Dialyzable Calcium and Phosphorus in Mexican Diets High in Insoluble Fiber

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Adequate calcium (Ca) intake is important to prevent osteoporosis. Beans, corn, and flour tortillas are the principal energy sources in the typical Mexican diet and Ca comes primarily from corn tortillas. Total dietary fiber intake is about 45 g/d. Intake of dairy products is low and only occasional. The objective of this study was to determine by in vitro methods, the percent dialyzable Ca and P in typical Mexican diets. Tortillas made from a mixture of lime-treated corn and a commercially prepared corn flour had 162.1 mg of Ca/100 g, dry weight basis, compared to tortillas made with corn flour only with a value of 106.2 mg/100 g. Phosphorus (P) content was 119.2 and 101.8 mg/100 g, respectively. The diet formulated with corn tortillas as the only source of Ca had 6% dialyzable Ca and 3% dialyzable P. The diet containing a mixture of animal and vegetable products showed significant ($\alpha = 0.05$) higher Ca dialyzability, 8%. P dialyzability in this diet was 4%. The results indicate that Ca in the typical Mexican diet is not very available.

Keywords: *Dialyzable calcium; dialyzable phosphorus; diets high in fiber*

INTRODUCTION

A large proportion of the world's population, especially in developing countries, consume diets consisting primarily of plant foods. Corn tortillas and beans are the primary sources of energy and protein in the typical Mexican diet. Inclusion of animal products is occasional and highly variable, but as income improves more animal protein products are included in the diet, as well as fresh fruits and vegetables (Wyatt et al., 1995).

An adequate intake of (Ca) throughout life is important for bone homeostasis and the prevention of several chronic diseases. Osteoporosis is responsible for hip and wrist fractures and vertebral compression's with serious health outcomes (Van Dokkum et al., 1996). An adequate intake of Ca-rich foods is the natural way to meet Ca requirements. Studies have shown that levels of Ca in the Mexican diet are adequate (1100 mg/d) (Wyatt et al., 1995). In other countries dairy foods are the main providers of Ca in the diet but not in Mexico, where corn tortillas are the principal source. CaO is used in the lime treatment process of the corn kernels to produce tortillas and this treatment increases the Ca content 15-fold (Serna-Saldivar, 1991). The important question is, to what extent is this Ca absorbed? Major determinants of Ca absorbability are the amount of free ionic Ca, soluble Ca complexes in the intestine, gastric pH, and competition for absorption sites by other minerals. Studies have shown diets high in fiber, oxalates, and phytates have low availability of minerals primarily due to the formation of insoluble complexes (Sandstrom et al., 1993). In published studies from this center, it was shown that the typical regional diet is very high in phytates but surprisingly, over 80% of the Ca was found to be in the soluble fraction by an in vitro method simulating gastric absorption (Wyatt and Triana, 1994). Graf in 1983 reported that certain Ca−phytate complexes are soluble. The ideal ratio of Ca:P in the diet is 1:1 or 2:1 for optimum conditions of Ca absorption. Ca availability can be affected by interactions with P in the diet, when the latter is present in the form of phytates (Allen, 1982). Animal studies have shown deleterious effects of high P to Ca ratios on Ca absorption, but for humans the data is not so clear (Spencer et al., 1988). Therefore, the objective of this study was to determine by in vitro methods, percentage dialyzable Ca and P in typical Mexican diets where the Ca was primarily from vegetable sources and high P content, simulating gastrointestinal conditions.

MATERIALS AND METHODS

Diets. Two typical diets were prepared, one where the Ca was solely from corn tortillas, which was referred to as the vegetable diet and the other a mixture of corn tortillas and dairy products, referred to as the mixed diet. The basic composition of these diets was determined in an earlier study (Wyatt et al., 1995) and modified slightly to meet the objectives of this study. All ingredients were weighed and mixed using a blender (Waring Products Corporation of America, New Hartford, CT). Food items were prepared according to established traditional recipes and then dried at 40 °C in a convection oven (Blue M. Blue Island, IL). Ca:P ratio of 1:1.5 was established on the basis of analysis of the traditional diet and quantities of corn tortillas and dairy products were adjusted to maintain this relation. Two control diets were included in the study, and these were purchased (Ralston Purina Mills, St. Louis, Mo). In both control diets, Ca existed in the form of $Ca(H_2PO_4)_2$ and the control test diet had a Ca:P ratio of 1:1.5. Additional P was added in the form of NaH_2PO_4 to meet this ratio.

Proximate Analysis. Proximate analysis of the diets, including, moisture, ash, protein, and fat content, were determined according to AOAC methods (AOAC, 1990, Sec 220.1; 923.03; 960.52; and 920.39, respectively).

Ca and P determination. The diets (0.5 g) were digested using a microwave oven (CEM Model 2000, Falcon Instrument, Mexico) with 10 mL of concentrated nitric acid (65%, Merck of Mexico), following the digestion procedure recommended by

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CEM (1991). After the samples were cooled on ice, they were brought to 25 mL volume with deionized water (Barnstead, Water Technology, Hermosillo, Mexico) and then an aliquot was taken for mineral determinations.

Ca was determined by atomic absorption method (AOAC, 1990, Sec 3,045), using 5% La_2O_3 (Sigma Chemical Co., St. Louis, MO) (w/v) to eliminate interferences in the air acetylene flame. Calibration curves were prepared using standard mineral solutions (Sigma Chemical Co., St. Louis, MO).

P was determined by the AOAC 1990 method (Sec 931.01) using a UV−vis Spectrophotometer (Perkin-Elmer, Junior Model 35, Oak Brook, IL).

Ca and P determination was validated using the certified standard, SRM, NBS bovine liver 1577b (Gaithersburg, MD). The certified standard was digested in the same manner as described as above for the diets. The certified values for Ca and P were 116.00 ± 4.0 μg/g and 1.10 ± 0.03 g/100 g, respectively. The values obtained in this study were 115.44 ± 1.33 μg of Ca/g and 1.10 ± 0.02 g of P/100 g with 1% CV. The percent recovery was 99.7% for Ca and 99.9% for P.

Dietary Fiber Determination. Soluble, insoluble, and total dietary fiber were determined using fat free dried samples according to the the AOAC method (section 985.29, 1990) utilizing dietary fiber kits (TD-FAB-1, Sigma Chemical Co., St. Louis, MO) and Tecator filtering and incubation equipment (Fibertec System E 1023, Tecator, Sweden). The method was validated using a Hard Red Wheat Bran Certified Standard (AACC, St. Paul, MN).

In Vitro Estimation of Availability. The amount of dialyzed Ca and P were determined in the experimental and control diets using the in vitro gastrointestinal digestion method of Shen et al. (1995). Digestive enzymes and bile salts were purchased from Sigma Chemical Co., St. Louis, MO, and deionized water (Barnsted, Water Technology, Hermosillo, Mexico) was used throughout the study. All glassware was soaked overnight in 10% (v/v) nitric acid (Merck of Mexico) and thoroughly rinsed with deionized water prior to use. The dialysis tubing (Spectra Por, 15 000 MW, 45 mm × 28.6 mm, VWR, South Plainfield, NJ), was treated to remove any trace mineral impurites. The samples were dissolved in deionized water, pH adjusted to 2 and then incubated at 37 °C for 2 h with the enzyme solution. To an aliquot of this digest, the pancreatic enymes were added, pH adjusted to 7.5 and then subsequently dialyzed against the membrane at 37 °C for 30 min. Once the dialysate was obtained, Ca and P were determined according to previously described methods. The percentage of dialyzability was calculated using the formula of Shen et al. (1995). The method was validated by using whole cow's milk and comparing the value obtained in this study with that reported by Shen et al. (1995).

Statistical Analysis. Data were statistically analyzed using one-way ANOVA analysis (NCSS, 1996).

RESULTS AND DISCUSSION

Table 1 presents the food items and quantity (g) of the two experimental diets used in this study. The quantity of corn tortillas was adjusted and dairy and egg products were completely eliminated in the vegetable diet, so that the primary source of Ca was from corn tortillas. The traditional Mexican diet in the northern part of Mexico, mimics the diet of industrially developed countries with its high fat content; however, it differs greatly in its dietary fiber content.The diet is characterized as being high in fat and dietary fiber. The total dietary fiber intake is about 45 g/d and the three basic food items, beans, corn, and flour tortillas constitute 68% of the total dietary fiber intake with a daily per capita consumption of 217, 128, and 180 g, respectively. The distribution of energy is 50% from carbohydrates, 14% from protein, and 36% from fat. The intake of dairy products is low and only occasional. The two dairy products that do appear in the traditional diet (that were eliminated from the experimental vegetable diet for the purpose of this study) are milk and cheese. Average milk intake is 228 g, slightly less than one glass a day and 40 g of cheese, approximately 1.5 oz, not meeting the recommended two servings of dairy products per day (USDA,1985).

Several commercial varieties of corn tortillas were analyzed. The most common formulation for corn tortillas in Mexico nowadays is a combination of lime-treated corn kernels and a commercially prepared corn flour. The tortillas used in this study had 162.1 mg of Ca/100 g and 119.2 mg of P/100, on a dry weight basis (151.7 mg of Ca/100 g and 111.6 mg of P/100 g on a wet wt basis). The Ca:P ratio was 1.4:1. An earlier study from this center reported Ca values in tortillas ranging from 111 to 201 mg/100 g (Wyatt and Triana, 1994). Weber et al. (1993) also reported a wide range of Ca and P values in commercial tortillas ranging from 112 to 391 mg of Ca/100 g and 38 to 127 mg P/100 g, on a wet weight basis. Obviously the lack of quality control in the lime treatment of the corn kernels is responsible for these wide ranges. Tortillas that are made entirely from the commercial corn flour, usually have lower Ca values.

Table 2 presents the proximate analysis of the experimental and control diets in this study. The control diets were identical except the P content was elevated in one to have a ratio of 1:1.5 to meet the criteria of this study. The impact of this will be discussed latter. The vegetable and mixed diets varied in their composition with the mixed diet having a higher protein and fat content, due to the inclusion of animal products in this diet.

Figure 1 presents the total, soluble, and insoluble dietary fiber in the experimental and control diets. The source of fiber in the control diets was cellulose which

Table 1. Food Components of the Experimental Diets

food item	mixed diet (g)	vegetable diet (g)
eggs, fried	69	
beans, refried	70	218
tortillas, flour	52	132
tortillas, corn	82	180
potatoes, fried	47	98
rice, steamed	111	112
meat, fried	104	60
milk, whole	376	
beef, dried	43	
chili, green	5	10
onions	27	25
tomatoes	45	68
lettuce	42	
carrots	19	
celery	5	
avocado	92	
oranges	100	
soda	270	380
sugar	23	23
beer	710	
bread, white	74	
oil, vegetable	19	10
coffee	97	397

Table 2. Proximate Analysis of the Experimental and Control Diets

diet	moisture (g/100 g)	protein (g/100 g)	fat (g/100 g)	ash (g/100 g)	carbohydrate (g/100 g)
control	7.23	18.40	5.00	4.37	65.00
Ca:P 1:1.5	6.54	18.40	5.00	6.76	63.30
vegetable	5.56	16.06	15.26	5.23	57.89
mixed	9.29	22.53	19.44	5.15	43.59

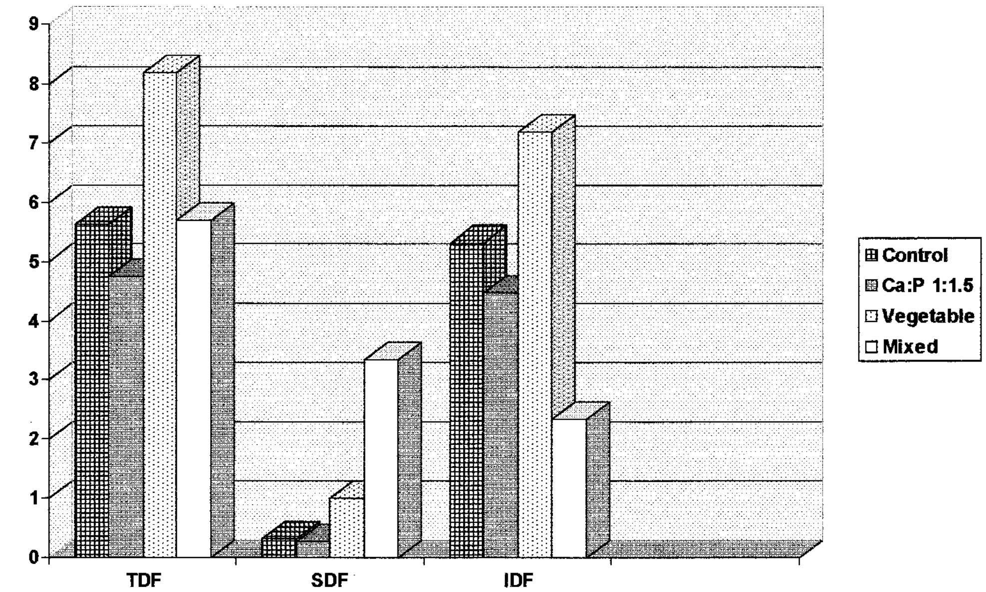

Figure 1. Total (TDF), soluble (SDF), and insoluble (IDF) dietary fiber in experimental and control diets (grams per 100 g, dry weight basis).

Figure 2. Total, dialyzable, and nondialyzable Ca in experimental and control diets (milligrams per 100 g, dry weight).

is almost completely insoluble. The vegetable diet had 8.20 g per 100 g of total dietary fiber compared to 5.70 g per 100 g for the mixed diet. Ninety percent of the fiber was insoluble in the vegetable diet. The mixed diet was lower in both total and insoluble fiber due to its higher content of animal products and other sources of soluble fiber such as, oranges (Hughes, 1991). High fiber intake is traditionally reported in diets from developing countries where the energy sources are primarily from cereal grains and legumes (Nolan et al., 1987; Ferguson et al., 1988). Wyatt et al. (1996) reported that in the traditional diet, 72% of the total dietary fiber was insoluble.

Figure 2 presents total, dialyzable, and nondialyzable Ca in the experimental and control diets. Approximately 70% of the Ca was nondialyzable in the control diets. This is surprising since the form of Ca in the diets was in the form of $Ca(H_2PO_4)_2$, which was assummed to be highly soluble and thus highly absorbable. Garcia-Lopez and Miller (1991) showed that the source of Ca has a significant effect on bioavailability in rats more so when diets are insufficient in Ca than when Ca is adequate. For low levels of Ca they noticed differences in the amount of Ca retained in rats. $Ca(H_2PO_4)_2$ had a lower retention than other Ca sources such as citrate or carbonate; however, no differences were noted in percentage of Ca in the femur of rats. Ninety-four percent of the Ca in the vegetable diet and 92% in the mixed diet was nondialyzable. Total Ca for both of the experimental diets were equal, although milk was the primary Ca provider in the mixed diet and corn tortillas in the vegetable diet. Dialyzability of individual ingredients was not the subject of this study, as the interactions in a complex diet system were felt to be of greater

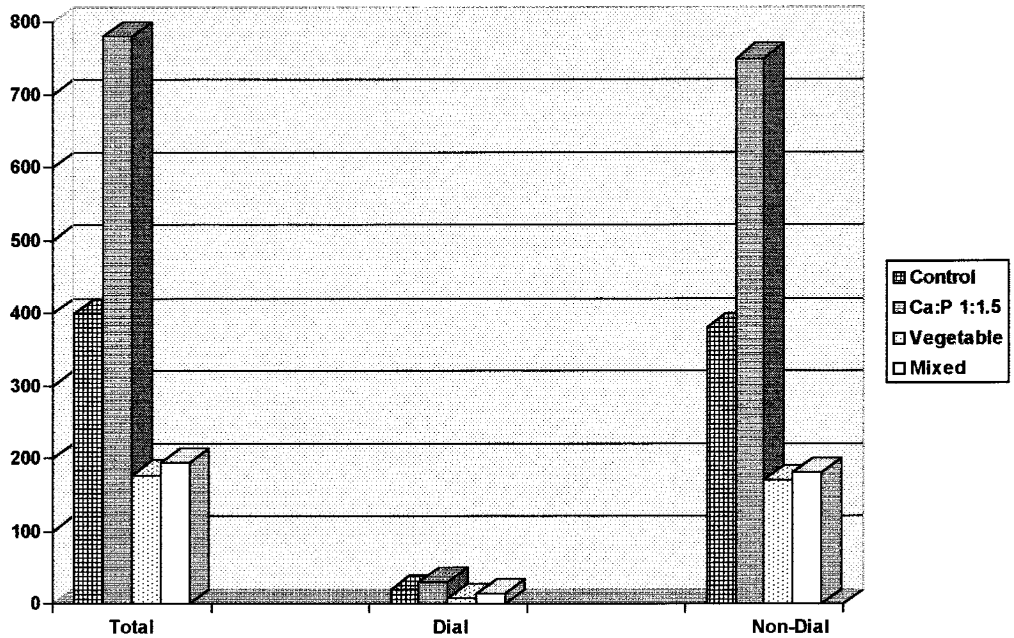

Figure 3. Total, dialyzable, and nondialyzable P in the experimental and control diets (milligrams per 100 g, dry weight).

importance. However, the in vitro system was validated with whole cow's milk, obtaining a value of 26% by itself, comparable to that reported by Shen et al. (1995). The solublity of the Ca in the lime treatment depends on the temperature of the cooking process. The solubility of lime in water decreases with the increase in temperature. Since cooking temperatures of corn may vary from 85 to 100 °C, this could affect calcium solubility (Serna-Saldivar et al., 1990). A higher dialyzability of Ca in the mixed diet was expected, but it appears that the amount of milk used in the formulation was insufficient to note a difference.

Reykdal and Lee (1993) reported that ionic dialyzed calcium is the best chemical test for bioavailability and they reported higher dialyzed Ca percentage values than ionic dialyzed Ca in cottage cheese. The data reported in this study for percent dialyzed Ca is comparable to the data in the Reykdal and Lee (1993) study. They also reported a high percentage of soluble Ca in cottage cheese although the percentage of soluble Ca is not a good indicator of availability. Earlier studies from this center (Wyatt and Triana, 1994) reported high solublility of Ca in corn tortillas and regional diets, as determined by an in vitro method simulating gastric conditions.

The corn tortilla supplies approximately 22% of the total RDA for Ca and 19% of the RDA for P (NAS, 1989). In animal studies, when corn tortillas were fed alone, the Ca was available; however these data are somewhat difficult to interpret because as a sole source of Ca in the diet, tortillas are very deficient and in most studies the tortillas were fortified with a Ca supplement (Braham and Bressani, 1966; Poneros and Erdman, 1988). Serna-Saldivar et al. (1991) showed a low intake of Ca from tortillas, only 9.6 mg/d, very much below the requirements for rats, but 86% of it was absorbed. Rosado et al. (1992) in a balance study with women showed that in a rural mexican diet, which would be comparable to the vegetable diet in this study, had a content of 745 mg Ca/d, almost meeting the requirement of 800 mg/d. However, the Ca was poorly absorbed and a negative balance was found.

Figure 3 presents the total, dialyzable, and nondialyzable P in the experimental and control diets. Even though the total P content differed greatly between the control and experimental diets, no difference in dialyzability was observed. All four diets had low dialyzability of P. The sources of P also varied. In the control diet, the source is $Ca(H_2PO_4)_2$ and in the Ca:P 1:1.5 control diet the P was elevated by the addition of NaH_2PO_4. In the vegetable diet, probably the major source of P is phytates and in the mixed diet, both animal sources of P like $Ca(H_2PO_4)_2$ and phytate P exist. None of this had any effect on the dialyzability. Only the control diet had a higher Ca content than P, with a ratio of 1.3:1. The experimental diets had a ratio of 1:1.4. Again ratio did not seem to affect dialyzability of Ca or P.

CONCLUSIONS

The typical diet in northern Mexico is high in fat and dietary fiber and the primary energy sources are beans, corn, and flour tortillas. Corn tortillas provide 22% of the RDA of Ca and 19% of the P. This Ca in corn tortillas is added during the lime treatment process. By an in vitro method, simulating gastrointestinal conditions, it was found that only 6% of Ca in a typical diet where the Ca is primarily from vegetable sources was dialyzable, compared to 8% of the Ca dialyzable when animal products were included in the diet. Because of the high phytate content in these diets, P content was also high and only 3% and 4% in the vegetable and mixed diets, respectively, was dialyzable. Recommended Ca:P ratios for maximum Ca absorption, are 1:1 or 2:1. Unfavorable Ca:P ratios were found in the experimental diets of this study. The vegetable diet had a Ca:P ratio of 1:1.4 and the mixed diet 1:1.7. The results of this study indicate that Ca in diets where the Ca source was primarily corn tortillas, was not readily available for absorption, if an in vitro test, is used as the indicator. Additionally, the small amount of milk included in the diet was not sufficient to note an improvement in the dialyzability. This study reinforces the importance of having a diet adequate in Ca and most importantly, a source that is readily available. It would be prudent for most Mexicans to improve their intake of absorbable Ca by attempting to include more dairy products in their diet.

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