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Modification of Intracellular Calcium and Plasma Renin by Dietary Calcium in Men

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A double blind, placebo-controlled, parallel study was conducted on the effect of a high daily oral calcium supplementation of 1 g elemental calcium, given twice daily for 16 weeks in normal male subjects, on plasma renin, aldosterone, kallikrein, cGMP, cAMP, and calciotropic hormones, intracellular calcium concentrations, and plasma total and ionized calcium. After a 1-month run-in period on a limited use of dairy products, the subjects (n = 32) were allocated to a placebo or a calcium group. Placebo or 1 g elemental calcium was administered twice daily in the morning and evening for 16 weeks. All subjects were investigated at baseline and after 1, 2, 4, 8, and 16 weeks of placebo or calcium administration.

A decreased intraerythrocyte and intraplatelet Ca^{2+} concentration was observed in the calcium-treated subjects. Compared with the placebo group, an increase in the plasma renin activity (PRA) in the calcium group was observed after 4, 8, and 16 weeks of oral calcium administration. However, plasma aldosterone and urinary excretion of aldosterone, kallikrein, cGMP, and cAMP were not changed during calcium administration. Oral calcium supplementation in these men was also accompanied by a reduction in the plasma concentration of intact parathyroid hormone and 1,25-dihydroxyvitamin D_3, and an increase in 24-h urinary calcium excretion, but no change in the plasma total Ca^{2+} concentration, serum ionized Ca^{2+} level, and plasma phosphate or 25-hydroxyvitamin D_3.Our data show that the increase in PRA observed in men during oral calcium supplementation is accompanied by a reduction in the intracellular free and total Ca^{2+} concentration in platelets and erythrocytes and by a decrease in the plasma concentration of intact parathormone and 1,25-dihydroxyvitamin D_3.
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KEY WORDS: **Calcium supplementation, renin, cytosolic calcium.**

Discordant findings are reported on the in vivo effect of calcium supplementation on plasma renin activity (PRA). Zemel et al[1] found an increased PRA after a high salt/high calcium diet in normotensive and hypertensive black adults, whereas Rich et al[2] found similar PRA values after a high salt/high calcium or high salt/low calcium diet in hypertensive white patients. In spontaneously hypertensive rats, Wuorela et al[3] found a suppression of the plasma renin-angiotensin system after oral calcium supplementation. According to Kotchen and Guthrie,[4] a high calcium chloride intake in normotensive rats on a normal salt intake did not affect PRA, whereas a high calcium chloride intake in rats on a low salt diet decreased PRA. During acute calcium infusions into the renal artery of mongrel dogs, peripheral calcium concentrations increased and renal venous PRA decreased.[4] A decrease in PRA was

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seen after calcium infusion in normal sodium-depleted men,[5] whereas calcium infusion had no effect on PRA in subjects on an unspecified dietary sodium intake.[6] In these in vivo studies, however, no measurements of intracellular calcium were performed.

The present double blind, placebo-controlled, parallel study was therefore conducted on the effect of a high daily oral calcium supplementation of 1 g elemental calcium, given twice daily for 16 weeks in normal men, on the intracellular calcium concentrations in platelets and erythrocytes, plasma total, ionized calcium, and calciotropic hormones such as intact parathyroid hormone (PTH), 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 and on plasma renin, aldosterone, kallikrein, cAMP, and cGMP. The aim of the present study was to investigate whether in vivo alterations in intracellular calcium concentrations or in plasma ionized calcium and calciotropic hormones participate in the stimulation of the renin secretion during oral calcium supplementation.

METHODS

Subjects A total of 32 normal male volunteers gave written consent for participation in the study after the procedure and purpose of the study had been explained. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Leuven. The age of the subjects averaged 24 ± 1 (SEM) years; height, 179.5 ± 1.1 cm; and weight 75.9 ± 1.3 kg.

Design All subjects (n = 32) received dietary advice for a limited use of dairy products throughout the whole study period. After a 1-month run-in period on a limited use of dairy products, baseline measurements were performed. The subjects then were allocated randomly either to a placebo (n = 16) or a calcium (n = 16) group. Placebo or 1 g elemental calcium as a powder was administered twice daily, in the morning and evening, for 16 weeks. All subjects were reinvestigated after 1, 2, 4, 8, and 16 weeks of oral placebo or calcium administration. They collected their 24-h urine samples the day immediately preceding the investigation.

The formulation of calcium used in this study was calcium gluconate, citric acid, aspartame, and an orange aroma; that of placebo consisted of β-lactose, citric acid, aspartam, and an orange aroma and was kindly provided by Boehringer Ingelheim.

Blood Sampling After a 10-min rest, blood was collected from an antecubital vein in each subject after an overnight fast preceded by a light evening meal. Blood was collected into heparinized or acid citrate-dextrose tubes, the plasma was separated, the buffy coat discarded, and the fresh cells handled as described previously.[7] PRA,[8] aldosterone concentrations,[9] and 24-h excretion of aldosterone,[10] and cAMP and cGMP[11] were measured by radioimmunoassay. The urinary excretion of kallikrein was measured spectrophotometrically.[12]

The intraerythrocyte total Ca^{2+} concentration was determined by atomic absorption spectrophotometry.[13] The platelet cytosolic free Ca^{2+} concentration was assayed by measuring the fluroescence of quin-2 entrapped by platelets.[14] Plasma Na^+, K^+, urea, uric acid, phosphate, creatinine, chloride, bicarbonate, alkaline phosphatase, and protein were measured by a multichannel autoanalyzer. Plasma Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometry. Serum ionized calcium and pH were measured by an ICA2 ionized calcium analyzer (Radiometer, Copenhagen, Denmark). Plasma intact PTH was measured by an immunoradiometric assay,[15] plasma 25-hydroxyvitamin D_3 by a competitive protein binding assay, and plasma 1,25-dihydroxyvitamin D_3 by a radioreceptor assay.[16]

Statistical Methods Values are expressed as arithmetic means ± SEM. Serial measurements in the two treatment groups were compared using the method of Matthews et al.[17] This method considers the individual as the basic unit and uses the average response over time for each subject as a summary statistic of that subject's response curve. The summary measures in the two treatment groups were then compared using the Student *t* test. The single *t* value obtained corresponds to the average difference in the treatment effect between the placebo- and calcium-treated groups. Repeated measures of analysis of variance also shows no interaction effect of group and time for the various variables.

RESULTS

At randomization there were no significant differences in demographic, anthropometric, and clinical characteristics between the groups allocated to placebo and calcium (Table 1). No significant differences between the two groups were observed at randomization in the erythrocyte or platelet intracellular calcium concentrations or in the plasma and urinary variables.

Blood Pressure Compared with the placebo group, standing systolic blood pressure was decreased in the calcium group and the standing diastolic blood pressure also tended to decrease (Figure 1). Compared with the baseline value, the average change in the standing systolic blood pressure in the calcium group (−7.8 mm Hg, range −16 to +4) was different (P = .01) from the changes observed in the placebo group (−2.1 mm Hg, range −15 to +7).

Standing diastolic blood pressure in the calcium group (−2.6 mm Hg, range −14 to +6) tended to be lower than that in the placebo group (+0.9 mm Hg, range −4 to +16). The changes in supine systolic and

TABLE 1. GENERAL CHARACTERISTICS OF THE SUBJECTS AT RANDOMIZATION

	Placebo Group	Calcium Group
Age (years)	24.4 ± 1.4	24.2 ± 1.1
Weight (kg)	74.8 ± 1.7	77.4 ± 2.0
Height (cm)	178.4 ± 1.6	180.5 ± 1.7
Blood pressure (mm Hg)		
Systolic	114 ± 2	114 ± 2
Diastolic	72 ± 2	74 ± 2
Heart rate (beats/min)	72 ± 2	72 ± 1
24-h urinary excretion of:		
Sodium (mmol)	139 ± 13	147 ± 16
Potassium (mmol)	68 ± 5	64 ± 6
Calcium (mmol)	4.79 ± 0.50	4.69 ± 0.62
Magnesium (mmol)	4.20 ± 0.37	4.52 ± 0.45
Phosphate (mmol)	26.1 ± 1.9	29.7 ± 2.6
Creatinine (mmol)	14.3 ± 0.9	14.9 ± 1.1
Plasma		
Sodium (mmol/L)	140.4 ± 0.6	140.4 ± 0.7
Potassium (mmol/L)	3.82 ± 0.04	3.93 ± 0.05
Magnesium (mmol/L)	0.81 ± 0.02	0.81 ± 0.02
Phosphate (mmol/L)	1.04 ± 0.05	1.11 ± 0.03

Blood pressure and heart rate were measured with the subjects recumbent. Values are expressed as arithmetic means ± SEM (n = 16). No significant differences were observed between the placebo and calcium groups using paired t *test.*

diastolic blood pressures did not differ between the calcium and the placebo groups.

Plasma Renin-Aldosterone Compared with the placebo group, on average an increase in the PRA was observed in the calcium group (Figure 2), whereas plasma aldosterone concentration did not change during calcium administration (Table 2). Unpaired *t* tests revealed no significant change in PRA after 1 and 2 weeks of oral calcium supplementation. A significant rise in PRA was seen after 4 weeks of calcium supplementation ($P < .05$) and remained elevated after 8 weeks ($P < .02$) and 16 weeks ($P < .005$) of calcium administration.

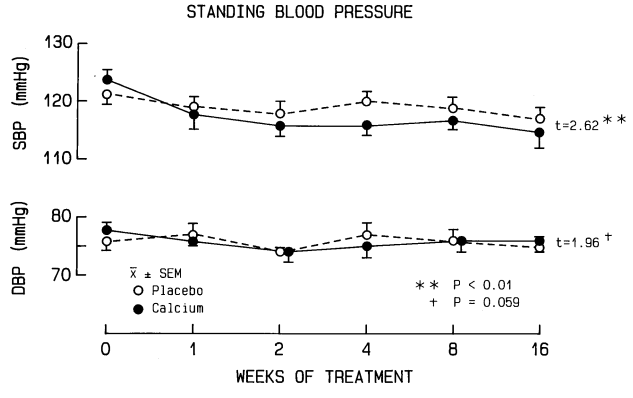

FIGURE 1. *Standing systolic and diastolic blood pressures (means ± SEM) before and during oral administration of calcium or placebo in 16 men. SBP, systolic blood pressure; DBP, diastolic blood pressure.*

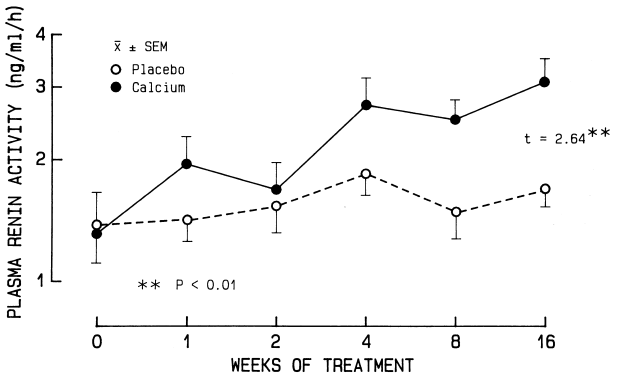

FIGURE 2. *Plasma renin activity before and during oral administration of calcium (filled circles) or placebo (open circles) in 16 men. The* t *value corresponds to the average difference in the treatment effect between the placebo and calcium groups (see Reference 17). Geometric means ± SEM are given.*

Intracellular Calcium Concentrations Compared with the placebo group, intraerythrocyte total Ca^{2+} concentration and intraplatelet free cytosolic Ca^{2+} concentration were reduced in the subjects given calcium (Figure 3). The decrease in intracellular Ca^{2+} concentration in the erythrocytes and platelets was already present after 1 week of oral calcium administration and persisted throughout the 16 weeks of calcium supplementation, whereas the rise in PRA occurred only after 4 weeks of oral calcium administration, and then remained elevated up to 16 weeks of calcium administration (Figure 2).

Urinary and Plasma Variables Compared with the placebo group, the 24-h urinary excretion of calcium increased ($P < .05$) in the calcium group (Figure 4), whereas no differences were found in the 24-h urinary excretion of sodium, potassium, magnesium, and phosphate. No significant differences were found in serum ionized calcium and total plasma calcium (Figure 5) or in plasma phosphate, magnesium, sodium, and potassium between the calcium and placebo group. The 24-h urinary excretion of aldosterone, kallikrein, cAMP, and cGMP did not change during oral calcium supplementation (Table 2).

The plasma concentration of intact PTH and 1,25-dihydroxyvitamin D_3 decreased in the calcium group (Figure 6). However, no significant difference was found in the plasma concentration of 25-hydroxyvitamin D_3 between the placebo- and calcium-treated groups (Figure 6).

TABLE 2. PLASMA ALDOSTERONE CONCENTRATION AND URINARY EXCRETION OF ALDOSTERONE, KALLIKREIN, cGMP, AND cAMP BEFORE (0) AND DURING (1, 2, 4, 8, 16 WEEKS) ORAL ADMINISTRATION OF PLACEBO OR CALCIUM IN 16 MEN

	Group	0	1	2	4	8	16	*t* Value
Plasma aldosterone (ng%)	Placebo	22.7 ± 3.0	23.1 ± 2.3	27.5 ± 4.6	24.8 ± 4.1	21.4 ± 3.5	26.3 ± 3.5	0.28^{NS}
	Calcium	24.8 ± 3.3	25.2 ± 3.4	23.5 ± 2.7	29.0 ± 4.0	23.8 ± 2.8	37.8 ± 6.0	
Urinary aldosterone (μg/24 h)	Placebo	10.4 ± 1.5	10.0 ± 2.0	8.2 ± 1.6	8.6 ± 1.4	8.4 ± 1.4	9.5 ± 1.4	0.53^{NS}
	Calcium	8.9 ± 1.2	8.7 ± 1.2	7.0 ± 1.1	6.2 ± 0.9	6.3 ± 1.1	9.3 ± 1.4	
Urinary kallikrein (U/24 h)	Placebo	0.788 ± 0.165	0.884 ± 0.223	0.763 ± 0.142	0.839 ± 0.178	0.771 ± 0.145	0.980 ± 0.188	0.85^{NS}
	Calcium	0.624 ± 0.074	0.623 ± 0.088	0.601 ± 0.069	0.661 ± 0.076	0.545 ± 0.046	0.621 ± 0.086	
Urinary cGMP (μmol/24 h)	Placebo	0.492 ± 0.067	0.470 ± 0.067	0.448 ± 0.060	0.526 ± 0.049	0.510 ± 0.048	0.499 ± 0.049	1.00^{NS}
	Calcium	0.436 ± 0.041	0.482 ± 0.044	0.448 ± 0.030	0.495 ± 0.042	0.469 ± 0.040	0.433 ± 0.047	
Urinary cAMP (μmol/24 h)	Placebo	4.92 ± .33	4.65 ± 0.36	4.79 ± 0.41	4.77 ± 0.30	4.46 ± 0.26	4.53 ± 0.31	0.90^{NS}
	Calcium	4.51 ± 0.38	4.66 ± 0.29	4.57 ± 0.28	3.72 ± 0.31	4.22 ± 0.38	4.00 ± 0.31	

Values are arithmetic means ± SEM.

NS = not significant. The t *value corresponds to the average difference in treatment effect between the placebo and calcium groups. (See Reference 17)*

DISCUSSION

The present study shows that, in men, oral supplementation with 2 g elemental calcium per day in a divided dose (1 g twice daily) is associated with a reduction in intraerythrocyte total Ca^{2+} concentration and intraplatelet free cytosolic Ca^{2+} concentration and with an enhancement of PRA. However, plasma and urinary aldosterone, as well as the urinary excretion of kallikrein, cAMP, and cGMP, did not change during calcium supplementation. The ability of dietary calcium to stimulate plasma renin activity in both animal and clinical studies was first reported by Resnick et al.[18–20]

Dietary calcium supplementation has indeed been reported to decrease the intracellular free Ca^{2+} concentration in lymphocytes and vascular smooth muscle cells in spontaneously hypertensive rats (SHR) and in stroke-prone SHR (SHRSP)[3,21–25] as well as the intracellular total Ca^{2+} concentration in erythrocytes of diabetic hypertensive patients.[26] In the present study we also observed a reduction in the free cytosolic Ca^{2+} concentration in platelets and in the total intracellular Ca^{2+} content in erythrocytes during oral calcium supplementation in men. An activation of calcium trans-

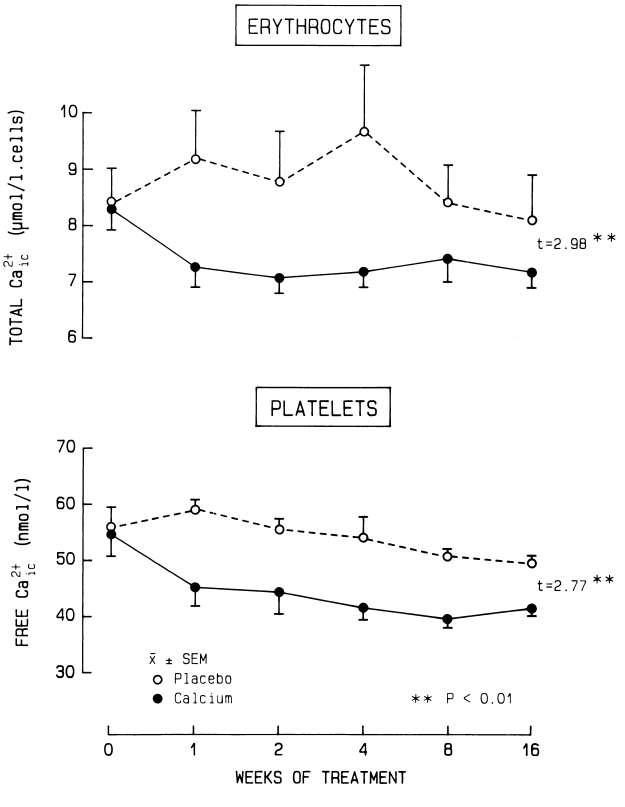

FIGURE 3. *Erythrocyte total intracellular Ca^{2+} concentration (Ca^{2+}_{ic}) and platelet intracellular free cytosolic Ca^{2+} concentration (Ca^{2+}_{ic}) before and during oral administration of calcium (filled circles) or placebo (open circles) in 16 men. The* t *value corresponds to the average difference in the treatment effect between the placebo and calcium groups (see Reference 17).*

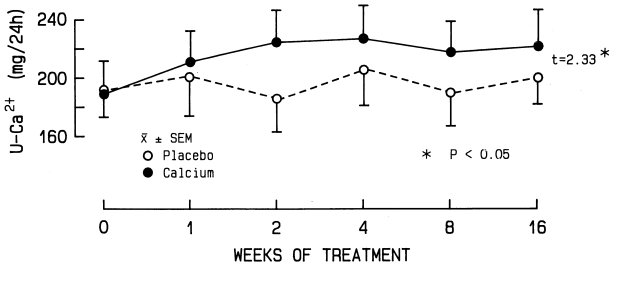

FIGURE 4. *Twenty-four-hour urinary excretion of calcium (U-Ca^{2+}) before and during oral administration of calcium (filled circles) or placebo (open circles) in 16 men. The* t *value corresponds to the average difference in the treatment effect between the placebo and calcium groups (see Reference 17).*

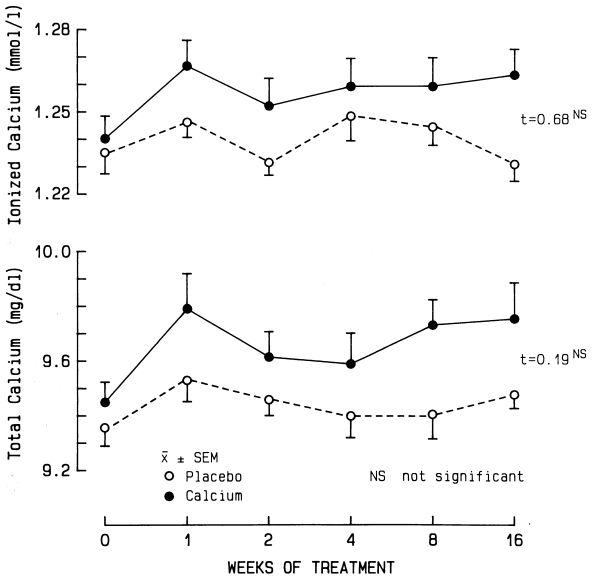

FIGURE 5. *Serum ionized Ca^{2+} and plasma total Ca^{2+} concentrations before and during oral administration of calcium (filled circles) or placebo (open circles) in 16 men. The* t *value corresponds to the average difference in the treatment effect between the placebo and calcium groups (see Reference 17).*

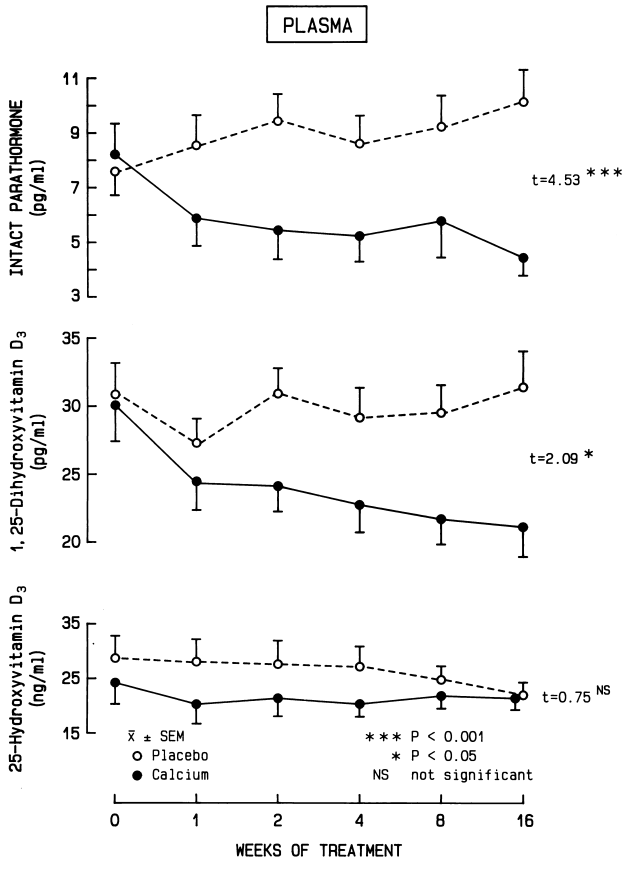

FIGURE 6. *Plasma concentration of intact parathormone, 1,25-dihydroxyvitamin D_3, and 25-hydroxyvitamin D_3 before and during oral administration of calcium (filled circles) or placebo (open circles) in 16 men. The* t *value corresponds to the average difference in the treatment effect between the placebo and calcium groups (sees Reference 17).*

port mechanisms or a direct effect on cell membrane could have contributed to the reduction in intracellular Ca^{2+} concentration. Indeed, calcium supplements significantly increase the erythrocyte Ca-ATPase activity and reduce intracellular calcium compared with the placebo group.[26]

Increased dietary calcium has been reported to reduce the plasma digitalis-like immunoreactive factor in rats.[27] Dietary calcium supplementation in men could thus have increased the activity of Na,K-ATPase in erythrocytes and platelets or in vascular smooth muscle cells[24] by reducing the amount of circulating sodium pump inhibitor. The latter inhibitor was, however, not measured in the present study.

An increased Na-K-pump activity observed during high calcium intake can lead to a decreased intracellular Na^+ concentration, thus increasing the driving force for Na^+-Ca^{2+} and Na^+-H^+ exchange mechanisms, which have a central role in controlling the intracellular free Ca^{2+} level[28,29] and, consequently, leading to a reduced free cytosolic Ca^{2+} concentration. Furthermore, the effect of high calcium intake on cytosolic Ca^{2+} is not mediated by its possible natriuretic[30] and sympathoinhibitory[31] actions.

Because hormone secretion is usually stimulated by calcium, it is of interest that, in the entire endocrine system, probably only three situations exist in which hormone secretion is inhibited by calcium.[32] They are parathyroid secretion of PTH and plasma hypertensive factor (PHF), juxtaglomerular secretion of renin and renal production of 1,25-dihydroxyvitamin D_3.

In the present study oral calcium supplementation in men is indeed accompanied by a reduction in the plasma concentration of intact PTH and 1,25-dihydroxyvitamin D_3. The circulating 1,25-dihydroxyvitamin D_3, the major determinant of intestinal absorption of calcium, is indeed reduced when calcium intake is high.[33,34] According to Basile et al,[35] increasing dietary calcium intake increases urinary calcium excretion and reduces plasma 1,25-dihydroxyvitamin D_3, but total and ionized serum Ca^{2+} and plasma 25-hydroxyvitamin D_3 are unchanged, as observed in the present study.

According to Pang et al,[32] the secretion of PHF in SHR is inhibited by dietary calcium loading. Whether PHF has a direct effect on the intracellular free Ca^{2+} concentration is not yet elucidated. However, 1,25-dihydroxyvitamin D_3 directly increased radiolabeled calcium uptake in vascular smooth muscle cells[36] and

PTH also enhanced calcium influx in red blood cells.[37] The decrease in intraerythrocyte and intraplatelet Ca^{2+} concentration observed after oral calcium supplementation in men (Figure 3) could probably be attributed to a reduced cellular influx of Ca^{2+}, induced by the suppressed circulating levels of intact PTH and 1,25-dihydroxyvitamin D_3. If the intracellular free Ca^{2+} concentration is also reduced in the juxtaglomerular cells, this could lead to an elevated secretion of renin. Indeed, exposure of rat renal cortical slices to low-Ca^{2+} EGTA buffer did not alter the β-adrenergic stimulation of renin release by isoprenaline, but blocked the inhibition of the renin release by angiotensin II. The Ca^{2+}-channel blocker nifedipine produced a dose-related increase in renin release in these cortical slices, suggesting that reduction of Ca^{2+} entry into juxtaglomerular cells is a potent stimulating signal for renin release. The agent TMB-8, which inhibits intracellular Ca^{2+} release, also produced a significant dose-dependent increase in renin release,[38] indicating that intracellular Ca^{2+} levels are key signals for renin release. Calmidozolium, a specific calmodulin inhibitor, is a potent stimulator of renin release, indicating that intracellular Ca^{2+} interacts with the Ca^{2+} binding protein calmodulin as an inhibiting signal for renin secretion by blocking an early step in the cellular events that lead to renin secretion such as pH gradient-dependent swelling of renin secretory granules.[39] The increased PRA during oral calcium supplementation can, however, also be related to intravascular volume depletion. Indeed, Addison[40,41] first suggested that oral calcium loading possesses diuretic properties. Walser[42] also reported a link between renal calcium and sodium excretion.

In the present study, PRA started to rise after 4 weeks of oral calcium supplementation in normal men and remained elevated after 8 and 16 weeks of calcium administration. No significant change in plasma renin was observed after 1 or 2 weeks of oral calcium administration in these men. In vivo, there seems thus to be a lag period before the renin secretion in the juxtaglomerular cells is stimulated by the reduced intracellular Ca^{2+} concentration, provoked by the high dietary calcium intake. In vitro, in primary cultures of mouse renal juxtaglomerular cells, an increase in extracellular calcium had a dual effect on renin secretion[43]: an inhibitory one that lasted for $\geq$1 h, and a powerful stimulatory one that occurred with a delay of approximately 1 to 3 h.

When Ca^{2+} entry into juxtaglomerular cells is inhibited by a calcium-channel antagonist such as verapamil, diltiazem, or dihydropyridines, renin release is stimulated from isolated juxtaglomerular cells,[44] isolated glomeruli,[45] kidney slices,[46–48] isolated perfused kidneys,[49,50] and in vivo.[51–53] In some studies, however, blockers of calcium entry, such as verapamil or diltiazem, failed to stimulate renin release when infused into the renal artery[54] or did not attenuate the inhibition of renin release from rat kidney slices by angiotensin II.[55]

These observations do not, however, contradict the basic concept of an inverse relationship between calcium and renin secretion,[56] as other actions of calcium-channel antagonists probably counteract their stimulatory effects on renin secretion. A possible explanation for the occasional lack of divergent effects of calcium-channel antagonists and of variations in extracellular calcium on renin is that the interplay of mobilization and sequestration of intracellular free calcium may primarily control renin release and that extracellular calcium becomes important only when the intracellular calcium homeostasis is disturbed.

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