 4, 6, and 8 and in Study 2 before the study and on days 6, 12, 14, and 24. Blood pressure measurements were taken in unanesthetized rats using an indirect rat tail-cuff blood pressure system (Harvard Apparatus, South Natick, MA), as described previously (6). To increase the reliability of blood pressure measurements, rats were familiarized with the blood pressure apparatus

before measurement, and a heating pad maintained animals at an ambient temperature of 35–40°C. At least five readings were averaged for each rat.

Sample Collection. Rats were weighed twice weekly. The animals were placed in Nalgene metabolic cages for 2-day collections of urine and feces during week 5 of Study 1. Urine was acidified with 50% nitric acid (Fisher Scientific, Fair Lawn, NJ) for a final concentration of 0.5% to prevent mineral precipitation, diluted, and frozen. Feces were dried to a constant weight, cleaned of foreign adhering matter, and ground to a fine powder for analysis.

In Study 1, rats were anesthetized with methoxyflurane (Pittman-Moore, Inc., Mundelein, IL) in weeks 2, 4, and 6, and blood samples were obtained via cardiac puncture. At the conclusion of Study 1 and at each of the four time points in Study 2, rats were fasted overnight and sacrificed by exsanguination. Tissues were excised, cleansed, weighed fresh, and frozen in acid-washed plastic containers.

All blood samples were obtained via cardiac puncture using EDTA-treated syringes. Blood was collected in prechilled plastic tubes containing the proteolytic enzyme inhibitors EDTA (1.0 mg/ml; Sigma Chemical Co., St. Louis, MO) and phenylmethanesulfonylfluoride (2×10^{-5} *M*; Sigma Chemical Co.). Plasma was stored at −70°C for subsequent analysis.

Samples were analyzed for sodium, potassium, calcium, and magnesium by atomic absorption spectroscopy (model 3100, Perkin-Elmer Corp., Norwalk, CT) (17) and for chloride (18) and phosphorus (19) calorimetrically. Certified milk standard (SRM 1549) and urine standard (SRM 2670) were obtained from the National Institute of Standards and Technology. Values for chloride (107 ± 1%; $n = 28$), sodium (105 ± 2%; $n = 45$), potassium (95 ± 1%; $n = 42$), calcium (103 ± 1%; $n = 48$), magnesium (98 ± 1%; $n = 50$), and phosphorus (96 ± 1%; $n = 21$) fell within the range of the stated certified values for the milk standard. Values for chloride (104 ± 2%; $n = 6$) fell within the range of certified values for the urine standard.

Radioimmunoassays were used to monitor plasma renin activity (DuPont Co., Billerica, MA) and plasma concentrations of atrial natriuretic peptide (Peninsula Laboratories, Belmont, CA). Samples of pooled rat plasma were measured with each assay. Urinary creatinine and blood urea nitrogen concentrations were determined (20, 21).

The effects of dietary treatments were assessed within the framework of general linear models for analysis of variance (ANOVA) (22). In Study 1, the effects of protein type and chloride and sodium levels were assessed by three-way ANOVA. In Study 2, the effects of chloride and sodium levels were evaluated by two-way ANOVA. Tests for least significant differences were applied when differences among treatments were significant ($P < 0.05$) as determined by ANOVA.

Results

In Study 1, rats fed supplemental chloride had higher systolic blood pressures than those fed basal levels of chloride at weeks 2–8 (Fig. 1). Ingestion of supplemental sodium was statistically associated with elevated blood pressures at weeks 6 and 8. Rats fed the diets containing cottage cheese as a protein source had higher blood pressures than rats fed the lactalbumin-based diets at week 8. In Study 2, rats fed supplemental chloride had higher blood pressures than rats fed basal levels of chloride on days 14 and 24.

Plasma Hormones. In Study 1, rats fed supplemental chloride rather than the basal level of chloride had significantly reduced plasma renin activity by week 2; this effect was sustained through 8 weeks (Fig. 2). The ingestion of supplemental sodium rather than the basal level of sodium was associated with a significant increase in plasma renin activity at weeks 2, 6, and 8.

In Study 2, as in Study 1, rats fed supplemental chloride rather than the basal level of chloride had depressed ($P < 0.05$) plasma renin activity during week

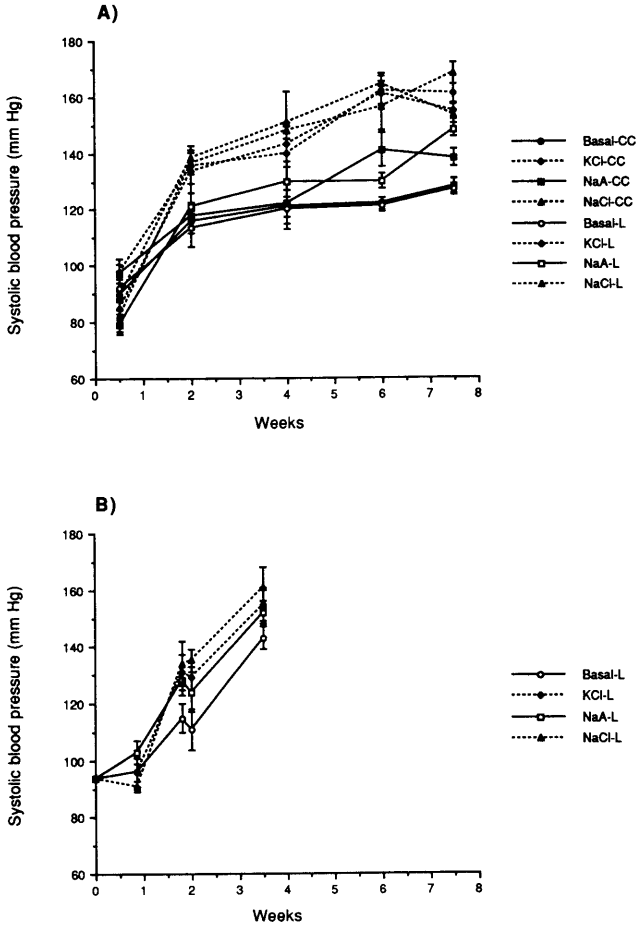

Figure 1. Blood pressures of rats fed supplemental chloride and sodium in lactalbumin- and cottage cheese-based diets in Study 1 (A) and Study 2 (B). In Study 1, ANOVA showed significant ($P < 0.05$) effects due to dietary chloride at weeks 2, 4, 6, and 8; dietary sodium at weeks 6 and 8; dietary protein source at week 8; and Na × Cl interaction at weeks 6 and 8. In Study 2, ANOVA showed significant effects due to dietary chloride at weeks 2 and 3.

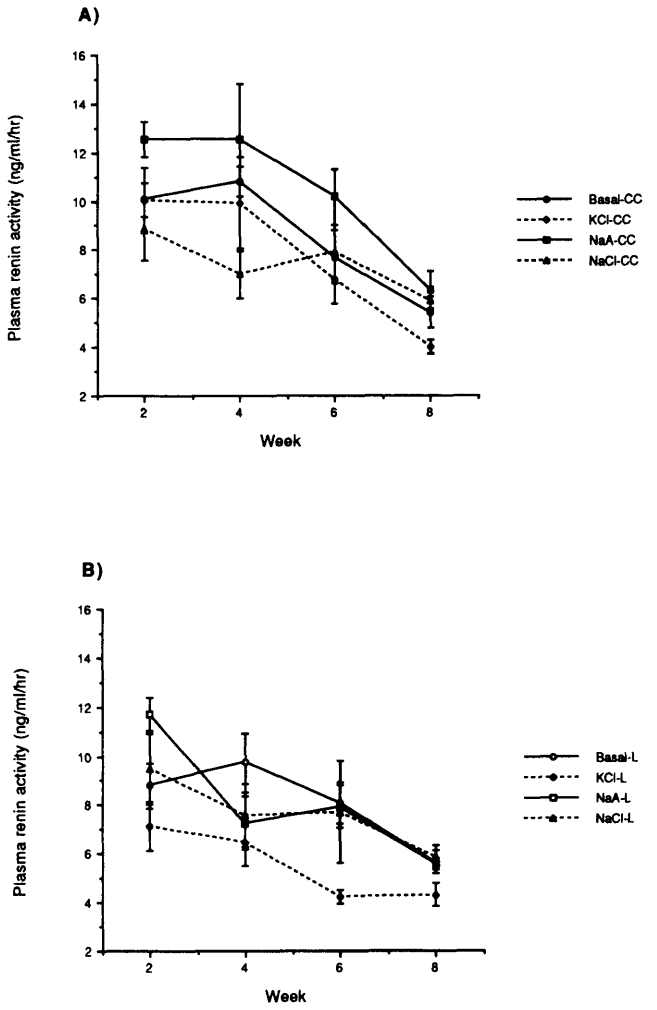

Figure 2. Plasma renin activity in rats fed supplemental chloride and sodium in lactalbumin-based diets (A) and cottage cheese-based diets (B) in Study 1. ANOVA showed significant ($P < 0.05$) effects due to dietary chloride at weeks 2, 4, 6, and 8; dietary sodium at weeks 2, 6, and 8; dietary protein source at week 4; and Na × Cl interaction at week 8.

2. Rats fed the basal and KCl-, sodium acetate-, and NaCl-supplemented diets had average (±SE) plasma renin activities of 6.6 ± 1.0, 4.2 ± 0.5, 5.8 ± 0.5, and 4.8 ± 0.7 ng/ml/hr, respectively, during week 2 (day 12). Only dietary sodium affected ($P < 0.01$) plasma renin activity during week 1. Rats fed the basal and KCl-, sodium acetate-, and NaCl-supplemented diets had average plasma renin activities of 5.3 ± 1.0, 3.1 ± 0.7, 6.8 ± 0.9, and 6.9 ± 1.0 ng/ml/hr, respectively, during week 1 (day 6). The effect of a Na × Cl interaction was significant ($P < 0.05$) only during week 3. Rats fed the basal and KCl-, sodium acetate-, and NaCl-supplemented diets had average plasma renin activities of 6.1 ± 0.4, 4.8 ± 0.5, 5.6 ± 0.9, and 7.8 ± 0.8 ng/ml/hr, respectively, during week 3 (day 24).

Plasma atrial natriuretic peptide concentrations were not significantly affected by dietary treatment at 8 weeks in Study 1 or at 1, 2, or 3 weeks in Study 2 (Table II). However, rats fed the supplemental rather than the basal level of sodium tended ($P < 0.07$) to have depressed plasma atrial natriuretic peptide concentrations during week 1 in Study 2.

Table II. Plasma ANP Levels in Rats Fed Supplemental Chloride and Sodium in Studies 1 and 2

Treatments	Study 1, 8 weeks (pmol/liter)	Study 2 (pmol/liter)		
		Week 1	Week 2	Week 3
Basal-CC	368 ± 51[a,b]			
KCl-CC	283 ± 52			
NaA-CC	272 ± 36			
NaCl-CC	282 ± 34			
Basal-L	318 ± 41[b,c]	370 ± 18	378 ± 21	385 ± 27
KCl-L	294 ± 38	317 ± 34	351 ± 24	359 ± 33
NaA-L	268 ± 34	312 ± 19	376 ± 32	326 ± 15
NaCl-L	271 ± 24	278 ± 24	370 ± 22	360 ± 22

[a] Values are the mean ± SE ($n = 8$ rats/treatment).
[b] No statistically significant interactions among treatments, as determined by ANOVA.
[c] Values are the mean ± SE ($n = 6$ rats/treatment/time).

Growth and Kidney Function. Rats weighed 57 ± 0.8 g at the beginning of Study 1 and 71 ± 0.6 g at the beginning of Study 2. By day 22 in Study 1 and day 14 in Study 2, rats fed supplemental chloride weighed significantly ($P < 0.05$) less than rats fed the basal level of chloride. After day 36 in Study 1, rats fed supplemental sodium weighed significantly ($P < 0.05$) less than rats fed the basal level of sodium. At the conclusion of Study 1 (day 58), ingestion of either supplemental chloride or sodium was associated with reduced weights in rats (Table III); at the conclusion of Study 2 (day 24), ingestion of supplemental chloride was associated with reduced weight of the rats (Table IV).

The average daily feed intake during the 58 days of Study 1 was 16.5 ± 0.1 g/day, and that during the 24 days of Study 2 was 15.5 ± 0.2 g/day. Differences in dietary chloride and sodium concentrations did not affect total feed intakes in Study 1 or 2.

In Study 1, animals fed supplemental sodium had larger kidneys (g fresh wt/100 body wt) than rats fed the basal level of sodium. Rats fed lactalbumin rather than cottage cheese also tended to have enlarged kidneys. Blood urea nitrogen, a measure of kidney function, was elevated at 8 weeks among rats fed supplemental chloride.

In Study 2, rats ingesting supplemental chloride had larger kidneys by as early as 6 days. On day 12, both dietary chloride and sodium affected kidney size, but the differences were no longer significant on day 24. Blood urea nitrogen levels were elevated by dietary chloride on day 12 and dietary sodium on day 24.

Rats fed supplemental chloride excreted more urine over a 2-day period (during week 5 of Study 1) than rats fed the basal level of chloride. The changes in urine volume could have resulted in changes in the size of fluid compartments and, hence, the early changes in

Table III. Body Weight and Kidney Size and Function in Rats Fed Supplemental Chloride and Sodium in Study 1

Treatments	Final body wt (g)	Kidney wt (g of fresh wt/100 g of body wt)	Blood urea nitrogen[a] (mmol/liter)	Urine volume (ml/2 days)
Basal-CC	377 ± 7[b,c]	0.35 ± 0.004[d]	4.3 ± 0.2[e]	25 ± 2[e]
KCl-CC	358 ± 9[c,e]	0.37 ± 0.007[e]	6.8 ± 0.5[b,c]	46 ± 4[b]
NaA-CC	366 ± 10[b,c,e]	0.38 ± 0.007[c,e]	6.7 ± 0.5[b,c]	31 ± 2[c,e]
NaCl-CC	360 ± 7[b,c,e]	0.37 ± 0.005[e]	6.2 ± 0.4[b,c]	48 ± 6[b]
Basal-L	381 ± 7[b]	0.37 ± 0.006[e]	5.8 ± 0.3[c]	27 ± 2[e]
KCl-L	357 ± 6[c,e]	0.39 ± 0.006[b,c]	6.9 ± 0.4[b]	39 ± 4[b,c]
NaA-L	363 ± 9[b,c,e]	0.40 ± 0.007[b]	6.0 ± 0.4[b,c]	32 ± 3[c,e]
NaCl-L	348 ± 8[e]	0.40 ± 0.007[b]	5.8 ± 0.2[c]	39 ± 4[b,c]
Statistical effect determined by ANOVA[f]				
Pro	NS	0.0001	NS	NS
Na	0.05	0.0001	NS	NS
Cl	0.001	NS (0.07)	0.005	0.0001
Na × Cl	NS	0.005	0.0001	NS

Values are the mean ± SE (n = 8 rats/treatment).
[a] Blood urea nitrogen measured in the fasted state.
[b–e] Means in a column not sharing a common superscript letter are significantly ($P < 0.05$) different.
[f] NS = not significant.

Table IV. Body Weight and Kidney Size and Function of Rats Fed Supplemental Chloride and Sodium in Study 2

Treatments	Body wt, day 24 (g)	Kidney size (g of fresh wt/100 g of body wt)			Blood urea nitrogen[a] (mmole/liter)		
		Day 6	Day 12	Day 24	Day 6	Day 12	Day 24
Basal-L	241 ± 3[b]	0.52 ± 0.01[c]	0.47 ± 0.02[c]	0.46 ± 0.01	3.9 ± 0.1	3.8 ± 0.2[c]	3.5 ± 4.1[c]
KCl-L	214 ± 4[c]	0.53 ± 0.01[b,c]	0.49 ± 0.01[b,c]	0.48 ± 0.01	4.7 ± 0.3	4.5 ± 0.3[b,c]	4.1 ± 0.3[b,c]
NaA-L	223 ± 4[c]	0.53 ± 0.00[b,c]	0.50 ± 0.01[b]	0.48 ± 0.01	4.6 ± 0.4	4.1 ± 0.2[c]	4.3 ± 0.2[b]
NaCl-L	226 ± 7[c]	0.56 ± 0.01[b]	0.51 ± 0.01[b]	0.48 ± 0.01	4.2 ± 0.2	5.1 ± 0.2[b]	4.5 ± 0.2[b]
Statistical effect determined by ANOVA[d]							
Na	NS	NS	0.005	NS	NS	NS (0.06)	0.05
Cl	0.05	0.05	0.05	NS	NS	0.005	NS (0.09)
Na × Cl	0.01	NS	NS	NS	NS (0.06)	NS	NS

Values are the mean ± SE (n = 6 rats/treatment/time).
[a] Blood urea nitrogen measured in fasted state.
[b,c] Means in a column not sharing a common superscript letter are significantly ($P < 0.05$) different.
[d] NS = not significant.

hematocrits noted in Study 2. Rats fed supplemental sodium had elevated hematocrits on days 6 and 12 (Table V). However, any changes in the size of fluid compartments appeared to be transient, because no long-term effects were observed. Hematocrits were not affected by dietary treatments at week 8 in Study 1 (49 ± 0.1%). Cardiac hypertrophy was not induced by the treatments. The relative sizes of hearts in rats in Study 1 on day 58 (0.36 ± 0.001 g fresh wt/100 g of body wt) or Study 2 on day 24 (0.44 ± 0.01 g fresh wt/100 g of body wt) were similar among treatments.

Electrolyte Metabolism. Apparent absorption of chloride, sodium, and potassium was very efficient (>90%), but still remained sensitive to dietary alterations (Table VI). The ingestion of supplemental sodium increased the apparent absorption of sodium and potassium. The ingestion of supplemental chloride increased the apparent absorption of chloride and potassium. Rats fed the cottage cheese-based diets absorbed sodium and potassium more efficiently than rats fed the lactalbumin-based diets.

The primary factors affecting urinary excretion of chloride and sodium were chloride and sodium intakes, respectively. Rats fed the cottage cheese-based diets excreted significantly more potassium in the urine than rats fed the lactalbumin-based diets. Overall, at 5 weeks of age, retentions ([intake-fecal-urine] × 100/intake) of chloride, sodium, and potassium by growing rats fed the basal diet were 47%, 62%, and 44%.

Despite the large variation in chloride, sodium, and

Table V. Hematocrits of Rats Fed Supplemental Chloride and Sodium in Study 2

Treatments	Day 6 (liters)	Day 12 (liters)	Day 24 (liters)
Basal-L	0.44 ± 0.008[a,b]	0.43 ± 0.003[a,b]	0.47 ± 0.015
KCl-L	0.43 ± 0.001[b]	0.42 ± 0.004[b]	0.45 ± 0.011
NaA-L	0.44 ± 0.006[a,b]	0.45 ± 0.013[a]	0.48 ± 0.009
NaCl-L	0.46 ± 0.002[a]	0.43 ± 0.004[a,b]	0.49 ± 0.008
Statistical effect determined by ANOVA[c]			
Na	0.05	0.05	NS (0.06)
Cl	NS	NS (0.06)	NS
Na × Cl	NS (0.06)	NS	NS

Values are the mean ± SE (n = 6 rats/treatment/time).
[a,b] Means in a column not sharing a common superscript letter are significantly ($P < 0.05$) different.
[c] NS = not significant.

potassium intakes, levels of chloride, sodium, and potassium remained constant in most tissues during Study 1. Sodium (68.7 ± 1.3 mmol/g [mean ± SE]) and potassium (44.0 ± 1.0 mmol/g) concentrations in the kidney were unaffected by dietary treatments. Kidney chloride concentrations, however, were significantly increased in animals fed diets containing supplemental sodium (Table VI). Dietary treatments did not affect sodium (161 ± 4 mmol/g), chloride (36.7 ± 0.2 mmol/g), or potassium (29.5 ± 0.2 mmol/g) concentrations in bone. Similarly, in Study 2, there were no differences among treatments in the concentrations of chloride, sodium, and potassium in bones or kidneys when rats were sacrificed after 6, 12, or 24 days of dietary treatments (data not shown to conserve space).

The apparent absorption of calcium and phosphorus was not affected by dietary treatments. Absorption of calcium ranged from 66% to 75% of dietary intake; the absorption of phosphorus ranged from 80% to 85% of dietary intake (data not shown in tabular form to conserve space). The ingestion of supplemental sodium increased the apparent absorption of magnesium (Table VII). Rats fed the lactalbumin-based diets absorbed more magnesium than the rats fed the cottage cheese-based diets.

Urinary excretion of calcium, phosphorus, and magnesium was significantly affected by chloride intake. Rats fed supplemental sodium also excreted more calcium and phosphorus. Rats fed lactalbumin rather than cottage cheese excreted less phosphorus and more magnesium.

Overall, rats fed the basal diet in Study 1 retained approximately 67%, 52%, and 36% of their daily intakes of calcium, phosphorus, and magnesium, respectively, at 5 weeks of age. Despite increased urinary excretion of calcium, phosphorus, and magnesium when supplemental chloride was fed, the dietary treatments did not affect kidney and bone calcium and phosphorus concentrations or kidney magnesium concentrations (kidney data not shown to conserve space).

Bone magnesium concentrations were significantly lower in rats fed supplemental sodium in Study 1 after 58 days (Table VII) and in Study 2 after 24 days (Table VIII). In Study 2, the dietary treatments had no effect on bone or kidney phosphorus concentrations after 6, 12, or 24 days of treatment. Transient changes in kidney calcium and magnesium concentrations were observed in Study 2.

Table VI. Electrolyte Utilization by Rats Fed Supplemental Chloride and Sodium in Study 1

Treatments	Apparent absorption[a] (% of intake)			Urinary excretion (% of intake)			Kidney Cl (μmol/g of fresh wt)
	Cl	Na	K	Cl	Na	K	
Basal-CC	98.7 ± 0.1[b]	97.2 ± 0.5[c]	96.3 ± 0.6[b]	50 ± 3[c]	30 ± 1[c]	59 ± 3[b–d]	53.7 ± 2.0[b–d]
KCl-CC	99.3 ± 0.2[d]	97.6 ± 0.4[b,c]	98.3 ± 0.2[d]	84 ± 5[b]	31 ± 3[c]	66 ± 3[b,d]	51.7 ± 0.6[c,e]
NaA-CC	98.4 ± 0.1[b]	98.6 ± 0.2[b,d]	94.6 ± 0.4[b,c]	50 ± 3[c]	60 ± 3[d]	69 ± 5[d]	54.2 ± 0.6[b–d]
NaCl-CC	99.3 ± 0.2[d]	98.7 ± 0.2[d]	94.9 ± 0.4[b,c]	93 ± 2[d]	68 ± 3[d]	58 ± 2[b,c]	55.9 ± 1.4[d]
Basal-L	98.8 ± 0.1[b]	95.6 ± 0.6[e]	93.7 ± 0.9[c,e]	53 ± 1[c]	39 ± 3[b,c]	43 ± 2[e]	52.8 ± 0.6[b–e]
KCl-L	99.4 ± 0.1[d]	96.8 ± 0.5[c]	98.5 ± 0.2[d]	93 ± 3[d]	44 ± 4[b]	68 ± 4[b,d]	50.6 ± 0.6[e]
NaA-L	98.6 ± 0.1[b]	98.7 ± 0.2[d]	95.1 ± 0.7[b,c]	56 ± 2[c]	66 ± 4[d]	51 ± 4[c,e]	54.8 ± 1.1[b,d]
NaCl-L	99.4 ± 0.1[d]	98.6 ± 0.2[b,d]	92.5 ± 1.1[e]	95 ± 7[d]	59 ± 5[d]	53 ± 6[c,e]	52.0 ± 1.1[b,c,e]
Statistical effect determined by ANOVA[f]							
Pro	NS	0.05	0.05	NS	NS (0.08)	0.005	NS (0.07)
Na	NS	0.0001	0.0001	NS	0.0001	NS	0.05
Cl	0.0001	NS	0.05	0.0001	NS	0.05	NS (0.07)
Na × Cl	NS	NS	0.0001	NS	NS	0.001	NS

Values are the mean ± SE (n = 8 rats/treatment).
[a] Apparent absorption = $\left(\frac{[\text{intake} - \text{fecal losses}]}{\text{intake}} \times 100\right)$.
[b–e] Means in a column not sharing a common superscript letter are significantly ($P < 0.05$) different.
[f] NS = not significant. No other interactions were statistically significant.

Table VII. Calcium, Phosphorus, and Magnesium Utilization by Rats Fed Supplemental Chloride and Sodium in Study 1

Treatments	Apparent absorption Mg[a] (% of intake)	Urinary excretion (% of intake)			Bone		
		Ca	P	Mg	Ca (mmol/g of fresh wt)	P (mmol/g of fresh wt)	Mg (μmol/g of fresh wt)
Basal-CC	$77 \pm 2^{b,c}$	$3.0 \pm 0.3^{c,d}$	$35 \pm 2^{b,c}$	$38 \pm 1^{d,e}$	4.06 ± 0.05	2.20 ± 0.06	116 ± 2^{f}
KCl-CC	75 ± 3^{c}	$3.8 \pm 0.5^{c,d}$	44 ± 1^{f}	$43 \pm 1^{c,d}$	4.00 ± 0.04	2.28 ± 0.06	109 ± 2^{b}
NaA-CC	$78 \pm 1^{b,c}$	2.3 ± 0.4^{d}	$33 \pm 2^{b,c}$	35 ± 1^{e}	4.04 ± 0.09	2.26 ± 0.07	$101 \pm 3^{c,d}$
NaCl-CC	$79 \pm 2^{b,c}$	8.2 ± 0.7^{f}	43 ± 1^{f}	$49 \pm 1^{f,b}$	4.02 ± 0.06	2.25 ± 0.10	$107 \pm 2^{b,c}$
Basal-L	$81 \pm 2^{b,f}$	$3.6 \pm 0.7^{c,d}$	$28 \pm 1^{d,e}$	$49 \pm 2^{b,f}$	4.00 ± 0.04	2.19 ± 0.08	117 ± 1^{f}
KCl-L	$80 \pm 2^{b,c}$	4.4 ± 0.6^{c}	37 ± 2^{b}	52 ± 2^{f}	4.05 ± 0.06	2.24 ± 0.09	108 ± 2^{b}
NaA-L	86 ± 1^{f}	$3.1 \pm 0.4^{c,d}$	26 ± 2^{e}	$47 \pm 2^{f,b,c}$	4.08 ± 0.04	2.24 ± 0.09	100 ± 1^{d}
NaCl-L	$82 \pm 2^{b,f}$	6.0 ± 0.6^{b}	$31 \pm 1^{c,d}$	$46 \pm 3^{b,c}$	4.10 ± 0.06	2.24 ± 0.09	108 ± 2^{b}
Statistical effect determined by ANOVA[g]							
Pro	0.001	NS	0.0001	0.0001	NS	NS	NS
Na	0.05	0.005	0.05	NS	NS	NS	0.0001
Cl	NS	0.0001	0.0001	0.0001	NS	NS	NS
Na × Cl	NS	0.0001	NS	NS	NS	NS	0.0001

Values are the mean ± SE (n = 8 rats/treatment).

[a] Apparent absorption = $\left(\frac{[\text{intake} - \text{fecal losses}]}{\text{intake}} \times 100\right)$.

[b–f] Means in a column not sharing a common superscript letter are significantly ($P < 0.05$) different.

[g] NS = not significant. No other interactions were statistically significant.

Table VIII. Calcium and Magnesium Utilization by Rats Fed Supplemental Chloride and Sodium in Study 2

Treatments	Bone Mg, day 24 (μmol/g of fresh wt)	Kidney Mg (μmol/g of fresh wt)			Kidney Ca (μmol/g of fresh wt)		
		Day 6	Day 12	Day 24	Day 6	Day 12	Day 24
Basal	92 ± 1^{a}	7.5 ± 0.3	8.0 ± 0.1	7.4 ± 0.1^{b}	0.93 ± 0.04^{a}	1.5 ± 0.1	1.8 ± 0.1^{b}
KCl	$88 \pm 3^{a,b}$	7.4 ± 0.1	7.9 ± 0.2	7.2 ± 0.2^{b}	0.80 ± 0.01^{b}	1.4 ± 0.1	1.6 ± 0.1^{b}
NaA	86 ± 2^{b}	7.6 ± 0.1	8.2 ± 0.1	7.6 ± 0.1^{a}	$0.91 \pm 0.03^{a,b}$	1.6 ± 0.2	2.2 ± 0.2^{a}
NaCl	85 ± 3^{b}	7.2 ± 0.2	8.2 ± 0.2	$7.5 \pm 0.1^{a,b}$	0.82 ± 0.08^{b}	1.4 ± 0.1	$1.9 \pm 0.2^{a,b}$
Statistical effect determined by ANOVA[c]							
Na	0.05	NS	NS	0.05	NS	NS	0.05
Cl	NS	NS	NS	NS	0.005	NS	NS (0.08)
Na × Cl	NS	NS	NS	NS	NS	NS	NS

Values are the mean ± SE (n = 6 rats/treatment/time).

[a,b] Means in a column not sharing a common superscript letter are significantly ($P < 0.05$) different.

[c] NS = not significant.

Discussion

In these studies we demonstrated that Sprague-Dawley rats fed supplemental chloride developed elevated blood pressures within 2 weeks of initiation of dietary treatments. These data confirm our hypothesis that dietary chloride had a more rapid and greater effect on blood pressure than dietary sodium. Moreover, the protein source fed (i.e., lactalbumin or cottage cheese) did not alter the effects of supplemental chloride and sodium on the early development of elevated blood pressure in these rats.

These observations were unique for several reasons. We did not use genetically sensitive or surgically or chemically altered rats as were used by others (7, 9, 14, 23, 24). The level of supplemental chloride that we fed (~14.6 mg of Cl/g of diet or ~0.4 mmol of Cl/g of diet) was more moderate than those fed by others (6–8% NaCl) (7, 8, 23) and was roughly equivalent to the amount of chloride found in human metabolic diets (0.2–0.3 mmol of Cl/g of dry diet) (25).

The effect of the dietary treatments was more rapid than we expected; in previous studies we did not begin to assess blood pressure changes until 7 weeks after the initiation of dietary treatments (5, 6). This rapid re-

sponse probably occurred because we initiated the dietary treatments shortly after the rats were weaned, and other investigators studied more mature rats (7, 9, 14, 23). Zicha *et al.* (26) observed that young (3- to 5-week-old Dahl salt-sensitive rats were more sensitive to dietary salt than mature rats.

Etiology of Chloride-Induced Hypertension. Usually, blood pressure and body weight are positively correlated in humans and animals (27), but there were no significant differences among treatments in terms of weights of rats until week 3 in Study 1. Blood pressures differed among treatments during week 2. Moreover, rats fed supplemental chloride tended to be smaller than rats fed the basal diet.

In these studies we wanted to determine whether changes in plasma renin activity occurred in response to changes in blood pressure or whether they preceded changes in blood pressure and were, therefore, part of the etiology of chloride-induced hypertension. In the first study, rats fed supplemental chloride had depressed plasma renin activity and elevated blood pressure by week 2. In Study 2, we observed significant differences in both plasma renin activity and blood pressure during week 2, but not during week 1. Thus, the antecedent occurrence could not be established. However, dietary sodium appeared to have a more rapid effect on plasma renin activity than on blood pressure. The ingestion of supplemental sodium was associated with an increase in plasma renin activity at 2 weeks and an increase in blood pressure at 6 weeks in Study 1. This suggests that the mechanisms by which sodium and chloride induce hypertension may differ, with renin being a more important mediator in the etiology of hypertension induced by sodium than in that induced by chloride.

Ingestion of supplemental chloride resulted in large (22–54%) increases in urine volume. These changes in urine volume might be expected to result in transient changes in the size of fluid compartments. Such a decrease in plasma volume could result in an elevated hematocrit. After 6 and 12 days of dietary treatment in Study 2, rats fed supplemental sodium had elevated hematocrits.

We, therefore, hypothesized that the dietary treatments might induce changes in plasma atrial natriuretic peptide (ANP), but we observed no significant difference in plasma ANP concentrations at 6, 12, or 24 days in Study 2. Perhaps we did not measure plasma ANP levels soon enough after the initiation of dietary treatments. Kato *et al.* (28) observed that plasma ANP levels were elevated when Wistar rats were fed a high salt diet for 1 day, but not when rats were fed a high salt diet for 3 days or more. The type of rat studied may also have affected the responsiveness of plasma ANP to dietary treatments. Kohno *et al.* (29) found that spontaneously hypertensive, but not Wistar-Kyoto, rats had elevated plasma ANP when given supplemental salt for 2 weeks.

When we observed in previous studies (5, 6) and in Study 1 that rats fed supplemental chloride had larger kidneys (grams/100 g of body wt) and higher blood urea nitrogen concentrations than rats fed the basal diet for 8 or more weeks, we assumed that the hypertension preceded the changes in kidney size and function. However, in Study 2 we noted kidney hypertrophy on days 6 and 12 and a tendency for the blood urea nitrogen concentration to be elevated on days 12 and 24 in rats fed supplemental sodium or chloride. This indicates that kidney changes preceded the development of hypertension in this animal model.

Based on our observations, we now hypothesize that ingestion of excess chloride induced hypertension in Sprague-Dawley rats through changes in kidney function. Transient changes in the volume of fluid compartments (as indicated by changes in hematocrits) may also have been important in the development of the hypertension.

Mineral Interactions and Hypertension. We demonstrated that rats fed supplemental chloride for 5 weeks excreted significantly more calcium, magnesium, and phosphorus in urine. This confirmed previous observations (12, 13, 30, 31). Although tissue concentrations of calcium and phosphorus were unchanged, bone magnesium concentrations were depressed in rats fed supplemental sodium in Study 1 and after 24 days in Study 2. No changes in bone magnesium were noted after 6 or 12 days of dietary treatment.

Kidney degeneration and hypokalemia are symptoms of magnesium deficiency (31, 32). However, the observed relative magnesium depletion occurred after the initial kidney hypertrophy and after the initial elevation of blood pressure induced by the ingestion of excess chloride. Thus, it is unlikely that magnesium depletion affected the early development of hypertension induced by chloride in this model.

The elevation of blood pressure that was statistically attributable to dietary sodium occurred after 6 weeks; the depression of bone magnesium concentrations that was statistically attributable to dietary sodium occurred after 24 days in Study 2. Thus, we hypothesize that ingestion of excess sodium by increasing urinary magnesium loss may have induced a relative magnesium deficiency that further exacerbated kidney degeneration and, hence, helped to maintain hypertension in this animal model. Moreover, Altura *et al.* (33) found that magnesium-deficient rats had reduced capillary, postcapillary, and venular blood flow because of constriction of blood vessels. Furthermore, magnesium has been found to counteract the effects of angiotensin II on blood pressure by preventing elevation of plasma aldosterone concentrations (34).

More work is needed to elucidate further the impact of changes in kidney function on the etiology of chloride-induced hypertension. The potential role of a relative magnesium deficiency, as induced by ingestion

of excess sodium, in the maintenance of hypertension in this model also deserves study.

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