Calcium Bioavailability from Calcium Fortified Food Products

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Summary The calcium balance of 12 presumed healthy human young adult subjects was assessed. Subjects consumed a constant laboratory-controlled diet supplemented with one of four calcium-fortified food products: orange juice (OJ), milk (M), experimental pasteurized processed cheese (T), soda (S), or a calcium carbonate plus vitamin D tablet (CC). Study length was 6 weeks with seven-day experimental periods (2-days allowed for adjustment with 5-days combined for purposes of analysis). All urine and fecal samples were collected by the subjects for the duration of the study. Blood samples were drawn at the end of each experimental period. Urine and fecal calcium contents were determined. Blood samples were analyzed for alkaline phosphatase. Results of this study indicate a higher fecal calcium content (mg/day) when subjects consumed CC and T, and when subjects consumed self-selected diets, than when given S, M, or OJ. Urinary calcium excretion was significantly lower when subjects consumed OJ than when they consumed M, T, or their self-selected diets. A significantly larger positive calcium balance was demonstrated when subjects consumed OJ as compared to T. Fecal transmit time did not vary significantly. Serum alkaline phosphatase was significantly lower when subjects consumed T than when they consumed self-selected diets.

Key Words calcium balance, calcium bioavailability, fortified food products

Recently, much attention has been given to osteoporosis, a debilitating disease which is associated primarily with bone mineral losses. Much controversy exists as to its cause(s) and treatment, although research has indicated calcium nutriture is an influencing factor. In addition, dairy product consumption (milk in particular)
has decreased steadily over the past two decades and seems to have been replaced with an increased consumption of carbonated beverages (1). There is widespread belief that increased dietary calcium may prevent or slow the processes of this disease, yet the question of which age group would most benefit from increased dietary calcium remains unanswered. Studies have shown that a greater dietary intake of calcium throughout life results in greater bone density, thus delaying the onset of osteoporosis (2). A few studies have shown that osteoporosis can at least be slowed by increasing dietary calcium even in postmenopausal and elderly individuals (3, 4). An increased calcium intake has also been shown to lead to a more positive calcium balance (5, 6). Another study conducted to test calcium balance as a predictor of bone mineral loss has indicated that subjects with negative calcium balances lose bone mass at a faster rate than those subjects with the more positive calcium balances (7). These findings might indicate that all age groups would benefit from increased dietary calcium and, thus, slow or prevent osteoporosis.

Due to the high incidence of osteoporosis, consumers are being advised by many scientific organizations to consume at least the Recommended Dietary Allowance for calcium (i.e., Surgeon General and National Academy of Sciences). Increased consumer awareness of osteoporosis, thanks largely to the mass media, has sparked an incredible increase in calcium supplement use (8). However, very little attention has been given to documenting the biological availability (absorption potential) and value (absorption and retention potential) of these supplements in humans. Research suggests that differences do exist in calcium bioavailability and value from different calcium supplements in animals (9, 10) and in humans (11). However, a few studies have not shown significant differences among calcium bioavailability of several different calcium sources (12-14). Further, it is very likely that calcium supplements are not utilized equally by all individuals.

Recently, food manufacturers have added calcium to food products such as breakfast cereals, carbonated beverages, frozen orange juice, and milk. Uncertainty exists as to whether calcium-fortified food products or calcium supplements attain their intended purpose (a better calcium balance and thus, increased biological value).

Gastrointestinal irritation has been reported when certain calcium supplements were taken (11). Since calcium fortification of food is a new process, gastrointestinal responses to this form of calcium supplementation have not been reported. Consumers who wish to increase their dietary calcium need to be aware of differences that may exist in biological availability and/or value of the calcium supplement they have chosen to consume. Consumers also need information on potential side effects of calcium supplements.

Dairy products account for approximately 57% of American calcium consumption (15). It would therefore be possible to increase consumer calcium intake by fortifying dairy or other food products. The purpose of this study was to investigate human calcium balance and gastrointestinal response when dietary calcium...
calcium supplements via food sources (two dairy products, one non-dairy product and one carbonated beverage) of calcium tablets were provided to a sample of young adults.

RESEARCH METHODS

Twelve adult human volunteers (Miami University students), average age 22 years, 10 females and 2 males, were the subjects of this study. The experimental plan for this study is shown in Table 1. The study protocol was reviewed by the Human Subjects Committee of Miami University with subsequent approval to conduct the study. All subjects consumed and kept dietary records of their self-selected diets (control) for 7 days prior to the experimental periods of this study. Nutrient analysis of the self-selected diets were conducted using the Nutritionist Three program. Subjects completed a subject activity form every day during the control and experimental periods for subsequent assessment of subject complaints and activity levels. All subjects received experimental treatments according to a randomized block design. Subjects were housed in their own living quarters and ate at least one meal of three daily, meals controlled in the nutrition metabolic laboratory of the Family and Consumer Sciences department. The other two daily meals supplied for this study were packaged and could be taken outside the metabolic unit for consumption by the subjects. Subjects were allowed to consume only food that was provided for the study. The study length was 42 days (Table 1), divided into five experimental periods of 7 days each, and one control period (self-selected diet). The first two days of each experimental period were

| Period     | Number of days | Diet type      | Supplement                      |
|------------|----------------|----------------|---------------------------------|
| Pre-period | 7              | Self-selected  |                                 |
| Adj. 1     | 2              | Lab-controlled | Calcium fortified milk          |
| Expt. 1    | 5              | Lab-controlled | Calcium fortified milk          |
| Adj. 2     | 2              | Lab-controlled | Calcium carbonate tablet        |
| Expt. 2    | 5              | Lab-controlled | Calcium carbonate tablet        |
| Adj. 3     | 2              | Lab-controlled | Calcium fortified               |
| Expt. 3    | 5              | Lab-controlled | Test cheese                     |
| Adj. 4     | 2              | Lab-controlled | Calcium fortified               |
| Expt. 4    | 5              | Lab-controlled | Orange juice                    |
| Adj. 5     | 2              | Lab-controlled | Calcium fortified soda          |
| Expt. 5    | 5              | Lab-controlled | Calcium fortified soda          |

Calcium fortified milk, C. F. Burger Creamery, Pleasantville, NJ. Calcium carbonate tablets, Marion Laboratories Inc., Kansas City, MO. Calcium fortified test cheese, Kraft Foods Inc., Glenview, IL. Calcium fortified orange juice, Procter & Gamble, Cincinnati, OH. Calcium fortified soda=diet soda with the addition of liquid calcium by the investigator. Liquid calcium, General Nutrition Corporation, Pittsburgh, PA.
used as adjustment days, as suggested by previous research with rats (16). The following five days were data collection days. During all experimental periods, subjects received a measured, laboratory-controlled diet composed of ordinary foods which met all nutrient needs of subjects, except for calcium, as published by the RDA (1980). Subjects were required to consume all of the foods given to them during the study. Calcium content of the controlled diet was analyzed and calculated to contain 545 mg Ca per day. This controlled diet was manipulated by the addition or removal of high-calorie, low-nutrient dense foods to maintain subject calorie needs. In each experimental period all subjects consumed the controlled diet plus one of the calcium-fortified food products under study (orange juice OJ, milk M, soda S, and experimental cheese T) or a calcium carbonate plus vitamin D tablet (CC) with breakfast. The calcium sources used to fortify these food products were different. For example: S contained CaCO₃ + Ca gluconate, T contained Ca₃PO₄, OJ contained Ca citrate malate and the milk contained CaCO₃. Supplemented calcium in experimental treatments supplied an additional 555–567 mg/day of calcium. Due to the vast differences in the vitamin A and D contents of a few of the calcium-fortified food products, vitamin A and D were added to the experimental periods where needed to keep these nutrients equal throughout the study.

All urine and feces were collected by the subjects for the 42 days of the study. Urine and fecal samples were combined and processed for analysis for each of the five data-collection days of each period. Urine was diluted and fecal samples ashed. The calcium contents were determined using a Varian atomic absorption spectrophotometer. Fasting blood serum samples were collected and analyzed for alkaline phosphatase (Azurechrone method) at the end of each experimental period by phlebotomist and technicians at McCullough-Hyde Hospital, Oxford, Ohio. Fecal transit times (mouth to anus) were determined with the use of fecal dye markers (Brilliant Blue and Carmine Red) supplied to subjects at the beginning and end of each experimental period and were consumed with breakfast. Statistical analysis was conducted on data using STATAN program for ANOVA and Duncans Multiple Range Test.

RESULTS AND DISCUSSION

Results of this study are expressed as excretion levels as a percent of intake because individual and group calcium intakes were significantly ($p < 0.05$) different (Table 2). Mean fecal calcium as a percent of intake of subjects while receiving each of the dietary treatments, and while consuming their self-selected diets, is shown in Table 2. Percent fecal calcium excretions were not found to be statistically significantly different, but fecal calcium tended to be lower when subjects consumed M, OJ, and S as compared to control, CC, and T (39.4, 55.0, and 43.2% versus 50.1, 54.4, and 55.5%, respectively). A few recently-published studies reported that the form of calcium in the orange juice used in this experiment
Table 2. Mean calcium intake, urinary calcium, fecal calcium, calcium balance of subjects while receiving dietary treatments and during self-selected diet.

| Treatment          | Mean Ca (intake mg/day ± SD) | Mean urinary Ca (% of intake ± SD) | Mean fecal Ca (% of intake ± SD) | Mean Ca (balance % of intake ± SD) |
|--------------------|-------------------------------|----------------------------------|----------------------------------|-----------------------------------|
| Self-selected diet | 941.6±141.2^d                 | 27.0±15.2^a                      | 50.1±13.1^a                      | 12.9±24.9^b                       |
| Calcium carbonate | 1,086.9±15.0^b                | 14.8±5.7^b                       | 54.4±23.4^a                      | 19.2±25.6^ab                      |
| Soda               | 1,082.9±17.9^b                | 15.8±8.8^b                       | 42.9±14.9^a                      | 28.8±22.9^ab                      |
| Orange juice       | 1,221.4±18.8^e                | 14.2±6.5^b                       | 55.0±18.7^a                      | 36.4±14.6^a                       |
| Milk               | 1,028.5±21.9^f                | 21.5±9.9^g                       | 39.4±17.7^a                      | 13.9±17.3^b                       |
| Experimental cheese| 1,006.5±18.0^g                | 20.2±7.1^h                       | 55.5±22.5^a                      | 10.0±9.6^b                        |

Values with different letter superscripts are significantly different from one another (p<0.05).

(calcium citrate malate) was absorbed an average 30% better than milk calcium (17, 18). Another publication reported a significant increase in calcium absorption from calcium citrate malate as compared to calcium carbonate (19). In the present study these dramatic differences were not demonstrated, possibly because the calcium carbonate tablet used in this study was combined with vitamin D.

Table 2 indicates urinary calcium excretion as a percent of intake which was highest when subjects consumed self-selected diets (average 27.0%). Urinary calcium excretion as a percent of intake was significantly lower when subjects consumed OJ, CC, and S than when they consumed self-selected diets (14.2, 14.8, 15.8% versus 27.0%, respectively) (p<0.05). While the percent urinary calcium excretion was not shown to be significantly different when subjects consumed CC, OJ, S, M, or T, urinary calcium excretion as a percent of intake tended to be lower when subjects consumed OJ, CC, and S.

Table 2 demonstrates mean calcium retention as a percent of intake [(intake - urinary and fecal excretion)/intake] of subjects consuming the various dietary treatments and self-selected diets. Calcium balance was significantly lower when subjects consumed self-selected diets, M and T than when subjects consumed OJ (12.9, 13.9, and 10.0% versus 36.4% retained). While not significantly different, the consumption of OJ, S, and CC resulted in a greater calcium retention as when compared to M and T (36.4, 28.8, and 19.2% versus 13.9, 10.0%, respectively). Researchers have reported that the calcium in rat diets supplemented with nonfat dry milk, yogurt, and rennet-precipitate casein was more efficiently utilized than was the calcium carbonate typically added to rat diets (20). Similar results were found in a study of 22 postmenopausal women in which one-half of these subjects were given an extra 24 oz. of milk per day for two years and the other half received no intervention (21). The treatment group had a significant increase in urinary calcium, decrease in bone accrual, and decrease in bone resorption. Calcium balance in the milk-supplemented group improved and suppression of bone remodeling was less than in a previous study conducted by the same group in 1977,
when a calcium carbonate supplement was used. These results suggest that milk was a better calcium source than calcium carbonate because it does not suppress bone remodeling as much as calcium carbonate. It further suggests that calcium balance is a reliable indicator of bone remodeling activity.

Other studies have reported that calcium balances as influenced by calcium from food sources, or by calcium from calcium salts, were not significantly different (22, 23). These studies and the present study utilized a controlled feeding regimen in which a constant background diet was used. This is important because recent literature indicates that many food constituents (protein, fiber, phosphorus, magnesium, fat, phytic acid, and oxalic acid) interfere with or at least influence calcium absorption and retention (24–38). Thus, the potential for false results of actual bioavailability of calcium is great if a constant diet is not used when calcium bioavailability comparisons are made. Because certain food constituents influence calcium nutriture it is apparent that the calcium-fortified food products used in this study would differ in their contents of calcium-influencing factors.

Two food constituents shown to influence calcium absorption and retention are fat and protein (27, 34, 36). The present study utilized two calcium-fortified food products higher in fat and protein (T and M) than the other experimental calcium supplemental groups (CC, S, and OJ). This fact may explain the lower calcium balances found in these two experimental treatments. However, the effects of fat and protein on calcium nutrition are still unclear. It is important to note that there are also published reports that did not find significant influences of fat and protein on calcium absorption and/or retention (39, 40). Attention has been drawn to the effects of fat malabsorption on calcium balance (37, 38). Fat malabsorption did not seem to be the factor for the lower calcium balances experienced by the consumption of M or T food products based on fecal transit times which were increased during the T but decreased during the M periods. Decreased fecal transit times would be indicative of fat malabsorption and were not present in this study.

Calcium-fortified soda (S) (in this study 95% of the subjects consumed a diet cola), is typically acidified with phosphoric acid, creating a calcium-fortified product high in phosphorus (60.0 mg/12 oz.), a mineral known to negatively influence calcium absorption (36), yet calcium balances of subjects consuming this food product were found to be more positive than when subjects consumed their self-selected diets, CC, M, or T. In the present study this increased phosphorus level in the diet did not seem to affect calcium absorption, nor did it appear to significantly affect urinary calcium excretion. In this study the soda was fortified with 540 mg Ca, creating a 9:1 ratio of Ca to P. The results of this study indicate that the ratio of Ca to P was high enough to counterbalance the negative effects of dietary P, as seen in past research.

Fecal transit times were not found to be significantly different (Table 3). However, M consumption tended to result in the fastest fecal transit time as compared to self-selected diets, CC, S, OJ, and T (34.3 versus 50.4, 46.6, 44.8, 47.0, and 52.8 h, respectively). Decreased fecal transit times have also been noted with
increased consumption of milk in another study (10) without resulting in decreased calcium absorption. In the present study, the decreased fecal transit time noted in the milk experimental period did not significantly affect the Ca balance of these subjects. Also, no differences were noted in the number or physiological origin of subject complaints (cramps, nausea, gas, headache, fatigue, diarrhea, and constipation) during any of the experimental periods of this study.

Serum alkaline phosphatase (Table 3) was shown to be significantly lower when subjects consumed T (64.2 U/liter) than when subjects consumed their self-selected diets (69.7 U/liter). Several recent reports have assessed serum alkaline phosphatase as an indicator of bone accretion (41, 42) and serum acid phosphatase as an indicator of bone resorption (43). All of these studies found significant correlations between actual bone status and these blood enzyme levels. Therefore, the significantly higher serum alkaline phosphatase levels seen in subjects consuming their self-selected diets would seem to indicate a greater rate of bone accretion than when these subjects consumed T. However, serum acid phosphatase levels were not determined in this study, and future studies of this type should include this measurement.

CONCLUSION

The calcium bioavailability of the calcium-fortified food products used in this study was shown to differ, with calcium-fortified orange juice resulting in a significantly more positive calcium balance than calcium-fortified milk and experimental pasteurized processed cheese. The calcium supplement (CaCO3 plus vitamin D), calcium-fortified milk and calcium-fortified soda were not shown to yield significantly different calcium balances than calcium-fortified orange juice or calcium-fortified experimental cheese. It seems that of the calcium supplements or calcium-fortified food products already on the market, calcium-fortified orange juice (Citrus Hill Plus Calcium) resulted in the best calcium balance for the subjects.
tested in this study. Whether the positive results shown here are the result of the calcium form or the food product used for calcium fortification is yet unclear. It is believed by this investigator that the form of calcium used for fortification of the experimental pasteurized processed cheese food in this study (Ca₃PO₄) was the main cause for the lower calcium balances in these subjects. Serum alkaline phosphatase was significantly higher during the control period (self-selected diet) than when subjects consumed calcium-fortified experimental cheese, suggesting that either the experimental cheese itself or the Ca₃PO₄ used to fortify the experimental cheese influenced alkaline phosphatase level and, therefore, bone accretion. Since fecal transit times and subject gastrointestinal complaints were not significantly different between treatments, this suggests that none of the calcium forms or the food products tested created more frequent gastrointestinal distress than the others under the conditions of this study. All calcium sources in this study were different, thus making it difficult to reach conclusions on calcium bioavailability based on calcium source alone. Calcium balances of the calcium-fortified foods tested here varied enough to raise the question whether this was a result of calcium source, the food product that the calcium was added to, or both. Further study is necessary to identify food constituents and calcium sources that yield maximum benefit to human calcium nutriture.

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