Partial Protection From Salt-Induced Stroke and Mortality by High Oral Calcium in Hypertensive Rats

Jacob D. Peuler, PhD, and Robert L. Schelper, MD, PhD

Background and Purpose: **Repeated demonstration of an antihypertensive effect of high oral calcium in stroke-prone spontaneously hypertensive rats led us to determine whether it also protects such rats from premature mortality and stroke-related lesions.**

Methods: **Female stroke-prone rats (11–13 per diet) were fed high- and low-calcium (2.0% and 0.4%, respectively) diets with both high and low salt (7.0% and 0.3%, respectively) content from age 4 weeks until spontaneous death. In addition to life span, other variables measured included blood pressures, plasma chemistries, and histological characterization of stroke-related lesions.**

Results: **Life span was increased from 51±4 to 68±1 weeks ($p<0.05$) by high versus low oral calcium in rats fed high-salt diets; it was further increased to ≥82 weeks ($p<0.05$) in rats fed low-salt (±added calcium) diets. As seen previously, high oral calcium attenuated salt-induced hypertension but did not affect blood pressure in rats fed low-salt diets. High versus low oral calcium exerted contrasting effects ($p<0.05$) on brain lesions (hemorrhages and infarctions) in rats fed high-salt diets, decreasing lesion size (242±21 versus 712±276 microns per rat [diameters seen in histological sections]) but increasing lesion number (8.9±2.4 versus 3.4±2.2 per rat); it exerted little influence on the few brain lesions that appeared in rats fed low-salt diets.**

Conclusions: **High oral calcium may protect stroke-prone hypertensive rats from early salt-induced mortality at least partially by decreasing severity (size) of stroke-related lesions, an effect which may relate to decreased blood pressure. However, this protection may be limited by increased number (incidence) of such lesions, an effect which suggests that high oral calcium may increase the number of brain vessels susceptible to stroke-related injury independent of change in blood pressure. (*Stroke* 1992;23:532–538)**

KEY WORDS • **calcium** • **hypertension** • **mortality** • **rats**

Recent stroke epidemiology fails to support previously accepted primary dependence of stroke incidence on blood pressure.[1–4] Currently, several other factors are under consideration as potentially critical determinants of stroke.[2] Two among them that recently received epidemiological attention are changes in dietary cation intake, particularly potassium,[5,6] and the level of maternal (and related neonatal) health.[7,8]

The stroke-prone spontaneously hypertensive rat (spSHR) has emerged in the last two decades as the foremost animal model of hypertensive stroke in humans. A number of dietary factors (particularly cations) influence stroke in this model, some by altering blood pressure. In spSHR, for example, stroke incidence is strongly dependent on high levels of sodium in the diet (in the form of sodium chloride), and high salt intake increases their blood pressure.[9–13] On the other hand, high potassium intake decreases stroke incidence in spSHR with or without reduction in blood pressure,[10–13] an effect resembling recent epidemiological evidence of oral potassium–related protection from stroke-associated mortality in older men and women, independent of blood pressure.[5]

Unlike sodium and potassium, little attention has been given to effects of dietary calcium on stroke, despite several actions of supplemental oral calcium that might alter appearance of stroke-related lesions in the brain. High oral calcium could potentially decrease such lesions by decreasing blood pressure,[9,14,15] the level of saturated lipids in the circulation,[16] and the rate of platelet aggregation.[17] On the other hand, high oral calcium could potentially increase such lesions by increasing incidence of vascular mineral deposits (calcification of the vessel wall) and by decreasing intestinal availability of other nutrients[18] that might be important to development and maintenance of normal vascular structure and function. Because we and others have clearly demonstrated that high oral calcium substantially decreases blood pressure in young spSHR,[9,14] we conducted the present study to determine whether lifelong high calcium intake would also protect aging spSHR from premature cardiovascular mortality and related histological signs of stroke.

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Materials and Methods

Female spSHR from the colony at the University of Iowa were fed either low- (0.3%) or high- (7.0%) salt diets (sodium chloride) with either low (0.4%) or high (2.0%) calcium content at each level of salt from age of 4 weeks until spontaneous (natural) death. Calcium in the low-calcium diets consisted only of calcium phosphate, and calcium in the high-calcium diets consisted of calcium phosphate plus calcium carbonate. In all diets (obtained from Nutritional Biochemicals, Cleveland, Ohio), total phosphorus was 0.9%, magnesium 0.1%, and potassium 0.5%.

At the ages of 22 and 65 weeks, a representative number of rats from each group were instrumented with nonthrombogenic (Teflon) abdominal aortic catheters[19] inserted through either tail artery (at age 22 weeks) or femoral artery (at age 65 weeks) to monitor mean arterial pressure and obtain blood samples 4–6 days later. Subsequently, catheters were removed to avoid death due to infection or other long-term complications related to indwelling catheters. Both insertion and removal of catheters were performed under anesthesia with methohexital sodium (40 mg/kg i.p.). Blood plasma from each rat was analyzed for sodium, potassium, calcium, and other minerals by standard methods.[19]

After spontaneous death, whole organs (brain, heart, kidney) were removed from each rat, weighed, and stored in buffered formaldehyde (10% formalin) for later analysis. Each brain was cut at identical locations into eight coronal sections of similar thickness (approximately 2,000 μm each). Four of these gross sections (same sections for each rat) were embedded in paraffin, further sectioned and stained with hematoxylin and eosin in the histology laboratory of the Department of Pathology (University of Iowa), and then examined by light microscopy (×10–×1000) for identification of stroke-related lesions as described previously[20–22] and for evidence of gross mineral deposits in the form of dark, solid blue spots. Lesions were counted and approximated in terms of age, location, and size (observed cross-sectional diameters).

A lesion was classified as a hemorrhage if there was evidence of extravasation of red blood cells. If hemosiderin deposition and gliosis were absent, the hemorrhage was classified as acute (1–2 days old); otherwise, with hemosiderin or gliosis present, it was classified as subacute or chronic (1–2 or >3 weeks old), respectively. A lesion was classified as an infarction of ischemic origin if there was edema with vascular congestion, eosinophilic neurons with swollen cytoplasm (sometimes with small pyknotic nuclei), reactive astrocytosis (gliosis) and infiltrating mononuclear cells, or disappearance of neuronal tissue altogether but no evidence of extravasated blood elements. In less specific cases, if there was clearly necrotic (infarcted) tissue present but accompanied by evidence of extravasated plasma proteins (proteinaceous debris) or a few red blood cells (with or without hemosiderin), the lesion was classified as an infarction of nonspecific origin. As with hemorrhages, all infarctions were also classified as acute, subacute, or chronic, depending largely on appearance of eosinophilic neurons, macrophages, or astrocytes, respectively.

Enumerative data (e.g., percentage of rats in a given group bearing one or more lesions in the sampled brain tissue) were subjected to χ^2 analysis to determine differences between groups. All other quantitative data were summarized as mean±SEM for each group of rats, transformed if necessary either by natural logarithm (lesion sizes) or by square root (lesion numbers) to achieve normality and homogeneity of variances, and subjected to multiple mean comparison tests subsequent to Bonferroni adjustments and analysis of variance. Heart weight (ventricular weight per body weight) or blood pressure versus lesion size (average cross-sectional diameter) or number per rat were subjected to simple linear correlation analysis. Statistically significant differences were so designated if the probability of error was <0.05.

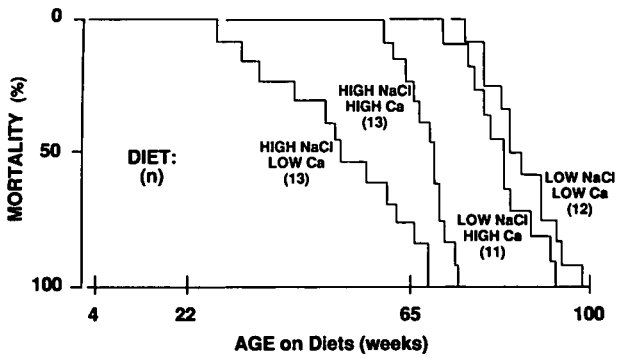

FIGURE 1. *Graph of mortality as a function of age in stroke-prone spontaneously hypertensive rats fed high- and low-calcium diets (2.0 and 0.4%, respectively) with high and low sodium chloride content (7.0 and 0.3%, respectively) from age 4 weeks until spontaneous death. Average life span increased (p<0.05) from 51±4 weeks in rats fed low-calcium, high–sodium chloride diet to 68±1 weeks in rats fed high-calcium, high–sodium chloride diet to 82±2 and 86±2 weeks in rats fed low–sodium chloride, high- and low-calcium diets, respectively.* n, *Number of rats.*

Results

In rats fed high-salt diets, life span was increased from 51±4 weeks in those fed low calcium to 68±1 weeks in those fed high calcium (Figure 1). Life span was further increased to 82±2 weeks and 86±2 weeks in rats fed low-salt diets with or without added calcium. Thus, life span was increased 33% ($p<0.05$) in spSHR fed high-salt, high-calcium diet compared with spSHR fed high-salt, low-calcium diet; it was increased ≥60% ($p<0.05$) by feeding low-salt diets with or without added calcium.

High oral calcium did not affect mean arterial pressure in rats fed low-salt diets but reduced mean arterial pressure in rats fed high-salt diets (Figure 2). At 22 weeks, high oral calcium completely prevented salt-induced hypertension; at 65 weeks it slightly attenuated such hypertension. Salt-induced ventricular enlargement, measured postmortem, was prevented by high oral calcium (Figure 3).

Generally, either the relative number of rats bearing lesions (percentage of rats with one or more lesions) or the number lesions per rat were increased by high oral calcium in rats fed high-salt diets (Table 1), both in terms of infarctions and hemorrhages. The latter index of incidence (number of lesions per rat) was increased nearly threefold for all lesions combined. The total

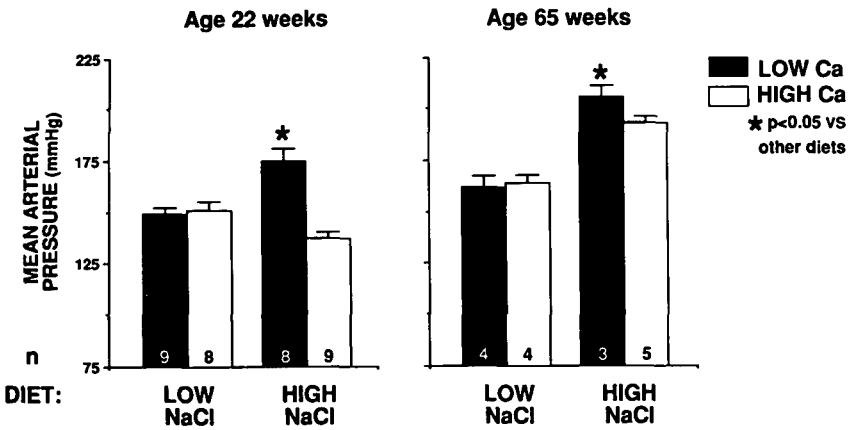

FIGURE 2. *Bar graph of blood pressure in young (age 22 weeks) and old (age 65 weeks) adult stroke-prone spontaneously hypertensive rats fed diets described in Figure 1 legend.* n, *Number of rats.*

number of lesions was also increased at least twofold by high oral calcium in those high-salt-fed rats with overlapping life span (10.5±4.1 versus 5.3±3.4 lesions per rat, $n=6$ versus 6; $p<0.05$ after square root transformation of data in combination with data from rats fed low-salt diets). High oral calcium also slightly increased the percentage of ischemic infarction in rats fed low-salt diets, but it had no effect on other indices of lesion incidence in these rats.

Although several infarctions were clearly ischemic in origin, many others, because of the presence of proteinaceous debris, were more difficult to classify in terms of origin and thus labeled nonspecific. They may have been caused by extravasation of plasma protein. Conversely, deposits of plasma protein may have resulted from reperfusion of infarcted tissue after an initial ischemic event (perhaps the most likely explanation). In all rats, most infarctions were relatively old lesions; ischemic infarctions were mostly chronic in age (≥3 weeks old), and nonspecific infarctions were mostly subacute (1–2 weeks old). Lesions that were clearly hemorrhagic (extravasation of red blood cells) were mostly acute in age (1–2 days old). In all rats across diets, lesions were found mostly in the cerebral cortex, although several were also present elsewhere (e.g., brain stem, cerebellum, basal ganglia).

Boundaries of lesions were sufficiently visible microscopically on hematoxylin and eosin–stained sections of brain tissue (Figure 4) to permit approximation of lesion

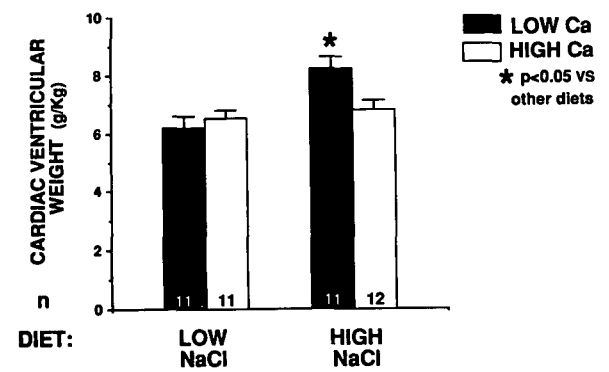

FIGURE 3. *Bar graph of heart weight/body weight ratio seen after spontaneous death in stroke-prone spontaneously hypertensive rats fed diets described in Figure 1 legend.* n, *Number of rats.*

dimensions. Altogether, lesions were reduced in size (approximate cross-sectional diameter as observed microscopically) by high oral calcium in spSHR fed high-salt diets (Figure 5). Indeed, it appeared as though high oral calcium prevented a salt-induced increase in lesion size in spSHR. Across diets, there was a significant positive correlation between postmortem cardiac ventricular weight/body weight and average lesion size per rat ($r=+0.48$, $n=24$, $p<0.05$). No significant correlation was seen between cardiac ventricular weight/body weight and average lesion number per rat ($r=-0.32$) in the same rats. No significant correlation was seen between average mean arterial pressure and average lesion size per rat ($r=+0.39$) or number per rat ($r=-0.27$) in a slightly smaller sample of rats ($n=20$).

Plasma calcium levels remained unaffected by dietary calcium in either young or old adult rats (Table 2). Evidence of gross mineral deposits was essentially absent in brain tissue from all spSHR in this study. We observed no obvious outward signs of calcium-induced nutritional deficiencies. In particular, circulating levels of key indicators of long-term nutritional deficiencies, phosphorus and magnesium, remained unchanged with advancing age and high calcium feeding (Table 2). Overall growth (Table 3) and food consumption (10–15 gm/day per rat in adult rats) were not affected by high oral calcium, although high salt inhibited growth without reducing food intake.

Creatinine increased from age 22 to 65 weeks in nearly all rats alike (Table 2). Postmortem, outer surfaces of both kidneys from all rats alike were not smooth but granulated, indicating underlying ischemic damage. With increasing age at death, we found increased incidence of fluid accumulation in chest and abdominal cavities ($p<0.05$), specifically in rats fed low-salt diets (82% with or without high calcium intake versus 31% in rats fed high-salt, high-calcium diet and 17% in rats fed high-salt, low-calcium diet). Finally, plasma potassium concentration decreased from age 22 to 65 weeks only in rats fed high-calcium diets (Table 2).

Discussion

There are two major new findings from this study. One is that lifelong high calcium intake provides significant but limited protection from early salt-induced mortality in spSHR. The other is that such high oral calcium exerts contrasting effects on stroke-related

TABLE 1. Incidence by Diet of Various Forms of Brain Lesions Seen After Spontaneous Death in Stroke-Prone Spontaneously Hypertensive Rats

	Low-NaCl diets		High-NaCl diets	
	Low Ca (n=11)	High Ca (n=11)	Low Ca (n=12)	High Ca (n=13)
Lesions (all forms)				
Rats with lesion(s) (%)	8	45	58*	85*
Lesions per rat	0.4±0.3	0.6±0.3	3.4±2.2*	8.9±2.4*†
Hemorrhages				
Rats with lesion(s) (%)	18	18	42	77*†
Lesions per rat	0.4±0.3	0.4±0.3	2.2±1.8	4.0±1.2*
Ischemic infarction				
Rats with lesion(s) (%)	0	27†	33*	54
Lesions per rat	0.0±0.0	0.4±0.2	0.6±0.3	1.7±0.6*
Nonspecific infarction				
Rats with lesion(s) (%)	0	0	25*	77*†
Lesions per rat	0.0±0.0	0.0±0.0	0.7±0.4	3.2±1.4*†

Values are mean±SEM. n, Number of rats. Diets: low NaCl, 0.3% NaCl; high NaCl, 7.0%; low Ca, 0.4% Ca; high Ca, 2.0%.
*p<0.05 greater than low-NaCl diet.
†p<0.05 greater than low-Ca diet.

brain lesions in high-salt-fed spSHR, decreasing their size but increasing their number (incidence).

Despite increased number of all forms of brain lesions, life span was increased 33% by high oral calcium in spSHR fed high-salt diets. Although this increase in life span was not as great as that achieved by feeding low-salt diets (with or without added calcium) it was nonetheless significant. Indeed, although the large number of lesions may explain earlier mortality in these rats compared with those fed low-salt diets, it obviously does not explain partial protection afforded by high oral calcium against the even earlier high salt–induced mor-

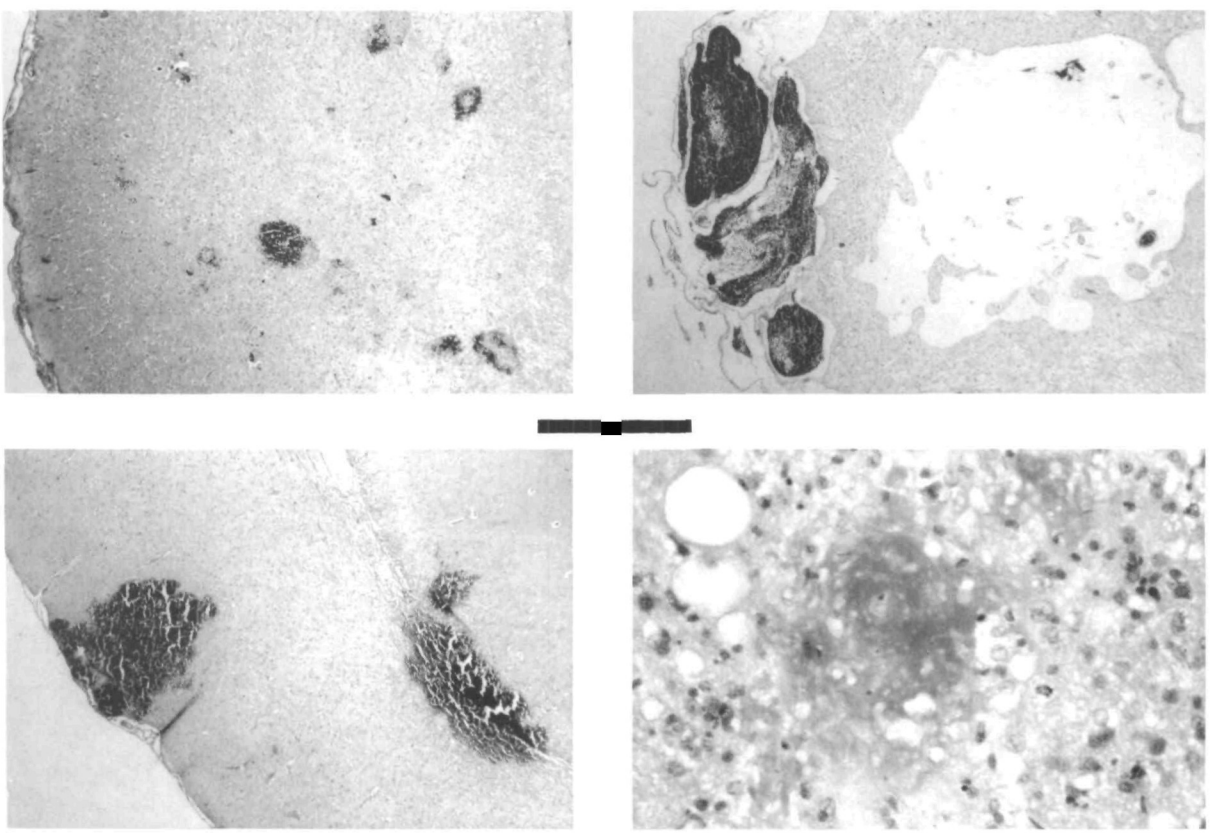

FIGURE 4. *Photomicrographs of stained coronal sections of brain tissue obtained after spontaneous death from stroke-prone spontaneously hypertensive rats (spSHR) fed diets described in Figure 1 legend.* Upper *and* lower left panels *(bar length, 1,000 μm each): Multiple hemorrhages of different sizes in two spSHR fed high-calcium, high–sodium chloride diet.* Upper right panel *(bar length, 1,000 μm): Massive hemorrhage and massive ischemic infarction in one spSHR fed a low-calcium, high–sodium chloride diet.* Lower right panel *(bar length, 100 μm): Small infarction showing proteinaceous debris surrounded by macrophages in one spSHR fed a high-calcium, high–sodium chloride diet.*

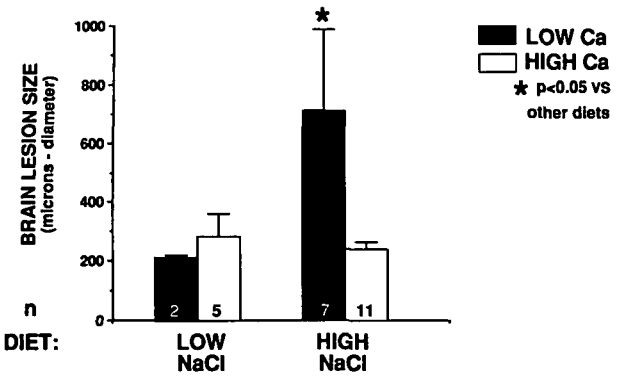

FIGURE 5. *Bar graph showing size of lesions (diameters in histological cross-sections, averaged for each rat) seen in stained coronal sections of brain tissue obtained after spontaneous death from stroke-prone spontaneously hypertensive rats fed diets described in Figure 1 legend.* n, *Number of rats.*

tality. Other unlikely mechanisms for this partial protection may be decreases in incidence or severity of renal and cardiac disease. Kidneys from these rats demonstrated postmortem signs of ischemic renal vascular disease in the form of irregular, granulated surfaces, but such signs appeared to be present in all rats alike, independent of diet. We also found postmortem signs of fatal cardiac failure in the form of pleural effusion and ascites, invariably accompanied by severe respiratory distress (congestion) 1–2 days before death. However, such signs were most frequent in rats fed low-salt diets, much less frequent in rats fed high-salt diets, and not affected by the level of oral calcium. Conceivably, high oral calcium might have delayed the onset of fatal renal and cardiac events in rats fed high-salt diets. However, we suspect another more likely mechanism whereby high oral calcium provided spSHR some protection against premature salt-induced mortality is the decrease in size, and therefore severity, of brain lesions. High oral calcium prevented a salt-induced increase in the average cross-sectional diameter of such lesions in spSHR fed high-salt diets.

Despite limitations associated with such an estimate of lesion size, it was possible because of the large number of lesions to separate by analysis of variance not only this significant dietary source of variability but also a significant interanimal variability from the inherent error of the measurement. Accordingly, there was also a significant, direct correlation between heart weight (per body weight) as a postmortem, lifelong index of blood pressure and average lesion diameter in all lesion-bearing rats across diets. However, such correlation was not seen between blood pressure and average lesion diameter in nearly the same number of rats, perhaps because our measures of blood pressure were not frequent enough to disclose such correlation. Thus, we can only speculate that the decrease in lesion size by high oral calcium in spSHR fed high-salt diets in the present study relates to attenuation of their salt-induced hypertension. Although lesion size in spSHR has been reported previously,[20,22] no others to our knowledge have considered its relationship to blood pressure in this animal model. Nonetheless, one recent survey of patients with cerebral hemorrhage revealed a 50% incidence of lesion sizes >4 cm (maximum diameter by computed tomographic scan) in 18 hypertensive patients compared with 6% of such incidence in 32 normotensive patients.[23] Thus, we cannot discard the possibility of a relationship of blood pressure to lesion size.

Although we can at least speculate that decreased blood pressure by high oral calcium in spSHR fed high-salt

TABLE 2. Plasma Chemistries by Diet in Young (Age 22 Weeks) and Old (Age 65 Weeks) Stroke-Prone Spontaneously Hypertensive Rats

	Low-NaCl diets		High-NaCl diets	
	Low Ca	High Ca	Low Ca	High Ca
Calcium (mg%)				
22 weeks	10.1±0.2	10.1±0.3	9.9±0.1	9.8±0.2
65 weeks	9.8±0.2	10.4±0.3	9.9±0.0	9.9±0.2
Magnesium (mg%)				
22 weeks	1.70±0.05	1.56±0.05	1.58±0.07	1.52±0.07
65 weeks	1.59±0.03	1.46±0.06	1.60±0.01	1.50±0.01
Phosphorus (mg%)				
22 weeks	4.7±0.2	5.2±0.4	4.6±0.1	4.7±0.2
65 weeks	3.9±0.2	4.3±0.4	4.8±0.2	4.7±0.3
Creatinine (mg%)				
22 weeks	0.51±0.03	0.45±0.02	0.43±0.02	0.41±0.01
65 weeks	0.58±0.03	0.58±0.03*	0.59±0.03*	0.58±0.02*
Potassium (meq/l)				
22 weeks	5.0±0.4	5.3±0.4	4.3±0.3	5.4±0.6
65 weeks	4.2±0.4	4.0±0.1*	4.4±0.1	3.6±0.2*
Sodium (meq/l)				
22 weeks	142±1.0	143±0.8	144±0.5	144±0.6
65 weeks	143±0.5	141±0.4	144±0.5	144±0.6
No. rats				
22 weeks	9	8	8	9
65 weeks	4	4	3	5

Values are mean±SEM. Diets: low NaCl, 0.3% NaCl; high NaCl, 7.0%; low Ca, 0.4% Ca; high Ca, 2.0%.
*$p<0.05$ vs. age 22 weeks.

TABLE 3. Body Weight With Increasing Age in Stroke-Prone Spontaneously Hypertensive Rats by Diet

	Low-NaCl diets				High-NaCl diets			
	Low Ca		High Ca		Low Ca		High Ca	
Age	Weight (g)	*n*	Weight (g)	*n*	Weight (g)	*n*	Weight (g)	*n*
4 weeks	58±3	12	56±2	11	52±2	13	53±2	13
14 weeks	196±4	12	183±3	11†	171±4	13*	160±3	13*†
22 weeks	229±3	12	222±3	11	194±4	13*	185±2	13*
31 weeks	228±3	12	250±3	11‡	197±7	12*	200±3	13*
47 weeks	254±6	12	266±6	11	206±5	10*	209±3	13*
56 weeks	284±7	12	295±8	11	224±5	5	213±3	11*
Postmortem	255±7	11	247±9	11	172±13	11*	178±12	12*

Values are mean±SEM. *n*, Number of rats. Diets: low NaCl, 0.3% NaCl; high NaCl, 7.0%; low Ca, 0.4% Ca; high Ca, 2.0%.
*$p<0.05$ less than low-NaCl diet.
†$p<0.05$ less than low-Ca diet.
‡$p<0.05$ greater than low-Ca diet.

diet may explain decreased size of brain lesions, it clearly does not explain increased number of such lesions. Neither does increased average life span explain this phenomenon, because the number of lesions was also increased by high versus low oral calcium among those specific high-salt diet spSHR with overlapping (and therefore matching average) life spans. High oral calcium might increase stroke incidence by decreasing the balance of potassium and its protective role in spSHR (intake of potassium was not supplemented in our study). There was an age-related reduction in the plasma concentration of potassium among spSHR fed high-calcium diets; this decrease was most notable in rats fed high-salt, high-calcium diet (i.e., those with highest incidence of stroke-related lesions). There was no parallel change of equal specificity in any other plasma chemistries, no evidence of calcium toxicity such as hypercalcemia with vascular (gross) mineral deposits, and no evidence of other nutrient deficiencies (decreased growth or decreased plasma phosphorus and magnesium) that might predispose vascular tissue to such damage. Mild but significant hypokalemia has been seen previously in both hypertensive rats and humans, noticeably after long-term,[24–26] but not short-term,[19,27–29] feeding of supplemental calcium. This effect is independent of change in blood pressure[24,26] and is preceded by a kaluretic action of high calcium intake.[24,25] Tobian and others[10–13] have repeatedly demonstrated that high oral potassium decreases incidence of stroke in spSHR fed high-salt diets; it appears to do so independent of its antihypertensive action,[11,13] and it can, in contrast to high oral calcium, increase plasma potassium.[10] Additional findings from the same and other investigators implicate vascular endothelium as one possible site of this protective influence of potassium.[30,31]

A second possible mechanism whereby high oral calcium might increase stroke incidence is by suppressing the circulating level of the secosteroid 1,25-dihydroxyvitamin D_3 (1,25 D), or, more precisely, by preventing a salt-induced increase in circulating 1,25 D and its newly-discovered vascular trophic actions.[32–34] When salt intake is high (without added calcium), the plasma level of this sterol increases,[35] no doubt because of renal wasting of calcium.[15] Indeed, prevention of such an increase by high oral calcium[35] might explain our previous finding that high oral calcium prevents salt-induced vascular hyperresponsiveness in spSHR.[9] However, even on normal diets, plasma 1,25 D is much higher in young versus adult humans and animals.[36–38] It is particularly high in very young (1 month old) spSHR compared with age-matched rats from the parent strains, namely SHR and Wistar rats.[37] A high circulating level of 1,25 D at a critical young age could protect spSHR against high stroke incidence at later ages much as their sympathetic innervation does, that is, by way of an early trophic action on cerebral arterioles and small (but not large) cerebral arteries.[20,21] This neural trophic action and its protective influence in spSHR can be abolished by regional sympathetomy but only when performed in very young spSHR, that is, at age 1 month,[21] when the vasculature in rats and other small animals is still in a state of rapid proliferative development.[39] Recently, a proliferative action of 1,25 D at concentrations even below the high plasma levels seen in 1-month-old spSHR[37] has been demonstrated in cultured arterial smooth muscle cells from both SHR and Wistar rats.[32,33] If such a trophic action of 1,25 D is operative as well in young spSHR (particularly in their smooth muscle–rich cerebral arterioles[40,41]), it may provide long-term protection, similar to that of the sympathetic innervation, against high incidence of stroke-related lesions.

We conclude from these findings that the extent to which high oral calcium protects spSHR from salt-induced mortality may depend in part on a balance between its effects on severity versus incidence of stroke, and, although decreased blood pressure by high oral calcium could conceivably explain decreased lesion size, it clearly does not explain increased lesion number in these rats. We suspect that high oral calcium increases lesion number in spSHR by either increasing susceptibility of all brain vessels to stroke-related injury or shifting such susceptibility from large to smaller vessels. The latter is a particularly attractive hypothesis because it explains decreased size as well as increased number of stroke-related lesions by a single mechanism.

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