SUMMARY To assess the consequences of hypercalcemia on systemic and renal hemodynamics, vasoactive hormones, and water and electrolyte excretion in intact, conscious mongrel dogs, measurements in 10 dogs receiving 100 mg/kg calcium gluconate and 10,000 U/kg vitamin D daily for 2 weeks were compared with measurements made in 10 time-control dogs not receiving calcium or vitamin D. Hypercalcemia induced by dietary supplementation with calcium and vitamin D resulted in profoundly reduced glomerular filtration rate (40 vs 78 ml/min in controls; p<0.005), estimated renal plasma flow (145 vs 267 ml/min in controls; p<0.005), and renal blood flow (254 vs 441 ml/min in controls; p<0.005). Renal resistance was significantly increased in the hypercalcemic dogs (0.57 ± 0.07 vs 0.28 ± 0.01 mm Hg/ml/min; p<0.005). Hypercalcemia also resulted in increased fractional excretion of water (4.8 vs 1.4% in controls; p<0.005), sodium (1.4 vs 0.6% in controls; p<0.005), calcium (1.7 vs 0.7% in controls; p<0.01), and magnesium (10.2 vs 4.1% in controls; p<0.005). Systolic blood pressure (160 vs 172 mm Hg in controls; p<0.05) and stroke volume were lower (0.024 vs 0.036 L/beat in controls; p<0.005) in hypercalcemic dogs, presumably because of the diuresis, while total peripheral resistance was higher (36 vs 31 mm Hg/L/min; p<0.05) in controls. Magnesium levels were significantly lower in the experimental group (1.3 vs 1.7 mg/dl in controls; p<0.0005). Aldosterone levels, plasma renin activity, and urinary prostaglandin excretion were not significantly affected. These results suggest that hypercalcemia induced by chronic dietary supplementation increases total peripheral resistance and renal vascular resistance, which may be due to the direct effects of increased calcium ions or to indirect effects on other vasoactive humoral systems including reductions in magnesium ions. (Hypertension 8: 975-982, 1986)

KEY WORDS • dietary calcium • hemodynamics • vitamin D

PREVIOUS studies of the effect of calcium on blood pressure have provided conflicting data. Evidence supporting the hypothesis that dietary calcium acts to decrease blood pressure comes from various sources. Epidemiological data link low calcium intake by dietary recall with high systolic blood pressure.1 More direct studies on the effect of altering dietary calcium in spontaneously hypertensive rats (SHR) have shown that the typical increase in systolic blood pressure that occurs with age is accelerated by low calcium diets and attenuated by high calcium diets. It is of importance, however, that serum total and ionized calcium levels in these animals were minimally affected by high calcium diet.2 In other studies, dietary supplements of calcium have been reported to reduce blood pressure in humans.3,4 Resnick et al.5 have shown that such a reduction in blood pressure in hypertensive humans is inversely related to initial serum calcium and plasma renin activity levels. There is also evidence that the calcium ion itself promotes hypertension. Substances that prevent the entry of calcium into cells have been reported to reduce blood pressure in hypertensive humans,7 and calcium is required in the external medium for the action of vasoconstrictors such as norepinephrine and vasopressin.8,9 Our preliminary studies with mongrel dogs have shown that intravenous calcium chloride infusions, which resulted in hypercalcemia, caused an increase in peripheral resistance and a decrease in serum magnesium levels.10 We undertook the present study using mongrel dogs to gain more insight into the effect of dietary calcium on blood pressure. Systemic and renal hemodynamics and water and electrolyte excretion were investigated after chronic hypercalcemia was induced by a dietary supplement of calcium and vitamin D.
Materials and Methods

All animal studies were performed in accordance with the guidelines of the Veterans Administration Research Committee of the Royal C. Johnson VA Medical Center, Sioux Falls, South Dakota.

Protocol

Twenty female mongrel dogs weighing between 14 and 25 kg were fed commercial dog chow (Purina, St. Louis, MO, USA) and water ad libitum. In 10 of these dogs, designated as experimental animals, hypercalcemia was induced by giving them daily oral doses of calcium gluconate, 100 mg/kg, and vitamin D, 10,000 U/kg, for 2 weeks. The remaining 10 dogs, designated as control animals, did not receive the calcium and vitamin D.

The animals were anesthetized with sodium pentobarbital (Nembutal), 30 mg/kg body weight, before surgical placement and externalization of catheters in the right femoral artery and vein and placement of a thermolocatheter in the pulmonary artery through the right jugular vein. During the operation each animal was ventilated using a Harvard pump respirator (Millis, MA, USA) set for 300-ml strokes at a rate of 12 strokes/min and received 200 to 300 ml of 0.9% NaCl by intravenous drip. After the operation each animal was given 600,000 units of procaine penicillin G intramuscularly and allowed to recover overnight.

The next day, the animal was placed in a sling, which supported it in the standing position, and an 8F Foley catheter (Bard, Murray Hill, NJ, USA) was inserted into the bladder. Baseline measures of blood pressures, cardiac output (CO), and cardiac index (CI) were made. Blood was drawn from one of the femoral catheters for later determination of plasma renin activity, plasma aldosterone concentration, whole blood ionized calcium (iCa), ionized potassium, ionized sodium, total serum calcium, and serum magnesium (sMg).

An infusion of a bolus dose of inulin (50 mg/kg) and p-aminohippuric acid (PAH), 20 mg/kg, in 50 ml of 0.9% NaCl then was initiated at a rate of 3.3 ml/min and was followed by a maintenance dose of inulin (20 mg/min) and PAH (5 mg/min) in 125 ml of 0.9% NaCl administered at a rate of 0.44 ml/min. Both doses were infused into the catheter in the femoral vein. One hour was allowed for equilibration, and then a graduated cylinder was attached to the end of the Foley catheter for urine collection. Thirty minutes later, blood was drawn for determination of plasma renin activity, plasma aldosterone, iCa, ionized potassium and sodium, serum calcium, sMg, and plasma inulin and PAH concentrations. Blood pressures, CO, and CI were also measured at this time. The urine volume for the first hour was recorded, and the urine was frozen for subsequent determination of urine osmolality; calcium, magnesium, sodium, potassium, and prostaglandin E2 (PGE2) concentrations; and calculation of electrolyte excretion rates. Two more such 1-hour urine collections followed; each time, blood pressures, CO, and CI were measured and blood samples were obtained half-way through the collection period.

Measurements

Systolic blood pressure, diastolic blood pressure, and mean arterial pressure in the femoral artery were determined using pressure transducers filled with 0.9% NaCl attached to a physiological recorder (Gould, Cleveland, OH, USA) that was calibrated periodically with a mercury manometer. The CO and CI were determined by thermodilution using a cardiac index computer (Gould, Cleveland, OH, USA), which computes CO using a modified form of the Stewart-Hamilton indicator dilution equation; 10 ml of room temperature 0.9% NaCl was used as the injectate. The computer monitors the change in blood temperature over time and uses this value as well as the volume of injectate to determine CO and gives a digital readout of the CI. The CI was calculated by dividing the CO by the body surface area of the animal. Body surface area is calculated by the computer using the Dubois height-weight equation. Digital readouts of both CI and body surface area are given by the computer.

Stroke volume was calculated by dividing the CO for a given period by the heart rate for that period. Total peripheral resistance (TPR) was calculated by dividing the mean arterial pressure for a given recording period by the corresponding CO. Renal resistance was calculated by dividing the mean arterial pressure for a given recording period by the renal blood flow for that period.

Ionized calcium, sodium, and potassium, and urinary excretion of potassium and sodium were determined using ion-selective electrodes with a Nova 6 electrolyte analyzer (Nova Biomedical, Newton, MA, USA). Serum calcium, urinary calcium excretion, blood urea nitrogen, and plasma creatinine were determined colorimetrically with an American Monitor KDA analyzer (Indianapolis, IN, USA). Serum magnesium and urinary magnesium excretion were determined colorimetrically using a Dupont ACA analyzer (Wilmington, DE, USA).

Plasma renin activity and plasma aldosterone level were measured as described previously by our group.

Plasma renin activity was calculated as the amount of angiotensin I generated in a maleate buffer for 90 minutes, which was determined by competition with 125I-angiotensin for binding to antibody-coated tubes.

To obtain the urinary PGE2 concentration, 1-ml urine samples were acidified to a pH of 3 with formic acid. Then 10 ml of chloroform was added, and the mixture was centrifuged. The upper (nonpolar) layer was aspirated, and the chloroform then was removed by nitrogen evaporation at 37°C, leaving the PGE2 adhered to the walls of the tube. The PGE2 was then reconstituted with 1 ml of gelatin-phosphate buffer, and the PGE2 concentration was determined by radioimmunoassay as described previously by our group. The PGE2 in an aliquot of the extracted and
purified experimental sample competes with added tritium-labeled PGE$_2$ (THPGE$_2$) for binding with the antibody (Sigma, St. Louis, MO, USA). Separation of bound from free PGE$_2$ is accomplished by adding a cold dextran charcoal solution, followed by centrifugation. Supernatant scintillation counts are interpreted from a standard curve generated from scintillation counts of standard samples in which [THPGE$_2$] is displaced from the antibody by the addition of known quantities of cold (non-tritium-labeled) PGE$_2$. There was less than 26% variation of results in 40 replicate measurements of a control sample.

Osmolarity was determined using a Fiske Osmatic-automatic Osmometer (Uxbridge, MA, USA). Inulin and PAH concentrations in plasma and urine and glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined as previously described by our group. Inulin concentrations in urine and plasma samples were determined by the anthrone colorimetric method. The PAH concentration also was determined by a spectrophotometric method. Both of these are standard methods for measuring inulin and PAH clearances. Renal blood flow was calculated from the formula renal blood flow = ERPF/(1 - hematocrit).

Fractional excretion was calculated by the following formula: fractional excretion = UV/GFR × P, where UV is the excretion rate of x and P is the plasma concentration of x. Fractional excretion of water was calculated from the formula fractional excretion of water = 1 divided by U/P inulin. Hematocrit was determined with a Coulter S Plus analyzer (Hialeah, FL, USA).

Statistical Analysis

The values reported for each dog are the average of the baseline and three ensuing recording periods. In the case of the urine parameters the values reported are the average of the three results from the separate urine collections. Statistical analysis was by the Mann-Whitney U test. Parameters designated significant in the text are those in which the Mann-Whitney test showed significance with 95% or greater confidence. Coefficients of correlation were calculated by regression analysis. Significant results are again set at 95% or greater confidence limits.

Results

Figures 1 through 7 illustrate the mean values of the various measurements for each group. As shown in Figures 1 and 2, heart rate and TPR were significantly higher in the experimental group (p<0.025 and p<0.05, respectively) and systolic blood pressure was significantly lower (p<0.05). Diastolic blood pressure, mean arterial pressure, CO, and CI were not significantly different in the two groups. Stroke volume was significantly lower in the hypercalcemic animals (0.024 ± 0.006 vs 0.036 ± 0.008 L/beat; p<0.005; data not shown).

Figures 3 and 4 show the mean results obtained by various analyses of blood. Total serum calcium, iCa, and serum creatinine were significantly higher (p<0.0005 for each) and sMg was significantly lower (p<0.0005) in the experimental group. Whole blood ionized potassium, whole blood ionized sodium, plasma renin activity, plasma aldosterone, blood urea nitrogen, and hematocrit were not significantly different in the two groups.

Figures 5 and 6 show the means of various measures of renal function in the two groups. The hypercalcemic group had a significantly lower GFR, ERPF, renal blood flow, and osmolarity (p<0.005 in all cases). Renal resistance was also calculated but is not shown in the figures. At 0.57 ± 0.07 mm Hg/ml/min in the experimental group versus 0.28 ± 0.01 mm Hg/ml/min in the control group, it was significantly higher (p<0.005) in the experimental group. Potassium excretion rate was also significantly lower (p<0.025) and urinary calcium excretion was significantly higher (p<0.05) in the experimental group. Urinary sodium and magnesium excretion rates were not significantly different in the two groups.

Figure 7 gives the mean fractional excretion rates for water and electrolytes in the two groups. Fractional excretion of sodium (p<0.005), calcium (p<0.01), magnesium (p<0.005), and water (p<0.005) were all significantly higher in the experimental group, as was urine flow rate (p<0.05). Fractional excretion of potassium and urinary PGE$_2$ excretion were not different in the two groups.

Correlation coefficients were calculated to determine the relation between different factors that may have been affected by high dietary calcium and vitamin D. These are given in Table 1. Ionized calcium showed significant positive correlations with serum calcium (p<0.001), renal resistance (p<0.05), heart rate (p<0.05), and fractional excretion of sodium (p<0.01), calcium (p<0.001), magnesium (p<0.001), and water (p<0.001) and significant negative correlations with sMg (p<0.05), stroke volume (p<0.01), renal blood flow (p<0.01), GFR (p<0.001), ERPF (p<0.01), and osmolarity (p<0.01). Serum magnesium showed significant negative correlations with serum calcium (p<0.05), iCa (p<0.05), diastolic blood pressure (p<0.05), heart rate (p<0.05), urine flow rate (p<0.05), and fractional excretion of water (p<0.05).

Discussion

The effect of dietary supplementation with calcium and vitamin D on systemic and renal hemodynamics in conscious dogs was studied. The experimental animals had a greater urine flow rate and higher fractional excretion of water, sodium, magnesium, and calcium. Increased calcium plus vitamin D therefore seems to have a diuretic effect, which acts to decrease blood volume and thereby blood pressure. Evidence for a lower blood volume in the experimental animals is a
Figure 1. Mean values for systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HRT) in normal and experimental groups. Bars represent SEM. An asterisk indicates that the difference between the groups for that parameter is significant (p<0.05).

Figure 2. Mean values for cardiac index (CI), cardiac output (CO), and total peripheral index (TPR) in normal and experimental groups. Bars represent SEM. An asterisk indicates that the difference between the groups for that parameter is significant (p<0.05).

Figure 3. Mean values for whole blood ionized potassium (iK), whole blood ionized calcium (iCa), total serum calcium (SCa), serum magnesium (SMg), serum creatinine (CREAT), and plasma renin activity (PRA) in normal and experimental groups. Bars represent SEM; if no bar is shown, SEM is less than 0.10. An asterisk indicates that the difference between the two groups for that parameter is significant (p<0.05).
FIGURE 4. Mean values for whole blood ionized sodium (INa), plasma aldosterone (Aldo), hematocrit (HCT), and blood urea nitrogen (BUN) in normal and experimental groups. Bars represent SEM; if no bar is shown, SEM is less than 0.10.

FIGURE 5. Mean values for renal blood flow (RBF), urine osmolarity (OSM), glomerular filtration rate (GFR), and effective renal plasma flow (ERPF) in normal and experimental groups. Bars represent SEM. An asterisk indicates that the difference between the groups for that parameter is significant (p<0.05).

FIGURE 6. Mean values of excretion rates of sodium (UNa), potassium (UK), calcium (UCa), and magnesium (UMg) in normal and experimental groups. Bars represent SEM. An asterisk indicates that the difference between the groups for that parameter is significant (p<0.05).
reduced stroke volume and a slightly higher hematocrit. A greatly reduced GFR and ERPF may also be an indicator of lower blood volume or may be due to renal vasoconstriction or a combination of both.

Although a decrease in systolic blood pressure was seen (in part due to the decrease in blood volume), there was no change in diastolic blood pressure, and TPR and renal resistance were higher, suggesting peripheral and renal vasoconstriction. The higher TPR and renal resistance in experimental animals also could be due to reactive mechanisms attempting to compensate for this reduced blood volume. Stern et al. have also reported a natriuretic response to increased dietary calcium in SHR during correction of initial reduced ionized serum calcium, which reduced blood pressure in adult SHR.

These hemodynamic findings are identical to our previous findings during acute hypercalcemia produced by calcium infusions. Since there are no known vascular effects of vitamin D, we believe the findings in the current study, when compared with identical hemodynamics during calcium infusion, are due to hypercalcemia per se. Indeed, in other studies in our laboratory, animals receiving calcium and vitamin D had elevated vascular resistances while animals receiving increased dietary calcium alone that did not become hypercalcemic did not have elevated vascular resistances. The elevated iCa could possibly increase vascular tone by increasing the gradient for iCa entry into smooth muscle cells. Also, this increased gradient could potentiate the action of vasoconstrictor substances such as antidiuretic hormone and norepinephrine, which cause vasoconstriction by a mechanism requiring entry of extracellular calcium.

In opposition to the idea that increased extracellular calcium could potentiate vasoconstriction is evidence that increased extracellular calcium acts to stabilize membranes by binding to them and preventing fluxes through calcium channels, thus inhibiting contraction. However, calcium from the extracellularly bound pool has been shown to enter smooth muscle cells upon activation of the contractile response, and it has been hypothesized that released extracellularly bound calcium facilitates contraction by increasing the gradient for calcium entry. Thus, it is uncertain whether increased extracellular calcium would inhibit or facilitate smooth muscle contraction. Our results of increased TPR support the latter hypothesis.

The lowered sMg could also account for the higher TPR, since acute magnesium withdrawal increases vascular tone in isolated arteries and potentiates the action of adrenergic amine-induced contraction. In the present study, the fact that sMg showed a significant inverse relation to diastolic blood pressure supports the hypothesis that magnesium deficiency contributes to the increased vascular resistances. The apparent vasoconstriction could also be a result of a synergistic action by magnesium and calcium. Arteries exposed to magnesium-free medium develop increased tone that is directly dependent on extracellular calcium, and it has been hypothesized that low magnesium causes vasoconstriction by allowing calcium entry into cells. In these hypercalcemic, hypomagnesemic animals, the gradient for calcium entry is increased and there is less magnesium present to prevent entry. The lower sMg may also account for the increase in heart rate in the hypercalcemic group, since hypomagnesemia has been associated with tachycardia in rats. The tachycardia could also be a response to low blood volume.

The cause of the lower sMg appears to be an increase in the fractional excretion of magnesium, which is related more closely to the level of iCa in the blood than to excretion of calcium. Increased iCa seems to result in magnesium excretion despite hypomagnesemia — evidence that calcium and magnesium excre-
mental group. This impairment of renal concentrating ability has previously been noted in hypercalcemia and seems mainly due to decreased reabsorption of sodium in the loop of Henle.23,24

The role of parathyroid hormone (PTH) and the sympathetic nervous system in the vascular changes caused by chronic hypercalcemia resulting from increased dietary calcium and vitamin D must also be considered. We did not measure changes in PTH levels in the animals made chronically hypercalcemic by dietary calcium and vitamin D supplementation. As acute administration of PTH is vasodilative,27 it may contribute to changes in vascular resistance produced by altering calcium levels. In our previous studies in dogs, however, we have shown little contribution of changes in PTH to elevated vascular resistances seen during hypercalcemia.25 Rather, PTH participates more in reduced vascular resistances during hypocalcemia.26 Anderson et al.27 found conversely that a high calcium diet in SHR led to greater sensitivity and prolonged response to vasodilator effects of PTH infusion. Difference in species may be important, as Mulvany et al.28 suggests that vascular responses of SHR are unique because of an intrinsic defect of their vascular smooth muscle.

The chronic effect of PTH on blood pressure seems to be different than that of the acute effect. Parathyroidectomy slows the development of hypertension in SHR and deoxycorticosterone acetate and salt–treated rats independently of the calcium level.29,30 Long-term PTH administration would seem to potentiate the effects of increased vascular resistance in hypercalcemia since it may act as a calcium ionophore.21 During calcium and vitamin D administration as produced in this study, PTH would be suppressed and would be unlikely to play a role in the increased vascular resistance. Suppressed PTH levels could explain in part why our model of hypercalcemia does not produce hypertension while chronic hyperparathyroid hypercalcemia does. Another difference may be that hyperparathyroidism often produces milder elevations of blood calcium33 than those produced in this study. We also did not measure catecholamine levels in this study. Vlachakis et al.34 and others have clearly shown that parathyroidism often produces milder elevations of blood calcium than those produced in this study. We also did not measure catecholamine levels in this study. Vlachakis et al.34 and others have clearly shown that hypercalcemia does elevate catecholamine levels, which can result in increased vascular resistance.

In conclusion, increased dietary calcium and vitamin D leading to increased iCa in the blood appear to result in increased diuresis and decreased blood volume, which act to decrease blood pressure. This decrease was evidenced by a lower systolic blood pressure in the experimental group. Increased iCa also led to decreased serum magnesium, while TPR was increased. These results resolve some of the conflict over the effect of dietary calcium on blood pressure — a diuretic effect of increased blood calcium does act to decrease blood pressure, but this decrease is opposed by an increase in TPR. We conclude that this increase in TPR is related to increased blood iCa, a lowered serum magnesium level, or an interaction between the two.

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