Effects of Lime Treatment on the Bioavailability of Calcium in Diets of Tortillas and Beans: Bone and Plasma Composition in Rats

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Cereal Chem. 69(1):78–81

ABSTRACT

The properties and composition of femurs and plasma of weanling rats fed (for eight weeks) regular corn, raw grains of quality protein maize (QPM) or sorghum, or tortilla-based diets supplemented with and without Ca were determined. The femurs of rats fed tortillas weighed more; were thicker and longer ($P < 0.05$); contained more ash, Ca, P, and Mg; and had less moisture and organic matter ($P < 0.05$) than those of rats fed raw grains. Femurs of rats fed tortillas were denser ($P < 0.05$) and at least five times stronger ($P < 0.05$) than those of rats fed raw grains. Among rats fed tortillas, QPM produced denser, stronger, longer, and thicker bones with more ash and Ca, followed by sorghum and regular corn tortillas. Supplementation of raw grain diets with Ca considerably improved the properties and mineral composition of the femur and serum Ca levels. These changes were not as marked when tortilla-based diets were supplemented with Ca. Serum Ca levels were approximately 30% lower for rats fed raw grains without supplemented Ca. Ca supplementation of QPM and sorghum tortillas had a marginal effect on serum Ca levels. Hypocalcemia was related to low serum albumin levels. Rats fed Ca-supplemented QPM products had the highest serum albumin levels ($P < 0.05$), probably because of the improved dietary protein quality.

The lime-cooking process for production of tortillas considerably upgrades its calcium content (Serna-Saldivar et al 1990, 1991). This source of Ca is extremely important for people who rely on tortillas as their staple food. In a preceding experiment, we determined that the bioavailability of Ca in tortillas prepared from regular corn, quality protein maize (QPM), and sorghum was high, as estimated by growth and Ca balance studies (Serna-Saldivar et al 1991). Rats fed tortillas grew better and absorbed and retained more Ca than their counterparts fed raw grains.

Effects of dietary Ca levels and/or availability is effectively studied by the evaluation of bone properties in rats, especially bone mass and breaking strength. This is because rats are subject to osteoporosis (i.e., loss of bone mass) and changes in the skeleton (i.e., cortical thinning, loss of minerals and organic matrix) that are analogous to those observed in humans (Mnakwe and Kies 1985).

The maintenance of proper Ca and phosphorus homeostasis is of critical importance to the proper functioning of organisms because these minerals are involved in a wide spectrum of biological processes. Plasma Ca regulation is the result of three vectors: net Ca input from the gastrointestinal tract, net Ca loss in urine, and the net Ca deposited in or removed from bone (Sammon et al 1970). In both normal and parathyroidectomized rats, bone exerts by far the major control of plasma Ca levels. Besides being the predominant mineral in bone, Ca has important roles in muscle contraction, blood clotting, and regulation of enzyme activities and acts as a second messenger in many responses mediated by c-adenosine 5′-monophosphate. The plasma concentration of Ca is stringently controlled, whereas P levels fluctuate in response to endocrine signals, which have the main objective of manipulating plasma Ca concentration.

The purpose of this experiment was to determine the availability of Ca in tortillas from regular corn, QPM, and sorghum via study of the changes in properties of femurs and plasma of weanling rats.

MATERIALS AND METHODS

Treatments and Feeding Experiment

Eighty four weanling rats were fed (for eight weeks) diets based on raw grains (regular corn, QPM, or sorghum) or their corresponding tortillas supplemented with the same amount of pinto beans. Each type of product (raw grain or tortilla) was supplemented with a Ca-free or Ca-rich mineral premix. Thus, the experiment consisted of 12 experimental treatments (three × two × two; grains, processing, and Ca supplementation). Details of diet formulation and composition and feeding protocols were described earlier (Serna-Saldivar et al 1991).

Blood Sampling and Femur Removal

One block of rats for seven consecutive days (12 rats per day, one of each treatment) were successively anesthetized via intramuscular injection in the left hind limb with rompun (0.064 ml/100 g of weight) and ketamine (0.085 ml/100 g of weight). Rats were immediately returned to their cages for approximately 15 min. Then, 5–7 ml of blood was withdrawn from the anesthetized rat via intracardiac puncture.

Immediately after blood sampling, rats were terminated via intracardiac injection of an overdose of barbiturates. Then the right and left femurs were surgically removed with a scalpel by first eliminating adjacent muscle followed by a careful ligament rupture and subsequent bone detachment from both the acetabulum in the ventral pelvis and from the knee (tibia-patella-femur) joint. The muscle tissue still attached to the femur was removed with scalpel blades. Then cleaned, fresh femurs were measured in length and thickness at the medial part and placed in plastic bags. Femur density was determined in left and right fresh femurs by weighing the specimens and determining their volume using a multipycnometer (model MVP-1, Quantachrome Corp., Syosset, NY). The density of the dry left femur was determined using the same procedure. Bone moisture content was determined after weighing femurs subjected to drying at 65°C for 48 hr and at 100°C for 24 more hr.

The load (in newtons) required to break the right fresh and left dry femurs and the corresponding plastic strains (mm/mm) were determined with an Instron series IX Automated Materials Testing System linked to a personal computer (using Instron series IX software, version 4.01). Femurs were placed on top of two separated metal sheets joined by an adjustable metal bridge. The separation between support sheets was 2.10 cm, so approximately 30% of the total bone length (15% from each bone end) rested on the support sheets. All tests were conducted under the following conditions: static load cell 1 kN, operated in a compression mode with a drill bit attachment; crosshead speed 5.0 mm/min, operated to stop at bone auto break. The load and plastic strain were calculated by the computer software as follows: load at auto break L (N) = peak force; plastic strain at break = distance (mm) divided by gauge length (mm). The first term gave the force necessary to break the bone; the second term was an indication of the bone bendability.

The dried right and left femurs were incinerated at 500°C for 6 hr to calculate percent ash and organic matter (100 − percent ash). Then ash contents were solubilized with nitric and perchloric

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acids on a hot plate. Ash solutions were diluted to 100 ml with deionized water and further analyzed for Ca, P, and Mg. Ca and Mg were quantified by atomic absorption spectrometry (Sandel 1950). For Ca analysis, an aliquot of the ash solution was diluted with 2% lanthanum chloride solution. P was estimated after reaction with molybdate and 1-amino-2-naphthol-4-sulfonic acid and quantified with a spectrophotometer (Fiske and Subarrow 1925).

Blood was centrifuged at 3,000 rpm for 15 min in a refrigerated (−5° C) centrifuge. Serum was removed and kept in frozen storage until needed. Serum analyses included Ca, inorganic P, and albumin, as suggested by Fiske and Subarrow (1925), Sandel (1950), and Kodak (1986). The serum aliquot used for inorganic P was previously treated with tricarboxylic acid.

Statistical Analysis

Data was statistically analyzed as a complete randomized block design. Treatment sum of squares were partitioned following a trifactorial design, in which factor A (three levels) was the type of grain; factor B (two levels), type of product (raw grain or tortilla); and factor C (two levels), Ca supplementation. Duncan's tests were used to determine statistical differences ($P < 0.05$) among means of the 12 experimental treatments and among levels within factors. The general linear model procedure of SAS (1979) was used for all analyses.

RESULTS AND DISCUSSION

Femur Composition

The low dietary Ca intake in rats fed raw grain diets without supplemented Ca caused poor weight gains (Serna-Saldivar et al 1991) and produced femurs that were lighter ($P < 0.05$) with higher moisture ($P < 0.05$) than femurs of rats fed unsupplemented tortillas (Table I). Rats fed the Ca-supplemented QPM diets had the heaviest, longest, and thickest femurs (most likely because of the higher feed and Ca intake), more apparent Ca absorbed and retained, and better dietary protein quality (Serna-Saldivar et al 1991). Femur length, density, and thickness were lowest for rats fed raw grains, followed by rats fed tortillas and rats given Ca-supplemented treatments (Table I).

Bone density values were directly related to percent ash and inversely related to organic matter content. Femurs of rats fed raw grains contained the highest percentage of organic matter ($P < 0.05$) and the lowest ash content ($P < 0.05$), followed by bones of rats fed unsupplemented tortillas (Table I). Rats fed Ca-supplemented raw grain and tortilla diets had bones with the lowest organic matter ($P < 0.05$) and highest ash content ($P < 0.05$). This was especially true for the raw and tortilla QPM Ca-supplemented diets. Femurs of rats fed raw grains contained one half and one fourth of the ash femur content of rats fed tortillas and Ca-supplemented diets, respectively.

Interestingly, the femur ash of rats fed raw grain diets without supplemented Ca contained 4% less total Ca and 1.5–2% and 0.1% more total P and Mg, respectively, than the femur ash of rats fed the Ca-supplemented diet (Table II). There was no difference ($P > 0.05$) in bone Ca and P composition when the various types of grains were compared. Values for Ca and P in bone ash and the Ca-P ratio in rats fed Ca-supplemented diets are similar to the ones reported by Anderson and Draper (1972) for adult rats. Rats fed tortillas had bones with a mineral composition similar to that of rats fed the Ca-supplemented diets except for those fed the regular corn tortillas, which contained bones with intermediate Ca, P and Mg values.

The lower bone ash; Ca, P, and Mg weight; and bone density in rats fed raw grains was clearly related to the bone breaking strength (Table II). Femurs of these rats required approximately one 10th the force required to break the bones of rats fed the Ca-supplemented diets. Bones of rats fed regular corn, QPM, and sorghum tortillas required four, six, and seven times more breaking force, respectively, than did bones of rats fed the corresponding raw grains, indicating that the Ca present in tortillas reduced symptoms of hypocalcemia. Among tortilla diets without

TABLE I
Effect of Tortilla Processing and Calcium Supplementation on Rat Femur Measurements[a]

Diet[b]	Moisture Content (%)	Weight (mg)	Length (cm)	Thickness (mm)	Density		Organic Matter (%)	Ash Weight (mg)
					Fresh (g/cm^3)	Dry (g/cm^3)		
Treatments								
RC								
G −	52.4 a	243 d	2.88 f	2.43 e	1.23 e	2.32 bc	69.2 a	97 d
G +	36.7 e	377 bc	3.08 b	2.99 ab	1.72 a	3.38 a–c	43.5 d	277 a
T −	44.1 b	340 cd	2.97 d–f	2.71 c	1.50 cd	3.81 a	53.0 b	159 c
T +	36.4 e	411 bc	3.07 bc	2.95 ab	1.59 a–c	3.22 a–c	42.5 d	274 a
QPM								
G −	52.1 a	247 d	2.91 ef	2.45 de	1.29 e	2.26 c	68.9 a	81 d
G +	35.8 e	532 a	3.20 a	3.06 a	1.62 a–c	2.58 bc	44.0 d	300 a
T −	39.5 cd	432 a–c	3.12 ab	2.89 b	1.57 b–d	2.38 bc	49.3 bc	220 b
T +	35.9 e	516 a	3.13 ab	2.96 ab	1.67 ab	3.12 a–c	42.3 d	293 a
SOR								
G −	53.2 a	273 d	2.92 ef	2.57 d	1.27 e	2.34 bc	67.9 a	84 d
G +	36.5 e	456 ab	3.13 ab	2.95 ab	1.62 a–c	3.07 a–c	44.4 d	280 a
T −	41.3 c	380 bc	3.04 b–d	2.85 b	1.48 d	2.96 a–c	51.6 b	181 c
T +	37.5 de	441 a–c	2.99 c–e	2.86 b	1.62 a–c	3.93 a	44.2 d	248 b
Grains								
RC	42.4 a	343 b	3.00 b	2.77 b	1.51 a	3.24 a	47.9 a	201 b
QPM	40.8 b	432 a	3.09 a	2.84 a	1.53 a	2.60 b	48.9 a	223 a
SOR	42.1 a	388 ab	3.02 b	2.81 ab	1.49 a	3.10 a	47.7 a	196 b
Products								
Grain	44.3 a	355 b	3.01 b	2.74 b	1.46 b	2.71 a	43.7 b	184 b
Tortilla	39.1 b	422 a	3.06 a	2.88 a	1.57 a	3.24 a	52.9 a	231 a
Calcium supplementation								
−	47.3 a	318 b	2.98 b	2.65 b	1.38 b	2.70 b	39.5 b	135 b
+	36.5 b	453 a	3.10 a	2.95 a	1.64 a	3.21 a	56.4 a	276 a

[a] Means with different letters within columns are statistically different at the $P = 0.05$ level.
[b] RC = regular corn, QPM = quality protein maize, SOR = sorghum, G = grain, T = tortilla, + = calcium-rich supplement, − = calcium-free mineral supplement.

TABLE II
Effect of Tortilla Processing and Calcium Supplementation on Rat Femur Strength and Mineral Contents[a]

Diet[b]	Mineral Content (mg/dry femur)			Mineral Composition (% total ash)			Load (N)		Plastic Strain
	Ca	P	Mg	Ca	P	Mg	Fresh	Dry	(mm/mm)
Treatments									
RC									
G −	30	20	0.7	31.4 c	20.1 ab	0.76 a	8.0 f	13.8 f	0.43 ab
G +	100	54	1.7	36.0 a	19.4 bc	0.63 ef	89.3 ab	67.4 bc	0.05 c
T −	53	31	1.0	33.1 b	19.4 bc	0.66 de	33.4 e	52.8 d	0.18 c
T +	99	53	1.8	35.7 a	19.2 c	0.64 de	85.1 bc	75.7 ab	0.09 c
QPM									
G −	25	16	0.6	31.1 c	20.0 ab	0.69 bc	11.0 f	19.1 ef	0.36 b
G +	106	57	1.8	35.2 a	19.1 c	0.61 f	106.0 a	81.4 a	0.08 c
T −	77	43	1.4	34.8 a	19.5 bc	0.64 de	69.8 cd	74.3 a–c	0.07 c
T +	102	56	1.8	34.9 a	19.2 c	0.63 ef	83.7 bc	79.4 a	0.05 c
SOR									
G −	27	63	0.6	32.0 bc	20.3 a	0.71 b	7.9 f	24.3 e	0.54 a
G +	98	54	1.8	35.1 a	19.4 bc	0.64 de	98.7 ab	80.7 a	0.05 c
T −	65	35	1.2	35.8 a	19.6 a–c	0.67 cd	59.4 d	65.0 c	0.06 c
T +	89	48	1.6	35.7 a	19.2 c	0.56 g	80.3 bc	72.9 a–c	0.05 c
Grains									
RC	68	39	1.3	34.0 a	19.5 a	0.67 a	57.5 b	53.9 b	0.17 a
QPM	76	43	1.4	34.0 a	19.4 a	0.64 b	67.6 a	63.5 a	0.14 a
SOR	68	38	1.3	34.6 a	19.6 a	0.67 a	61.6 ab	60.7 a	0.18 a
Products									
Grain	62	36	1.3	33.5 b	19.7 a	0.68 a	56.1 b	49.3 b	0.23 a
Tortilla	64	45	1.5	35.0 a	19.4 a	0.65 b	68.6 a	70.2 a	0.08 b
Calcium supplementation									
−	44	27	0.9	32.9 b	19.8 a	0.69 a	31.8 b	41.0 b	0.27 a
+	98	53	1.8	35.4 a	19.2 b	0.64 b	90.1 a	76.3 a	0.06 b

[a]Means with different letters within columns are statistically different at the $P = 0.05$ level.
[b]RC = regular corn, QPM = quality protein maize, SOR = sorghum, G = grain, T = tortilla, + = calcium-rich supplement, − = calcium-free mineral supplement.

TABLE III
Effect of Tortilla Processing and Calcium Supplementation on Serum Measurements[a]

Diet[b]	Ca (mg/dl)	Inorganic P (mg/dl)	Ca-P Ratio	Albumin (g/dl)
Treatments				
RC				
G −	5.80 e	12.6 ab	0.46	2.66 c
G +	9.19 a–c	11.6 a–c	0.79	2.74 a–c
T −	8.82 bc	11.5 a–c	0.77	2.77 ab
T +	9.26 a–c	10.8 bc	0.86	2.86 a–c
QPM				
G −	6.54 de	13.1 a	0.50	2.64 b
G +	9.42 a–c	10.1 c	0.93	2.93 ab
T −	8.76 c	10.6 bc	0.83	2.96 a
T +	9.83 a	10.6 bc	0.93	2.93 ab
SOR				
G −	6.82 d	10.8 bc	0.63	2.71 bc
G +	9.78 ab	11.0 a–c	0.89	2.84 a–c
T −	9.23 a–c	11.2 a–c	0.82	2.84 a–c
T +	9.51 a–c	11.4 a–c	0.83	2.83 a–c
Grains				
RC	8.26 b	11.5 a	0.72	2.75 b
QPM	8.63 ab	11.1 a	0.78	2.86 a
SOR	8.91 a	11.0 a	0.81	2.81 ab
Products				
Grain	7.98 b	11.5 a	0.69	2.76 b
Tortilla	9.23 a	10.9 a	0.85	2.85 a
Calcium supplementation				
−	7.64 b	11.6 a	0.66	2.75 b
+	9.49 a	10.9 a	0.87	2.87 a

[a]Means with different letters within columns are statistically different at the $P = 0.05$ level.
[b]RC = regular corn, QPM = quality protein maize, SOR = sorghum, G = grain, T = tortilla, + = calcium-rich supplement, − = calcium-free mineral supplement.

Ca supplement, the regular corn tortilla produced the weakest bones, followed by sorghum and QPM. This difference is related to the lower Ca content of regular corn tortillas, which produced bones with poorer ash and Ca contents, and to the higher intake of feed and Ca observed in rats fed QPM tortillas (Serna-Saldivar et al 1991).

Interestingly, the fresh bone plastic strain, which is an index of the bone bendability, was highest ($P < 0.05$) for femurs of rats fed unsupplemented raw grains, followed by femurs of rats fed regular corn tortillas (Table II). More bendable bones, as occur in osteomalacia (lack of bone mineralization), had a characteristic higher moisture and organic matter content, with correspondingly lower ash and Ca contents. The break force and plastic strain indexes were closely related to the other factors used to study Ca bioavailability. Dry bones required less breaking force than the fresh counterparts and had negligible plastic strain values. For testing purposes, we recommend working with fresh bones, because break force values and densities are more discriminatory and reliable and testing for plastic strain or bone bendability is possible.

Serum Composition

Serum of rats fed raw grain diets without supplemented Ca contained less Ca ($P < 0.05$) and more P than serum of rats fed tortillas (Table III). A low plasma Ca level is considered to be a clear sign of hypocalcemia because serum levels are stringently controlled in mammals. Low serum Ca values are difficult to use in determining Ca status because mammalian systems mobilize bone Ca (99% of total Ca) to maintain intracellular and extracellular Ca homestasis (1% of total Ca). Serum Ca is maintained at a relatively constant level and is regulated by a hormonally induced mechanism involving parathyroid hormone, calcitonin, and 1α,25-dihydroxycholecalciferol (vitamin D-3). In spite of these mechanisms, plasma Ca levels were suboptimal in rats fed raw grains without supplemented Ca. These values are similar to the ones reported by Sammon et al (1970) in hypocalcemic rats that consumed diets containing 0.05% Ca. Rats fed tortillas had better bone properties and plasma Ca levels because they adapted to low dietary Ca by almost doubling the percentage of Ca absorption (Serna-Saldivar et al 1991).

There was no apparent difference in serum Ca and P values when the Ca-supplemented raw grain and tortilla diets were compared. The ratio of serum Ca and inorganic P for rats fed raw grains unsupplemented with Ca was considerably lower than ratios obtained for rats fed unsupplemented tortillas. Supplementation of tortillas with the Ca-rich mineral mix slightly improved the ratio (Table III).

Hypocalcemia was evident in animals fed unsupplemented raw grains, especially during the last two weeks of the experiment. One of the rats showed symptoms of tetany, another had a broken-recalcified femur. In addition, most of these animals had watery eyes, consumed little food, and drank more water.

CONCLUSIONS

Results of this study complement those obtained in a preceding experiment (Serna-Saldivar et al 1991), which confirmed that lime cooking for tortilla preparation considerably increases dietary Ca and that such Ca is highly bioavailable, as estimated with Ca balance and growth studies. In the current study, rats fed tortillas had better bone properties (density, ash, Ca, breaking strength, etc.) and higher serum Ca than rats fed raw grains. Rats fed QPM tortillas had better and stronger femurs than rats fed sorghum or regular corn tortillas.

ACKNOWLEDGMENTS

We wish to thank R. Young and G. Guzman for assistance with the operation of the Instron; H. Almeida for help in statistical analyses; and R. Bressani, INCAP, and K. Kubena and R. Waniska, Texas A&M University, for critically reviewing the manuscript. This research was supported in part by Grant AID/DSAN/1254 G-TS-5065 from the Agency for International Development, Washington, DC.

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[Received April 18, 1991. Accepted August 12, 1991.]