

Effect of oral calcium supplementation on intracellular calcium and plasma renin in men

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Abstract

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 calcitropic 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 , 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 . Lacidipine tended to increase PRA in the placebo-treated subjects and to decrease it in the calcium-treated subjects: this difference in lacidipine effect between the placebo and calcium group was significant ($P<0.05$). 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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Introduction

An inverse association between calcium concentration in the incubation medium and renin release was first recognized by Vandongen & Peart (1974) in the isolated perfused kidney of the rat. Such an inverse relationship can be observed under a variety of *in vitro* conditions in which the intracellular free calcium concentration is changed, or can be assumed to be changed, by pharmacological or physical manipulations.

An increase in renin release was indeed observed when extracellular calcium was drastically lowered in renal cortical cell suspensions, renal cortical slice incubations, isolated glomeruli and isolated perfused rat kidneys (Hackenthal *et al.* 1990). The agent 8-*N,N*-diethylaminodioctyl-3,4,5-trimethoxybenzoate (TMB-8), which acts as an intracellular calcium antagonist by preventing the stimulus-induced release of calcium from the endoplasmic reticulum, has been found to increase renin release from

kidney slices (Antonipillai & Horton 1985, Baxter *et al.* 1985, Heinrich & Campbell 1986) and in isolated perfused rat kidneys (Hackenthal & Taugner 1986).

An inhibition of renin release from kidney slices and from isolated rat kidneys was found with the calcium-channel agonist BAY K8644 and CGP 28392 which induce a rise in intracellular calcium (Hackenthal & Taugner 1986, Churchill & Churchill 1987, Matsumura *et al.* 1987). Inhibition of renin release is also observed *in vitro* with ouabain which by inhibiting sodium transport out of the cells facilitates calcium entry in the epitheloid cells (Lyons & Churchill 1974, Cruz-Soto *et al.* 1984, Churchill & Churchill 1980). There is thus a clear association between calcium influx and renin secretion *in vitro*; renin secretion is inhibited by increased transport of calcium into juxtaglomerular cells and inhibition of calcium transport stimulates renin secretion (Blaine *et al.* 1970, Fray 1980, Baumbach & Scott 1981, Park *et al.* 1981, Matsumura *et al.* 1987).

Discordant findings are, however, reported on the *in vivo* effect of calcium on plasma renin activity (PRA). Zemel *et al.* (1986) found an increased PRA after a high salt/high calcium diet in normotensive and hypertensive black adults, while Rich *et al.* (1991) 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.* (1992) found a suppression of the plasma renin-angiotensin system after oral calcium supplementation. According to Kotchen & Guthrie (1988) a high calcium chloride intake in normotensive rats on a normal salt intake did not affect PRA, while 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 (Kotchen & Guthrie 1988). A decrease in PRA was seen after calcium infusion in normal sodium-deplete men (Kisch *et al.* 1976), while calcium infusion had no effect on PRA in subjects on an unspecified dietary sodium intake (Maxwell & Kleeman 1972). 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 D₃ and 1,25-dihydroxyvitamin D₃ 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.

Materials and Methods

Subjects

Thirty-two normal male volunteers gave written consent for participation in the study after the procedure and purpose of the study had been explained. This study has been approved by the Ethics Committee of the Faculty of Medicine, University of Leuven. The age of the subjects averaged 24 ± 1 (S.E.M.) 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. Then the subjects 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. After 16 weeks of placebo or calcium administration, the 16 subjects in each group were treated at random with the calcium-channel blocker lacidipine (2 mg twice daily; $n=8$) or placebo ($n=8$) for an additional period of 8 weeks. The subjects were reinvestigated after 4 and 8 weeks of additional lacidipine or placebo administration.

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

Blood sampling

After 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 (Lijnen *et al.* 1986).

PRA (Fyhrquist & Puutula 1978), aldosterone concentrations (Lijnen *et al.* 1978) and 24-h excretion of aldosterone (Malvano *et al.* 1976), cAMP and cGMP (Tovey *et al.* 1974) were measured by radioimmunoassay. The urinary excretion of kallikrein was measured spectrophotometrically (Overlack *et al.* 1980).

The intraerythrocyte total Ca²⁺ concentration was determined by atomic absorption spectrophotometry (Zemel *et al.* 1988b). The platelet cytosolic free Ca²⁺ concentration was assayed by measuring the fluorescence of quin-2 entrapped by platelets (Tsien *et al.* 1982).

Plasma Na⁺, K⁺, urea, uric acid, phosphate, creatinine, chloride, bicarbonate, alkaline phosphatase and protein were measured by a multichannel autoanalyser. Plasma Ca²⁺ and Mg²⁺ were determined by atomic absorption spectrophotometry. Serum ionized calcium and pH were measured by an ICA2 ionized calcium analyser (Radiometer, Copenhagen, Denmark).

Plasma intact PTH was measured by an immunoradiometric assay (Bouillon *et al.* 1990), plasma 25-hydroxyvitamin D₃ by a competitive protein binding assay (Edelstein *et al.* 1974) and plasma 1,25-dihydroxyvitamin D₃ by a radioreceptor assay (Hollis 1986).

Statistical methods

Values are expressed as arithmetic means \pm S.E.M. Serial measurements in the two treatment groups were compared using the method of Matthews *et al.* (1990). This

TABLE 1. General characteristics of the subjects at randomization. Blood pressure and heart rate were measured with the subjects recumbent. Values are expressed as means \pm S.E.M. ($n=16$). No significant differences were observed between the placebo and calcium groups using paired t -test

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

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 Student's 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 and body weight

Compared with the placebo group, standing systolic blood pressure was decreased in the calcium group, while the standing diastolic blood pressure tended to decrease

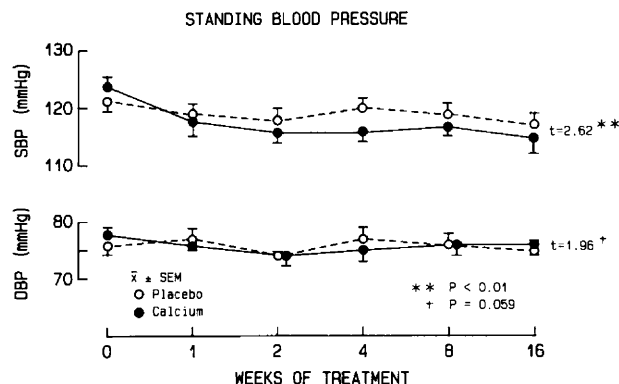


FIGURE 1. Standing systolic (SBP) and diastolic blood pressure (DBP) before and during oral administration of calcium (●) or placebo (○) in 16 men. The t -value corresponds to the average difference in the treatment effect between the placebo and calcium groups (Matthews *et al.* 1990).

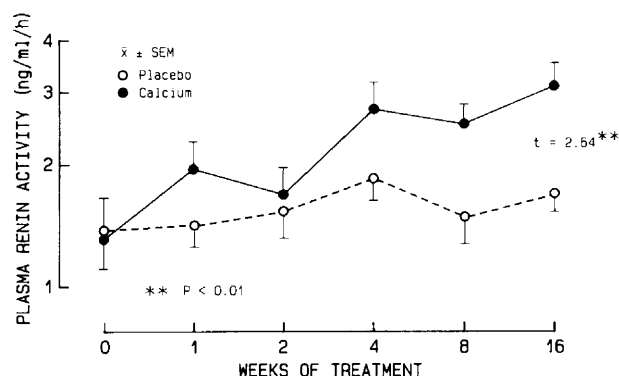


FIGURE 2. PRA before and during oral administration of calcium (●) or placebo (○) in 16 men. The t -value corresponds to the average difference in the treatment effect between the placebo and calcium groups (Matthews *et al.* 1990). Geometric means \pm S.E.M. are given.

(Fig. 1). Compared with the baseline value, the average decrease in the standing systolic blood pressure in the calcium group (-7.8 mmHg, range from -16 to $+4$ mmHg) was different ($P=0.013$) from the changes observed in the placebo group (-2.1 mmHg, range from -15 to $+7$ mmHg). A tendency ($P=0.059$) for a reduction in standing diastolic blood pressure in the calcium group (-2.6 mmHg, range from -14 to $+6$ mmHg) compared with the placebo group ($+0.9$ mmHg, range from -4 to $+16$ mmHg) was also observed. No significant difference was found in body weight between the calcium and placebo group.

No significant difference in the effect of lacidipine versus placebo on standing systolic or diastolic blood pressure or on body weight was found between the calcium- and placebo-treated subjects.

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. Values are means \pm S.E.M.

	Group	0	1	2	4	8	16	t-value
Plasma aldosterone (ng%)	Placebo	22.7 \pm 3.0	23.1 \pm 2.3	27.5 \pm 4.6	24.8 \pm 4.1	21.4 \pm 3.5	26.3 \pm 3.5	0.28 ^{NS}
	Calcium	24.8 \pm 3.3	25.2 \pm 3.4	23.5 \pm 2.7	29.0 \pm 4.0	23.8 \pm 2.8	37.8 \pm 6.0	
Urinary aldosterone (μ g/24 h)	Placebo	10.4 \pm 1.5	10.0 \pm 2.0	8.2 \pm 1.6	8.6 \pm 1.4	8.4 \pm 1.4	9.5 \pm 1.4	0.53 ^{NS}
	Calcium	8.9 \pm 1.2	8.7 \pm 1.2	7.0 \pm 1.1	6.2 \pm 0.9	6.3 \pm 1.1	9.3 \pm 1.4	
Urinary kallikrein (U/24 h)	Placebo	0.788 \pm 0.165	0.884 \pm 0.223	0.763 \pm 0.142	0.839 \pm 0.178	0.771 \pm 0.145	0.980 \pm 0.188	0.85 ^{NS}
	Calcium	0.624 \pm 0.074	0.623 \pm 0.088	0.601 \pm 0.069	0.661 \pm 0.076	0.545 \pm 0.046	0.621 \pm 0.086	
Urinary cGMP (μ mol/24 h)	Placebo	0.492 \pm 0.067	0.470 \pm 0.058	0.448 \pm 0.060	0.526 \pm 0.049	0.510 \pm 0.048	0.499 \pm 0.049	1.00 ^{NS}
	Calcium	0.436 \pm 0.041	0.482 \pm 0.044	0.448 \pm 0.030	0.495 \pm 0.042	0.469 \pm 0.040	0.433 \pm 0.047	
Urinary cAMP (μ mol/24 h)	Placebo	4.92 \pm 0.33	4.65 \pm 0.36	4.79 \pm 0.41	4.77 \pm 0.30	4.46 \pm 0.26	4.53 \pm 0.31	0.90 ^{NS}
	Calcium	4.51 \pm 0.38	4.66 \pm 0.29	4.57 \pm 0.28	3.72 \pm 0.31	4.22 \pm 0.38	4.00 \pm 0.31	

NS, not significant. The *t*-value corresponds to the average difference in treatment effect between the placebo and calcium groups.

Plasma renin-aldosterone

Compared with the placebo group, on average an increase in the PRA was observed in the calcium group (Fig. 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 ($P < 0.05$) and remained elevated after 8 ($P < 0.02$) and 16 ($P < 0.005$) weeks of calcium administration.

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 (Fig. 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, while the rise in PRA occurred only after 4 weeks of oral calcium administration, and then remained elevated up to 16 weeks of calcium administration (Fig. 2).

Urinary and plasma variables

Compared with the placebo group, the 24-h urinary excretion of calcium increased ($P < 0.05$) in the calcium group (Fig. 4), while 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 (Fig. 5) or in plasma phosphate, magnesium, sodium and potassium between the calcium and placebo group. The 24-h urinary

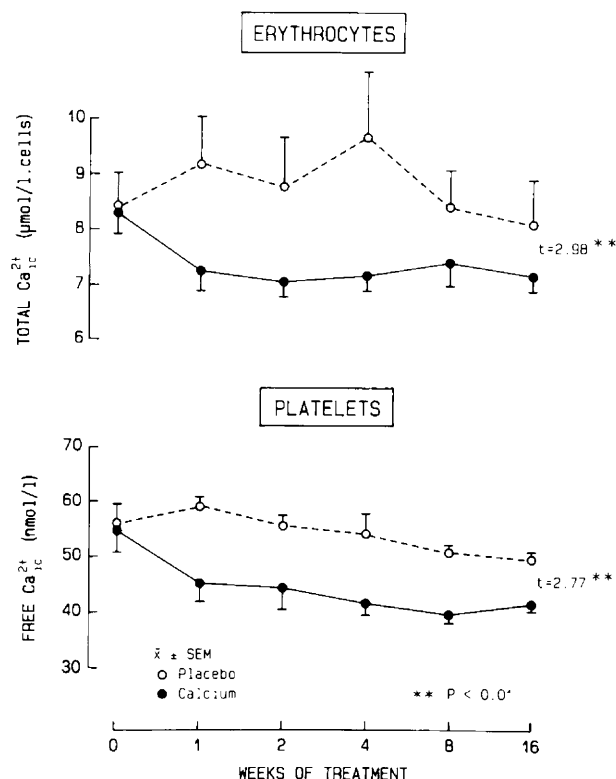


FIGURE 3. Erythrocyte total intracellular Ca^{2+} concentration ($\text{Ca}^{2+}_{\text{ic}}$) and platelet intracellular free cytosolic Ca^{2+} concentration ($\text{Ca}^{2+}_{\text{ic}}$) before and during oral administration of calcium (●) or placebo (○) in 16 men. The *t*-value corresponds to the average difference in treatment effect between the placebo and calcium groups (Matthews *et al.* 1990).

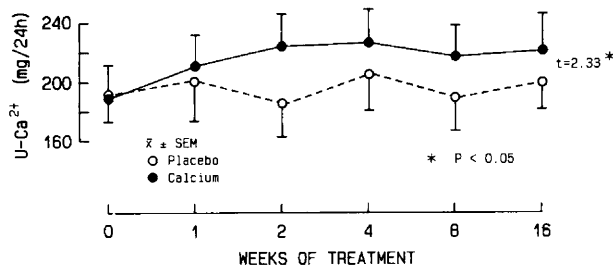


FIGURE 4. 24-h urinary excretion of calcium (U-Ca^{2+}) before and during oral administration of calcium (●) or placebo (○) in 16 men. The t -value corresponds to the average difference in treatment effect between the placebo and calcium groups (Matthews *et al.* 1990).

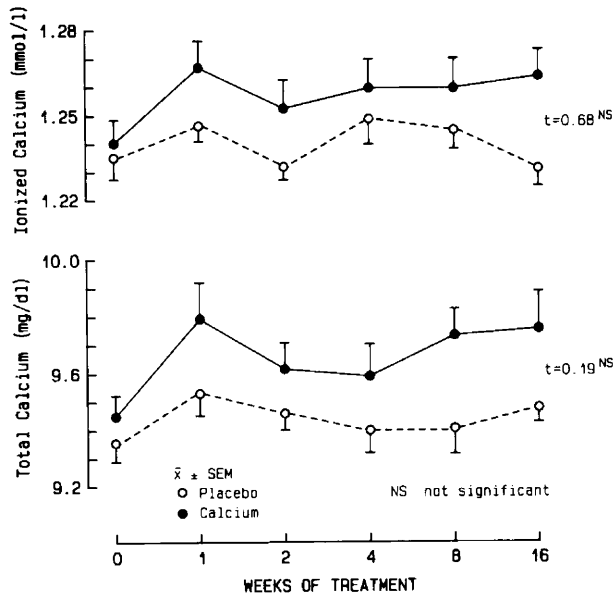


FIGURE 5. Serum ionized Ca^{2+} and plasma total Ca^{2+} concentrations before and during oral administration of calcium (●) or placebo (○) in 16 men. The t -value corresponds to the average difference in treatment effect between the placebo and calcium groups (Matthews *et al.* 1990).

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 (Fig. 6). However, no significant difference was found in the plasma concentration of 25-hydroxyvitamin D_3 between the placebo- and calcium-treated groups (Fig. 6).

Additional calcium-channel blockade in calcium- and placebo-treated subjects

The effect of lacidipine or placebo administration on PRA in the placebo- or calcium-treated subjects is given in Table 3. PRA was increased in the calcium group com-

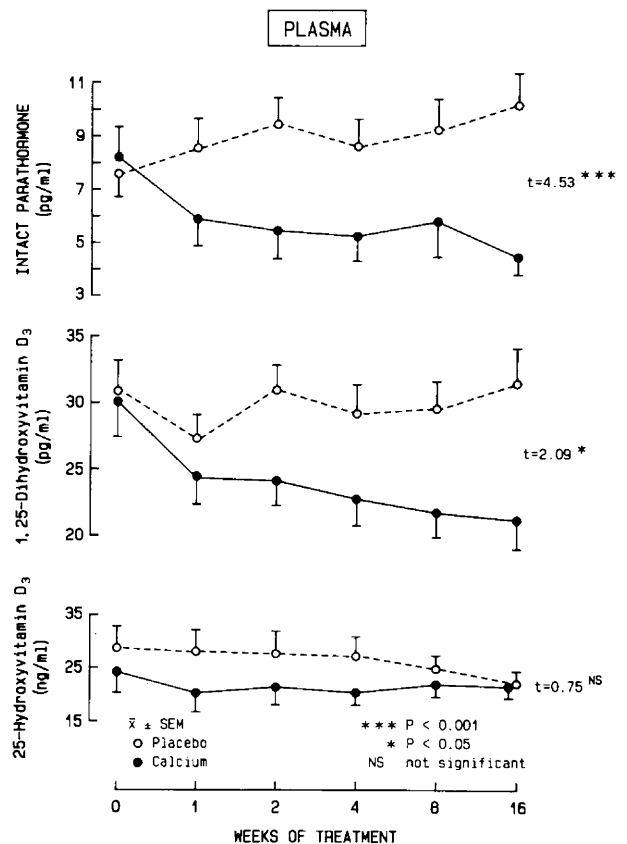


FIGURE 6. Plasma concentration of intact parathormone, 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 before and during oral administration of calcium (●) or placebo (○) in 16 men. The t -value corresponds to the average difference in treatment effect between the placebo and calcium groups (Matthews *et al.* 1990).

pared with the placebo group. Lacidipine tended to increase PRA in the placebo-treated subjects and to decrease PRA in the calcium-treated subjects. This difference in lacidipine effect between the placebo and calcium group was statistically significant ($t = -2.38$; $P < 0.05$). However, no significant difference in the lacidipine effect was found for plasma or urinary aldosterone, urinary excretion of kallikrein, cAMP and cGMP, intraerythrocyte or intraplatelet Ca^{2+} concentration, plasma total Ca^{2+} , ionized serum calcium or plasma calcitropic hormones.

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 an enhancement of

TABLE 3. Effect of additional administration of lacidipine or placebo, for 4 and 8 weeks, in placebo- and calcium-treated normal men. The average change in PRA during placebo or lacidipine administration (Matthews *et al.* 1990) is shown. Values are means \pm S.E.M. ($n=8$)

PRA (ng/ml per h) after 16 weeks of (A) placebo or (B) calcium administration	PRA (ng/ml per h) after additional administration of lacidipine or placebo for			Average change in PRA
		4 weeks	8 weeks	
A. Placebo group				
2.02 ± 0.27	Placebo	1.82 ± 0.34	1.71 ± 0.27	− 0.33 ± 0.19
1.77 ± 0.31	Lacidipine	2.35 ± 0.28	3.29 ± 1.17	+ 1.05 ± 0.65
B. Calcium group				
2.84 ± 0.52	Placebo	1.63 ± 0.30	1.88 ± 0.41	− 1.09 ± 0.38
3.36 ± 0.77	Lacidipine	2.65 ± 0.48	2.07 ± 0.36	− 1.00 ± 0.89

The difference in lacidipine effect between the placebo and calcium groups: $t = -2.38$; $P < 0.05$.

PRA. However, plasma and urinary aldosterone as well as the urinary excretion of kallikrein, cAMP and cGMP did not change during calcium supplementation. A modest but significantly lower systolic blood pressure in standing position during oral calcium supplementation was also observed (Fig. 1), while the standing diastolic blood pressure tended to be lower (Lijnen & Petrov 1995).

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) (Furspan *et al.* 1989, Dominiczak & Bohr 1990, Pörsti 1992, Pörsti *et al.* 1992, Wuorela *et al.* 1992, Arvola *et al.* 1993) as well as the intracellular total Ca^{2+} concentration in erythrocytes of diabetic hypertensive patients (Zemel *et al.* 1988a). 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 transport 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 (Zemel *et al.* 1988a).

Increased dietary calcium has been reported to reduce the plasma digitalis-like immunoreactive factor in rats (Doris 1988). 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 (Pörsti *et al.* 1992) 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

$\text{Na}^+ - \text{Ca}^{2+}$ and $\text{Na}^+ - \text{H}^+$ exchange mechanisms, which have a central role in controlling the intracellular free Ca^{2+} level (Ashida & Blaustein 1987, Beck *et al.* 1988), 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 (Pörsti *et al.* 1991) and sympathoinhibitory (Young *et al.* 1988) 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 (Pang *et al.* 1992). 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 (Adams *et al.* 1979, Gallagher *et al.* 1979). According to Basile *et al.* (1993) 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. The rise in 24-h urinary calcium excretion ($+16.8 \pm 1.5\%$) observed in the present study during oral calcium supplementation (50 mmol/day for 16 weeks) is in agreement with the increase reported in various studies (Grobbee & Hofman 1986, Luft *et al.* 1986, Nowson & Morgan 1986, Strazzullo *et al.* 1986, Van Beresteyn *et al.* 1986, Cappuccio *et al.* 1987, Meese *et al.* 1987, Siani *et al.* 1988, Zoccali *et al.* 1988, Belizan *et al.* 1991). In these trials with a duration of 8.2 ± 1.3 weeks and an oral supplementation of 26.5 ± 3.6 mmol of elemental calcium

per day the 24-h urinary calcium excretion increased by $17.9 \pm 1.6\%$.

According to Pang *et al.* (1992) 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 radiolabelled calcium uptake in vascular smooth muscle cells (Bukoski *et al.* 1987) and PTH also enhanced calcium influx in red blood cells (Bogin *et al.* 1982). The decrease in intraerythrocyte and intraplatelet Ca^{2+} concentration observed after oral calcium supplementation in men (Fig. 2) 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 (Nadler & Antonipillai 1986), indicating that intracellular Ca^{2+} levels are key signals for renin release. Calmidazolium, 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 (Park *et al.* 1992). The increased PRA during oral calcium supplementation can, however, also be related to intravascular volume depletion.

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 (Schricker *et al.* 1993): an inhibitory one that lasted for at least 1 h and a powerful stimulatory one that occurred with a delay of approximately 1–3 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 (Kurtz 1986), isolated

glomeruli (Baumbach & Scott 1981), kidney slices (Park *et al.* 1981, Naftilan & Oparil 1982, Heinrich & Campbell 1986), isolated perfused kidneys (Marre *et al.* 1982, Loutzenhiser *et al.* 1985) and *in vivo* (Abe *et al.* 1983, Roy *et al.* 1983, Imagawa *et al.* 1986).

In some studies, however, blockers of calcium entry, such as verapamil or diltiazem, failed to stimulate renin release when infused into the renal artery (Cho *et al.* 1987) or did not attenuate the inhibition of renin release from rat kidney slices by angiotensin II (Churchill 1987).

These observations do not, however, contradict the basic concept of an inverse relationship between calcium and renin secretion (Hackenthal *et al.* 1990), as other actions of calcium-channel antagonists probably counteract their stimulatory effects on renin secretion. Also, in the present study, a divergent effect of lacidipine, either an increase or a decrease, on PRA is observed in the placebo- and calcium-treated subjects.

A possible explanation for the occasional lack of or 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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