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DIETARY LEAD AND CALCIUM: EFFECTS ON BLOOD PRESSURE AND RENAL NEOPLASIA IN THE RAT

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The enclosed manuscript is the product of our research for last year. It has been reviewed by the Journal of Nutrition, and this is the revised copy, which we expect will be accepted for publication shortly. In this paper we describe our first chronic experiment with lead and calcium, in which two concentrations of calcium in the diet were used , viz., 0.2% and 4.0%. We found a graded rise in blood pressure in the low calcium group with increasing levels of lead in the drinking water. The high calcium group had consistently higher blood pressures than the low calcium group, and there did not appear to be any ability of calcium to prevent the hypertensive effects of lead. A very exciting finding was the fact that the high calcium diet appeared to enhance the tumorigenic potential of lead on the kidney. There were, in addition, very significant changes in trace metal concentrations other than lead in several organs. The high calcium diet did not prevent the accumulation of lead in any of the 6 tissues studied, but did cause significant decreases in kidney copper, femur and plasma magnesium, and iron in kidney, liver and testis.

Since then, we instituted a larger multifactorial experiment, begun in October, 1989, to examine in more detail the actions of various calcium and lead concentrations in the diet on blood pressure and neoplasia. In this experiment, which will last about 1 year, we are studying the actions of 3 dietary concentrations of calcium (0.1, 0.5, and 2.5%) in combination with 3 levels of lead in the drinking water (0, 50 and 100 ppm). Since we found previously that the 4.0% calcium diet produced considerable toxicity (nephrolithiasis), the present 2.5% calcium concentration appears to be better tolerated. We are finding in our

preliminary studies on blood pressure that the 0.1% calcium group given the highest concentration of lead (100 ppm) appears to consistently have the highest blood pressures and that there is a tendency toward lower blood pressures as the concentration of calcium in the diet increases. This experiment will continue until September, 1990, so that the absolute values of pressure will not be available until then. At that time we shall examine the tumorigenic actions on the kindey and do trace metal analysis.

We estimate that this component of our study will be completed by February 1991, if sufficient funds are available to us. Without additional funding , we may not be able to satisfactorilly complete this project.

Dietary Lead and Calcium: Effects on Blood Pressure and Renal Neoplasia in the Rat

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ABSTRACT

We examined the potential of increased Ca in the diet to modify the effects of Pb on tissue metal concentrations, blood pressure, and the incidence of renal tumors. Male Wistar rats (n=48, 5 weeks old) were randomly assigned to one of 6 treatment groups. They were fed a low (0.2%) or high (4.0%) Ca diet for 31 weeks and given 0, 1.0 or 100 ppm Pb in drinking water. In the low Ca groups, increasing concentrations of Pb produced graded increases in mean blood pressure. Rats receiving 4.0% Ca had higher mean blood pressures than the animals fed the 0.2% Ca diet. The 4.0% Ca diet also caused renal and urinary bladder stones to develop in some rats. The high Ca diet did not prevent dose-dependent increases in tissue Pb accumulation, but caused significant decreases in kidney copper, femur magnesium, and iron in kidney, liver and testis. Femur magnesium and iron and liver iron concentrations were lowest in rats receiving 4.0% Ca and 100 ppm Pb. Precancerous and cancerous renal lesions occurred to the greatest extent in the rats receiving 100 ppm Pb and the high Ca diet. These results suggest that very high dietary Ca does not protect against Pb-induced increases in blood pressure or Pb accumulation in tissues and may often produce nephrocalcinosis. In addition, high dietary Ca in the presence of Pb may increase the incidence of renal tumors.

Key Words: lead, calcium, blood pressure, nephrocalcinosis, kidney tumors, trace metals

INTRODUCTION

Two independent types of evidence suggest that there may be an association between low level lead exposure and mild essential hypertension. First, in several studies low level exposure to lead in drinking water produced increases in blood pressure in the rat or the dog (1-7). This has not been a consistent finding since, in other studies as reviewed by Victery (7), lead exposure did not result in increases in blood pressure. Second, several epidemiologic studies have demonstrated a significant association between blood lead concentrations and blood pressure, while other studies have found no association between these two variables (8-12). The reasons for the contrasting results of the various experimental and epidemiological studies are not known. One possible explanation is that dietary calcium may directly influence blood pressure and/or alter the effects of lead on blood pressure, and this was not considered in the design of prior studies.

Harlan, et al (8) studied about 3000 men and women, and found significantly higher blood lead concentrations in subjects who had elevated diastolic blood pressures than in those with diastolic pressures of less than 90 mm Hg. No data on lead exposure were presented other than blood levels. However in the subjects they studied, higher blood lead and blood pressure were associated with a significantly lower dietary calcium intake. These data suggest the importance of evaluating lead-calcium-blood pressure interactions.

There is both experimental and epidemiologic evidence that increases in dietary calcium may favorably influence blood pressure in laboratory animals and man (13-19). Furthermore, it has been reported that diets deficient in calcium can augment the toxic effects of lead and promote increased lead absorption and decreased excretion (18,19). In addition, several recent articles suggest that the effects of lead on blood pressure are related to lead-induced alterations in intracellular calcium metabolism (20-22). Thus, lead/calcium interactions may in part account for the differing results of studies of the relationship of each of these metals to blood pressure.

In addition to effects on blood pressure, lead can induce the development of kidney tumors in the rat. These tumors are often bilateral and multiple, being either adenomas or adenocarcinomas of the renal cortex (23). The adenomas can be solid, papillary, tubular, or mixed, and can occur in both male and female rats. Lead dose and duration of exposure, as well as lead accumulation in the kidney, influence the development of renal tumors, with higher doses resulting in a greater incidence of tumors in a shorter period of time (23). Because of the central role of the kidney in the control of blood pressure, studies of lead effects on blood pressure should include histopathologic evaluation of the kidneys, including examination for renal neoplasms.

The major objective of the current investigation was to study the effects of 2 dietary calcium concentrations, one below normal and the other substantially above normal, in combination with several levels of lead exposure on both blood pressure and the development of renal tumors in the rat.

METHODS

Forty-eight male weanling Wistar rats (50-75g) (Charles River; No. Wilmington, MA) were housed individually in stainless steel cages and allowed to adapt to the laboratory environment for one week. Twelve hour light-dark cycles were maintained. The rats were randomly assigned to one of 6 treatment groups, with 8 rats per group. Animals were fed diets containing either 0.2% or 4.0% calcium and were given drinking water containing 0, 1.0, or 100 ppm (ug/ml) Pb. The choice of these calcium and lead concentrations was based on the results of previously reported studies (7,24-27) using identical concentrations to separately assess the effects of Ca or Pb on blood pressure in the rat. It was anticipated that the lead doses used would produce blood lead concentrations similar to those found in United States residents with low to moderate lead exposures.

Diets containing 0.4%-0.5% Ca are required to support maximum calcification during growth, but diets 50% lower in calcium can support maximum weight gain (28). The 0.2% Ca diet was intended to be a diet containing the lowest calcium concentration needed to support maximum weight gain in the rat, but greater than the concentration (< 0.1%) that could produce symptomatic calcium deficiencies (28). The 4.0% Ca diet was the highest concentration shown by other investigators to reduce blood pressure in the rat (27). Use of these concentrations resulted in a 20-fold difference between the high and low calcium diets; this large difference was intended to maximize the ability to detect effects of diet calcium on lead-induced changes in blood pressure and the incidence of renal tumors. The study of intermediate calcium concentrations was reserved for future investigations. The phosphorus and vitamin D contents of both diets were not varied to prevent the introduction of other variables that could complicate interpretation of the study results.

Introduction of the modified calcium diets was done one week after receiving the rats; distilled water containing sodium acetate trihydrate (0 ug/ml Pb) (J.T. Baker, Phillipsburg, N.J.) or lead acetate trihydrate (1.0 and 100 ug/ml Pb) (Fisher Scientific, Fair Lawn, N.J.) was also introduced at the same time. The control groups received sodium acetate in the drinking water at an acetate concentration equal to that of the groups receiving 100 ug/ml Pb. Drinking water bottles were fitted with silicone stoppers (VWR, Scientific, San Francisco, CA).

Rats were fed a modified AIN-76 diet (Table 1). Calcium carbonate ($CaCO_3$) was used to prepare the 2 different dietary levels of calcium. The diets contained no calcium phosphate. Diets were prepared by Research Diets, Inc., New Brunswick, NJ. Analysis in triplicate of the diets showed a mean calcium concentration of 0.23% for the low calcium diet and 4.04% for the high calcium diet.

Drinking water consumption was monitored throughout the study. Rats were weighed every two weeks.

At the end of 31 weeks, final systolic and diastolic blood pressures were measured directly by use of an indwelling catheter inserted in a tail artery that was connected to a Statham-type physiological pressure transducer (Model CP21, Century Technology Company, Inglewood, CA). The transducers were initially calibrated using a mercury manometer. The catheter was inserted under ether anesthesia and the rats allowed to recover from anesthesia for two hours prior to blood pressure measurement. Measurements of rat blood pressure during recovery from anesthesia demonstrated that waiting 1 1/2 hours was sufficient to prevent any lowering of blood pressure by the anesthesia used. Blood pressures were measured in triplicate and averaged; replicate values were within $\pm$ 5 mm of mercury. The data obtained were used to calculate a mean blood pressure (MBP= diastolic blood pressure + 1/3 (systolic - diastolic blood pressure) for each rat.

Following these measurements, approximately 5 ml of blood was withdrawn from the catheter and the rats were killed by decapitation. The heart, kidneys, liver, right hind femur, brain, and testes were removed and saved for trace metal analyses. One kidney from each rat was preserved in 10% buffered formalin (Fisher Scientific, Fair Lawn, N.J.). This kidney was bisected and embedded in paraffin. Five-micrometer-thick sections of the tissue were made and stained by the hematoxylin and eosin (Fisher Scientific, Fair Lawn, N.J.) technique and evaluated by light microscopy.

Organ concentrations of zinc, copper, iron, calcium, and magnesium were determined by previously described techniques using flame atomic absorption spectrophotometry (Perkin-Elmer Model 403, Norwalk, CT) after digestion with nitric (GFS Chemicals, Columbus, OH) and perchloric (GFS Chemicals, Columbus, OH) acids (29). Lead concentrations were determined by a flameless atomic absorption procedure (Perkin-Elmer Model 503 and HGA-2100, Norwalk, CT). Plasma calcium and magnesium were determined by flame atomic absorption after

dilution with a 0.1% lanthanum solution. National Bureau of Standards bovine liver (SRM 1577a, Gaithersburg, MD) was used as a quality control sample for all tissue metal analyses. Assays of this sample gave results within 7% of certified values.

Data were analyzed by analysis of variance (ANOVA). If ANOVA indicated that there were significant ($p < .05$) differences among the 6 treatment groups, then pair-wise comparisons were made using Duncan's test (30). Data on tissue lead concentrations were log-transformed prior to evaluation by ANOVA.

In statistical evaluation of the data on tissue metal concentrations, 36 ANOVAs were done. To compensate for multiple comparisons, the level of significance at which the null hypotheses were tested was set at $p < 0.01$ instead of $p < 0.05$ for evaluation of tissue concentrations.

RESULTS

Growth curves for the 6 treatment groups are shown in Figure 1. Rats fed the 4.0% calcium diet appeared to grow more slowly than animals consuming the 0.2% Ca diet, but this difference was significant only for the group of rats fed the 4.0% Ca diet and 100 ppm lead in the drinking water. Daily drinking water consumption for rats fed the low calcium diet was 45±7 ml, 37±3 ml, and 40±5 ml, respectively, for rats receiving 0, 1.0 or 100 ppm lead. Consumption for rats fed the high calcium diet was 40±5 ml, 46±3 ml, 43±5 ml. These values do not differ significantly among the 6 treatment groups; thus, lead consumption was comparable in corresponding treatment groups fed the low and high calcium diets.

None of the 24 rats fed the 0.2% calcium diet died prior to the termination of the study at 31 weeks, but 10 of the 24 rats consuming the 4.0% calcium diet did. These included 4 rats from the 0 ppm Pb group, 3 from the 1 ppm Pb group, and 3 from the 100 ppm Pb group. Autopsies of these ten rats showed kidney and/or bladder stones.

Blood Pressure

Mean blood pressures (MBP) at the termination of the study are shown in Figure 2. For rats fed the low calcium diet, the control (0 ppm Pb) group had the lowest MBP and the rats consuming 100 ppm Pb in the drinking water had the highest MBP. Only the values for the control and 100 ppm Pb groups were significantly ($p < 0.05$) different.

Rats fed the high calcium diet and 0 or 1.0 ppm Pb had significantly higher MBP than animals fed the low calcium diet. Although rats fed this diet and 100 ppm lead had lower MBP than their counterparts fed the low calcium diet, this difference was not statistically significant.

Kidney Histopathology

The eight rats in the group fed 0.2% calcium and 0 ppm Pb did not have any pathological findings in the kidneys.

Of eight rats in the group fed 0.2% calcium and 1 ppm Pb, two showed focal lymphocytic inflammatory cell infiltration of interstitium.

In the group fed 0.2% calcium and 100 ppm Pb, 7 of 8 rats showed scattered tubular cell atypia with cellular enlargment, nuclear hypertrophy, hyperchromasia, coarse chromatin clumping and single or multiple enlarged nucleoli (Figure 3). One rat showed intranuclear inclusions with nuclear vacuolization in convoluted tubules of the kidney. Three rats showed focal lymphocytic inflammatory cell infiltration of interstitium.

Of four rats examined from the group receiving 4.0% calcium and 0 ppm Pb, one rat showed a stone in the caliceopelvic system with atypical transitional cell hyperplasia and dilatation of the tubular lumen. Three rats revealed focal mild lymphocytic inflammatory cell infiltration of interstitium.

Of the five rats examined from the group fed 4.0% calcium and 1 ppm Pb, one rat showed a stone in the caliceopelvic system along with papillary transitional cell carcinoma of the pelvis. Three rats showed focal lymphocytic inflammatory cell infiltration of interstitium.

Of five rats examined from the group consuming 4.0% calcium and 100 ppm Pb, four rats showed a stone in the dilated caliceopelvic system. Three rats had transitional cell hyperplasia and two had invasive transitional cell carcinoma of the pelvis (Figure 4). Two rats with stones showed dilatation of convoluted tubules. Tubular epithelial atypia with enlarged cells and nuclei, hyperchromasia, and single or multiple enlarged nucleoli were seen in three rats having a stone. Convoluted tubules were dilated in two rats. In three rats, there were focal lymphocytic inflammatory cell infiltrations and in one rat there was polymorphonuclear leukocytic infiltration of interstitium, interstitial fibrosis, and edema with renal and perinephric abscess formation.

Tissue Metal Concentrations

Metal concentrations for blood, whole brain, kidney, liver, femur, heart, and testis are provided in Tables 2-8, respectively.

Concentrations of lead in whole blood, heart, and brain were significantly increased in rats consuming 100 ppm but not 1.0 ppm lead. Testicular lead concentrations were significantly increased only in rats receiving 100 ppm Pb and the high calcium diet. For kidney, liver, and femur, Pb concentrations were significantly increased in rats receiving 1.0 ppm Pb in the drinking water and were further increased in animals consuming 100 ppm Pb in their water.

Plasma magnesium concentrations were reduced by the high calcium diet. Plasma calcium was significantly elevated only in the group of rats receiving 4.0% Ca and 100 ppm Pb. The high calcium diet resulted in large increases in the calcium content of the kidney, but did not significantly increase calcium concentrations in any other organ. Kidney concentrations of copper and iron, but not magnesium and zinc, were substantially reduced by the high calcium diet.

Concentrations of iron in testis and liver were also significantly decreased by the high calcium diet, the latter especially in animals consuming

100 ppm Pb in the drinking water. Feeding the high calcium diet also resulted in decreased femur magnesium and iron concentrations, particularly in the group of animals receiving 100 ppm lead.

DISCUSSION

Blood Pressure

The results of this study are in agreement with prior investigations (1-7) that demonstrate that chronic low-dose exposure to lead can increase blood pressure in the rat. However, the results do not support prior studies that show that high dietary calcium concentrations (up to 4.0%) reduce blood pressure in the rat. In contrast, the rats fed the 4.0% calcium diet and 0 or 1.0 ppm Pb in the present study had higher MBP than rats consuming a low-normal (0.2%) calcium diet and the same Pb concentrations. Only the rats consuming the 4.0% calcium diet and 100 ppm Pb had lower MBP than their counterparts on the low Ca diet, but this difference was not statistically significant and is based on blood pressure in only three rats in the 4.0% Ca/100 ppm Pb group.

The higher mean blood pressures in the rats consuming 4.0% calcium may have developed as a result of the nephrocalcinosis observed in these animals. Alternatively, other factors may be responsible for the differences between these results and those of other studies, including differences in duration of the study, other dietary components besides calcium, strain of rats studied, or whether the blood pressure was measured indirectly or directly, as in the current study.

Nephrocalcinosis

The development of nephrocalcinosis in rats is dependent upon the dietary ratio of calcium to phosphorus as well as the magnesium content of the diet (31,32,33). Hoek et al. (31) have shown that increases in dietary calcium from 0.13 to 0.50% and phosphorus from 0.20 to 0.40% increased the Ca and

phosphorus content of the kidney and the extent of nephrocalcinosis. However, further increases in dietary Ca to 0.75% actually reduced the degree of nephrocalcinosis. The phosphorus content of all diets in our study was held constant at 0.4%, and the magnesium content was 0.05%; at these levels, an increase in dietary calcium from 0.2% to 4.0% led to the development of marked nephrocalcinosis. However, from these results it cannot be determined if the nephrocalcinosis was the result of the high diet Ca concentration, its ratio to phosphorus or magnesium, or the combined influence of these factors.

Greger et al. (32) have shown that feeding dibasic calcium phosphate to rats results in 20 fold higher kidney calcium concentrations when compared to other calcium salts. For this reason, we used calcium carbonate instead of calcium phosphate in our diets. Nevertheless, the 4.0% calcium diet did produce substantial nephrocalcinosis.

Evans and Weaver (34) have found that a diet containing 2.5% calcium as the carbonate produces nephrocalcinosis in spontaneously hypertensive rats consuming diets containing 0.01% or 0.05%, but not 0.75%, magnesium. Blood pressure was lowered by increasing dietary calcium despite the nephrocalcinosis, an observation that puzzled these authors. The above results suggest that the development of nephrocalcinosis should be monitored in all blood pressure studies in the rat, especially those in which dietary calcium, phosphorus, or magnesium are modified.

Renal Tumors

The occurrence of kidney lesions including renal tumors in rats as a result of lead exposure is well known (23,35,36). All rats studied in the group fed 4.0% calcium and 100 ppm Pb had hyperplasia or invasive transitional cell carcinoma, but none of the rats fed 0.2% calcium and 100 ppm Pb exhibited these changes. Thus, the high calcium diet promoted the development of kidney tumors in the rats receiving 100 ppm lead in the drinking water. This result is in agreement with the observations of Kasprzak et al. (37), who found that

lead-induced renal carcinogenesis was enhanced by increases in dietary calcium despite the fact that higher diet calcium concentrations reduced kidney lead concentrations. These results are also in agreement with the observation that a high calcium diet is more likely to promote chemical carcinogenesis than to inhibit it (38).

Renal tumors developed in our rats receiving 100 ppm Pb and 4.0% Ca after 7 months. This period of time is shorter than that reported by other authors (39,40) using diets of normal calcium content even though they used higher doses of lead. Kasprzak et al. (37) did not find renal tumors in their rats until 58 weeks after beginning lead exposure. This may be due to the greater promoting ability of the high calcium diet that we used, which had a higher calcium content (4.0%) than the approximately 2.4% Ca diet used by Kasprzak and co-workers. Alternatively, the tumors that we observed in transitional cells of the kidney pelvis may develop more quickly than the tubular cell neoplasms observed by Kasprzak et al. (37). The fall in body weight at the end of the study of the rats receiving 4.0% calcium and 100 ppm lead could be related to the renal tumors that developed in this treatment group.

Typical rodent diets have high calcium concentrations in comparison to human diets. Little is known about the role of dietary calcium in promoting chemically-induced tumors, but it is possible that other chemicals besides lead acetate that have been shown to be animal carcinogens may also require promotion by calcium. It may be that prior studies of chemically-induced cancer in rodents overestimate the risk to humans because of promotion by the usually high calcium content of rodent diets. This may have important public health implications, since judgements about the safety of many chemicals are based on carcinogenicity studies in rodents.

Male rats have unique urinary proteins, alpha-2 microglobulins, that may be a factor that predisposes them to the development of kidney tumors (41-43). Thus, the applicability of these findings to female rats is uncertain.

Several studies suggest that lead can act synergistically with other carcinogens, including chromate, 2-acetylaminofluorene, cadmium, and benz(a)pyrene, to produce renal tumors (23). Other types of tumors may also be produced by these combined exposures. Lead stimulates proliferation of renal tubular epithelium in rats and mice, a finding that may have pre-neoplastic significance (23). A consistent effect of lead is to cause atypical hyperplasia of kidney tubular epithelium, with nuclei that are enlarged and irregular in shape. The renal tumors that develop may arise from the hyperplastic nodules. However, the effects of high dietary and kidney calcium on this process are not known. There are no reports that lead causes kidney cancer in humans (23).

Tumors of the renal pelvis have been reported to account for 7-8% of kidney cancers, and most of these are transitional cell tumors (44). There are reports that kidney stones are associated with the development of squamous and possibly transitional cell kidney carcinoma in humans (44-47). It has been suggested that kidney stones irritate the mucosa, transforming the transitional epithelium to squamous epithelium that may progress to leukoplasia and cancer (45). Nevertheless, the relevance of our results to human kidney cancer is uncertain.

Tissue Metal Concentrations

Lead concentrations in whole blood and the 6 organs analyzed were not reduced by the 4.0% calcium diet in comparison to the 0.2% calcium diet. Thus, differences in kidney lead accumulation cannot explain the tumor enhancing effects of the high Ca diet. These results support prior studies that suggest that high dietary calcium does not protect against organ lead accumulation when compared to normal or low-normal diet calcium concentrations (48). However, we cannot exclude the possibility that such protection might have been observed if we had studied other dietary calcium concentrations, particularly comparison of a low concentration less than 0.1% with higher concentrations, as has been

reported by other investigators (48,49). Mylroie et al. (50) have found that rats fed semipurified diets are more susceptible to lead toxicity than rats fed stock diets. Our use of semipurified diets of different calcium content may be responsible for differences between our results and those of some other investigators. In particular, use of the AIN-76 diet can enhance the development of nephrocalcinosis in the rat (51); thus our use of a modified AIN-76 diet may have contributed to the development of nephrocalcinosis in the rats fed the 4.0% Ca diet.

The nephrocalcinosis observed in the rats consuming the 4.0% calcium diet was associated with a four-fold increase in kidney calcium. Kidney copper and iron concentrations were reduced two-fold by the high calcium diet, but kidney magnesium and zinc concentrations were not affected. It is possible that calcium accumulation in the nephron resulted in the displacement of iron and copper. However, if this were the reason for the decrease in kidney copper and iron, it is surprising that magnesium and zinc were not similarly decreased. Alternatively, the decreases in kidney iron and copper may be secondary to the previously described pathologic changes that occurred in the kidneys of our rats fed the 4.0% Ca diet.

The high calcium diet produced a two-fold decrease in the femur magnesium concentration, as well as decreases in plasma magnesium, but it did not affect the magnesium concentrations of any other organ. The decrease in femur magnesium was more pronounced in rats also consuming lead. Thus, simultaneous exposure to both lead and high dietary calcium appears to be particularly effective in reducing bone magnesium. Rats fed the high calcium diet and 100 ppm Pb also had the lowest femur and liver iron concentrations; this suggests that iron stores can be reduced by this combination of exposures.

There were relatively small but statistically significant differences among treatment groups for liver magnesium and zinc. However, it is unlikely that these differences are physiologically important.

Rats fed the high calcium diet and 100 ppm lead had significantly higher plasma calcium concentrations than rats in the other treatment groups. Although based on a small number of observations, this result suggests that the combination of lead exposure and high dietary calcium may compromise homeostatic mechanisms that control plasma calcium concentrations.

The rats receiving 1.0 ppm lead in the drinking water had whole blood lead concentrations lower than those of most United States residents (52,53), yet experienced an increase in mean blood pressure. The rats consuming 100 ppm lead had blood concentrations similar to humans with moderate to high lead exposures. Thus, the concentrations of lead used produced blood lead levels comparable to these found in man.

Both tumor development and increases in blood pressure are associated with increases in free intracellular calcium (Ca^{2+}_i) (26,54). It may be that the effects of the high calcium diet on blood pressure and renal tumor development found in this study are the results of increases in (Ca^{2+}_i). However, additional study will be needed to address this hypothesis.

In summary, the present study demonstrates that high dietary calcium does not protect against lead-induced increases in blood pressure. In addition, high dietary calcium may itself increase blood pressure, produce nephrocalcinosis, reduce tissue concentrations of some essential minerals, and promote lead induced renal carcinogenesis in the rat. The relevance of these findings to lead exposure of humans is uncertain. Nevertheless, these results suggest caution in the use of calcium supplements, particularly by those exposed to excessive quantities of lead, and suggest the need for additional studies of tumor promotion by dietary calcium.

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Table 1

Composition of Diets

Ingredient	Low Ca diet	High Ca diet
	grams	
Casein	200	200
DL-methionine	3	3
Salt mix	30	30
Vitamin mix	10	10
Choline bitartrate	2	2
Cellulose	50	50
Cornstarch	550	550
Sucrose	100	100
Corn oil	50	50
Calcium carbonate	5	110
Total Weight	1000	1105

1. Salt mix contained (in gm/857 gm): monobasic potassium phosphate - 500, magnesium sulfate heptahydrate - 72.5, magnesium oxide - 11.8, sodium chloride - 74, chromium potassium sulfate ($12\ H_2O$) - 0.55, cupric carbonate - 0.3, potassium iodate - 0.01, ferric citrate monohydrate -6.0, manganous carbonate - 3.5, sodium selenite - 0.01, zinc carbonate -1.6 and sucrose - 186.8.

2. The vitamin mix consisted of the following components in gm/kg: vitamin A palmitate - 0.8, vitamin D3 - 0.25, vitamin E acetate - 10.0; menadione sodium bisulfate - 0.08, biotin (1.0%) - 2.0, cyanocobalamin (0.1%) - 1.0, folic acid - 0.2, niacin - 3.0, calcium pantothenate - 1.6, pyridoxine hydrochloride - 0.7, riboflavin - 0.6, thiamin hydrochloride - 0.6, and sucrose - 979.2.

Table 2

BLOOD CONCENTRATIONS

Drinking Water Pb Conc.	Lead	Plasma Calcium	Plasma Magnesium	N
Low Calcium Diet				
0 ppm	1.87 ± 0.72^{a}	9.73 ± 0.24^{a}	1.66 ± 0.10^{a}	(8)
1 ppm	3.50 ± 0.60^{a}	9.61 ± 0.19^{a}	1.65 ± 0.10^{a}	(8)
100 ppm	39.1 ± 3.87^{b}	9.55 ± 0.29^{a}	1.58 ± 0.04^{a}	(8)
High Calcium Diet				
0 ppm	2.00 ± 1.00^{a}	9.65 ± 0.55^{a}	0.69 ± 0.07^{b}	(8)
1 ppm	3.20 ± 0.58^{a}	10.79 ± 0.40^{a}	0.89 ± 0.10^{b}	(3)
100 ppm	53.3 ± 19.4^{c}	13.18 ± 1.26^{b}	1.36 ± 0.39^{a}	(3)

Data are mean $\pm$ SE, n = 3-8
Pb - ug/dl, Ca and Mg - mg/dl
Values with different superscripts are significantly ($p < 0.05$) different.

Table 3

Brain Metal Concentrations

Low Calcium Diet

Drinking Water Lead Concentration	Lead	Copper	Iron	Magnesium	Zinc
0 ppm	0.03 ± 0.02^{a}	2.67 ± 0.09	22.1 ± 0.8	160 ± 3	11.4 ± 0.4
1 ppm	0.03 ± 0.01^{a}	2.90 ± 0.14	21.9 ± 0.8	161 ± 3	11.5 ± 0.4
100 ppm	0.44 ± 0.09^{b}	2.68 ± 0.07	23.3 ± 0.8	155 ± 3	11.2 ± 0.2

High Calcium Diet

	Lead	Copper	Iron	Magnesium	Zinc
0 ppm	0.02 ± 0.01^{a}	2.86 ± 0.13	21.0 ± 1.7	152 ± 3	12.3 ± 0.4
1 ppm	0.03 ± 0.01^{a}	2.56 ± 0.06	23.0 ± 1.7	147 ± 3	11.2 ± 0.2
100 ppm	0.79 ± 0.14^{b}	2.57 ± 0.09	18.6 ± 1.3	153 ± 3	11.5 ± 0.2

Concentration units are μg/g wet tissue weight.
Data are mean $\pm$ SE
n = 6-8 for all values.
Values with different superscripts are significantly ($p < 0.05$) different.

Table 4

Kidney Metal Concentrations

Low Calcium Diet

Drinking Water Lead Concentration	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.05 ± 0.03^{a}	85.3 ± 7.8^{a}	8.77 ± 0.65^{a}	88.9 ± 4.8^{a}	200 ± 3	23.9 ± 0.8
1 ppm	0.32 ± 0.06^{b}	104 ± 29^{a}	7.91 ± 0.46^{a}	83.7 ± 5.8^{a}	196 ± 3	23.4 ± 0.5
100 ppm	16.0 ± 2.63^{c}	124 ± 16^{a}	7.55 ± 0.33^{a}	84.7 ± 4.9^{a}	197 ± 3	23.0 ± 0.5

High Calcium Diet

	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.01 ± 0.01^{a}	459 ± 267^{b}	3.90 ± 0.45^{b}	41.8 ± 4.9^{b}	188 ± 10	23.1 ± 2.2
1 ppm	0.43 ± 0.18^{b}	451 ± 205^{b}	3.74 ± 0.58^{b}	46.0 ± 5.4^{b}	187 ± 10	20.8 ± 1.0
100 ppm	13.7 ± 3.10^{c}	384 ± 172^{b}	3.29 ± 0.67^{b}	38.9 ± 6.9^{b}	187 ± 10	21.3 ± 2.1

Concentration units are g/g wet tissue weight.
Data are mean ± SE.
n = 6-8 for all values.
Values with different superscripts are significantly ($p < 0.05$) different.

Table 5

Liver Metal Concentrations

Low Calcium Diet

Drinking Water Lead Concentration	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.00±0.00[a]	26.4±1.1	4.83±0.12	168±22[a]	235±3[ab]	28.0±0.7[bc]
1 ppm	0.09±0.03[b]	25.5±0.9	5.32±0.74	138±14[ab]	224±3[bc]	24.9±0.6[c]
100 ppm	0.35±0.13[c]	26.0±0.7	4.49±0.08	184±15[a]	233±3[ab]	26.3±0.4[c]

High Calcium Diet

	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.00±0.00[a]	38.9±6.4	5.25±0.33	89±18[bc]	229±10[bc]	31.3±1.7[ab]
1 ppm	0.03±0.02[b]	36.7±8.1	6.70±2.13	89±27[bc]	214±3[c]	29.1±1.3[abc]
100 ppm	0.68±0.23[c]	34.2±2.5	5.46±0.45	65±12[c]	247±10[a]	32.7±3.9[a]

Concentration units are g/g wet tissue weight.
Data are mean ± SE.
n = 6-8 for all values.
Values with different superscripts are significantly ($p < 0.05$) different.

Table 6

Femur Metal Concentrations

Low Calcium Diet

Drinking Water Lead Concentration	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.31 ± 0.11^{a}	137 ± 11	2.73 ± 0.07	48.4 ± 4.3^{a}	2.80 ± 0.07^{a}	156 ± 6
1 ppm	3.76 ± 0.93^{b}	113 ± 7	2.75 ± 0.06	48.9 ± 4.0^{a}	2.50 ± 0.08^{a}	151 ± 5
100 ppm	308 ± 38.0^{c}	129 ± 6	2.81 ± 0.03	59.7 ± 4.1^{a}	2.76 ± 0.09^{a}	145 ± 5

High Calcium Diet

	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.05 ± 0.05^{d}	154 ± 10	3.21 ± 0.27	$42.7\pm9.5^{a,b}$	1.56 ± 0.16^{b}	158 ± 5
1 ppm	2.56 ± 0.46^{b}	138 ± 7	2.97 ± 0.22	$44.1\pm8.5^{a,b}$	1.47 ± 0.10^{bc}	151 ± 6
100 ppm	335 ± 74.0^{c}	125 ± 11	2.59 ± 0.12	28.7 ± 1.9^{b}	1.22 ± 0.07^{c}	145 ± 3

Concentration units are g/g wet tissue weight for Pb, Cu, Fe, and Zn and mg/g wet tissue weight for Ca and Mg. n = 6-8 for all values.
Data are mean ± SE.
Values with different superscripts are significantly ($p < 0.05$) different.

Table 7

Heart Metal Concentrations

Low Calcium Diet

Drinking Water Lead Concentrations	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.06 ± 0.03^{a}	21.7±1.0	4.62±0.16	76.2±2.5	147±3	14.3±0.5
1 ppm	0.12 ± 0.05^{a}	23.9±0.8	4.63±0.14	82.3±3.1	142±3	14.2±0.3
100 ppm	0.23 ± 0.05^{b}	24.4±1.0	4.50±0.10	85.1±4.0	140±3	14.0±0.3

High Calcium Diet

	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.07 ± 0.03^{a}	25.2±2.8	4.55±0.19	74.9±6.4	155±3	14.6±0.6
1 ppm	0.05 ± 0.01^{a}	25.1±1.8	4.31±0.17	68.2±2.0	143±3	14.6±0.4
100 ppm	0.37 ± 0.15^{b}	25.6±2.4	4.21±0.19	71.6±5.1	149±10	13.4±0.4

Concentration units are g/g wet tissue weight.
Data are mean ± SE.
n = 5-8 for all values.
Values with different superscripts are significantly ($p < 0.05$) different.

Table 8

Testis Metal Concentrations

Low Calcium Diet

Drinking Water Lead Concentration	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.03 ± 0.01^{b}	28.8 ± 1.8	1.95 ± 0.02	37.4 ± 1.6^{a}	145 ± 3	23.4 ± 0.5
1 ppm	0.03 ± 0.02^{b}	26.6 ± 2.8	2.22 ± 0.20	38.5 ± 2.9^{a}	146 ± 3	23.2 ± 0.4
100 ppm	0.04 ± 0.01^{ab}	25.2 ± 0.9	2.01 ± 0.08	39.2 ± 0.6^{a}	151 ± 3	23.2 ± 0.4

High Calcium Diet

	Lead	Calcium	Copper	Iron	Magnesium	Zinc
0 ppm	0.00 ± 0.00^{c}	29.7 ± 3.3	2.30 ± 0.29	26.6 ± 5.7^{b}	152 ± 10	23.1 ± 0.7
1 ppm	0.01 ± 0.00^{bc}	34.1 ± 3.7	2.01 ± 0.05	20.6 ± 1.6^{b}	155 ± 3	25.1 ± 1.2
100 ppm	0.13 ± 0.03^{a}	29.1 ± 1.2	1.89 ± 0.07	20.2 ± 1.6^{b}	148 ± 10	23.5 ± 0.8

Concentration units are g/g wet tissue weight.
Data are mean + SE.
n = 5-8 for all values.
Values with different superscripts are significantly ($p < 0.05$) different.

FIGURES

1. Growth curves for rats in the six treatment groups. LoCa = 0.2% diet calcium content. HiCa = 4.0% diet Ca content.
2. Mean blood pressures (MBP) $\pm$ SE of rats in the six treatment groups after 31 weeks.
3. Photomicrograph of renal proximal convoluted tubules with scattered tubular epithelial cells showing nuclear hypertrophy, hyperchromasia, coarse chromatic clumping and enlarged nucleoli. (hematoxylin - eosin, x400)
4. Histopathologic section of transitional cell carcinoma of the renal pelvis. The tumor is papillary in configuration in the right upper portion of photograph and confluent tumor masses are compressing underlying stroma. (hematoxylin - eosin, x100)

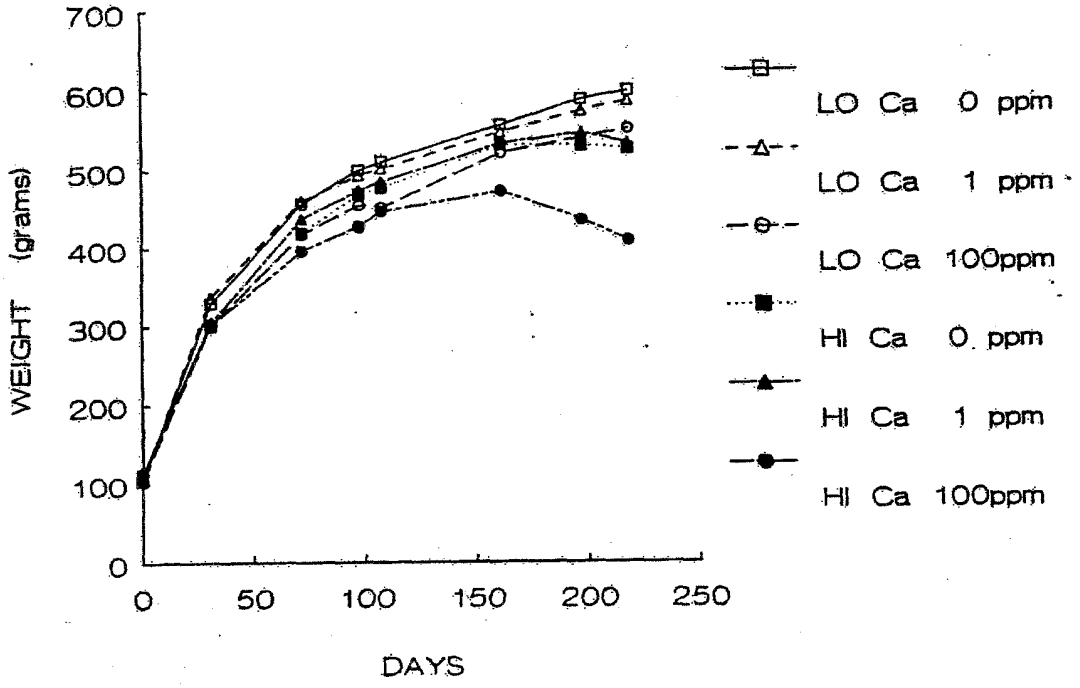
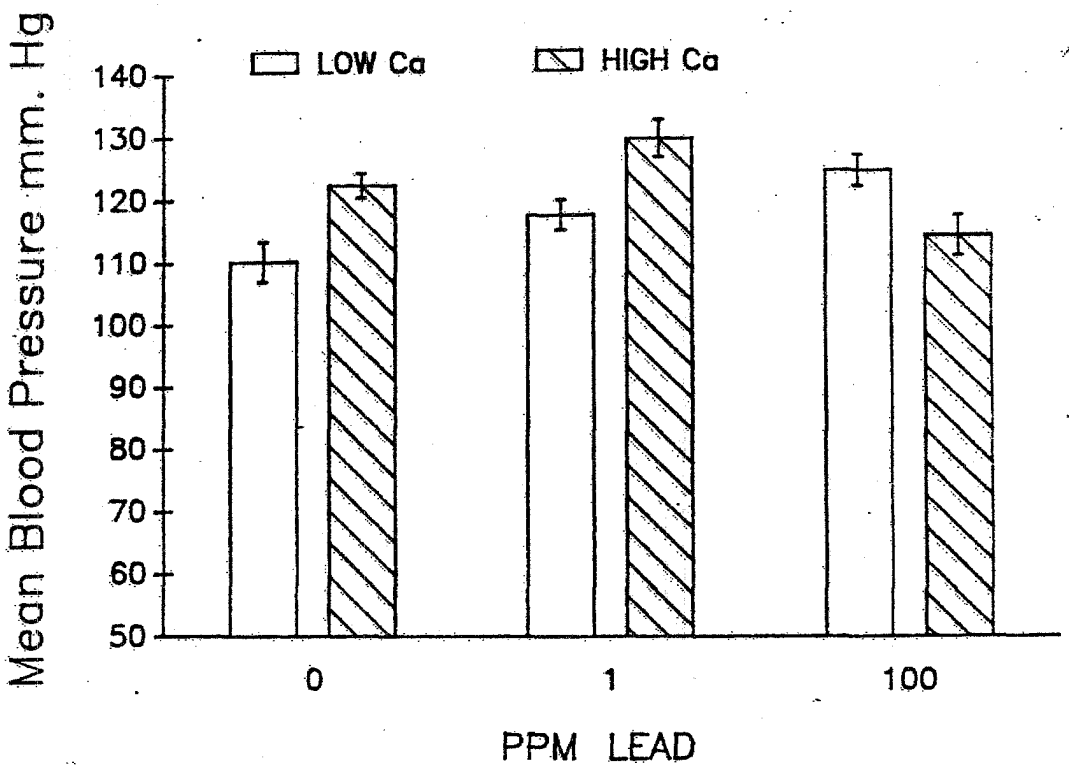

700
600
500
400
300
200
100
0
WEIGHT (grams)
0
50
100
150
200
250
DAYS
LO Ca 0 ppm
LO Ca 1 ppm
LO Ca 100ppm
HI Ca 0 ppm
HI Ca 1 ppm
HI Ca 100ppm

Figure 1


Mean Blood Pressure mm. Hg
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HIGH Ca
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130
120
110
100
90
80
70
60
50
0
1
100
PPM LEAD

Figure 2

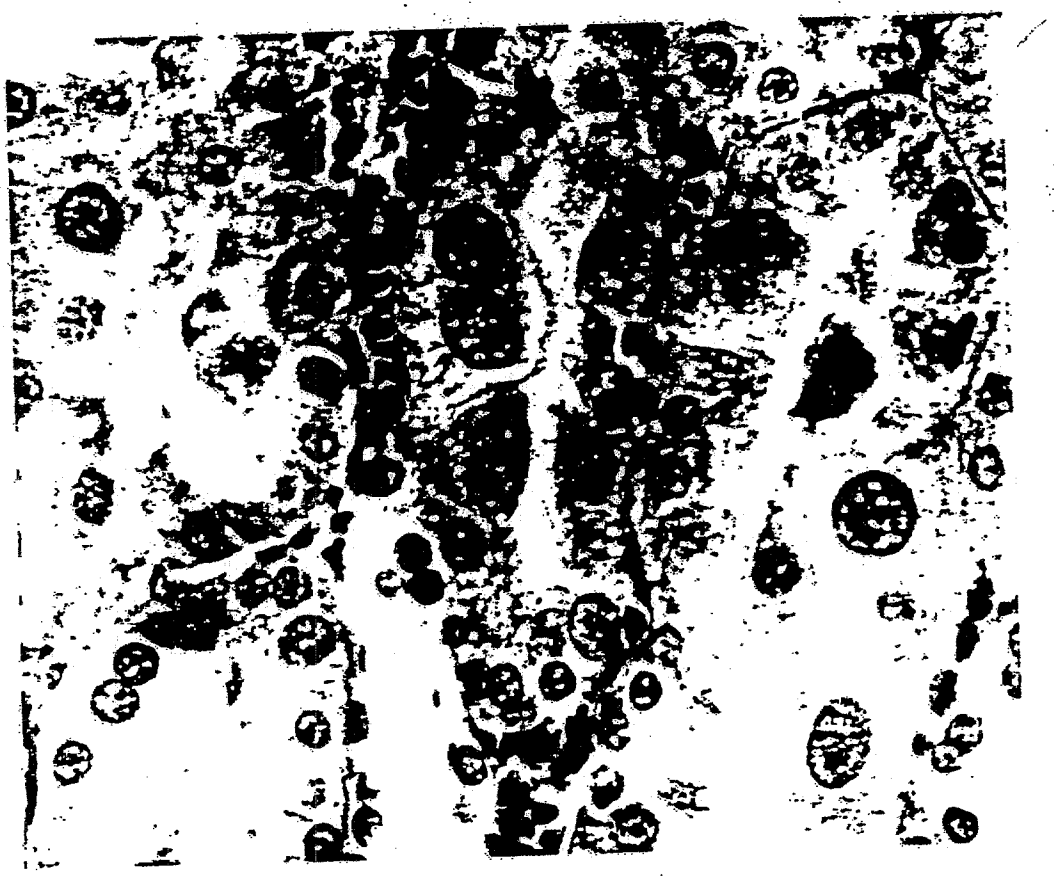

Figure 3

Figure 4