Evidence Against the Role of Calcium Deficiency in Genetic Hypertension

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SUMMARY  Epidemiological studies suggest an association between reduced calcium uptake and hypertension, while clinical trials and rat experiments indicate a small but significant hypotensive effect with oral calcium supplements. These data imply that calcium deficiency has a role in genetic hypertension. We reasoned that if the hypothesis is correct, the hypertension should be aggravated by further reducing calcium balance but attenuated by augmenting calcium balance. We tested this hypothesis by evaluating the blood pressure response in spontaneously hypertensive rats (SHR) as calcium balance was decreased by dietary restriction of calcium or increased by supplementation with magnesium or 1α,25-dihydroxycholecalciferol (calcitriol). A low calcium diet within the physiological range did not accentuate the hypertension in SHR during the 11 weeks of treatment, even though calcium balance was reduced by half. Similar results were obtained with dietary calcium restriction in parathyroidectomized SHR, which excludes any offsetting effects of changes in parathyroid hormone levels. Conversely, 7 weeks of a high magnesium diet, which increased calcium balance without reducing PO4 balance, did not correct the hypertension of SHR. Similarly, long-term administration of calcitriol failed to reduce the blood pressure of parathyroidectomized SHR and normotensive Wistar-Kyoto (WKY) controls, despite the presence of increased serum calcium levels comparable to those produced by oral calcium loading. Finally, external calcium balance was measured directly in 25-day-old, prehypertensive SHR. As a result of the increased calcium absorption and reduced calcium excretion, SHR retained more calcium than did the normotensive WKY, which directly refutes the existence of calcium deficiency at this normotensive stage. These data do not support the role of calcium deficiency in genetic hypertension. (Hypertension 8: 45-49, 1986)

KEY WORDS • 1α, 25-dihydroxycholecalciferol • increased gut absorption • prehypertensive spontaneously hypertensive rats

ALTHOUGH clinical1-6 and animal7-10 studies suggest that calcium deficiency has a role in genetic hypertension, a cause-and-effect relationship has not been established from epidemiological studies,7 which did not exclude many confounding variables, or from inferences using indirect indices of calcium metabolism.1-3,7-9 For example, hypercalciuria1-2,7,11-12 does not necessarily imply calcium deficiency, since the role of hypercalcemia,12 feeding (vs fasting in control),1 and increased sodium intake was never vigorously excluded.2,11 A balance study found that the hypercalciuria of spontaneously hypertensive rats (SHR) was absorptive and associated with increased calcium retention and skeletal calcium deposits.12 Similarly, the reduced serum ionized calcium in genetic hypertension may not reflect calcium deficiency because these studies did not exclude the role of respiratory alkalosis, calcium, and sodium intake.3,7,8,14 Likewise, interpreting the mildly increased serum parathyroid hormone levels in isolated samples from hypertensive subjects1-3 and SHR7 is difficult without definition of volume status and magnesium homeostasis. In one study, 24-hour urinary excretion of cyclic adenosine 3′,5′-monophosphate (cAMP) was actually decreased in SHR.12

Although perfusion studies in anesthetized SHR and in everted gut sac have yielded conflicting results,9,15 net intestinal calcium absorption in the conscious SHR was actually increased, which argues against the postulate of calcium malabsorption and calcium deficits.13
Extreme dietary calcium deprivation was associated with increased blood pressure, but firm conclusions were impossible since the concomitant secondary hyperparathyroidism and magnesium depletion also could have altered the blood pressure. Although a high calcium diet has been associated with reduced blood pressure, hypercalcemia of a comparable magnitude, achieved by long-term calcium injections, did not reproduce these results. More importantly, when the reduction in PO₄ balance that accompanied oral calcium loading was prevented, the antihypertensive effect was also abolished. In view of these uncertainties, and in view of the inability of a recent study to confirm the hypotensive effect of oral calcium supplement, the present study attempted to directly test the calcium deficiency hypothesis.

Materials and Methods

Group 1 comprised 22 male SHR (Charles River Breeding Laboratories, Wilmington, MA, USA), aged 11 weeks, equilibrated on a synthetic rat diet containing 0.87% calcium, 0.15% magnesium, and 2.2 IU of vitamin D₃ hereafter designated as a normal calcium diet. Systolic blood pressure was measured by the tail cuff method as detailed previously. At 13 weeks of age, the rats were randomized to either the normal calcium diet or a low (0.35%) calcium diet for the next 11 weeks, during which serial blood pressure was monitored. The calcium balance was determined as previously described; fasting blood calcium and magnesium levels were measured during the 5th week.

After equilibrating for 1 week on the normal calcium diet, thirty 8-week-old male SHR (Group 2) were parathyroidectomized (PTX) as previously described and allowed to recover over the ensuing 2 weeks. When the rats were 10 weeks of age, completeness of parathyroidectomy was confirmed after an overnight fast by a serum calcium level of 7.5 mg/dl or less. They were allowed a week to recover from the fast and bleeding. Between the 11th and 13th week of age, they were trained for tail cuff blood pressure measurement until a stable baseline measurement was obtained. They were then randomized into 13 pairs receiving either the normal (0.87%) or low (0.30%) calcium diet for the subsequent 7 weeks. Blood pressure and blood chemistry values were determined as for Group 1.

The 22 male SHR, aged 11 weeks, in Group 3 underwent the same procedures as Group 1 and were randomized to either a normal (0.15%) or a high (0.50%) magnesium diet for 7 weeks, during which blood pressure was compared. A high magnesium diet has been shown to augment calcium absorption and hence, calcium balance in growing rats. Serum calcium and magnesium levels were measured as in Group 1.

Group 4, comprised of 15-week-old male SHR and Wistar-Kyoto rats (WKY), was fed the normal calcium diet for 2 weeks before parathyroidectomy was attempted. Parathyroidectomy was confirmed in 19-week-old rats. They were then allowed to recover from the effects of fasting and bleeding. At 20 weeks of age, 20 stable PTX SHR and 12 PTX WKY were equilibrated respectively on high (1.35%) calcium and normal (0.87%) calcium diets and trained for tail cuff blood pressure measurements. At 23 weeks of age, the animals were injected subcutaneously with either propylene glycol vehicle (0.2 ml per rat) or calcitriol (3 ng/100 g body weight/day) for 17 days. Blood pressure and weight were compared on Days 12, 14, and 16 of treatment and the mean value of these three determinations for each animal was used for group analysis. Fasting serum calcium level was measured on Day 7 of treatment.

Group 5, comprised of 19-day-old WKY and prehypertensive SHR, was weaned and fed the normal diet for 6 days before balance studies. In separate groups of identically treated rats (n = 46), carotid cannulas were placed under light ether anesthesia at 28 days of age. After the rats had regained full consciousness and a rectal temperature of 37.0 ± 0.5 °C, direct blood pressure was recorded by a Hewlett-Packard monitor (Palo Alto, CA, USA). The sex was identified by laparotomy.

Results

In Group 1, the low calcium diet reduced calcium balance (31 ± 2 to 15 ± 1 mg/day; p < 0.001) compared with that in rats fed a normal calcium diet, primarily by decreasing absorption (32 ± 2 vs 16 ± 1 mg/day; p < 0.001). Fasting serum calcium (10 ± 0.1 vs 10 ± 0.2 mg/dl) and magnesium (2.3 ± 0.1 vs 2.1 ± 0.1 mg/dl) levels were comparable between groups. Body weight was similar before (267 ± 2 vs 264 ± 2 g), during the 5th week (339 ± 3 vs 335 ± 5 g), and after 11 weeks of diet treatment (346 ± 9 vs 348 ± 9 g), which indicates that the mildly restricted calcium diet was compatible with normal growth and normocalcemia. Systolic blood pressure was not affected during the entire 11 weeks (F < 1.0; Figure 1). Blood pressure rose similarly, as expected, with growth in the low calcium diet group (from 171 ± 4 to 220 ± 10 mm Hg) and in the control group (from 170 ± 4 to 218 ± 7 mm Hg).

Despite comparable baseline weights (225 ± 4 vs 228 ± 6 g) in Group 2, within a week of commencing the low calcium diet, anorexia, though temporary, produced marked weight loss in these PTX rats (216 ± 4 vs 259 ± 5 g; p < 0.001). To exclude a possible hypotensive action of stunted growth, food intake was restricted to 10 g/day in the control group during the 4th week of diet treatment. Four days later, body
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Figure 1. Effects of a low calcium diet on systolic blood pressure in intact SHR (Group 1). Values are means ± SE.

Figure 2. Effects of a low calcium diet on systolic blood pressure in parathyroidectomized SHR (Group 2). Values are means ± SE NS = not significant.

weight was equalized (249 ± 4 vs 252 ± 3 g) and remained so for the next 4 days (251 ± 4 vs 253 ± 3 g). Blood pressure remained similar in both groups (Figure 2). When ad libitum food intake was resumed, the difference in body weight reappeared by the 7th week of treatment (267 ± 4 vs 299 ± 5 g; p < 0.001). Blood pressure, however, remained virtually identical between groups (see Figure 2). In these PTX animals, fasting serum calcium level was reduced by the low calcium diet (4.9 ± 0.1 vs 6.2 ± 0.2 mg/dl; p < 0.001) but serum magnesium level (1.6 ± 0.1 vs 1.7 ± 0.1 mg/dl) was similar to that in animals fed the normal calcium diet.

The high magnesium diet increased fasting serum magnesium levels (2.39 ± 0.11 vs 2.14 ± 0.03 mg/dl; p < 0.03), calcium absorption (41.5 ± 1.9 vs 33 ± 3 mg/day; p < 0.02), and calcium balance (40.5 ± 2.0 vs 32 ± 2 mg/day, p < 0.02) in Group 3 rats, despite the presence of hypercalciriuria (1.00 ± 0.08 ± vs 0.71 ± 0.04 mg/day; p < 0.02). Fasting serum calcium levels tended to be increased compared with those in rats fed a normal magnesium diet, but the difference was not significant (10.3 ± 0.1 vs 10.0 ± 0.02 mg/dl). The increases in weight (69 ± 10 vs 71 ± 9 g) and blood pressure (15 ± 3 vs 14 ± 4 mm Hg) were unaffected by 7 weeks of the high magnesium diet. Systolic blood pressure was similar between groups before (170 ± 4 vs 168 ± 2 mm Hg) and during the 2nd (187 ± 3 vs 187 ± 4 mm Hg), 4th (176 ± 8 vs 174 ± 7 mm Hg), 5th (183 ± 3 vs 180 ± 4 mm Hg), and 7th week of treatment (185 ± 3 vs 182 ± 4 mm Hg).

In WKY of Group 4, vehicle and calcitriol injections were associated with comparable weight losses. Mean systolic blood pressure, determined by using the average of three separate measurements for each rat, was not affected by calcitriol in either group of WKY or SHR, even though serum calcium level was increased (Table 1).

In Group 5, direct systolic carotid blood pressure was not measurably different between conscious 28-day-old male WKY (107 ± 3 mm Hg; n = 7) and SHR (106 ± 4 mm Hg; n = 12) or between 28-day-old female WKY (103 ± 2 mm Hg; n = 15) and SHR (109 ± 3 mm Hg; n = 12). In separate groups of identically treated rats, balance studies indicated that at comparable calcium intake, the normotensive female SHR had increased net calcium absorption compared with that in WKY. They also excreted less calcium, resulting in a greater calcium retention even before the onset of hypertension (Table 2). Net calcium absorption tended to be higher in male SHR than in male WKY and reached statistical significance when expressed as a percent of ingested calcium (78 vs 72%). The calcium retention was also greater in the male SHR than in the male WKY (59.2 vs 50.8 mg/day; see Table 2).

Discussion

The calcium deficiency hypothesis, if correct, should predict a deterioration of the hypertension by decreasing calcium balance, an improvement by increasing calcium balance, and, most important, the existence of calcium deficiency in the SHR. Testing the first prediction, we found no adverse impact on blood pressure despite demonstrably reduced calcium balance with physiological calcium restriction in either
the intact or PTX SHR for up to 11 weeks of treatment once hypertension had been established. Similar studies, initiated in the prehypertensive phase of SHR, must be performed to exclude any possible deleterious influence of calcium restriction on blood pressure in the developing phase of hypertension. It is conceivable that calcium excess plays a contributory role in the pathogenesis, but more data are necessary to test this thesis.

Similar to findings with long-term CaCl₂ injections,¹⁸ the blood pressure was neither reduced in normotensive rats nor attenuated in established hypertension by magnesium or calcitriol supplement, maneuvers that are known to augment calcium absorption without reducing PO₄ balance.²⁰⁻²¹ These negative results are in sharp contrast to those found with oral calcium loading,⁵⁻⁶,¹⁸ which produced a hypotensive effect in young, growing rats only if reduced PO₄ balance was allowed to develop.¹⁸ A recent experiment revealed that a 4-week 3% calcium diet restored serum ionized calcium and parathyroid hormone levels in SHR to that found in WKY but failed to ameliorate the progressive hypertension experienced by SHR fed a 0.4% calcium diet.¹⁹ Although at odds with prior studies,⁵⁻⁶,¹¹,¹⁸ these results do not support the role of reduced serum calcium and elevated parathyroid hormone levels in genetic hypertension.

Our experiments using magnesium and calcitriol supplements yielded similarly negative findings, but they were more than confirmatory because of several important differences. First, we specifically measured calcium balance to directly establish decreases (with calcium restriction) and increases (with magnesium supplementation). Second, we augmented calcium balance by manipulations other than oral loading that are known not to reduce PO₄ balance.²⁰⁻²¹ Third, we studied PTX rats to eliminate parathyroid hormone as a confounding variable when calcium balance was altered. Fourth, most of our long-term experiments were more prolonged, extending beyond 5 to 7 weeks (Groups 1–3). Fifth, we initiated studies at or after the 13th week of age, which covers the period of established hypertension in SHR and thus extends beyond the earlier developing phase evaluated by Stern et al.¹⁹ Taken together, the results of Stern et al.¹⁹ and of our studies independently contradict the second prediction of the calcium deficiency hypothesis, namely that increasing calcium balance should reduce the blood pressure.

Preliminary studies in hypertensive humans, which also failed to document any hypotensive effects of calcitriol,²² corroborate our animal results and do not support a role of increased calcium balance or hypercalcemia in mediating the antihypertensive effects of calcium supplements. Although blood pressure fell in humans during oral CaCO₃ supplements,⁵,⁶ the effects were equivocal and inconsistent. In a double-blind, placebo-controlled trial, less than half of the hyperten-

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**Table 1. Effects of Calcitriol on Blood Pressure and Weight in Parathyroidectomized WKY and SHR**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight (g)</th>
<th>Systolic BP (mm Hg)</th>
<th>Serum Ca (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre-</td>
<td>Treatment</td>
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<tr>
<td></td>
<td>treatment</td>
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<tr>
<td>WKY</td>
<td></td>
<td></td>
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<tr>
<td>Vehicle (n = 6)</td>
<td>360 ± 22</td>
<td>339 ± 22*</td>
<td>−21.5 ± 2.6</td>
</tr>
<tr>
<td>Calcitriol (n = 6)</td>
<td>373 ± 13</td>
<td>352 ± 15†</td>
<td>−17.7 ± 6.4</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vehicle (n = 10)</td>
<td>330 ± 8</td>
<td>336 ± 8*</td>
<td>5.9 ± 1.5</td>
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<tr>
<td>Calcitriol (n = 10)</td>
<td>323 ± 10</td>
<td>319 ± 10*</td>
<td>−3.5 ± 1.5</td>
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</tbody>
</table>

Values are means ± SEM. BP = blood pressure
* p < 0.02, † p < 0.05, compared with pretreatment values
‡ p < 0.001, compared with vehicle values

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**Table 2. Calcium Metabolism in 25-Day-Old Prehypertensive SHR and in Control WKY**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ingested Ca mg/day</th>
<th>Fecal Ca mg/day</th>
<th>Net Ca absorbed mg/day</th>
<th>%</th>
<th>Urine Ca mg/day</th>
<th>%</th>
<th>Retained Ca mg/day</th>
<th>%</th>
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<tbody>
<tr>
<td>Female</td>
<td></td>
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<tr>
<td>WKY (n = 13)</td>
<td>77 ± 1</td>
<td>30.0 ± 3.0</td>
<td>47.1 ± 2.9</td>
<td>60.8 ± 3.1</td>
<td>3.19 ± 0.25</td>
<td>43.9 ± 2.9</td>
<td>56.6 ± 3.2</td>
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<tr>
<td>SHR (n = 13)</td>
<td>79 ± 2</td>
<td>23.8 ± 2.2*</td>
<td>55.4 ± 2.9*</td>
<td>69.8 ± 3.0*</td>
<td>1.84 ± 0.27†</td>
<td>53.6 ± 2.9*</td>
<td>67.4 ± 3.0§</td>
<td></td>
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<tr>
<td>Male</td>
<td></td>
<td></td>
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<tr>
<td>WKY (n = 5)</td>
<td>75 ± 3</td>
<td>21.2 ± 1</td>
<td>54 ± 2</td>
<td>72 ± 2</td>
<td>3.1 ± 1.1</td>
<td>50.8 ± 2.8</td>
<td>67.7 ± 2.6</td>
<td></td>
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<tr>
<td>SHR (n = 8)</td>
<td>79 ± 2</td>
<td>18 ± 1</td>
<td>61 ± 2</td>
<td>78 ± 1*</td>
<td>1.9 ± 0.7</td>
<td>59.2 ± 1.8§</td>
<td>75.2 ± 0.9§</td>
<td></td>
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</tbody>
</table>

Values are means ± SEM.
* p < 0.05, † p < 0.005, ‡ p < 0.03, § p < 0.02, compared with values in WKY
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...sive subjects and only 17% of normotensive subjects "responded," according to preliminary analysis. Even if statistically established, the mechanism may not be related to the undocumented increase in calcium balance, since the role of an initial natriuresis and suppressed parathyroid hormone levels could not be excluded by merely checking serum and urine chemistry values at 2- to 4-week intervals in these outpatient studies.

Evaluating the third prediction, we found that 25-day-old, prehypertensive SHR not only absorbed more calcium, similar to findings in older, adult SHR, but also excreted less calcium. This finding indicates that their external calcium balance is increased, which is contrary to the predictions made by isolated serum ionized calcium and parathyroid hormone values, and disputes the presence of calcium deficiency. It is still conceivable, though highly unlikely, that from Day 28 to Day 33 or Day 35 when definite hypertension was described, calcium balance was reduced in the SHR as a complete reversal to the changes in the first 10 days after weaning. However, persistence of the hyperabsorption has been established previously in the adult SHR by their high bone calcium content measured at 1 year of age, which refutes this possibility.

In summary, our results provide three lines of evidence arguing against the role of calcium deficiency in genetic hypertension. Although our results document transport abnormalities across the intestinal and renal epithelia, their precise role in genetic hypertension, if any, has yet to be defined.

Acknowledgments

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