

Calcium Absorption from Corn Tortilla Is Relatively High and Is Dependent upon Calcium Content and Liming in Mexican Women

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ABSTRACT Corn tortillas are the staple food of Mexico. During their preparation, calcium is added to the tortillas; therefore, tortillas are the main source of calcium for a large proportion of the population. The bioavailability of calcium from lime-treated tortillas in humans is not known. The objectives of the present study were to determine calcium absorption from corn tortilla, to determine the effect of lime treatment on calcium absorption from corn tortilla, and to compare calcium absorption from tortilla prepared with a commercial corn flour and tortillas prepared with the traditional lime treatment at home. Nonpregnant, nonlactating women ($n = 9$) were administered 3 different treatments: 1) 180 g of corn tortilla prepared from corn flour with no lime treatment (CF), 2) 180 g of corn tortilla prepared from lime-treated commercial corn flour (LTCCF), or 3) 180 g of corn tortillas prepared from lime-treated home-prepared corn flour (LTHCF). Calcium absorption was measured using an established dual-tracer stable isotope technique. Calcium absorption of CF, LTCCF, and LTHCF was (mean \pm SD): 44 ± 3.2 , 32 ± 4.4 , and $30 \pm 2.4\%$, respectively; the fractional calcium absorption from CF differed from that of either LTCCF or LTHCF ($P < 0.01$). The total amount of calcium absorbed per treatment was higher in LTHCF ($98.10 \text{ mg} \pm 21.7$) than in LTCCF ($59.9 \text{ mg} \pm 23.7$, $P < 0.001$) and CF ($3.78 \text{ mg} \pm 0.9$, $P < 0.001$). In conclusion, calcium absorption from corn tortillas is high and dependent on calcium concentration. The addition of calcium during lime treatment increases calcium concentration and total calcium absorption. J. Nutr. 135: 2578–2581, 2005.

KEY WORDS: • calcium absorption • tortillas • lime treatment • stable isotopes

In Mexico, corn tortilla is the most widely consumed staple food. The corn dough used for tortilla preparation is traditionally prepared by boiling corn in a lime solution that contains calcium, and letting it stand overnight. This process is called *nixtamalización* (1). Commercial corn flour is also available for the preparation of tortillas; it is usually lime-treated for only 1 h. Tortilla, either prepared in the traditional method or from commercial corn flour, represents up to 67% of the total energy intake of the Mexican population, with the highest intake in rural areas (1,2).

Studies showed that the amount of calcium absorbed from food sources is determined mainly by other food components, the amount of calcium in the food, and the form of calcium added (3–7). The efficiency of calcium absorption from most vegetable sources is as good as or better than that from dairy foods, unless high concentrations of inhibitors such as oxalic acid, phytic acid, or dietary fiber are present (8–14). In addition, physiological factors such as vitamin D and calcium deficiency, menopause, and age affect the efficiency of calcium absorption (4,15,16).

The nutritional status in the Mexican population for calcium is not known. For a number of years it was speculated that calcium deficiency is not a problem in this population mainly because of the high intake of calcium from lime-treated

tortilla. However, it is not clear how much of the calcium present in tortillas is actually absorbed. Two animal studies found that consuming tortillas with added calcium (as CaO) improved the mineral composition of the femur, increased serum calcium levels, and improved weight gain (17,18). A third study evaluated the physical properties and composition of femurs from rats fed corn tortillas processed differently. The rats fed the lime-treated tortillas with 1 and 0.25% lime solution had heavier, thicker, longer femurs, with the highest calcium content (19). No information on the bioavailability of calcium from lime-treated tortillas in humans is available.

Higher bioavailability of calcium from the diet was shown to improve bone health (20). A diet with a high content of bioavailable calcium may help improve calcium nutritional status and may prevent bone diseases associated with calcium deficiency.

The purposes of this study were to determine calcium absorption from corn tortilla, to determine the effect of lime treatment on calcium absorption from corn tortillas, and to compare calcium absorption from tortillas prepared from commercial corn flour and from those prepared with traditional lime treatment at home.

SUBJECTS AND METHODS

Subjects. Nonpregnant, nonlactating women ($n = 9$) participated in this study. They were healthy volunteers, 26 ± 5 y old, from

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the staff and students of the nutrition department of the National Institute of Medical Sciences and Nutrition in Mexico City (NIMSN).² All participants were informed about the study and they signed consent forms. The project was approved by the Committee of Biomedical Research in Humans of the NIMSN.

Experimental design. The dual-tracer isotope technique was used to measure calcium absorption from 3 different and successive treatments given on different occasions to each individual, so that each individual served as her own control (21). Treatments were administered following a Latin-square design to avoid a potential confounding effect of treatment order. The 3 different treatments evaluated were: 1) 180 g of corn tortilla made from corn flour with no lime treatment (CF), 2) 180 g of corn tortilla made from commercial lime-treated corn flour (LTCCF), and, 3) 180 g of corn tortilla made from lime-treated, home-prepared corn flour (LTHCF). The amount of tortilla (180 g) is equivalent to 6 tortillas. Each tortilla was extrinsically labeled with ⁴⁶Ca as calcium chloride on the previous evening to permit equilibration.

The test meal given to the subjects for breakfast and for lunch included 90 g of tortillas (3 tortillas) (806 kJ), 200 g of mashed potatoes (611 kJ), and 240 mL of apple juice (904 kJ). The total energy intake of the test meal was 2321 kJ. All meals were consumed under supervision to ensure that no food was left over. Tortillas in the different treatments varied only in their calcium concentration (Table 1) (22–25).

After baseline blood and urine samples were taken, the subjects consumed each of the test meal for breakfast and for lunch for 12 d (adaptation period). On d 13, the subjects consumed the test meal for breakfast with the addition of ⁴⁶Ca (0.0178 ± 0.001 mg). Immediately after breakfast, the subjects received an i.v. dose of ⁴²Ca (1.25 ± 0.02 mg) as calcium chloride. Beginning with breakfast, a complete 24-h urine collection was obtained. Four hours after breakfast, the subjects consumed the same test meal for lunch with the addition of the same amount of ⁴⁶Ca, and the subjects were instructed not to eat anything for 4 h after lunch (21).

Calcium absorption measurement. Ca⁴⁶ (4.1% enriched) and Ca⁴² (89.6% enriched) were obtained as the carbonate from Trace Sciences. They were converted to aqueous solutions of the chloride salt and tested for sterility and pyrogenicity before use. Urine and serum samples were prepared with the use of an oxalate precipitation technique to measure calcium absorption from the oral:i.v. tracer ratio by inductively coupled plasma-MS (ThermoFinnigan element 2) in the Nutrition Laboratory of the Children's Nutrition Research Center, Houston, TX. The method was described previously in detail (21). Samples were analyzed for the ratios ⁴²Ca:⁴³Ca and ⁴⁶Ca:⁴³Ca, correcting for fractionation to the reference ⁴⁴Ca:⁴³Ca. Accuracy and precision of this technique are 0.1 and 0.15%, respectively.

Tortilla preparation. Tortillas were hand made on a daily basis during the adaptation period and during the administration of the isotopes, at the Pilot Plant of the Food Science and Technology Department of the NIMSN. The CF tortillas were prepared by boiling 1 kg of corn for 50 min. The corn was then rinsed and drained for milling. The dough was prepared after mixing the milled corn with deionized water until consistency and texture were uniform. Tortillas from the LTCCF were handmade from dough prepared from a commercial corn flour (Minsa). Deionized water was added to the flour in a 1:1.75 ratio to form the dough. The commercial corn flour was prepared previously by the manufacturer by boiling the corn for 50 min in a lime solution, containing a 1% calcium hydroxide solution and left standing for 1 h at room temperature. The corn used to prepare tortillas for the LTHCF treatment was weighed (1 kg) and boiled for 50 min in a 1% calcium hydroxide solution according to the traditional method for the preparation of tortilla in Mexico called *nixtamalización* (1). After 18 h of standing at room temperature, the grain was separated and rinsed twice before milling. Deionized water was then added to the milled corn to prepare the dough. Tortillas in the 3 treatments were prepared by forming small balls of dough that

TABLE 1

Nutrient composition of the 3 different types of tortilla used in the 3 treatments

	CF	LTCCF	LTHCF
	unit/180 g		
Energy, ¹ kJ	1611	1611	1611
Calcium, mg	8.5	187.6	326.8
Fiber, ¹ g	9.9	9.9	9.9
Phytate, ¹ mg	1524	1524	1524

¹ From food composition tables and previous studies (22–25).

were pressed by hand and cooked on a hot surface to obtain tortillas of ~30 g each.

The main difference between the CF and LTHCF tortillas was the addition of the calcium hydroxide solution. The main difference between the LTCCF and the LTHCF tortillas was the time that the corn was left standing in the calcium hydroxide solution after boiling (1 vs. 18 h, respectively).

Biochemical determinations. Because vitamin D deficiency decreases calcium absorption, vitamin D status was determined by measuring 25-hydroxy vitamin D concentration in plasma using a RIA kit (Dia Sorin). The cutoff point to determine vitamin D deficiency was <25 nmol/L (<10 ng/L) (26).

To determine total calcium intake from the 3 treatments, calcium content was measured from a representative sample of each batch of tortilla using atomic absorption spectrophotometry (AAS) (Perkin Elmer Spectrometer, model no. 2380). Total calcium concentration in urine was measured by AAS.

Food intake data collection and analyses. Habitual calcium intake was assessed at the beginning of the study by a 24-h food intake questionnaire covering 3 d, including 2 weekdays and 1 weekend day. Food composition tables of the NIMSN were used to determine the calcium intake per day for each subject, as well as the nutrient composition of the test meals (27).

Data analyses. Statistical analyses were performed using SPSS version 11.0. Continuous variables were examined for conformity to the normal distribution. The differences among the percentage of calcium absorption, calcium intake, and total calcium absorbed from the different treatments were tested for significance with a repeated-measures ANOVA, with a level of significance of 5%. When within-subject effects for different treatments were significant, differences among treatments were explored with pairwise comparison using the least significant difference (LSD) test.

RESULTS

Baseline information of the 9 subjects who participated in the study is shown in Table 2. Mean calcium intake (711 ± 188) was below the recommended intake of 900 mg/d for women between 19 and 30 y of age (28). None of the subjects had 25-hydroxy vitamin D concentration < 25 nmol/L.

Calcium absorption from the 3 tortilla treatments ranged from 24.6 to 51.7%, with mean absorptions of 44 ± 3.2, 32 ± 4.4, and 30 ± 2.4% for the treatments CF, LTCCF and LTHCF, respectively. The absorption from CF was higher than that from either LTCCF or LTHCF ($P < 0.01$).

Figure 1 shows total calcium intake and total amount of calcium absorbed from each treatment. The amount of calcium ingested with the LTHCF (326.8 mg/180 mg of tortillas) was almost 40 times higher than the amount of calcium ingested with the CF (8.5 mg/180 mg of tortillas) ($P < 0.0001$). The difference in calcium content due to the lime treatment resulted in a lower amount of calcium absorbed from CF (3.78 mg/180 mg of tortillas) than from LTHCF (98.10

² Abbreviations used: AAS, atomic absorption spectrophotometry; CF, corn flour with no lime treatment; LTCCF, lime-treated commercial corn flour; LTHCF, lime-treated home-prepared corn flour; NIMSN, National Institute of Medical Sciences and Nutrition in Mexico City.

TABLE 2

Baseline anthropometric measurements, calcium and energy intake, and serum 25-hydroxy vitamin D of the 9 women

Index	
Age, y	26 ± 5.4
Weight, kg	52.4 ± 10
Height, cm	1.6 ± 0.05
BMI, kg/m ²	21.2 ± 2.9
Calcium intake, mg/d	711 ± 188
Energy intake, kJ/d	5395 ± 1463
Serum 25-hydroxy vitamin D, nmol/L	43 ± 9

¹ Values are means ± SD, *n* = 9.

mg/180 mg of tortillas) ($P < 0.001$). Compared with the LTHCF, the amount of calcium in the LTCCF (187.6 mg/180 mg of tortillas) was 43% lower ($P < 0.0001$), and this resulted in a lower amount of calcium absorbed from the LTCCF tortillas (59.9 mg/180 mg of tortillas) ($P < 0.001$).

DISCUSSION

In Mexico, tortillas constitute an important source of energy and other nutrients for a majority of the population. It has been assumed that the addition of calcium from the solution of calcium hydroxide during the lime treatment process (*nixtamalización*), increased the total amount of calcium available for absorption. In the present study, calcium absorption from tortillas was measured using stable isotope tracers, which are the most accurate and precise method for determining calcium absorbability from food sources (29).

The traditional method of lime treatment proved to be an effective way of increasing the amount of calcium available for absorption. Without lime treatment, tortillas are not a good source of calcium as observed in the amount of calcium absorbed in the CF treatment of this study. The amount of calcium present in the food determined the amount of calcium absorbed. Heaney et al. (30) demonstrated that different loads of calcium ranging from 15 to 500 mg are absorbed differently. The lowest loads had an average calcium absorption of 64% and the highest loads of 29%. This agrees with the results in the present study in which each treatment had a different calcium content. The tortillas in the CF treatment had the lowest amount of calcium (8.6 mg/180 g of tortillas) and the highest percentage of calcium absorption (44%), compared with the LTHCF tortillas with the highest amount of calcium (327 mg/180 mg of tortillas) and the lowest percentage of calcium absorbed (30%). The increased calcium load in the lime-treated tortilla led to lower fractional calcium absorption. However, the calcium content of the lime-treated tortillas was sufficiently high to overcome this effect, and significantly more calcium was absorbed from the lime-treated tortillas than from the untreated ones.

The addition of calcium to commercial corn flour also increases calcium content, but not to the extent of the traditional method of preparing tortillas. The main difference between the 2 is the time the grain is left soaking in the lime solution after boiling. Letting the grain soak for at least 12 h as in the traditional method allows for more contact time between the grain and the lime solution, which ultimately results in a higher, readily absorbable concentration of calcium in the tortillas (1).

In addition to calcium content, calcium absorption from

food sources is also determined by food composition. Food components such as oxalate, phytate, fiber, lactose, and protein, affect the efficiency of calcium absorption (3–7,30). In green leafy vegetables, almost all of the calcium is bound to oxalate. Studies showed that the absorption of calcium from food sources such as spinach is very poor because of the high content of oxalic acid (4,11,31). The percentage of calcium absorption from spinach is ~5% (31). Phytic acid is a food component that has the ability to chelate multivalent metal ions, especially zinc, calcium, and iron. The binding results in very insoluble salts, which are poorly absorbed from the gastrointestinal tract (32). However, although calcium phytate is a complex that is not absorbed, phytate is digested to some degree in the lower intestine by bacteria, and this might contribute to calcium bioavailability (4). Even though the amount of phytic acid in the tortillas is high (>1500 mg/180 g of tortilla), the percentage of calcium absorption was high. Among other possibilities, this may be due to a partial digestion of calcium phytate in the lower intestine, which allows the calcium to be free for absorption. In addition, some of the phytate in tortillas could be hydrolyzed during the process of tortilla dough preparation. These results agree with studies using animal models in which lime-treated tortillas proved to be a good source of calcium, despite the phytic acid content (17–19).

According to the results of the present study, lime-treated tortillas are a good source of calcium and thus may be useful to promote bone health among populations subsisting on corn diets. According to the WHO's criteria for osteoporosis, several studies reported that in Latin America, 12–18% of women ≥ 50 y old have vertebral osteoporosis and 8–22% have proximal femur osteoporosis. In Mexico, the prevalence of vertebral fractures in community-dwelling women ≥ 50 y old is 19.35% (33). A study in Mexico determined the prevalence of osteoporosis among women between 50 and 60 y of age to be 19.8% (95% CI: 14.3–25.2%) (34). This prevalence increased with age, reaching 35.29% in the group aged 65–69 y. In general, low calcium intake and low fractional absorption increase the risk of fractures in elderly women. Corn tortilla is a staple food in Mexico and is consumed by a majority of the population. The habitual intake of lime-treated tortillas may contribute to increasing calcium intake among Mexican women, and help to prevent bone loss and osteoporosis.

In conclusion, calcium absorption in corn tortillas is high and depend on calcium concentration. The addition of calcium during lime treatment increases calcium concentration

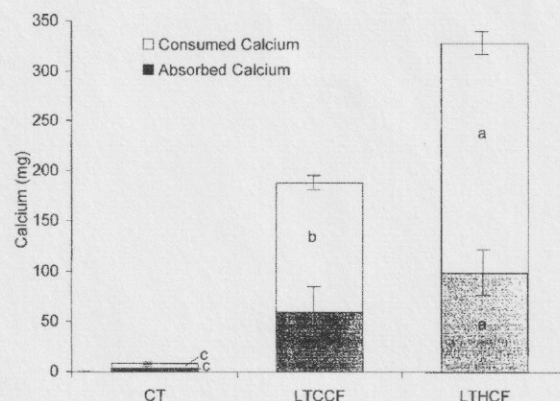


FIGURE 1 Total calcium intake and total calcium absorbed by women consuming CF, LTCCF, and LTHCF. Values are means ± SD, *n* = 9. Means without a common letter differ, $P < 0.01$.

and therefore total calcium absorbed. In the present study, lime-treated tortilla proved to be a good source of calcium, and calcium absorption was not seriously affected by phytate content. It is suggested that industrial lime treatment be modified to allow the corn to remain in the lime-solution for a longer period of time so as to reach the same calcium concentration as that in homemade tortillas.

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