Calcium and magnesium in essential hypertension

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SUMMARY

1. Because disturbances of calcium metabolism have been described in hypertension, measurements of plasma and serum concentrations of ionized calcium, total calcium, magnesium and renin were made in 38 patients with essential hypertension and age- and sex-matched control subjects. Urinary excretion of calcium, magnesium and sodium was also determined.

2. The mean serum concentration of ionized calcium was 1.23 ± 0.04 (SD) mmol/l in the hypertensive group and 1.21 ± 0.03 mmol/l in controls, and results were similar after correction for pH. There was a weak positive correlation between serum ionized calcium (pH 7.4) and systolic pressure (r = 0.26, P < 0.02), but no correlation with plasma renin concentration.

3. Although the difference between serum total calcium concentration in the hypertensive (2.29 ± 0.09 mmol/l) and control (2.26 ± 0.07 mmol/l) subjects was not significant, there was a significant correlation between total calcium and systolic pressure (r = 0.23, P < 0.05) which was maintained after correction for other variables.

4. There were no differences in plasma concentrations of parathyroid hormone or 1,25-dihydroxycholecalciferol between hypertensive and control subjects.

5. The hypertensive group showed higher urinary excretion of calcium (5.9 ± 3.0 mmol/24h) than controls (4.6 ± 1.7 mmol/24 h), but the difference was not maintained after correction for sodium excretion.

6. Serum concentrations of magnesium were similar in the two groups, but urinary excretion of magnesium was significantly lower in hypertensive (3.7 ± 1.3 mmol/24 h) than control (4.5 ± 1.6 mmol/24 h) subjects and there was an inverse correlation between magnesium excretion and blood pressure (r = 0.3–0.35, P < 0.01).

Key words: calciferol, calcium, hypertension, magnesium, parathyroid hormone, renin.

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Abbreviations: 25-(OH)D₃, 25-hydroxycholecalciferol; 1,25-(OH)₂D₃, 1,25-dihydroxycholecalciferol; PTH, parathyroid hormone.

INTRODUCTION

Force development in vascular smooth muscle is directly related to the concentration of calcium ions in smooth muscle cells [1]. Sustained hypertension is maintained by an increase in total peripheral resistance and attention has therefore focused on the possibility that changes in intracellular calcium are important in the pathogenesis of essential hypertension in man.

Vascular smooth muscle from patients with essential hypertension is inaccessible, but the concentration of free calcium is increased in platelets [2, 3] and alterations in extracellular calcium metabolism have also been described. Perhaps surprisingly, some epidemiological and clinical evidence has been presented to suggest that hypertension is linked with calcium deficiency [4], but this interpretation has been questioned [5, 6]. In essential hypertension, serum levels of ionized calcium may be slightly reduced [7–9] or normal [10–12] and a positive correlation with plasma renin activity has also been suggested [10].

Paradoxically, the concentration of total calcium in serum is positively correlated with blood pressure [13] but again the relationship has not been evident in all studies [8, 9]. A more consistent finding is an increase in urinary calcium excretion [9, 11, 14].

In the light of these uncertainties and contradictions, we undertook a comprehensive prospective study of calcium and magnesium metabolism in patients with untreated essential hypertension and in age- and sex-matched controls.

PATIENTS AND METHODS

Thirty-eight patients with essential hypertension, normal renal function and untreated outpatient diastolic blood
pressure readings of $>95$ mmHg (12.6 kPa) on at least two occasions were recruited from the Blood Pressure Clinic. Patients were either untreated or had not received any antihypertensive medication for at least 1 month before the study. Thirty-eight age- and sex-matched normal hospital workers with diastolic blood pressure $<90$ mmHg (12 kPa) were recruited as controls. Subjects were excluded from the study if taking regular medication or if there was a history or current evidence of renal calculi or other disorders of calcium metabolism. Nine borderline hypertensive subjects with diastolic blood pressure $>90$ mmHg and $<95$ mmHg, and six normotensive subjects, were also studied under identical conditions, making 91 patients in all. All were Caucasian subjects.

After fasting from 24.00 hours, each subject had an indwelling cannula inserted in an antecubital vein between 09.00 and 10.00 hours. After 30 min recumbency, blood pressure was measured using a Hawksley random zero sphygmomanometer and pulse rate was measured by palpation in each subject by the same observer (D.T.). These measurements were repeated after 2 min standing and after blood had been drawn.

Blood samples were taken via the cannula and without the use of a tourniquet. Samples for ionized calcium measurements were collected anaerobically using sealed vacutainers which were filled. Serum and plasma samples were separated by centrifugation at 4°C within 1 h of collection. Ionized calcium, pH and ionized calcium corrected to pH 7.4 were measured immediately thereafter using a Radiometer ICA-1 ionized calcium analyser [15] by the same observer (D.T.). Reproducibility was assessed using a Radiometer ICA-1 ionized calcium analyser [15].

The coefficient of variation for ionized calcium was 0.4% less than 1%.

Blood samples were also collected for the following measurements: packed cell volume, serum concentrations of creatinine, sodium, potassium, total calcium, protein, albumin, phosphate and magnesium, and plasma active renin concentration [16]. Serum concentrations of electrolytes, urea, creatinine, total protein, albumin, total calcium and serum and urinary phosphate were measured on a SMA-6 (Technicon Instruments Corporation, Basingstoke, U.K.), according to the manufacturer's protocol. Serum and urinary magnesium and urinary calcium concentrations were measured using a Video II atomic absorption spectrophotometer (Instrumentation Laboratories, Warrington, U.K.). Urinary concentrations of sodium and potassium were measured by flame photometry (Instrumentation Laboratories, 343 Flame Photometer). Urinary concentration of creatinine was determined by a Beckman creatinine analyser-2 (Beckman-RIIC Ltd, High Wycombe, Bucks, U.K.). Total serum calcium values were corrected for albumin concentration using the formula:

$$\text{Calcium (corrected)} = \text{measured calcium} + 0.02 / (40 - \text{serum albumin})$$

Serum parathyroid hormone (PTH) was measured using a double antibody radioimmunoassay which employs highly purified bovine PTH as standard and for radioiodination, and guinea-pig anti-(bovine PTH) serum which recognizes both ends of the intact PTH molecule. 23-Hydroxycholecalciferol [25-(OH)D$_3$] (normal range 15–100 nmol/l) was measured using a modification of the method of Preece et al. [17]. Extracts of serum were chromatographed on silicic acid and the vitamin D metabolite was quantified in a competitive protein-binding assay. 1,25-Dihydroxycholecalciferol [1,25(OH)$_2$D$_3$] (normal range 20–120 pmol/l) was measured using a radio-receptor assay [18] after purification by h.p.l.c. [19].

Statistical analysis was performed by unpaired Student's t-tests or Mann-Whitney U-tests. Simple linear regressions were calculated using the method of least squares, and multiple linear regression analysis was also performed.

RESULTS

Table 1 shows clinical details and mean ($\pm$ sd) values for serum biochemical variables in hypertensive and control subjects. Although the two groups were age- and sex-matched, the hypertensive subjects were significantly heavier than their controls. The respective mean ($\pm$ sd) values for supine blood pressure in the two groups were $167 \pm 25/108 \pm 11$ mmHg (22.2 $\pm$ 3.3/14.4 $\pm$ 1.5 kPa) and $118 \pm 10/74 \pm 8$ mmHg (15.7 $\pm$ 1.3/9.8 $\pm$ 1.1 kPa). The serum levels of urea, creatinine and other electrolytes were similar in the two groups (Table 1). Total serum protein concentration and packed cell volume were slightly but significantly higher in the hypertensive group, but there was no difference in serum albumin concentration.

Although the mean total serum calcium concentration in hypertensive ($2.29 \pm 0.09$ mmol/l) was slightly higher than in control ($2.26 \pm 0.07$ mmol/l) subjects, the difference was not significant ($P=0.06$) and was mainly accounted for by the small difference in serum albumin concentration (Fig. 1). Serum concentrations of phosphate and magnesiu were similar in both groups (Table 1).

As shown in Figs. 2 and 3, there were no significant differences in serum or plasma ionized calcium concentrations, either before or after correction for pH, although in each case the mean value in the hypertensive group was slightly higher than in the group with normal blood pressure. Plasma concentrations of ionized calcium were consistently lower than corresponding serum concentrations. Mean serum and plasma pH values were similar in the two groups.

Urinary calcium excretion over 24 h was significantly higher in the hypertensive group (Fig. 4). When this was corrected for sodium excretion, the difference between the two groups was no longer significant (Table 2). In the hypertensive group urinary excretion of potassium and
Calcium and magnesium in hypertension

Table 1. Characteristics of hypertensive patients and normal controls

Values are means ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive subjects</th>
<th>Control subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>38 (24 males, 14 females)</td>
<td>38 (24 males, 14 females)</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 ± 13</td>
<td>41 ± 11</td>
<td>—</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 ± 14</td>
<td>68 ± 12</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean blood pressure (diastolic plus one-third of pulse pressure) (mmHg)/(kPa)</td>
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<td></td>
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<tr>
<td>Supine</td>
<td>127 ± 15 (16.9 ± 2)</td>
<td>89 ± 7 (11.8 ± 9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Erect</td>
<td>125 ± 14 (16.6 ± 1.9)</td>
<td>88 ± 9 (11.7 ± 1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum sodium (mmol/l)</td>
<td>140 ± 2</td>
<td>141 ± 2</td>
<td>0.31</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>4.2 ± 0.4</td>
<td>4.2 ± 0.3</td>
<td>0.89</td>
</tr>
<tr>
<td>Serum bicarbonate (mmol/l)</td>
<td>26 ± 2</td>
<td>26 ± 2</td>
<td>0.80</td>
</tr>
<tr>
<td>Serum chloride (mmol/l)</td>
<td>106 ± 2</td>
<td>107 ± 2</td>
<td>0.068</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>4.7 ± 1.4</td>
<td>4.9 ± 1.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>83 ± 18</td>
<td>84 ± 14</td>
<td>0.78</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>1.02 ± 0.15</td>
<td>0.78 ± 0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum magnesium (mmol/l)</td>
<td>0.80 ± 0.06</td>
<td>0.78 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum protein (g/l)</td>
<td>65 ± 3</td>
<td>62 ± 3</td>
<td>0.21</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>42 ± 3</td>
<td>41 ± 2</td>
<td>0.21</td>
</tr>
<tr>
<td>Serum 25-(OH)D₃ (mmol/l)</td>
<td>34 ± 24</td>
<td>38 ± 21</td>
<td>0.36</td>
</tr>
<tr>
<td>Serum 1,25-(OH)₂D₃ (pmol/l)</td>
<td>51 ± 22</td>
<td>53 ± 14</td>
<td>0.69</td>
</tr>
<tr>
<td>Serum pH</td>
<td>7.43 ± 0.04</td>
<td>7.43 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>0.42 ± 0.04</td>
<td>0.40 ± 0.03</td>
<td>0.012</td>
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</tbody>
</table>

Fig. 1. Serum total calcium in hypertensive (●) and control (○) subjects before (a) and after (b) correction for serum albumin concentration. Mean ± s.d. is also shown for each group. (a) P = 0.06. (b) P = 0.11.

Fig. 2. Serum ionized calcium in hypertensive (●) and control (○) subjects before (a) and after (b) correction to pH 7.4. Mean ± s.d. is also shown for each group. (a) P = 0.07. (b) P = 0.09.

magnesium (Table 2 and Fig. 5) was significantly lower than in controls. Creatinine excretion was similar in the two groups but, expressed as a function of body weight, was lower in hypertensive subjects. These results are summarized in Table 2.

PTH concentrations were within the normal range (<600 ng/l) in all hypertensive subjects and in controls, and, as many values lay below the limit of detection of the assay, statistical comparisons between groups were not possible. Twenty-six of the 38 hypertensive patients had PTH levels below 250 ng/l, while 22 of the 38 control subjects had PTH values in this range. Small differences between hypertensive and control subjects cannot be excluded but the results do exclude hyperparathyroidism. Serum concentrations of 25-(OH)D₃ and 1,25-(OH)₂D₃ were also similar in both groups (Table 1) and there were no significant correlations with plasma concentrations of renin.

Regression analysis

When results from all the subjects (n = 91) were analysed there was a weak but significant positive correlation between total serum calcium concentration and
supine systolic blood pressure \((r=0.23, P=0.031)\). This relationship was maintained \((P<0.05)\) after correction for age, weight, serum albumin and protein concentrations, and packed cell volume. There were similar positive correlations with systolic pressure for serum \((r=0.26, P=0.014)\) and plasma \((r=0.28, P=0.008)\) ionized calcium after correction to pH 7.4, but significance was not maintained after correction for age, weight, serum albumin and protein concentrations and packed cell volume. Measured, but not pH-corrected, values for serum and plasma ionized calcium showed a significant positive correlation with serum albumin concentration \((r=0.24, P=0.019\) and \(r=0.26, P=0.012,\) respectively).

There was a weak, but significant, inverse correlation between serum phosphate concentration and mean supine \((r= -0.22, P=0.040)\) and mean erect \((r= -0.23, P=0.031)\) blood pressure.

There were no significant correlations between blood pressure and serum or plasma pH. Correlations between serum concentrations of ionized calcium or magnesium and plasma renin concentration were not significant in the hypertensive group \((n=38)\) or when results from all subjects \((n=91)\) were analysed.

Although hypertensive patients had relative hypercalciuria compared with controls, there was no significant

![Fig. 3. Plasma ionized calcium in hypertensive (●) and control (○) subjects before (a) and after (b) correction to pH 7.4. Mean±so is also shown for each group. (a) \(P=0.14\). (b) \(P=0.09\).](image)

![Fig. 4. Urinary calcium excretion in hypertensive (●) and control (○) subjects. Mean±so is also shown for each group. \(P=0.048\).](image)

<table>
<thead>
<tr>
<th>Table 2. Urinary excretion of cations, phosphate and creatinine</th>
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<tbody>
<tr>
<td>Values are means±sd.</td>
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<tr>
<td>Hypertensive subjects</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Sodium (mmol/24 h)</td>
</tr>
<tr>
<td>Potassium (mmol/24 h)</td>
</tr>
<tr>
<td>Calcium (mmol/24 h)</td>
</tr>
<tr>
<td>(10^}\times[Calcium/sodium]</td>
</tr>
<tr>
<td>Phosphate (mmol/24 h)</td>
</tr>
<tr>
<td>Magnesium (mmol/24 h)</td>
</tr>
<tr>
<td>Creatinine (mmol/24 h)</td>
</tr>
<tr>
<td>Creatinine (mmol 24 h⁻¹ kg⁻¹)</td>
</tr>
<tr>
<td>Creatinine clearance (ml min⁻¹ 1.73 m⁻²)</td>
</tr>
</tbody>
</table>
correlation between urinary calcium excretion and blood pressure when results from all subjects were considered. There were, however, highly significant inverse correlations between magnesium excretion and systolic and diastolic blood pressures both in the supine and upright positions \( (r=0.3-0.35, P=0.005-<0.001) \) (Fig. 6). These relationships remained significant after correction for age and calcium excretion \( (P<0.01 \text{ in all cases}) \).

DISCUSSION

No reduction in serum or plasma concentrations of ionized calcium was observed in our patients with untreated essential hypertension. The results, therefore, do not confirm the original observation by McCarron [7], but are more consistent with several subsequent studies [8, 10-12, 20]. In one study [20], treatment effects must be considered, since some of the patients were taking thiazide diuretics which increase serum concentrations of ionized calcium [21]; this effect may explain the higher levels of ionized calcium in members of stroke-cluster pedigrees in Utah [22].

Posture, ambulation and exercise are all factors which can influence ionized calcium concentration, and blood pH is critical. There is also significant diurnal variation in serum ionized calcium concentration. For accurate, reliable measurements, blood must be collected anaerobically, without stasis or forearm exercise, at the same time of day. Mild respiratory alkalosis from overbreathing will reduce serum concentrations of calcium ions. Measurements of pH were only reported in one previous study [12] and some adjusted pH with carbon dioxide before assay [20, 22]. It is sometimes unclear if reported calcium values were corrected for pH [7, 10, 11]. Our patients had blood sampled via an indwelling cannula rather than by direct venepuncture as in other studies [7-12, 20-22]; sampling via an indwelling cannula may be less likely to provoke hyperventilation. In contrast to the recent report by Shore et al. [12], we found no significant difference in mean serum or plasma pH values between hypertensive and control subjects and no significant correlation between serum or plasma pH and blood pressure. A negative correlation between ionized calcium concentration and blood pressure has recently been described [9], but, as no corrections for pH were made, a possible confounding effect of mild alkalosis in hypertensive subjects [12] is not excluded.

Ionized calcium and total magnesium concentrations were not significantly related to plasma concentrations of
renin. A positive correlation between plasma renin activity and serum ionized calcium, and a negative correlation between plasma renin activity and serum magnesium, have been reported in essential hypertension [10], a relationship that was determined to a large extent by the values in high renin patients. Very few hypertensive patients in our series had high plasma renin concentrations; in our experience, with the exception of patients with renovascular disease, high plasma renin concentrations are unusual and occur only in young patients with severe hypertension. The significant positive correlation between serum concentrations of ionized calcium and albumin observed in our study has been previously reported [23], but the relationship in our patients was not maintained after pH correction.

Serum total calcium concentrations appear weakly but significantly correlated with blood pressure in some [24-26] but not all [27] population surveys. Effects of high alcohol intake in hypertensives may be relevant [28] and some studies may have included subjects treated with thiazides. When results from all patients in our study were analysed together there was a significant positive correlation between total serum calcium concentration and supine systolic blood pressure, which was maintained after correction for age, weight, serum albumin and protein concentrations and packed cell volume. It is unlikely, therefore, that this effect is simply related to haemoconcentration, whereby younger patients with essential hypertension tend to have slightly reduced extracellular fluid volume [30]. Neither is it simply a result of differences in protein or albumin concentration. Indeed, in contrast to a previous report [25], the relationship between total calcium and blood pressure became more significant ($P = 0.031$ to $P = 0.007$) when correction for albumin concentration was introduced. There is uncertainty about the validity of the various algorithms for albumin correction, particularly when albumin values are not low [31]; it has been emphasized that the total calcium should not be adjusted downwards when albumin is increased due to venous occlusion [32]. In the population surveys, details regarding the use of tourniquets in blood sampling were not given.

In our series of patients with normal ionized calcium levels there was a weak but significant negative relationship between serum phosphate concentration and blood pressure. There have been previous reports of low serum concentrations of phosphorus in patients with essential hypertension [7, 14] and of an inverse correlation between serum phosphate concentrations and arterial pressure [9, 27]. The PTH assay used in our study was less sensitive than some, but the results certainly exclude the presence of hyperparathyroidism in both groups. In a previous study, slightly higher PTH levels were seen in patients with essential hypertension [14], although the difference was of borderline significance in a larger series [11]. Serum concentrations of 25-(OH)D$_3$ and 1,25-(OH)$_2$D$_3$ were similar in hypertensive and control subjects and were unrelated to renin. Increased levels of 1,25-(OH)$_2$D$_3$ in low renin hypertension have been reported [29].

There is general agreement that some patients with essential hypertension have increased rates of calcium excretion or hypercalciuria relative to sodium excretion [9, 11, 13, 14, 33] and this was our finding. The renal excretion of both cations is linked [34] and dietary intake of sodium is therefore a factor which influences calcium excretion [35]. Some studies in the spontaneously hypertensive rat suggest that an intestinal mechanism is responsible for the hypercalciuria also present in this animal model [36], but increased absorption of calcium is not firmly established [37, 38]. Dietary intake of calcium per se is also a determinant. In this regard there has been vigorous debate about the role of dietary calcium deficiency in the pathogenesis of hypertension in man [39]. This has centred on the interpretation of epidemiological studies, most of which show a significant, albeit weak, inverse relationship between calcium intake and blood pressure [40].

Urinary magnesium excretion was significantly reduced in the hypertensive group. When results from all patients were analysed there was an inverse correlation between blood pressure and magnesium excretion which remained highly significant after correction for age and calcium excretion. Factors known to decrease urinary magnesium excretion include extracellular volume depletion, hypomagnesaemia or hypocalcaemia, hypothyroidism and PTH, although the effect of the latter is complicated by its role in calcium metabolism [41]. In the present study serum concentrations of calcium and magnesium were similar in both groups of patients and there was no evidence of hyperparathyroidism in the hypertensive subjects. The differences are opposite to those expected if differences in alcohol intake were responsible, because the relationship between alcohol intake and blood pressure tends to be positive [42], and alcohol tends to increase magnesium excretion. A reduction in extracellular fluid volume, as evidenced by the slight but significant increase in packed cell volume in hypertensive subjects, may contribute to reduced magnesium excretion but seems unlikely to fully explain the relationship.

Despite convincing evidence that urinary excretion of calcium is increased in some patients with essential hypertension, we can find no evidence that the concentration of calcium ions in blood is abnormal. Because serum total calcium levels were correlated with blood pressure, it seems likely that bound calcium in serum is responsible for the difference. We have also shown a distinct but inverse relationship between magnesium excretion and blood pressure that may not be entirely explained by differences in extracellular fluid volume.

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