Effects of High Calcium Intake on Blood Pressure and Calcium Metabolism in Young SHR

NAFTALI STERN, DAVID B. N. LEE, VINCENT SILIS, FRANCES W. J. BECK, LEONARD DEFTOS, STAVROS C. MANOLAGAS, AND JAMES R. SOWERS

SUMMARY Increased dietary calcium intake in the adult spontaneously hypertensive rat (SHR) has been reported to correct low serum ionized calcium concentration ([Ca++] ), and to result in a significant amelioration of the prevailing hypertension. In the present study we examined several parameters of calcium metabolism in young (6-week-old) SHR and compared them with those observed in normotensive Wistar-Kyoto (WKY) rats fed equal amounts of a diet containing normal quantities of calcium (0.4%, wt/wt) for 4 weeks. A separate group of SHR was placed on an equal amount of a high calcium (2.8%, wt/wt) but otherwise identical diet. In SHR and WKY eating a normal calcium diet, serum total calcium concentration was not different, but [Ca++] was lower in SHR (1.58 ± 0.06 vs 1.91 ± 0.07 mmol/liter, p < 0.01). Serum immunoreactive parathyroid hormone (PTH) was increased in some, but not all, SHR. No difference was noted between the two groups in the following parameters: calcium intake, serum 1,25 dihydroxycholecalciferol (1,25(OH)2D3), urinary calcium excretion, fractional stool calcium content ([stool calcium/calcium intake] × 100), and in vitro 45Ca uptake by everted gut sacs constructed from segments of duodenum, mid-jejunum, ileum, and proximal colon. A high calcium diet corrected the abnormal serum [Ca++] and PTH but did not alter the progression or severity of the hypertension in SHR. A lower net weight gain was observed in SHR on a high calcium diet when compared to SHR eating normal calcium diet (9.1 ± 1.8 vs 27.0 ± 2.0 g). This was attributed, at least in part, to a consistently higher urinary sodium loss in the former group of rats (p < 0.05-0.001 throughout the study period). The progression of hypertension, even in the face of normalized serum [Ca++] in young SHR, suggests that low [Ca++] and hypertension do not have a cause-and-effect relationship. Sustained natriuresis caused by the augmented dietary calcium intake and increased urinary calcium excretion may contribute to the blood-pressure-lowering effect reported in adult SHR. (Hypertension 6: 639-646, 1984)

KEY WORDS • calcium • 1,25(OH)2D • sodium • spontaneously hypertensive rat

ALTERATIONS in calcium homeostasis in humans with essential hypertension and in spontaneously hypertensive rats (SHR) have been described in recent years.1-3 Similar findings in human and rat hypertension include reduced serum ionized calcium concentration [Ca++] , increased immunoreactive parathyroid hormone (PTH) levels, and enhanced urinary calcium excretion. Whereas a unifying theory for the observed abnormalities has not yet been fully evolved, recent evidence suggests that high calcium diet may ameliorate hypertension in humans and in SHR.3-5 In hypertensive patients, high calcium intake may perhaps reverse a habitual, long-standing dietary deficiency in calcium intake, particularly that derived from dairy products other than milk.6 Indeed, a number of epidemiological studies have suggested a consistent inverse relationship between 'hardness of water,' presumably a measure of water calcium content, and the incidence of mortality related to cardiovascular disease, including hypertension.7,8 Whether abnormalities in calcium metabolism play a role in the initial development of high blood pressure or are merely consequences of the hypertensive process is unknown. This study investigates the effects of high calcium intake on blood pressure and calcium metabolism at the prehypertensive and early hypertensive phases in young SHR.
Materials and Methods
Thirty 5-week-old male SHR and 20 5-week-old Wistar Kyoto (WKY) rats (Charles River Breeding Laboratories, Boston, Massachusetts) were housed in individual metabolic cages under controlled temperature (26°C), humidity (45%–50%), and lighting (14:10 cycle). After delivery, all rats were given ad libitum a normal calcium (NCa) diet containing 0.4% calcium. After 1 week of habituation, rats were segregated into three experimental groups: 10 SHR and 10 WKY rats were placed on a NCa diet, and 10 SHR were given a high calcium (HCa) diet containing a 2.8% calcium. The remaining 10 SHR and 10 WKY rats were sacrificed by guillotine, and blood was collected for analysis. The data obtained from these rats were used to represent prestudy parameters at 6 weeks of age.

The basic dietary formula used in this study was a sodium-free rat diet (ICN, Cleveland, Ohio) to which NaCl has been first added to yield a final concentration of 0.5% (0.2% for Na+). Subsequently, the formula was enriched with sufficient CaCO3 to yield final concentrations of 0.4% and 2.8% calcium for the normal and high calcium diets, respectively. Potassium content of the diet was 0.55%, and phosphate content was 0.9%.

A modified "pair feeding" method was used for the calcium balance studies in the three experimental groups by assigning all rats into 10 sets that each consisted of one SHR and one WKY rat on NCa (NCa-SHR, NCa-WKY) and one SHR on HCa (HCa-SHR). The amount of food consumed by the rat that was the least amount in each set during the preceding 24 hours was then measured into the three feeding pans in the set for the following day. All rats drank distilled water ad libitum. Urine was collected daily and stools every 2 to 3 days. The duration of the balance study was 4 weeks.

Systolic blood pressure was determined 1 day prior to the beginning of the study and every 4 to 5 days thereafter. Measurements were carried out between 0900 to 1100 hours in conscious rats by tail sphygmomanometry (Narco Biosystems, Houston, Texas), with the use of a temperature-controlled restraint cage. Each measurement represented the mean of five separate readings after equilibration (the intermeasurement difference not exceeding 10 mm Hg).

Analytical Methods
Plasma total calcium was measured by titration with ethylene glycol-bis (aminoethyl ethyl) N,N'-tetraacetic acid and the use of an automatic calcium analyzer (Calkette (R), Precision Scientific Instruments, Chicago, Illinois). [Ca2+] was measured in fresh whole blood using a NOVA 2 calcium analyzer (NOVA Biomedical, Newton, Massachusetts). Each measurement represented two consecutive readings, with a coefficient of variation of 2%. Plasma PTH was measured by previously described procedures for human and bovine PTH.30,31 A chicken antiserum, raised against bovine PTH (Inelox), and acetic acid extracts of rat parathyroid glands were used at a final dilution of 1:4000. Radioiodinated bovine PTH was used as tracer, and otherwise the assay conditions were as previously described.30-31 The 1,25 dihydroxycholecalciferol [1,25(OH)2D] was measured by cytoreceptor assay.13 Urine sodium and potassium were determined by atomic absorption.

The rate of 4Ca uptake by intestinal segments obtained from the duodenum, mid-jejunum, ileum, and proximal colon was determined as previously described.29 In brief, 5- to 6-cm-long pieces from these intestinal segments were removed, everted, rinsed in ice-cold isotonic saline, and ligated with silk thread into sacs 2 to 3 cm in length. Everted sacs were incubated for 30 minutes at 37°C in continuously gassed (95% O2, 5% CO2) bicarbonate-buffered Krebs-Ringer solution containing 1.25 mM calcium, 1.18 mM phosphate, 11 mM D-glucose, and 50 uCi of 4Ca as CaCl2 (New England Nuclear, Boston, Massachusetts). Sacs were then removed from incubation, trimmed, blotted, weighed, and digested overnight in 4 ml of 0.1 N HNO3. Aliquots (1 ml) were counted in duplicate in 10 ml of ACS (Amersham Corporation, Oakville, Ontario) with the use of a Beckman LS 250 dual-window liquid scintillation spectrophotometer. The total counts per minute for each sac were divided by wet weights of tissue in milligrams. Correction for differences in specific activity of 4Ca in the incubation solutions from one experiment to another was made by expressing the isotopic uptake as a function of 106 cpm/ml of incubation medium. The units of uptake were then expressed as counts per minute per milligram of wet weight per 06 cpm/ml per 0.5 hour.

Statistical assessment was made by analysis of variance (ANOVA) that included repeated measures. Fisher least significant difference test was applied for individual timepoints when the overall analysis showed significant differences between groups. Results are expressed as means ± SEM.

Results
Body Weight and Blood Pressure
The effect of the two dietary regimens on body weight are shown in Figure 1. Mean baseline body weight was similar for the three experimental groups (116 ± 5 g for NCa-SHR, 113 ± 5 g for NCa-WKY, and 127 ± 7 g for HCa-SHR). HCa-SHR gained significantly less weight (9.1 ± 1.8 g) throughout the study period than either NCa-SHR (27 ± 2 g; p < 0.001) or NCa-WKY rats (36.6 ± 5.4 g; p < 0.001). The different weight gain was not related to differences in food intake, as these three groups did not differ in mean daily food consumption in any of the study phases or in cumulative food consumption throughout the study period. Food intake on Days 7–8 and Days 19–20 of the study is represented in Figure 1 (lower panel).

In SHR 6 weeks of age, mean systolic blood pressure, although still in the normotensive range, was already higher (p < 0.01) than that measured in WKY rats (Figure 2). The progression of hypertension in SHR was unaffected by high calcium intake. Thus,
systolic blood pressure recorded in NCa-SHR and HCa-SHR did not significantly differ from each other in any of the study phases.

Calcium Balance

Mean daily dietary calcium consumed by NCa-SHR and NCa-WKY was indistinguishable during either Weeks 1–2 or Weeks 3–4 of the study (Figure 3). Likewise, cumulative calcium intake (throughout the study period) of these two experimental groups did not differ significantly (988.1 ± 27.1 mg for NCa SHR; 1,032.6 ± 41.0 mg for NCa-WKY). Cumulative calcium intake of HCa-SHR was 6891.7 ± 47.1 mg. Mean 24-hour urine calcium excretion of NCa-SHR and NCa-WKY was similar at both early (Weeks 1–2) and late (Weeks 3–4) phases of the study (Figure 3). It can be seen that a six- to sevenfold higher calcium intake in HCa-SHR resulted in an approximately 18-fold increase in urinary calcium, reflecting the fact that when on high calcium intake, SHR excreted a much larger fraction of their dietary calcium in the urine. Mean daily fecal calcium excretion of SHR and WKY rats on normal calcium intake was indistinguishable (32.1 ± 1.9 mg/day and 31.0 ± 1.8 mg/day, respectively). Mean daily fecal excretion expressed as a percentage of daily calcium intake was 82.8% ± 1.7% for NCa-SHR, 85.5% ± 3.6% for NCa-WKY, and 90.5% ± 1.8% for HCa-SHR. Based on individual balance analyses, mean cumulative net calcium balance was 180 ± 15 mg, 218 ± 24 mg, and 279 ± 12 mg in NCa-SHR, NCa-WKY, and HCa-SHR, respectively.

Serum Calcium, PTH, and 1,25(OH)2D Concentrations

Total serum calcium concentration s determined in 10-week-old rats was comparable for the three experi-
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FIGURE 4. Total serum calcium and serum ionized calcium in the three experimental groups by 10 weeks of age. SHR on a regular calcium diet had significantly lower ionized calcium ($p < 0.01$) compared to both WKY on a regular calcium intake and SHR on a high calcium diet.

Experimental groups (Figure 4). $[\text{Ca}^{++}]$ of NCa-SHR was, however, significantly lower than that observed in NCa-WKY ($1.58 \pm 0.06$ mmol/liter vs $1.91 \pm 0.07$ mmol/liter respectively; $p < 0.01$). HCa-SHR, on the other hand, had $[\text{Ca}^{++}]$ ($1.88 \pm 0.06$ mmol/liter) that was indistinguishable from that observed in NCa-WKY. NCa-WKY rats exhibited C-terminal PTH levels consistently lower than 150 pg/ml at 6 weeks as well as at 10 weeks of age (Figure 5). In contrast, by 6 weeks of age, some SHR already disclosed higher PTH levels (three of 10 rats). By 10 weeks of age, the proportion of NCa-SHR with high PTH levels increased (five of nine rats). However, after 4 weeks of high calcium intake, all HCa-SHR had PTH levels below 150 pg/ml. No significant differences in serum $[1,25 (OH)_{2}\text{D}]$ existed between WKY or SHR at either 6 or 10 weeks of age (Figure 6).

In Vitro Uptake of $^{45}\text{Ca}$ by Gut Segments

$^{45}\text{Ca}$ uptake by everted gut sacs from duodenum mid-jejunum, ileum, and proximal colon disclosed no significant differences between SHR and WKY on normal calcium intake (Figure 7). Further, in vitro $^{45}\text{Ca}$ uptake by these gut segments obtained from HCa-SHR was also not different from those observed in the other two groups.

FIGURE 5. Serum C-terminal PTH levels of SHR and WKY rats at 6 weeks and again at 10 weeks of age, by the end of the study.

FIGURE 6. $1,25$ dihydroxycholecalciferol levels of SHR and WKY rats at 6 weeks and again at 10 weeks of age, by the end of the study (N.S. = no significant difference).
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DuOdtnum Jf lunurn Colon
FIGURE 7. In vitro uptake of 45Ca by gut segments obtained from SHR on high and regular calcium intake and WKY on regular calcium diet on the day of sacrifice (10 weeks of age).

Sodium Balance

No significant differences in sodium intake were evident among the three experimental groups (Table 1). Compared to NCa-SHR, HCa-SHR exhibited significantly higher urinary sodium excretion (p < 0.05–0.001 throughout the study period), expressed as mean daily urinary sodium excretion, sodium excretion factored by creatinine excretion, or sodium excretion as a fraction of dietary sodium intake (Figure 8). This did not result from altered renal function, as the creatinine clearance determined on the day of sacrifice was not different among the three groups (Figure 9). Similarly, creatinine excretion (mg creatinine/100 g weight) of the three groups was indistinguishable (Table 2). Cumulative sodium balance calculated as the difference between sodium intake and the sum of urinary and fecal sodium excretion was 3.92 ± 0.47 mEq for WKY rats, 1.03 ± 0.26 mEq for NCa SHR, and -2.12 ± 0.25 mEq for HCa-SHR (p < 0.001 for the comparisons between WKY and NCa-SHR, as well as between HCa-SHR and NCa-SHR).

Discussion

This study investigated calcium metabolism and blood pressure during the early phases of the evolution of high blood pressure in young SHR. Multiple investigations have emphasized the possible role of factors such as sodium intake,14 exaggerated sodium retention,14 adrenal corticosteroids,15 the renin-angiotensin system,16 and sodium potassium ATPase17 in the time course of the hypertensive disease. In most reports the

TABLE 1. Daily Sodium Consumption of Wistar-Kyoto (WKY) Rats and Spontaneously Hypertensive Rats (SHR) on Normal Calcium (NCa) and High Calcium (HiCa) Intakes

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Week 1 (mEq/day)</th>
<th>Week 2 (mEq/day)</th>
<th>Week 3 (mEq/day)</th>
<th>Week 4 (mEq/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>0.65 ± 0.04</td>
<td>0.68 ± 0.05</td>
<td>0.77 ± 0.03</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td>NCa-SHR</td>
<td>0.53 ± 0.04</td>
<td>0.73 ± 0.05</td>
<td>0.88 ± 0.04</td>
<td>0.7 ± 0.05</td>
</tr>
<tr>
<td>HiCa-SHR</td>
<td>0.53 ± 0.03</td>
<td>0.60 ± 0.04</td>
<td>0.69 ± 0.07</td>
<td>0.7 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
The evolution of hypertension could be merely decelerated but not arrested when these determinants were modified or removed. This is compatible with a multifactorial etiology for primary hypertension or with the existence of additional, yet unidentified, factors critical to the evolution of high blood pressure.

Recent studies have documented a number of abnormalities in calcium metabolism in adult SHR: reduced serum [Ca++]1, increased PTH, relative hypercalciuria,3 and altered intestinal calcium absorption.18,19 In the present study, we were able to demonstrate the existence of low [Ca++] and relatively high PTH in young SHR.

Notably, the lower Ca++ was observed in the face of apparently normal intestinal absorption and urinary excretion of calcium in SHR. In this study, calcium intake was controlled so that WKY and SHR consumed similar amounts of calcium. Moreover, possible obscure variations in calcium handling resulting from sampling errors in collection of excreta or from unexplained interdaily inconsistencies were minimized by repetitive daily monitoring of calcium intake and excretion. Under these conditions, hypercalciuria was not detected at Weeks 6–10 in SHR. It should be pointed out in this context that hypercalciuria in SHR was observed by McCarron et al.,3 only in SHR older than 17 weeks of age and long after abnormalities in [Ca++] and PTH had been fully established.

Similarly, the low [Ca++] does not seem to result from altered intestinal absorption of calcium in SHR. Results of the in vitro uptake of 45Ca by duodenal, jejunal, ileal, and colonic segments fail to disclose any difference between SHR and WKY rats. This finding appears to be at variance with reports of increased18 or suppressed19 uptake of 45Ca by duodenal segments obtained from 12-week-old SHR. Methodological differences can account for the discordant observations, as we have used shorter incubation time (30 minutes rather than 90 minutes) and have assessed total gut tissue content of 45Ca rather than the ratio of 45Ca counts in the serosal fluid to that of the mucosal fluid. Also, a potential effect of age cannot be eliminated, since Torraisons and Wright18 have also observed no difference in duodenal 45Ca uptake between SHR and WKY in rats younger than 12 weeks of age. It is noteworthy that our 1,25(OH)2D measurements and balance data are consistent with the results of the 45Ca uptake studies performed on the same rats in this study. Thus, 1,25(OH)2D level in SHR was not different18 from that observed in WKY rats, both at 6 and 10 weeks of age.

Total fecal calcium excretion expressed as a percentage of calcium intake was not different between the two rat strains, suggesting that the net in vivo absorption of calcium by the alimentary tract in young SHR and WKY rats on normal calcium intake is similar.

Thus, the low [Ca++] in the face of normal total calcium concentration is more likely to reflect changes in the binding of calcium in plasma. The reduced calcium-binding capacity of plasma membranes of erythrocytes,20,21 adipocytes,22 cardiomyocytes, and arterial smooth muscle cells,23,24 with enhanced membrane permeability to calcium,20 have been described, but whether these alterations are related to the low ionized calcium observed in serum remains to be elucidated. The role of calcium in the contractile process24 as well as in membrane stabilization20 at the vascular smooth muscle is well recognized in in vitro studies. While short-term depletion of extracellular calcium may enhance the initial, fast phase and depress the second, prolonged phase of epinephrine-stimulated vascular muscle contraction,25 it is not known whether chronic low reduction of Ca++ affects vascular contractility and blood pressure in vivo. The results of this study do not support the existence of a simple relationship between low [Ca++] and hypertension in SHR. The high calcium diet normalized [Ca++] but failed to arrest or ameliorate the high blood pressure throughout the 4 weeks of this study. This differs from the blood-pressure-lowering effect of high calcium diet in adult SHR.3 Several points need to be considered for the interpretation of the discordant outcome. First, some mechanisms involved in amelioration of high blood pressure by a modified calcium balance in adult SHR may not be fully operative in the growing rat, where a considerable fraction of the absorbed calcium is diverted to skeletal and systemic growth. Thus, skeletal demands at this age may lead to reduced in vivo availability of calcium compared to mature, nongrowing SHR for which a hypotensive effect of high calcium intake has been demonstrated. Changes in blood pressure may therefore occur subsequent to the phase studied by us, as indeed has been previously shown.3,4 Secondly, whereas modified pair-feeding as used in this study ascertains close monitoring of dietary intake and a practically equal overall intake of nutrients by the different experimental groups, the rats were not allowed to freely seek their own daily dietary intake. Theoretically, an ad libitum intake with even higher amounts of calcium consumed by HCa-SHR might have induced a hypotensive effect. However, no particular group in...
this study could be singled out as imposing dietary restriction over the two groups by a consistently lower intake. Even under these circumstances, calcium intake of HCa-SHR was sevenfold higher than in NCa-SHR, and normalization of the low [Ca++] was achieved. Finally, although high calcium intake elicited hypotensive effects in the study by McCarron et al., a significant reduction in blood pressure was not apparent until 24 weeks after the initiation of a calcium-rich diet or 18 weeks after a normalized [Ca++] had first been recorded.

The evolution of high blood pressure in the face of corrected ionized calcium levels in HCa-SHR suggests that hypertension and low ionized calcium may represent two separate genetic traits. This disparity should stimulate exploration of metabolic pathways, in addition to their normalization of [Ca++], as possible underlying mechanisms for the blood-pressure-lowering effects of a high calcium diet observed by previous investigators. A striking natriuretic effect of the high calcium diet is evidenced in the present study, as total urinary sodium excretion or urine sodium related to creatinine excretion or sodium intake were consistently higher in SHR on high calcium diet compared to SHR on regular calcium intake. This natriuretic effect is not associated with changes in glomerular filtration rate, since creatinine clearance in SHR on the two different diets was similar. In fact, enhanced sodium excretion in HCa-SHR might have been anticipated on theoretical grounds, in view of the well-known close association between the handling of sodium and calcium at the proximal tubule and the observed positive correlation between net urinary sodium and calcium excretion.

The high calcium diet in this study resulted in a sustained negative sodium balance that could account, perhaps along with a relative sodium-volume depletion, for the lower rate of weight gain and growth in HCa-SHR. Although measurements of the extracellular and blood volume were not obtained, the negative sodium balance might have led to some contraction of the extracellular and intravascular compartments. However, as the glomerular filtration rate in HCa-SHR remained preserved, any changes in blood volume were probably only moderate. Interestingly, blood pressure remained unaffected even under these circumstances of sodium wasting. However, it is not inconceivable that sustained natriuresis over lengthy periods of time, as induced by high calcium load, may finally result in or contribute to amelioration of hypertension. In fact, in a single report, calcium-enriched diet prevented the development of high blood pressure in an experimentally induced salt-dependent form of hypertension, namely, DOCA-salt hypertension. This not only suggests a possible calcium-sodium exchange but also could mean that a high calcium diet may affect blood pressure via mechanisms unrelated to unique, genetically determined abnormalities.

The effect of a high calcium diet could also involve a number of pathways that may be modulated by alterations in [Ca++]. Potential determinants other than a direct effect on the vascular smooth muscle include a direct effect of changes in calcium levels on renin and aldosterone secretion, as well as on indirect stimulation of the renin-angiotensin-aldosterone system and catecholamines secretion, via induction of a negative sodium balance. Some of these countermechanisms may have been sufficiently stimulated by the sustained natriuresis to attenuate the potential blood-pressure-lowering effect of high calcium intake. In fact, preliminary observations suggest that sodium restriction may impede the hypotensive effect of calcium-enriched diet. Changes in circulating PTH induced by the altered calcium intake could also be relevant to modulation of blood pressure by virtue of the possible vasodilatory role of this hormone. The interplay of these multiple factors awaits direct investigation and might ultimately determine the net effect of calcium intake on blood pressure.

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Hypertension. 1984;6:639-646
doi: 10.1161/01.HYP.6.5.639

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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