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Dietary calcium and magnesium supplements in spontaneously hypertensive rats and isolated arterial reactivity

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1 High calcium diet attenuates the development of hypertension but an associated undesirable effect is that Mg^{2+} loss to the urine is enhanced. Therefore, we studied the effects of high calcium diet alone and in combination with increased magnesium intake on blood pressure and arterial function.

2 Forty-eight young spontaneously hypertensive rats (SHR) were allocated into four groups, the dietary contents of Ca^{2+} and Mg^{2+} being: 1.1%, 0.2% (SHR); 2.5%, 0.2% (Ca-SHR); 2.5%, 0.8% (CaMg-SHR); and 1.1%, 0.8% (Mg-SHR), respectively. Development of hypertension was followed for 13 weeks, whereafter electrolyte balance, lymphocyte intracellular free calcium ($[Ca^{2+}]_i$), and mesenteric arterial responses *in vitro* were examined. Forty normotensive Wistar-Kyoto (WKY) rats were investigated in a similar manner.

3 Calcium supplementation comparably attenuated the development of hypertension during normal and high magnesium intake in SHR, with an associated reduced lymphocyte $[Ca^{2+}]_i$ and increased Mg^{2+} loss to the urine.

4 Endothelium-dependent arterial relaxation to acetylcholine was augmented in Ca-SHR and CaMg-SHR, while the relaxations to isoprenaline and the nitric oxide donor SIN-1 were similar in all SHR groups. Relaxation responses induced by the return of K^+ to the organ bath upon precontractions in K^+-free solution were used to evaluate the function of arterial Na^+, K^+-ATPase. The rate of potassium relaxation was similar in Ca-SHR and CaMg-SHR and faster than in untreated SHR.

5 Contractile responses to high concentrations of potassium and noradrenaline, and the ability of vascular smooth muscle to sequester Ca^{2+}, which was evaluated by eliciting responses to caffeine or noradrenaline after loading periods in different Ca^{2+} concentrations, were comparable in all SHR groups. In SHR with increased magnesium intake, and in WKY rats with calcium or magnesium supplementation, no detectable effects on blood pressure and arterial function were observed.

6 In conclusion, high calcium diet attenuated the development of hypertension in SHR, with an associated augmented endothelium-dependent relaxation, promoted recovery rate of ionic gradients across the cell membrane via Na^+, K^+-ATPase, and reduced basal $[Ca^{2+}]_i$. Dietary magnesium supplementation, whether combined with normal or high calcium intake, had no beneficial effects on blood pressure or arterial function.

Keywords: Arterial function; blood pressure; dietary calcium; dietary magnesium; spontaneously hypertensive rat

Introduction

Increased dietary calcium intake lowers blood pressure and favourably affects arterial smooth muscle function in various forms of experimental hypertension (Resnick *et al.*, 1986; Bukoski & McCarron 1986; Pörsti *et al.*, 1992; Arvola *et al.*, 1993a). The mechanisms underlying the antihypertensive effect of oral calcium loading, however, remain to be clarified. High calcium diet may correct the generalized membrane defect observed in hypertension, and thus reduce plasma membrane permeability to Ca^{2+} and other ions (Furspan *et al.*, 1989). Calcium supplementation has also been shown to decrease the abnormally high Na^+, K^+-ATPase inhibitory activity in plasma from hypertensive rats (Doris, 1988; 1994). In arterial smooth muscle of deoxycorticosterone (DOC)-NaCl treated rats the blood pressure-lowering action of high calcium diet has been reported to reduce sensitivity to depolarization and voltage-dependent Ca^{2+} entry, and enhance function of Na^+, K^+-ATPase (Arvola *et al.*, 1993a). Furthermore, it has been suggested that increased dietary calcium augments vascular sensitivity to endogenous and exogenous nitric oxide (NO) in DOC-NaCl hypertension (Mäkynen *et al.*, 1994). While having beneficial effects on arterial function, calcium supplementation has been reported to increase the urinary excretion of Mg^{2+} (Wuorela *et al.*, 1992a; Arvola *et al.*, 1993a), and even to provoke signs of Mg^{2+} deficiency in rats receiving relatively low levels of magnesium in the diet (Evans *et al.*, 1990). Recently a 3 fold increase in calcium intake in spontaneously hypertensive rats (SHR) was found to lower blood pressure as effectively as a 4 fold increase. However, the higher level of supplemented calcium also induced higher excretion of Mg^{2+} in the urine, suggesting that exaggerated Mg^{2+} loss may be a limiting factor for the beneficial effects of high calcium diet (Wuorela *et al.*, 1992a).

Magnesium is an important cofactor in many enzymatic reactions and it has well-documented effects in the vasculature. Magnesium deficiency has been observed to elevate blood pressure and increase peripheral vascular resistance in rats (Altura *et al.*, 1983; Chrysant *et al.*, 1988; Altura & Altura, 1990). Low magnesium intake also causes insulin resistance (Nadler *et al.*, 1993), and reduces the release of atrial natriuretic peptide from the heart (Wong *et al.*, 1991), factors which possibly contribute to the elevation of blood pressure. Furthermore, intracellular free Mg^{2+} concentration in vascular smooth muscle cells (Ng *et al.*, 1992), as well as total Mg^{2+} content in several other tissues has been found to be lower in hypertensive than in normotensive rats (Henrotte *et al.*, 1991). Oral magnesium supplementation has also been reported to

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lower blood pressure in patients with essential hypertension (Motoyama *et al.*, 1989), and intravenous Mg^{2+} infusion to dilate coronary arteries in man (Vigorito *et al.*, 1991).

Since the long-term effects of magnesium supplementation on blood pressure during increased calcium intake are unknown, the present investigation was undertaken; we also examined the associated effects on arterial function and electrolyte balance in SHR and their normotensive control strain Wistar-Kyoto (WKY) rats.

Methods

Animals and experimental design

First study series: 48 male SHR of the Okamoto-Aoki strain and 12 age-matched WKY rats (Møllegaards Breeding Centre, Ejby, Denmark) were used. At the age of 8 weeks, SHR were divided into 4 groups ($n=12$) of equal mean systolic blood pressures. Thus the study groups were: control SHR on normal dietary calcium and magnesium (SHR), high calcium SHR (Ca-SHR), high calcium and high magnesium SHR (CaMg-SHR), high magnesium SHR (Mg-SHR), and control WKY rats on normal dietary calcium and magnesium.

Second study series: Forty 8-week old male WKY rats (Møllegaards Breeding Centre) were divided into 4 groups ($n=10$) of equal mean systolic blood pressures. The groups were: control WKY rats on normal dietary calcium and magnesium, high calcium WKY rats (Ca-WKY), high calcium and high magnesium WKY rats (CaMg-WKY) and high magnesium WKY rats (Mg-WKY).

The rats were housed four to a cage in a standard experimental laboratory room at 22°C with 12 h light-dark cycle and had free access to drinking fluid (tap water) and food pellets (Ewos, Södertälje, Sweden). The contents of Ca^{2+} and Mg^{2+} in the chow were 1.1% and 0.2% (SHR and WKY rats), 2.5% and 0.2% (Ca-SHR and Ca-WKY), 2.5% and 0.8% (CaMg-SHR and CaMg-WKY), 1.1% and 0.8% (Mg-SHR and Mg-WKY), respectively. The systolic blood pressures of conscious animals were measured at 28°C by the tail-cuff method (Model 129 Blood Pressure Meter; IITC Inc., Woodland Hills, Ca., U.S.A.). During study week 13, urine was collected for 24 h in metabolic cages, where the rats had free access to food and drinking fluid. Fluid and food consumption were determined by weighing the bottles and chow pellets. Urine volumes were measured and samples stored at −20°C until assayed. At the end of the study the rats were weighed, decapitated and exsanguinated. For plasma electrolyte and lymphocyte $[Ca^{2+}]_i$ measurements blood samples were drawn into polystyrene tubes containing heparin (100 units ml^{-1}) as anticoagulant. Lymphocyte $[Ca^{2+}]_i$ was measured with acetoxymethyl ester of quin-2 as previously described (Pörsti *et al.*, 1992; Wuorela *et al.*, 1992a). Plasma and urine concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometer (Spectr AA-30, Varian, Techtron Ltd., Victoria, Australia). For the Ca^{2+} measurements, 5 mM $LaCl_3$ was used as ionization suppressant. The hearts were removed and weighed, and superior mesenteric arteries carefully excised and cleaned of connective tissue. The experimental design of the study was approved by the Animal Experimentation Committee of the University of Tampere, Finland.

Mesenteric arterial responses in vitro

Three successive standard sections (3 mm in length) of the mesenteric artery were cut, beginning 1 cm distally from the mesenteric artery-aorta junction. The most distal ring was endothelium-intact and from another two the endothelium was removed by gently rubbing with a jagged injection needle (Arvola *et al.*, 1992). The rings were placed between two stainless steel hooks (diameter 0.3mm) and suspended in organ bath chamber (volume 20 ml) in physiological salt solution (PSS) (pH 7.4) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, and aerated with 95% O_2 and 5% CO_2. The rings were initially equilibrated for 1 h at 37°C with a resting tension of 1.5 g. The force of contraction was measured with an isometric force-displacement transducer and registered on a polygraph (FT03 transducer & model 7E Polygraph; Grass Instrument Co., Quincy, Ma., U.S.A.). The presence of intact endothelium in the most distal vascular preparation was confirmed by an almost complete relaxation response to 1 μM acetylcholine (ACh) in 1 μM noradrenaline (NA)-precontracted rings, and the absence of endothelium by the lack of this relaxation response.

Vascular preparation 1: After a 30-min stabilization period, the endothelium-denuded mesenteric arterial ring was contracted with 10 μM NA (reference contraction, normal PSS). When the maximal response was reached, the ring was rinsed with Ca^{2+}-free PSS. After a 10 min period in Ca^{2+}-free PSS, 1 mM ethylene glycol *bis*(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was introduced and the maximal response with 10 μM NA was elicited 1 min later. This induced a rapidly fading contraction, the peak of response reflecting the amount of Ca^{2+} liberated from cellular stores (Dohi *et al.*, 1990). Then the following cycle was used to evaluate Ca^{2+} sequestration into cellular stores: (1) The preparations were incubated for 6 min in Ca^{2+}-free PSS containing 1 mM EGTA (depletion of cellular stores). (2) The rings were allowed a 10 min period in PSS with Ca^{2+} (Ca^{2+} loading period; no EGTA in organ bath). (3) The Ca^{2+}-free PSS containing 0.1 mM EGTA was returned to the organ bath chamber and 1 min later contractions to 10 μM NA were elicited and maximal responses registered. The cycle was repeated 5 times and the following Ca^{2+} concentrations were tested: 0, 0.033, 0.1, 0.4 and 1.2 mM. After a 20 min recovery period similar cycles were repeated with 30 mM caffeine as the contractile agent.

Vascular preparation 2: After 30 min the rings were exposed to K^+-free buffer solution (pH 7.4, KH_2PO_4 and KCl substituted with NaH_2PO_4 and NaCl, respectively, on an equimolar basis). The omission of K^+ induced gradual contractions in all vascular rings. Once the contraction had reach a plateau, 1 mM K^+ was added and the subsequent relaxation registered.

Vascular preparation 3: In the most distal ring with intact endothelium, concentration-response curves for NA, and 30 min later for KCl, were determined cumulatively. After another 30 min recovery period, vascular responses to ACh, 3-morpholinosydnonimine (SIN-1), and isoprenaline were examined. The rings were precontracted with 1 μM NA, and after the contraction had fully developed, increasing concentrations of the relaxing agent were cumulatively added to the organ bath. The next concentration of the relaxant was added only after the previous level of relaxation was stable. The rings were allowed a 20 min recovery in resting tension between the study of each relaxant.

The KCl- and NA-induced contractile responses were expressed in g. The maximal contractile forces induced by 30 mM caffeine and 10 μM NA after each Ca^{2+} loading period were normalized by relating them to the previously determined reference contractions. After K^+ repletion upon K^+-free contraction, the greatest reduction in smooth muscle tone during a 1 min period was considered the maximal relaxation rate. The relaxations in response to K^+ repletion, ACh, SIN-1 and isoprenaline were presented as percentage of pre-existing contractile force.

Drugs

The following drugs were used: ammonium salt of heparin, acetylcholine chloride, EGTA, isoprenaline hydrochloride (Sigma Chemical Co., St. Louis, Mo, U.S.A.), quin-2 (Aldrich

Chemical Co., Milwaukee, Wis., U.S.A.), (−)-noradrenaline L-hydrogentartrate (Fluka Chemie AG, Buchs SG, Switzerland), 3-morpholinosydnonimine (GEA Ltd., Copenhagen, Denmark). The stock solutions of the compounds were dissolved in distilled water, with the exception of quin-2 (in dimethylsulphoxide). All solutions were freshly prepared before use and protected from light.

Analysis of results

Statistical analysis was carried out by one-way of analysis of variance (ANOVA) supported by Bonferroni confidence intervals when carrying out pairwise comparisons between the test groups. When appropriate, ANOVA for repeated measurements with Greenhouse-Geisser adjustment was applied for data consisting of repeated observations at successive time points. All results were expressed as mean ± s.e.mean, with P values <0.05 considered statistically significant.

Results

First study series: the SHR, Ca-SHR, CaMg-SHR, Mg-SHR and WKY rat groups

During the 13-week study the development of hypertension was significantly attenuated, and the final blood pressures remained approximately 12 mmHg lower in the Ca-SHR and CaMg-SHR groups when compared with control SHR. Magnesium supplementation alone did not affect blood pressure in SHR (Figure 1 and Table 1). Heart weights and heart: body weight ratios were similar in all SHR groups and higher than in WKY rats. Moreover, final body weights in the Ca-SHR and CaMg-SHR groups were somewhat lower than in control SHR and WKY rats (Table 1).

During the 24 h monitoring period in metabolic cages at the close of the study, calcium supplementation induced a 3–4 fold increase in Ca^{2+} intake, and magnesium supplementation a 6–7 fold elevation in Mg^{2+} ingestion in SHR. Furthermore, the high calcium diet clearly enhanced the excretion of Mg^{2+} in the urine, this effect being apparent in Ca-SHR when compared with control SHR, as well as in CaMg-SHR when compared with Mg-SHR. Magnesium supplementation alone also somewhat increased Ca^{2+} excretion to the urine (Table 2). The plasma concentrations of K^+, Ca^{2+} and Mg^{2+} were comparable in all SHR groups. However, plasma Na^+ concentration was slightly lower in CaMg-SHR, Mg-SHR, and WKY rats, and slightly higher in Ca-SHR than in the control SHR group (Table 1). Lymphocyte $[Ca^{2+}]_i$, measured with quin-2, was comparable in the Ca-SHR, CaMg-SHR, and WKY groups and lower than in the control SHR and Mg-SHR groups (Table 1).

In endothelium-intact mesenteric arterial rings the contractile responses to NA were comparable in all study groups. Contractile force generation to high concentrations of KCl did not differ between the SHR groups either, while the maximal responses to this agonist were higher in SHR than in WKY rats (Figure 2). The relaxation responses of endothelium-intact arterial rings to ACh were impaired in the control SHR and Mg-SHR groups when compared with WKY rats. Interestingly, endothelium-dependent relaxation to ACh was augmented in Ca-SHR and CaMg-SHR when compared with control SHR, the response being shifted towards that of WKY rats. However, no differences were found between the study groups in the relaxation responses induced by the NO donor, SIN-1 (Figure 3). Furthermore, arterial relaxation to isoprenaline was attenuated in all SHR groups when compared with WKY rats, and neither calcium nor magnesium supplementation had any effect on this response (Figure 4).

Mesenteric arterial relaxation induced by the addition of 1 mM K^+ (upon precontraction elicited by the omission of K^+

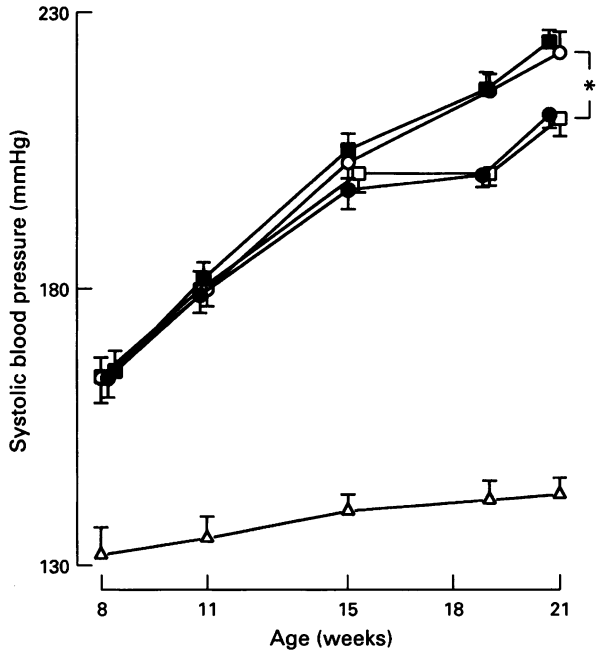

Figure 1 Systolic blood pressures in spontaneously hypertensive rats (SHR, ○), calcium supplemented SHR (Ca-SHR, ●), calcium and magnesium supplemented SHR (CaMg-SHR, □), and magnesium supplemented SHR (Mg-SHR ■), and untreated Wistar-Kyoto (WKY, △) rats during the 13-week study. Symbols indicate means with s.e.means, $n=12$ in each group; $*P<0.05$ SHR vs. Ca-SHR and CaMg-SHR, $*P<0.05$ Mg-SHR vs. Ca-SHR and CaMg-SHR, ANOVA for repeated measurements.

Table 1 Experimental group data at close of the 13-week study

Variable	*SHR*	*Ca-SHR*	*CaMg-SHR*	*Mg-SHR*	*WKY*
Blood pressure (mmHg)	223 ± 4	212 ± 2*	211 ± 3*	225 ± 2†#	143 ± 3*
Body weight (g)	374 ± 3	352 ± 4*	350 ± 3*	363 ± 5	379 ± 7
Heart weight (mg)	1322 ± 22	1331 ± 15	1342 ± 33	1329 ± 27	1221 ± 25*
Heart: body weight (mg g^{-1})	3.6 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.7 ± 0.1	3.2 ± 0.1*
Plasma electrolytes (mM)					
Na^+	143.4 ± 1.2	149.2 ± 0.9*	135.4 ± 1.1*†	137.8 ± 0.6*†	137.7 ± 1.1*
K^+	5.46 ± 0.17	5.47 ± 0.12	5.14 ± 0.19	5.19 ± 0.12	4.76 ± 0.15*
Ca^{2+}	2.38 ± 0.06	2.47 ± 0.04	2.46 ± 0.04	2.57 ± 0.09	2.38 ± 0.11
Mg^{2+}	0.85 ± 0.03	0.91 ± 0.03	0.95 ± 0.05	0.94 ± 0.03	0.90 ± 0.03
Lymphocyte intracellular free Ca^{2+} (nM)	97.7 ± 3.3	81.4 ± 5.7*	79.8 ± 2.4*	86.8 ± 7.7	72.0 ± 2.8*

Values are mean ± s.e.mean; $n=8-10$ for all groups. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Ca, calcium supplemented rats; Mg, magnesium supplemented rats. $*P<0.05$ compared with the SHR group, $†P<0.05$ versus Ca-SHR, $\#P<0.05$ versus CaMg-SHR (Bonferroni test).

Table 2 Metabolic data (per 24 h) at close of the 13-week study

Variable	*SHR*	*Ca-SHR*	*CaMg-SHR*	*Mg-SHR*	*WKY*
Chow intake (g)	8.0±1.3	14.0±1.0*	17.7±1.5*	13.9±1.8*	9.9±1.9
Fluid intake (ml)	18.4±2.0	26.9±1.4	23.8±2.1	27.8±2.8	19.0±2.1
Urine volume (ml)	13.5±1.9	14.2±1.0	14.9±2.6	14.8±1.3	9.4±1.0
Electrolyte intake (mmol)					
Na^+	1.37±0.18	2.30±0.15*	2.78±0.21*	2.30±0.28*	1.62±0.29
K^+	1.70±0.27	2.95±0.20*	3.70±0.30*	2.93±0.38*	2.08±0.40
Ca^{2+}	3.03±0.38	9.95±0.66*	12.28±0.95*	5.06±0.61*†#	3.57±0.62
Mg^{2+}	0.86±0.14	1.44±0.09*	6.11±0.49*†	4.87±0.63*†	1.02±0.18
Urinary excretion					
Na^+ (mmol)	1.24±0.14	2.04±0.30*	1.70±0.24	1.76±0.28	1.31±0.20
K^+ (mmol)	1.95±0.14	3.94±0.48*	2.40±0.18	2.57±0.25	1.7±0.23
Ca^{2+} (μmol)	18.5±1.9	391.8±50.7	419.9±121.8*	67.1±27.1*†#	17.0±5.0
Mg^{2+} (μmol)	213±37	504±58*	987±94*†	478±111*#	110±15*

Values are mean±s.e.mean; $n=8-10$ for all groups. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; Ca, calcium supplemented rats; Mg, magnesium supplemented rats. *$P<0.05$ compared with the SHR group, †$P<0.05$ versus Ca-SHR, #$P<0.05$ versus CaMg-SHR (Bonferroni test).

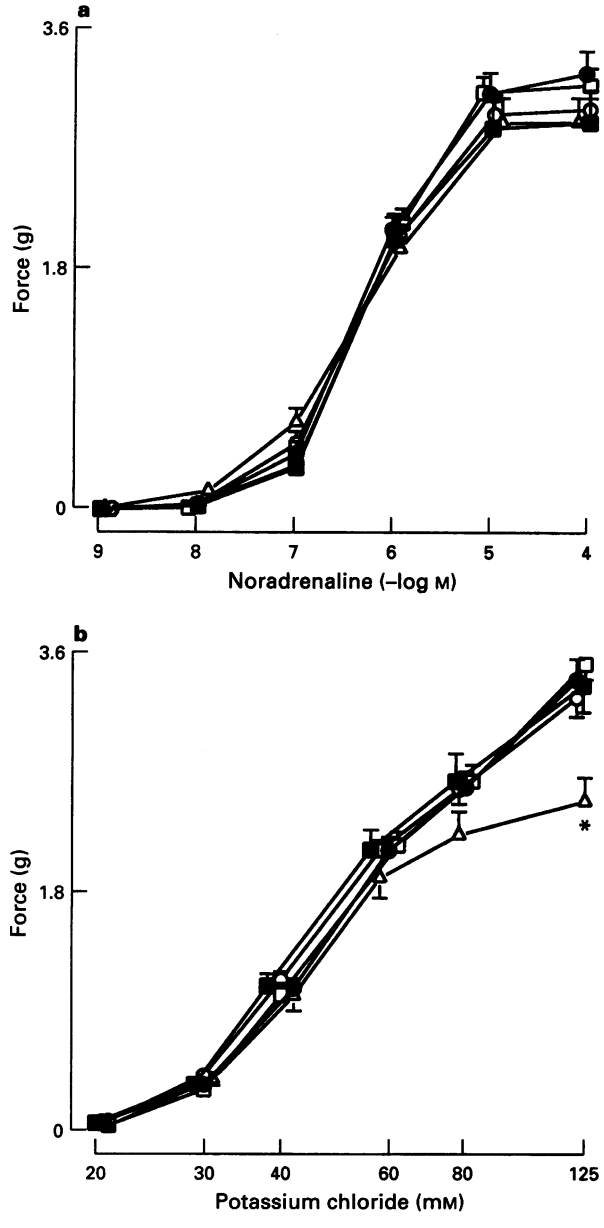

Figure 2 Concentration-response curves of endothelium-intact mesenteric arterial rings to noradrenaline (a) and potassium chloride (b) in spontaneously hypertensive rats (SHR, ○), calcium supplemented SHR (●), calcium and magnesium supplemented SHR (□), magnesium supplemented SHR (■), and untreated Wistar-Kyoto (WKY, △) rats. Maximal potassium chloride-induced contractile force generation was lower in WKY rats than in SHR. Symbols indicate means with s.e.means, $n=8$ in each group; *$P<0.05$, Bonferroni test.

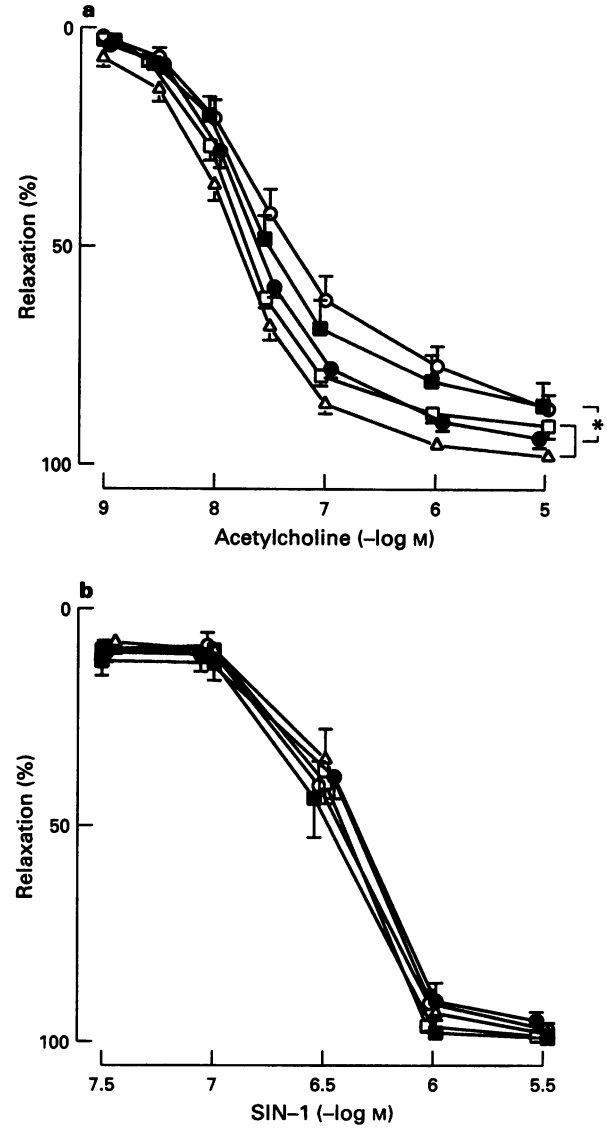

Figure 3 Cumulative relaxation responses to acetylcholine (a) and 3-morpholinosydnonimine (SIN-1) (b) after precontraction with 1 μM noradrenaline in isolated endothelium-intact mesenteric arterial rings from spontaneously hypertensive rats (SHR, ○), calcium supplemented SHR (●), calcium and magnesium supplemented SHR (□), magnesium supplemented SHR (■), and untreated Wistar-Kyoto (WKY, △) rats. Symbols indicate means with s.e.means, $n=10$ in each group; *$P<0.05$, ANOVA for repeated measurements.

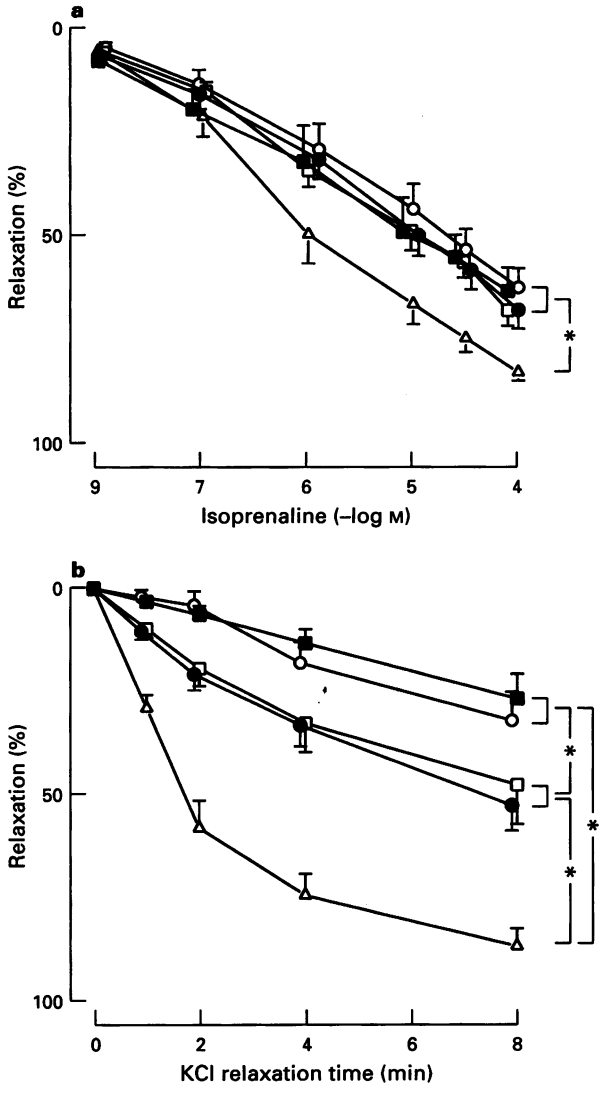

Figure 4 Cumulative relaxation responses to isoprenaline after full precontraction with 1 μM noradrenaline in isolated endothelium-intact mesenteric arterial rings (a), and relaxation responses to re-addition of 1.0 mM K^+ after full precontraction induced by K^+-free buffer solution in endothelium-denuded rings (b). The groups were spontaneously hypertensive rats (SHR, ○), calcium supplemented SHR (●), calcium and magnesium supplemented SHR (□), magnesium supplemented SHR (■), and untreated Wistar-Kyoto (WKY, △) rats. Symbols indicate means with s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

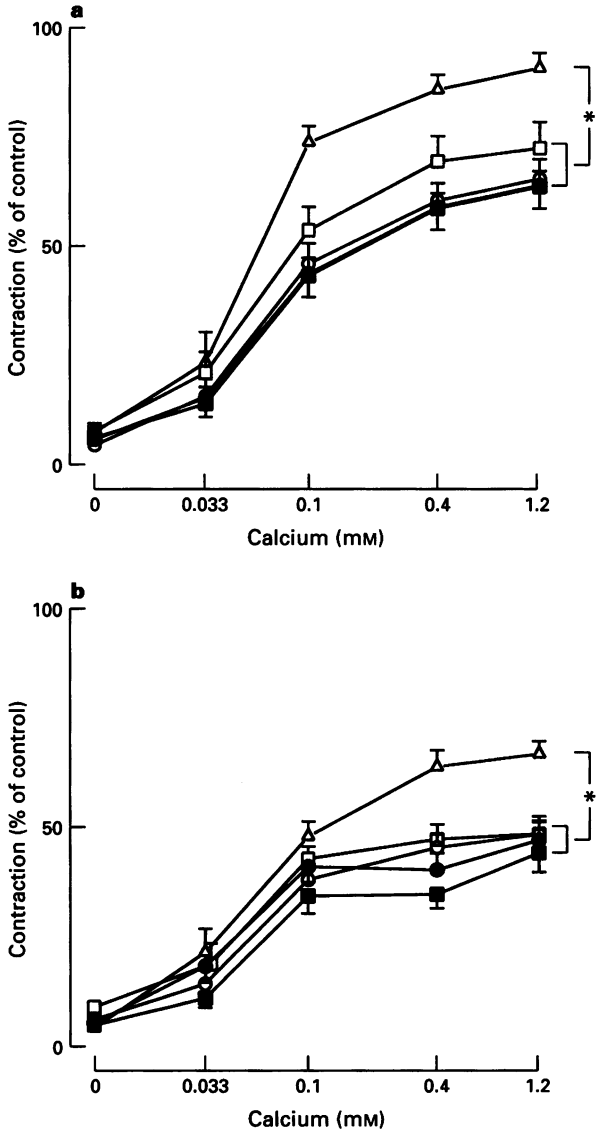

Figure 5 Contractile responses reflecting the ability to sequester calcium into cellular stores in endothelium-denuded mesenteric arterial rings from spontaneously hypertensive rats (SHR, ○), calcium supplemented SHR (●), calcium and magnesium supplemented SHR (□), magnesium supplemented SHR (■), and untreated Wistar-Kyoto (WKY, △) rats. Initially, calcium stores were depleted, after which calcium was returned to the organ bath for 10 min, concentrations of 0, 0.033, 0.1, 0.4, 1.2 mM being tested. The maximal contractions induced by 10 μM noradrenaline (a) and 30 mM caffeine (b) after each calcium loading period were normalized by comparing them to the previously determined reference responses. Symbols indicate means with s.e.means, $n=8-10$ in each group; $^*P<0.05$, ANOVA for repeated measurements.

from the organ bath) was clearly faster in WKY rats than in SHR. In addition, calcium- and calcium-magnesium-supplemented SHR showed promoted K^+ relaxation, while magnesium supplementation alone had no effect on K^+ relaxation rate (Figure 4). The contractile responses induced by 30 mM caffeine and 10 μM NA after loading periods in increasing organ bath Ca^{2+} concentrations were clearly less marked in SHR than in WKY rats. These contractions, reflecting the ability of arterial smooth muscle to sequester Ca^{2+} into cellular stores, were not improved by any of the dietary supplementations studied (Figure 5).

Second study series: calcium and magnesium supplementation in normotensive WKY rats

During the 24 h monitoring period in metabolic cages the calcium and magnesium supplementations increased the gain of these electrolytes by over 2 and 3 fold, respectively, in WKY rats. Corresponding with the above results in SHR, the clear magnesiuretic effect of increased dietary calcium intake was also observed in WKY rats (Table 3). In the second study series with normotensive WKY rats, the dietary regimens had no influence on blood pressure, body weight, lymphocyte $[Ca^{2+}]_i$, mesenteric arterial contractile (NA, KCl) and relaxation responses (ACh, SIN-1, isoprenaline, K^+ relaxation) (data not shown).

Discussion

The present study confirmed the earlier observations of our group and others that dietary calcium supplementation moderately attenuates the development of hypertension in SHR (Pörsti *et al.*, 1992; Wuorela *et al.*, 1992a; Doris 1994;

Table 3 Metabolic data (per 24 h) from normotensive rats at close of the 13-week study

Variable	*WKY*	*Ca-WKY*	*CaMg-WKY*	*Mg-WKY*
Chow intake (g)	18.0±0.6	20.7±0.6*	18.0±0.6†	18.1±0.6†
Fluid intake (ml)	29.6±1.7	28.7±1.0	29.9±0.8	27.2±0.7
Urine volume (ml)	7.8±1.2	8.6±0.9	10.3±0.6	9.1±0.8
Electrolyte intake (mmol)				
Na^+	2.86±0.10	3.20±0.09*	2.87±0.08†	2.83±0.09†
K^+	3.77±0.13	4.32±0.13*	3.77±0.12†	3.78±0.13†
Ca^{2+}	6.26±0.23	14.17±0.43*	12.54±0.37*†	6.17±0.20†#
Mg^2	1.80±0.06	2.01±0.06*	6.24±0.19*†	6.24±0.22*†
Urinary excretion				
Na^+ (mmol)	0.83±0.15	0.95±0.16	2.04±0.08*†	0.56±0.04#
K^+ (mmol)	1.32±0.15	1.76±0.18	1.94±0.09*	1.63±0.11
Ca^{2+} (μmol)	17.9±2.6	44.0±5.9*	153.8±15.7*†	23.6±1.3†#
Mg^{2+} (μmol)	109±13	219±25*	809±34*†	306±32*†#

Values are mean±s.e.mean; $n=8-10$ for all groups. WKY, Wistar-Kyoto rats; Ca, calcium supplemented rats; Mg, magnesium supplemented rats. *$P<0.05$ compared with the WKY group; †$P<0.05$ versus Ca-WKY, #$P<0.05$ versus CaMg-WKY (Bonferroni

McCarron *et al.*, 1985). Furthermore, the increase in urinary Mg^{2+} excretion during high calcium intake, which has previously been suggested to hamper the beneficial effects of increased dietary calcium on blood pressure (Wuorela *et al.*, 1992a), was observed in both hypertensive and normotensive rats in this study. Since plasma Mg^{2+} levels were not reduced by calcium supplementation, the associated magnesiuria did not appear to induce a significant Mg^{2+} deficiency in SHR. Nevertheless, plasma Mg^{2+} levels do not necessarily parallel the intracellular concentrations of this ion, and hypomagnesemia or changes in body Mg^{2+} status can exist, regardless of normal plasma Mg^{2+} (Reinhart, 1988). However, the fact that magnesium supplementation failed to induce a further reduction of blood pressure during high calcium intake supports the conclusion that these animals were not Mg^{2+} deficient, since Mg^{2+} deprivation has been reported to elevate blood pressure in rats (Altura *et al.*, 1983; Chrysant *et al.*, 1988; Altura & Altura, 1990). The present findings thus suggest that when the diet contains normal levels of magnesium (i.e. 0.2%) the increase in urinary Mg^{2+} excretion elicited by the high calcium diet does not induce a significant Mg^{2+} deficiency in SHR, and that the blood pressure-lowering effect of high calcium diet cannot be amplified by concurrent magnesium supplementation. It is also noteworthy that high magnesium diet alone failed to affect blood pressure in SHR, and none of the supplementary diets affected blood pressure in WKY rats. Therefore, normotensive animals do not appear to benefit from either increased calcium or magnesium intake.

Plasma K^+ and Ca^{2+} were not altered by dietary treatments in SHR, while in plasma Na^+ there was a slight rise in Ca-SHR and a small reduction in CaMg-SHR and Mg-SHR, the values, however, remaining well within the normal physiological range. Previously calcium supplementation has been shown to affect Na^+ balance in rats by promoting natriuresis and diuresis (Ayachi, 1979; Arvola *et al.*, 1993a). The reason for the small elevation in plasma Na^+ in Ca-SHR cannot be defined on the basis of the present experiments, but may be explained by alterations in plasma volume. Furthermore, Na^+ and Mg^{2+} excretion in the urine are also known to parallel each other (Altura & Altura, 1990; Arvola *et al.*, 1993a), and the effect of increased Mg^{2+} intake on plasma Na^+ may result from increased urinary Na^+ excretion. We want to stress that the evaluations of electrolyte metabolism in the study described here, where the rats were housed for 24 h in metabolic cages at close of the study, were carried out to verify the effect of increased dietary calcium intake on urinary Mg^{2+} excretion, and a more detailed approach would have been required to make definite conclusions about Na^+ balance in these animals.

In the present study the hypothesis, in particular, was tested, whether the dietary influences on blood pressure would be reflected as specific alterations in the responsiveness of the rat mesenteric artery. Calcium supplementation has previously been reported to promote vascular relaxation and exert favourable effects on the function of arterial cell membrane in experimental hypertension (Pörsti *et al.*, 1992; Arvola *et al.*, 1993a; Mäkynen *et al.*, 1994). Moreover, increased cell membrane permeability to ions, reduced Na^+, K^+-ATPase and Ca^{2+}-ATPase activity, and enhanced arterial contractility have been suggested as possible mechanisms mediating the blood pressure-elevating effect of dietary magnesium deficiency (Altura & Altura, 1990). Mg^{2+} has also been considered to function as a naturally occurring Ca^{2+} antagonist *in vivo* (Altura & Altura, 1990). Therefore, the present study protocol was designed to address vascular constrictor and relaxation sensitivity, and to evaluate the transfer of ionic gradients across the cell membrane as well as arterial calcium loading characteristics in the study groups.

Smooth muscle contractions were induced by activating α-adrenoceptors with NA and by depolarizing the smooth muscle by high concentrations of KCl. Noradrenaline releases Ca^{2+} from sarcoplasmic reticulum, prevents Ca^{2+} re-uptake, and increases Ca^{2+} influx through the receptor activated channel (Bülbring & Tomita, 1987; Karaki & Weiss, 1988). High concentrations of K^+ depolarize the smooth muscle membrane and liberate endogenous NA from adrenergic nerve-endings, the subsequent contraction depending mainly upon Ca^{2+} influx through the voltage-dependent Ca^{2+} channel and to a lesser extent upon α-adrenoceptor stimulation (Karaki & Weiss, 1988; Xiao & Rand, 1991). In the present study, NA- and KCl-induced contractions were not affected by any of the supplementary diets in either of the two strains, the only difference being that maximal contractile force generation to KCl was somewhat higher in standard sections of SHR mesenteric arteries than in those from WKY rats. Thus, the changes in arterial relaxation in this study (see below) cannot be attributed to alterations in smooth muscle contractile sensitivity, but rather represent specific changes in vascular dilator properties.

Agonist-induced arterial relaxation was studied in response to ACh, SIN-1 and isoprenaline, all acting through different cellular pathways. ACh relaxes arteries endothelium-dependently via the release of several factors from the endothelial cells, the most prominent autacoids being NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) (Moncada *et al.*, 1991; Fujii *et al.*, 1992). Subsequently, NO stimulates soluble guanylate cyclase elevating intracellular guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in smooth muscle, prostacyclin acts via adenylate cyclase and adenosine 3′:5′-cyclic monophosphate (cyclic AMP), while EDHF dilates arteries via the opening of K^+ channels (Moncada *et al.*, 1991; Fujii *et al.*, 1992). Previously several studies have reported impaired endothelium-dependent relaxation in both human

and experimental forms of hypertension, and reduced NO production has been suggested to underlie the attenuated endothelium-mediated dilatations (Treasure *et al.*, 1992; Arvola *et al.*, 1993b; Mäkynen *et al.*, 1994). However, in the mesenteric artery of SHR deficient endothelium-dependent hyperpolarization has recently been shown to account for the impaired relaxation to ACh, while the role of NO in the response was well preserved, and the participation of prostacyclin in the relaxation appeared to be marginal (Fujii *et al.*, 1992; Kähönen *et al.*, 1994). Furthermore, ACh is known to evoke contractions in blood vessels of SHR but not of WKY rats via the release of endothelium-derived contractile factors (Lüscher & Vanhoutte, 1986). Thus, impaired dilator response to ACh in SHR may not result from deficient dilator autacoid production alone, but from simultaneous release of contractile factors from the endothelium (Lüscher & Vanhoutte, 1986; Ito & Carretero, 1992). In the present study, endothelium-dependent relaxation to ACh was impaired in control SHR and improved in both hypertensive rat groups receiving the high calcium diet, while magnesium-supplementation was without effect on the response. Thus, the reduction of blood pressure by calcium supplementation had a favourable influence on endothelial function in SHR, but whether this effect could be attributed to increased NO generation, promoted endothelium-derived hyperpolarization, or reduced contractile factor release from the endothelium remains to be studied. Moreover, the production of NO is also the mechanism of action for the endothelium-independent vasodilator SIN-1 (Feelisch *et al.*, 1989). The finding that no significant differences in relaxations to SIN-1 were found between study groups suggests that the sensitivity of arterial smooth muscle to NO was comparable in hypertensive and normotensive rats, and was not altered by oral calcium or magnesium supplementation.

Isoprenaline, a non-selective β-adrenoceptor agonist, activates smooth muscle adenylate cyclase and increases intracellular cyclic AMP (Bülbring & Tomita, 1987). In addition, the stimulation of endothelial β-adrenoceptors increases cyclic AMP within the endothelial cells, which augments NO release and promotes vascular relaxation (Graves & Poston, 1993). Hence, β-adrenoceptor agonists are partially endothelium-dependent vasodilators, which may explain the more pronounced relaxation to isoprenaline observed in WKY rats than in SHR. On the other hand, arterial relaxation sensitivity to isoprenaline in SHR was not affected by any of the dietary supplementations used. Taken together, augmented relaxation to ACh together with no changes in vascular responses to SIN-1 and isoprenaline suggests improved endothelium-dependent dilatation in SHR following the high calcium diet.

Arterial contractions induced by Na^+, K^+-ATPase inhibition with K^+-free medium result from Na^+ leak to smooth muscle cells, which causes depolarization and increases Ca^{2+} influx through voltage-dependent channels. In addition, NA release from vascular adrenergic nerve-endings contributes to the response (Arvola *et al.*, 1992). In the present study, the function of vascular Na^+, K^+-ATPase was evaluated indirectly by the readdition of K^+ to the organ bath upon full K^+-free medium-induced precontractions. The return of K^+ activates Na^+, K^+-ATPase which repolarizes the cell membrane and initiates the relaxation of smooth muscle (Bonaccorsi *et al.*, 1977; Arvola *et al.*, 1992). We found that K^+ relaxation rate was markedly slower in SHR than WKY rats, in agreement with earlier observations (Pörsti *et al.*, 1992; Arvola *et al.*, 1992), and was clearly enhanced in SHR by dietary calcium supplementation whether combined with increased magnesium intake or not, whereas magnesium supplementation alone was without effect on the response. Enhanced K^+ relaxation in SHR following the high calcium diet indicates promoted recovery rate of ionic gradients across the cell membrane, an effect which is probably mediated via vascular Na^+, K^+-ATPase, since K^+ relaxation in the mesenteric artery of SHR can be inhibited completely by ouabain (Arvola *et al.*, 1992). The present findings, however, cannot explain the mechanism by which calcium supplementation could alter the activity of Na^+, K^+-ATPase in vascular smooth muscle. Interestingly, increased calcium intake has recently been shown to decrease blood pressure and the abnormally high Na^+, K^+-ATPase inhibitory activity in plasma from volume expanded rats as well as from SHR (Doris, 1988; 1994). Therefore, reduction of circulating Na^+ pump inhibitor concentration by calcium supplementation could provide a tempting explanation for the augmented K^+ relaxation in Ca-SHR and CaMg-SHR, but further work will be required to clarify the matter in detail.

The ability of arterial smooth muscle to sequester Ca^{2+} was evaluated by means of caffeine- and NA-induced contractions after loading periods in increasing organ bath Ca^{2+} concentrations. Out of these two agonists, caffeine directly and NA via the stimulation of inositol triphosphate accumulation open the Ca^{2+} channels in sarcoplasmic reticulum (Karaki & Weiss, 1988; Guild *et al.*, 1992). The refilling of the Ca^{2+} stores without cell activation and elevation of $[Ca^{2+}]_i$ presumably occurs via direct link between the extracellular space and cellular Ca^{2+} stores (Rink, 1990). After Ca^{2+} loading periods, the contractions to both caffeine and NA were lower in SHR than WKY rats, in agreement with previous reports (Dohi *et al.*, 1990), and neither dietary calcium nor magnesium supplementation affected these responses in SHR. Thus, the present findings suggest that these dietary treatments did not induce alterations in Ca^{2+} sequestration characteristics of arterial smooth muscle.

The concentration of cytosolic free Ca^{2+} is a primary determinant of arterial contraction (Rembold, 1992), and abnormalities of cellular Ca^{2+} metabolism have been found in both young and adult SHR (Sugiyama *et al.*, 1990; Pörsti *et al.*, 1992). Moreover, normalization of blood pressure in SHR by ACE-inhibitor therapy has been reported to normalize the elevated $[Ca^{2+}]_i$ in lymphocytes of these animals (Arvola *et al.*, 1993b). Previously, high dietary calcium intake has been found to lower blood pressure with an associated reduction in $[Ca^{2+}]_i$ in SHR (Furspan *et al.*, 1989; Pörsti *et al.*, 1992; Wuorela *et al.*, 1992a). In the present study, basal $[Ca^{2+}]_i$ in lymphocytes was clearly higher in SHR than WKY rats, and was reduced by dietary calcium supplementation, while increased magnesium intake did not affect $[Ca^{2+}]_i$. Thus, assuming that the observed changes in lymphocyte $[Ca^{2+}]_i$ also reflect cellular Ca^{2+} metabolism in other tissues, these results support the concept that high calcium diet improves cellular Ca^{2+} handling in hypertensive rats. Reduced $[Ca^{2+}]_i$ in lymphocytes by calcium supplementation, however, may seem to contradict the above results in that Ca^{2+} sequestration characteristics in arterial smooth muscle remained unaffected. However, the latter experiments reflected more the ability of sarcoplasmic reticulum to take up and store Ca^{2+}, and thus possible changes in plasma membrane Ca^{2+} handling properties may not have been revealed, i.e. alterations in the function of cell membrane Ca^{2+}-ATPase. Indeed, we have previously reported that high calcium intake activates red cell membrane Ca^{2+}-ATPase in SHR (Wuorela *et al.*, 1992b).

In conclusion, the moderate antihypertensive effect of high calcium diet in SHR was accompanied by enhanced endothelium-dependent arterial relaxation and promoted recovery rate of ionic gradients across the cell membrane via Na^+, K^+-ATPase. Dietary magnesium supplementation alone or in combination with increased calcium intake had no effects on blood pressure or arterial function. Therefore, the present results suggest that the blood pressure-lowering action of increased dietary calcium intake in SHR is associated with enhanced arterial relaxation, and no further advantage can be gained by concomitant magnesium supplementation.

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