Bioavailability of calcium supplements and the effect of vitamin D: comparisons between milk, calcium carbonate, and calcium carbonate plus vitamin D$^{1-3}$

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**ABSTRACT** Our aim was to examine a regimen for calcium supplementation because various factors seem to be important for its bioavailability, and to examine the effect of adding vitamin D to the supplement. The participants were 20 healthy women aged 28–59 y (3: 38 y). During the 3-d periods and 1 d before, the participants were consuming a calcium and energy-balanced diet as similar to their usual daily diet as possible. The study was designed as a randomized, placebo-controlled, partly blinded crossover study divided into four periods of 3 d each: 1) three tablets containing 1000 mg CaCO$_3$/d, 2) three tablets containing 1000 mg CaCO$_3$ plus 5 µg (200 IU) vitamin D/d, 3) 1 L more milk than in the usual daily diet, and 4) three placebo tablets daily. Bioavailability of the different calcium-supplement regimens were evaluated by changes in 24-h urinary excretion of calcium, phosphate, and magnesium. A significant increase in urinary calcium excretion was found during all periods of supplementation compared with the placebo period ($P < 0.01$). Excretion of calcium in the calcium carbonate period was not significantly higher than that in the milk period, but calcium carbonate plus vitamin D resulted in significantly higher calcium excretion compared with that in the milk period. We conclude that the examined calcium carbonate regimen is at least as good a calcium supplement as milk, and that addition of 600 IU vitamin D/d promptly resulted in an increase in urinary calcium excretion after an increase in calcium absorption, even in healthy women. _Am J Clin Nutr_ 1996;63:354–7.

**KEY WORDS** Bioavailability, intestinal absorption, calcium carbonate, milk, calcium, phosphate, magnesium, vitamin D, nongenome, calcium supplementation

**INTRODUCTION**

The number of different preparations of calcium supplements is expanding very fast as more and more subgroups in the population are recommended to increase their daily calcium intake, either by increasing their intake of milk or by taking a calcium supplement. The solubility of a certain calcium salt may change depending on the manufacturing process, and does not necessarily reflect its bioavailability (1). It is therefore necessary to test the bioavailability of the individual preparation (2). The means of administering the calcium supplement is also important to the bioavailability of a certain calcium salt (2-4). Finally, the addition of vitamin D to calcium preparations has been discussed. Some consider it unnecessary for normal, healthy persons with normal dietary habits to take vitamin D supplements because their vitamin D concentrations are assumed to be normal (4). However, it has been shown that vitamin D status is often insufficient in elderly people and that they can benefit by taking vitamin D with a calcium supplement to improve calcium absorption (5-8).

Isotopic methods are very accurate in measuring calcium absorption (4). However, in the present study the renal handling of minerals was assumed to be constant and bones were assumed to be in a steady state condition because the subjects were consuming a balanced diet, consequently, intestinal calcium absorption was estimated by urinary excretion of calcium (2).

The aim of this study was 1) to examine the bioavailability of a calcium supplementation regimen given as 1000 mg calcium carbonate (400 mg elementary calcium) at breakfast, at dinner in the evening, and at bedtime; 2) to compare the bioavailability of a calcium supplement with that of milk, the gold standard of calcium supplementation; and 3) to examine the effect on calcium absorption of a vitamin D supplement plus calcium in normal healthy women with normal dietary habits.

**SUBJECTS AND METHODS**

**Study population**

The participants were 20 healthy women with a mean age of 38 y (range: 28–59 y) who knew how to collect 24-h urine samples because they were employed in the healthcare system. None of the participants had ever suffered from any disease that might result in disturbances in calcium or bone metabolism, diseases with disturbances in parathyroid hormone, malabsorption, diabetes mellitus, rheumatoid arthritis, or renal, intestinal, or hepatic diseases. None of the participants received

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medication known to influence bone or mineral homeostasis. Before the study began, all participants had normal serum concentrations of calcium, phosphate, magnesium, creatinine, alanine aminotransferase, and parathyroid hormone. The participants had a mean daily calcium intake of 1115 mg (95% CI: 893, 1339 mg). The study was approved by the ethical committee of Aarhus County and performed according to the rules of the Helsinki II Agreement.

Methods

The study was a placebo-controlled, partly blinded crossover study in which subjects were randomly assigned to receive one of four treatments for 3 d each: 1) three tablets each containing 1000 mg CaCO₃ (400 mg elemental calcium)/d (Pharma-Vinci A/S, Frederiksvaer, Denmark); 2) three tablets each containing 1000 mg CaCO₃ (400 mg elemental calcium) plus 200 IU vitamin D (5 µg cholecalciferol) daily (Pharma-Vinci A/S); 3) 1 L more milk than in the usual daily diet, containing 1250 mg Ca, ~73% of which was bound as calcium phosphate, ~25% as calcium citrate, and ~2% as ionized calcium; and 4) three placebo tablets daily containing cellulose and no calcium.

The regimen of calcium carbonate alone and the placebo regimen were blinded to both the participants and the investigators. The milk regimen was not blinded to either the participants or the investigators. The regimen of calcium carbonate plus vitamin D was blinded to the participants but not to the investigators—we decided that this regimen should occur last for all participants to avoid any effect of vitamin D in a subsequent period. The technician analyzing the urine samples was unaware of whom and which regimen the samples came from. The blinded and randomization were performed by the manufacturer of the tablets, and the unblinding took place when all urine samples had been analyzed.

The calcium supplements were taken three times a day: at breakfast, at dinner in the evening, and at bedtime—one tablet or 0.333 L milk each time, corresponding to a daily elemental calcium intake of ~1200 mg. All tablets looked and tasted the same and a surplus was packed in each container.

Based on information obtained from an interview performed by a trained dietitian, a calcium- and energy-fixed diet was formulated that was comparable with the subjects' usual daily diet. Participants consumed the diet 4 d every week for 4 wk. On the last 3 d of the diet for each of the four treatment regimens, the participants collected 24-h urine samples and received calcium supplements or placebo according to the randomization code. The 24-h urine samples were delivered to the investigator each day. The urine was collected in plastic containers and the samples were acidified before being analyzed. The concentrations of calcium, phosphate, magnesium, and creatinine in the urine samples were measured in the hospital laboratory by using routine methods. The individual mean value was calculated as the mean of the values from the 3 d. Values from days for which there was incorrect sampling or improper medication was taken by the participants were not included. Because the participants were consuming a balanced diet the mean changes in 24-h urinary excretion of calcium, phosphate, and magnesium were used as an estimate of changes in intestinal absorption during the four periods.

Compliance with tablet intake was tested by checking the surplus of tablets. Compliance in the milk period was tested by asking the participants to mark the extra daily liter of milk with a label at the start of the day and to bring the empty container to the investigator the following day. Compliance with urine collection was tested by examining the constancy in 24-h urinary creatinine excretion. The study was completed within 6 wk, starting in the beginning of April. The participants were instructed to avoid sun exposure during the study.

Statistics

Data were tested by Friedman's test. Tukey's test was performed after the Friedman's test to control the type I experiment-wise error. Differences between urinary mineral excretion on each of the 3 d in the vitamin D period were tested by Friedman's test. Differences between urinary creatinine excretion on each of the 3 d for all regimens were also tested by Friedman's test. The level of significance was set at $P = 0.05$. Statistical analyses were performed with SAS software (SAS Institute Inc, Cary, NC).

RESULTS

All participants completed the study. Compliance was very good and mistakes during urine sampling or tablet intake were corrected when possible, because the participants usually contacted the investigator as soon as a mistake occurred. Compliance with urine sampling was good, no significant differences were found in 24-h urinary excretion of creatinine between days in each period or between periods and the intraday CV was low (4%, 2%, 2%, and 5% for the four regimens). Compliance with tablet intake was also very good. Only three participants missed one tablet each during the first regimen. No surplus of tablets was registered during the later periods for any of the participants.

Mean ($±$ SEM) 24-h urinary excretion of calcium, phosphate, and magnesium during the four treatment regimens are given in Figures 1, 2, and 3, respectively. There was a significant increase in 24-h urinary calcium excretion after calcium supplementation compared with excretion after placebo treatment ($P < 0.01$). Calcium excretion during the calcium car-

Figure 1. Mean ($±$ SEM) 24-h urinary excretion of calcium in four different treatment periods. $n = 20$. *** $P < 0.001$. 

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bonate period was not significantly higher than during the milk period. Addition of vitamin D to the calcium carbonate resulted in the greatest increase in calcium excretion; the increase was significantly higher than during the milk regimen (\( P < 0.01 \)).

The 24-h urinary excretion of phosphate increased significantly during the milk regimen (\( P < 0.00001 \)). Phosphate excretion decreased significantly in the calcium carbonate period (\( P < 0.001 \)) as well as in the calcium carbonate plus vitamin D period (\( P < 0.001 \)) compared with excretion in the placebo period. There was no significant difference between phosphate excretion in the two different calcium carbonate periods and no significant differences in 24-h urinary magnesium excretion between regimens. There was no significant difference in urinary calcium excretion over the 3 d of the calcium carbonate plus vitamin D regimen, by Friedman’s test.

**DISCUSSION**

Usually, milk is recommended as the best calcium supplement because it also contains other valuable nutrients such as proteins and vitamins. Unfortunately, milk consumption by children as well as adults has decreased in recent years, probably because of an increasing incidence of milk allergy and a general concern about weight gain. An increasing number of subgroups in the population are being recommended to increase their daily calcium intake. Calcium and vitamin D supplementation have been shown to be beneficial in the prevention of excessive bone loss in the perimenopausal and especially the postmenopausal period (5–9). For persons with allergy to milk, especially children, it is important to ensure their calcium supplementation with a proper regimen. Therefore it is important to examine the bioavailability (defined as the sum of factors affecting the intestinal calcium absorption) of a given regimen before it is recommended. The bioavailability of a given calcium salt may vary from preparation to preparation. Also, the means of administering a calcium supplement is important to the bioavailability of a certain calcium salt because the percentage absorption of a given calcium load decreases as the load increases (10), which means that a total daily dose should be divided into more doses. The time of intake is important because calcium taken with a meal is better absorbed than is calcium taken without a meal (11). Recker (3) found that a minority of subjects with achlorhydria might have difficulties absorbing a calcium carbonate preparation in a fasting state. However, this has been shown to be only a minor problem, which is fully compensated for when calcium carbonate is taken with a meal (11). Taking a dose of calcium at bedtime may also be favorable because it seems to inhibit calcium fluxes from bone during the night (12). We examined a well-absorbed and economically favorable calcium salt, calcium carbonate, given at meals and at bedtime to secure good absorption and compliance. Because urinary excretion of calcium is a qualitative estimate of its bioavailability, we compared its absorption with that in both a placebo period and a period in which the calcium source was the “gold standard”, i.e., milk.

The study subjects were normal, healthy women with normal dietary habits and a high calcium intake. Therefore, it was not because of a low calcium intake that the urinary calcium excretion increased by a high percentage. Second, there is no reason to believe that these women were vitamin D–deficient because they all had normal dietary habits, enjoyed sunbathing during the summer, and had normal 24-h urinary calcium excretion and serum parathyroid concentrations. The time of the study should not have been a problem in these women because McKenna (13) showed that the concentrations of vitamin D metabolites in the young Scandinavian population are normal in the spring and should be above the concentration at which the serum calcitriol concentration is influenced by the serum calcidiol concentration.

This study showed that a regimen of calcium carbonate given at two meals and at bedtime increased calcium uptake significantly. Calcium uptake by this regimen was at least as good as

**FIGURE 2.** Mean (± SEM) 24-h urinary excretion of phosphate in four different treatment periods. \( n = 20. *** P < 0.001 \).

**FIGURE 3.** Mean (± SEM) 24-h urinary excretion of magnesium in four different treatment periods. \( n = 20. *** P < 0.001 \).
the uptake of calcium from an equivalent amount of milk, i.e., calcium carbonate can be used as an alternative to milk in persons who do not drink milk or eat milk products. The increased urinary phosphate excretion during the milk period was not surprising because milk contains a substantial amount of phosphate. The decreased excretion of phosphate during calcium carbonate treatment might be explained by an increased formation of insoluble phosphate complexes in the intestine, which thereby lower intestinal phosphate absorption; calcium carbonate is a well-known inhibitor of phosphate absorption. It cannot be a vitamin D-induced effect because no change in urinary phosphate excretion was observed between the two calcium carbonate regimens. Another positive result was the lack of significant change in urinary magnesium excretion—calcium carbonate supplementation with or without added vitamin D had no effect on magnesium homeostasis, and the changes in urinary calcium and phosphate excretion were not related to changes in magnesium homeostasis.

In healthy persons, supplements of cholecalciferol will be absorbed and deposited until it is needed. It will then be further metabolized into the active vitamin D metabolite calcitriol. The circulating active metabolite increases active intestinal calcium absorption as well as phosphate absorption by a genomic action regulated by physiologic signals. No effect was observed on intestinal calcium absorption in animal studies when calcitriol or cholecalciferol were applied in the intestinal lumen. Vitamin D is also able to stimulate renal reabsorption of calcium and phosphate (14, 15). Usually, healthy people with normal dietary habits and exposed to sun during the summer, like the participants in this study, have a certain depot of vitamin D, and therefore addition of vitamin D to calcium supplements has been considered useless (4). It was therefore surprising that the addition of cholecalciferol improved the bioavailability of calcium in this study. Moreover, it was surprising that the addition of cholecalciferol seemed to have an immediate effect on calcium absorption—urinary excretion increased on the very first day and stayed at this same amount for all 3 d. These findings suggest that an increased calcium load in the intestinal lumen as well as the presence of a vitamin D metabolite directly stimulate intestinal calcium absorption, a nongenomic effect. This quick response may suggest that vitamin D has a direct effect on the intestinal absorption of calcium and that the effect may not be dependent on transformation into the active metabolite. The effect was an increase in the urinary calcium excretion to a certain amount because urinary calcium excretion remained constant during all 3 d of the investigation. This effect might be the result of a nongenomic action because the genomic-regulated process would be expected not to respond to vitamin D in these subjects.

We conclude that a regimen of calcium carbonate given daily at two meals and at bedtime significantly increased intestinal calcium uptake. The bioavailability of calcium carbonate was at least as good as that of calcium from milk. This regimen therefore is a useful alternative to milk as a calcium source. Addition of 15 μg (600 IU) cholecalciferol to the calcium supplement increased, even in healthy women, the short-term bioavailability of calcium.

REFERENCES