Serum Calcium Fractions in Essential Hypertensive and Matched Normotensive Subjects

AARON R. FOLSOM, CHARLES L. SMITH, RONALD J. PRINEAS, AND RICHARD H. GRIMM, JR.

SUMMARY Concentrations of serum total calcium and serum calcium fractions were compared between 28 hypertensive subjects and 28 race-sex-age-matched normotensive controls. Mean levels of serum total calcium were not different between the two groups. Hypertensive subjects had lower mean serum levels of ultrafilterable calcium (-0.32 mg/dl; \( p = 0.01 \)), ionized calcium (-0.07 mg/dl; \( p = 0.09 \)), and complexed calcium (-0.23 mg/dl; \( p = 0.04 \)) and higher levels of protein-bound calcium (+0.36 mg/dl; \( p = 0.07 \)). Estimated dietary calcium intake was similar in the two groups. These findings add to the evidence that essential hypertension is associated with perturbations in calcium metabolism. (Hypertension 8: 11-15, 1986)

KEY WORDS • blood pressure • dietary calcium • hypertension

ALTERATIONS in intracellular calcium are hypothesized to be involved in the pathogenesis of essential hypertension.1,2 Recent studies indicate that extracellular calcium concentrations also may differ between hypertensive and normotensive persons.3-11 Three extracellular calcium fractions — ionized, protein-bound, and complexed calcium — are in equilibrium with one another in the serum. Ionized calcium is believed to be the physiologically active form. Protein-bound calcium is apparently inactive. The function of complexed calcium, which is complexed with small negative ions such as citrate, phosphate, and lactate, is uncertain.12 The ionized calcium and complexed calcium fractions are diffusible across semipermeable membranes and are collectively measured as ultrafilterable calcium. The purpose of the present study was to compare these serum calcium fractions between 28 mild hypertensive subjects and 28 race-sex-age-matched normotensive controls.

Subjects and Methods

Subjects were recruited from two community-based population studies in Minnesota. The Minnesota Heart Survey measured coronary risk factors in 1980 through 1982 in a probability sample of persons aged 25 to 74 years in the seven-county Minneapolis–St. Paul metropolitan area (population, 2.0 million).13 The Minnesota Heart Health Program measured risk factors through mass screening of an entire community (population, 35,000) in 1981 through 1983.14 All subjects provided informed consent. Blood pressures and a 30-second radial pulse were measured in each subject after a 5-minute rest in a quite room. Two measurements of systolic and diastolic blood pressure (fifth phase) were recorded in the right arm of seated subjects by trained technicians15 using the bell of the stethoscope. In the Minnesota Heart Survey a random-zero sphygmomanometer (Hawksley & Sons, West Sussex, UK) was used; a standard mercury sphygmomanometer (Bau- manometer 0300, W. A. Baum, Copiague, NY, USA) was employed in the Minnesota Heart Health Program. Twenty-four hour dietary recalls were obtained by trained interviewers in the Minnesota Heart Survey. Recalls were coded using Version 5 of the food table of the National Heart, Lung, and Blood Institute’s Nutrition Coding Center (Minneapolis, MN, USA).

A random sample of participants who were not taking antihypertensive medication and whose mean diastolic blood pressures were 90 mm Hg or greater were invited to participate in a blood pressure rescreening during November 1983 to January 1984. In addition, a
random group of participants whose mean diastolic blood pressures were less than 85 mm Hg were invited to serve as potential controls. Blood pressure and heart rates were re-measured using techniques identical to those of the prescreening. The hypertensive group comprised 28 subjects whose diastolic blood pressure on both occasions was greater than 90 mm Hg and who were not taking antihypertensive medication. One normotensive control was matched to each hypertensive subject. Controls were required to be of the same race and sex as the matched hypertensive subject and within 5 years of age. Controls also were required to have a diastolic blood pressure less than 85 mm Hg on both occasions and not to be taking antihypertensive medication. To ensure that both groups were representative of their respective study populations, we compared the baseline characteristics of those who were selected with all subjects eligible for the rescreening. No significant differences were observed for baseline age, systolic blood pressure, diastolic blood pressure, heart rate, education, or body mass index (kg/m²). The blood pressure values reported herein are the mean of two readings taken at the rescreening visit.

During the rescreening, all subjects completed a standardized interview ascertaining their current medical status to rule out secondary causes of hypertension and to ascertain potential illnesses that might affect blood calcium levels. Questions also were asked about calcium supplementation, vitamins, and other medication use. Usual weekly calcium intake from dairy products, which accounts for nearly 80% of calcium intake in the American diet,16 was estimated using a quantitative food frequency interview. Food models and measuring cups were used to aid in estimating portion sizes. Calcium intake was computed using the nutrient database. Usual weekly calcium intake from dairy products, which accounts for nearly 80% of calcium intake in the American diet, was estimated using a quantitative food frequency interview. Food models and measuring cups were used to aid in estimating portion sizes. Calcium intake was computed using the nutrient database.

### TABLE 1. Calcium Coding for the Food Frequency Questionnaire

<table>
<thead>
<tr>
<th>Food item</th>
<th>Estimated calcium content (mg) per 8 oz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, cream, buttermilk</td>
<td>280</td>
</tr>
<tr>
<td>Yogurt</td>
<td>300</td>
</tr>
<tr>
<td>Cheese</td>
<td>1800</td>
</tr>
<tr>
<td>Ice cream, ice milk, frozen yogurt</td>
<td>200</td>
</tr>
<tr>
<td>Custard, pudding made with milk</td>
<td>300</td>
</tr>
<tr>
<td>Cream soups made with milk</td>
<td>175</td>
</tr>
<tr>
<td>Mixed dishes made with a cheese or white sauce</td>
<td>200</td>
</tr>
</tbody>
</table>

Source of data: Agricultural Research Service

As shown in Table 3, serum total calcium concentrations were not different between hypertensive subjects and controls. The hypertensive subjects had significantly lower serum ultrafilterable calcium levels ($p = 0.01$) and borderline significantly lower serum ionized calcium levels ($p = 0.09$) compared with levels in
normalotensive controls. Calculated serum concentrations of complexed calcium were significantly lower in hypertensive subjects (p = 0.04), while protein-bound calcium concentrations were higher (p = 0.07). Serum phosphorus and albumin concentrations, as well as estimated dietary calcium intake, were not different between the two groups. Despite serum differences between the two groups, there were no significant correlations between individual systolic or diastolic blood pressure levels and serum ionized or ultrafilterable calcium levels within each group.

When analyzed separately by sex, the serum differences between matched hypertensive and normalotensive subjects proved to be similar for men and women. Only the difference in serum ionized calcium showed any sex effect (hypertensive vs normalotensive subjects: women, −0.14 mg/dl; men, −0.02 mg/dl). The difference in dietary calcium intake between hypertensive and normalotensive subjects, although not significant, was mostly attributable to differences for the men.

Dietary Calcium in the Minnesota Heart Survey

Another estimate of the relationship between dietary calcium intake and hypertension was obtained from the 24-hour dietary recall data from the Minnesota Heart Survey, a reference population for this study. Subjects were 25 to 74 years of age and were in a probability sample of a Minnesota metropolitan area (see Methods). Hypertensive subjects (diastolic blood pressure ≥ 90 mm Hg or taking antihypertensive medication) had slightly lower intake of both calcium and phosphorus than did normalotensive controls (diastolic blood pressure < 90 mm Hg and not taking antihypertensive medication), but these differences were not statistically significant (Table 4). Furthermore, the correlations of these dietary variables with systolic and diastolic blood pressures (adjusted for age, body mass, and antihypertensive medication use) were not significantly different from zero.

Discussion

Evidence is growing that calcium physiology is altered in essential hypertension, but whether this is a secondary association or a causal relationship is unresolved. Intracellular calcium ions are known to have direct effects on peripheral vascular tone,1 and it has been reported recently that hypertensive persons have increased concentrations of intracellular free calcium.
that decrease to normal levels with antihypertensive
treatment.\textsuperscript{19} Data from animal models suggest further
that with hypertension smooth muscle is hyperresponsive
to changes in extracellular calcium concentrations
and that the vascular membrane permeability to calcium is increased.\textsuperscript{1} Blaustein\textsuperscript{2} hypothesizes that the
increase in intracellular calcium levels in hypertension is due to altered sodium-calcium exchange across the
acellular membrane of smooth muscle.

Epidemiological and clinical studies to measure the
effect of calcium on blood pressure are difficult, in part
because other factors, such as sodium, potassium,
magnesium, parathyroid hormone, and renin, may influence
the calcium–blood pressure association. Studies such as this investigation are needed to establish
differences in dietary calcium and serum calcium frac-
tions between hypertensive and normotensive persons
in the general population. The weaknesses of the present
investigation are its relatively small sample size, its
failure to measure the aforementioned factors, and the
unmatched differences that were observed between hypo-
ertensive subjects and controls (see Table 2). Sodium
and potassium intake were not measured but could
confound our results, because serum calcium levels have been reported to be negatively correlated with 24-
hour sodium excretion and positively correlated with potassium excretion.\textsuperscript{20} The higher heart rate in hyper-
tensive subjects observed here has been noted fre-
cently in epidemiological studies, but it seems unlikely to be related to the effects of calcium. The
erudication difference observed between hypertensive and normotensive subjects was unanticipated, but it
also would seem to be an unlikely explanation of our findings. The higher prevalence of antacid intake of
hypertensive subjects could have influenced our results, since antacids can raise the serum levels of their
major component cations if intake is heavy or if renal insufficiency exists. However, none of the hyperten-
sive subjects reported renal disease. Furthermore, if
their intake of calcium-containing antacids had been
heavy, it most likely would have resulted in higher serum concentrations of ultrafilterable calcium, rather
than the lower concentrations observed here.

Several other investigators have reported positive associations between blood pressure levels and concen-
trations of serum total calcium,\textsuperscript{6, 7, 9–11} as well as with 24-hour urinary calcium excretion.\textsuperscript{6, 8} Hyperten-
sion frequently accompanies hypercalcemic condi-
tions.\textsuperscript{21} Furthermore, drugs that block calcium entry clearly can lower blood pressure.\textsuperscript{22} On the other hand,
some\textsuperscript{5, 2–4} but not all,\textsuperscript{10, 20} investigators note that, compared with normotensive subjects, essential hyperten-
sive subjects have lower serum ionized calcium concen-
trations even when total calcium levels are similar. Our findings generally support this observation. We
found that serum concentrations of ultrafilterable calcium, which consists of the ionized and complexed
fractions of serum total calcium, were lower in hyperten-
sive subjects than in normotensive controls, whereas protein-bound calcium concentrations were higher.
It has been hypothesized that, due to aberrant trans-
membrane calcium transport, lower serum ionized cal-
caium levels in hypertensive subjects may in fact reflect increased levels of intracellular ionized calcium,
which would account for the arteriolar vasoconstric-
tion in hypertension.\textsuperscript{5}

Protein binding of serum calcium has been hypo-
thetized to be enhanced in essential hypertension.\textsuperscript{2, 23}
Our results, showing greater concentrations of protein-
bound calcium in hypertensive subjects than in normo-
tensive controls, support this hypothesis. On the other
hand, a primary increase in protein binding is not a
likely explanation for reduced serum concentrations of the ultrafilterable/ionized calcium fractions. If the
hormonal factors currently thought to modulate ionized calcium concentration continued to operate normally
around a normal set point in the hypertensive popula-
tion, then a primary increase in protein binding of serum calcium would not detectably alter ultrafilter-
able/ionized calcium concentrations. The only detect-
able serum difference would be in the concentration of protein-bound calcium.

Studies of dietary calcium intake and blood pressure have yielded mixed and seemingly contradictory re-

results. Pietinen et al.\textsuperscript{24} point out that both the hyperten-
sion rate and calcium intake in Finland are among the
highest in the world. Within populations, however,
calcium intake generally is negatively associated with blood pressure levels,\textsuperscript{9, 25–27} and calcium supplemen-
tation lowers blood pressure in animals\textsuperscript{28} and humans.\textsuperscript{29}
Using two sources, we found slightly lower estimated
mean dietary calcium intake in hypertensive subjects
than in normotensive controls, but this difference was
not significant. Low dietary calcium intake in hyper-
tensive subjects is seemingly consistent with their re-
duced serum ultrafilterable calcium levels. This lower intake, however, seems inconsistent with aforemen-
tioned hypotheses that high intracellular calcium concen-
trations cause essential hypertension. Low calcium intake also seems inconsistent with the high urinary
calcium excretion observed in essential hypertensive
subjects, although a primary renal calcium leak in hy-
ertension has been proposed.\textsuperscript{30}

In summary, essential hypertension is associated with a variety of perturbations in calcium physiology.
Evidence from a variety of sources, however, is con-
tradictory. Recent data from Resnick et al.\textsuperscript{5} suggest that discrepancies among studies of calcium and blood
pressure may relate to different renin profiles of study populations. To our knowledge, no epidemiological
study of essential hypertension has measured in one
population serum levels of calcium, renin, and para-
thyroid hormone, along with calcium intake and excre-
tion. Such investigations might prove informative.

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