Postprandial parathyroid hormone response to four calcium-rich foodstuffs\textsuperscript{1,2}

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ABSTRACT We studied the effects of four calcium-rich foodstuffs on postprandial parathyroid hormone secretion. Four hundred milligrams calcium from either Emmental cheese, milk, sesame seeds, spinach, or calcium salt (calcium lactate gluconate + calcium carbonate) or no additional calcium (control session) were given to nine female volunteers immediately after a first blood sample (at 0900) in random order with a light standardized meal containing 37 mg Ca. Blood samples were taken at 0900 (before the calcium load), 1000, 1100, 1300, and 1500 at every study session. Urine was collected during the sessions. Serum ionized calcium, phosphate, magnesium, intact parathyroid hormone, and urinary calcium excretion were measured. The serum ionized calcium concentration increased significantly after ingesting cheese ($P = 0.004$, contrast analysis) or calcium salt ($P = 0.05$, contrast analysis) compared with the control session. Compared with the control session, the serum phosphate concentration increased after the cheese session ($P = 0.004$, contrast analysis) and after the milk session ($P = 0.02$, contrast analysis). Calcium salt ($P = 0.007$, contrast analysis) and cheese ($P = 0.002$, contrast analysis) caused a significant decline in serum intact parathyroid hormone compared with the control session. The urinary calcium excretion with cheese was 141\% ($P = 0.001$), with milk was 107\% ($P = 0.004$), and with calcium salt was 75\% ($P = 0.02$) above that of the control session. Our results show that calcium from sesame seeds and spinach does not cause an acute response in calcium metabolism. Our results indicate that fermented cheese could be a better dietary source of calcium than milk when the metabolic effects of the foodstuffs are considered. Am J Clin Nutr 1997;65:1726–30.

KEY WORDS Parathyroid hormone, calcium salt, fermented cheese, spinach, sesame seed, bioavailability, milk, magnesium, phosphate

INTRODUCTION

The importance for bone health of an adequate calcium intake during the life span has been emphasized. Whereas calcium is a building material needed in achieving maximal peak bone mass, supplemental calcium, when given to elderly people for example, may be considered a weak antiresorptive agent. Few studies show the effects of calcium from foodstuffs on bone and calcium metabolism and bone density. It has been shown that 2-y milk supplementation improved calcium balance in postmenopausal women (1) and that 3-y milk supplementation prevented a decrease in bone mineral density in premenopausal women (2). Some retrospective studies have shown a positive effect of milk intake in youth on bone mineral density later in life (3).

Parathyroid hormone (PTH) is one of the major regulators of calcium and bone metabolism. PTH increases bone resorption and acutely decreases some stages of bone formation (4). An acute oral calcium load increases the serum ionized calcium concentration and suppresses PTH secretion (5–7) in a dose-dependent manner (8, 9).

Calcium from foodstuffs may have acute effects on calcium and bone metabolism, but this has not been studied. These metabolic effects could be important when different dietary calcium sources are discussed and recommended. The absorbability of calcium is one factor affecting calcium utilization (10). Oxalate and phytate may decrease calcium absorption (11, 12), whereas lactose may enhance it (13). In addition, phosphate (14, 15) and magnesium (16) have direct effects on calcium and bone metabolism. This study investigated the acute metabolic effects of some calcium-rich foodstuffs on calcium metabolism.

SUBJECTS AND METHODS

Subjects and protocol

Nine healthy female volunteers using oral contraceptives, aged 24–34 y, took part in the trial. All subjects gave their informed consent to the procedures, which were in accord with the Helsinki Declaration. The subjects took no medications known to affect calcium and bone metabolism. The basic characteristics of the subjects are presented in Table 1.

The amount of calcium administered in this study was chosen on the basis of a pilot study in which we noted a significant response in PTH secretion to 250 mg Ca administered as calcium salt (6810 mg calcium lactate gluconate, 300 mg calcium carbonate, 1350 mg acid citrate anhydrous, and 40 mg

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TABLE 1
Basic characteristics of the study group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Energy intake (MJ)</td>
<td>8.7 ± 0.6</td>
</tr>
<tr>
<td>(kcal)</td>
<td>2086 ± 146</td>
</tr>
<tr>
<td>Calcium intake (mg)</td>
<td>1293 ± 130</td>
</tr>
<tr>
<td>Phosphate intake (mg)</td>
<td>1554 ± 135</td>
</tr>
</tbody>
</table>

*p = SEM; n = 9 women.

aspartame, corresponding to 1 g Ca²⁺; Laboratoires Sandoz, SARL, France). Because the calcium in foodstuffs may not be absorbed as easily as calcium salts, the calcium dose we chose was 400 mg/portion. We wanted to keep the portion sizes as small as possible.

Four foodstuffs were selected for the experiment: fermented cheese (Emmental type, 30% fat), milk (1.5% fat), whole sesame seeds, and spinach. Milk and cheese are important sources of dietary calcium. Sesame seeds are considered to be a rich source of calcium in a vegetarian diet. Only whole sesame seeds, however, contain a large amount of calcium; decorticated sesame seeds contain only 10% of the calcium in whole seeds. The calcium content of spinach is high, but the absorbability of this calcium is poor (17). The biochemical responses to spinach calcium may be small. Calcium salt was used as a standard for calcium bioavailability. In addition, the daily variation in PTH secretion was assessed by a control session. The order of ingestion of foodstuffs, calcium salt, and the control was assigned randomly. The calcium and phosphate contents of the foodstuffs were analyzed in frozen specimens by routine methods before the trial (Valio Ltd, R&D Laboratory Services, Chemical Division, Helsinki). The portion sizes were 333 g milk, 43 g Emmental cheese, 36 g sesame seeds, and 190 g spinach. Each portion contained 400 mg Ca. The phosphate contents of the portions were 254 mg for cheese, 286 mg for milk, 231 mg for sesame seeds, and 86 mg for spinach. The calculated protein contents were 11 g for milk and 12 g for cheese. The foodstuffs were stored at −20 °C until divided into portions.

Sampling

The subjects fasted overnight. The first blood sample was drawn at 0900 at every study session. After the first blood sample, a light meal (0.2 L orange juice, 5 g butter, 0.2 L tea, 4 g sugar, 50 g cucumber, 25 g white bread, and 0.2 L water, except with milk) together with the experimental foodstuff or calcium salt was served at each session and at the control session. The next blood samples were drawn at 1000, 1100, 1300, and 1500. A standardized lunch, which consisted of 0.2 L orange juice, 5 g margarine, 50 g white bread, 50 g carrot, and 275 g potato and meat casserole, was served immediately after the blood sample at 1300. The breakfast contained 37 mg Ca, 71 mg P, 5 g fat, and 2 g protein, and the lunch contained 40 mg Ca, 209 mg P, 12 g fat, and 12 g protein by calculation. Serum was separated from the blood samples. Urine was collected 24 h before the test and during the study every 2 h from 0700 to 1500. The serum and urine samples were refrigerated at −20 °C until analyzed.

Biochemical analyses

The serum ionized calcium concentration was measured within 90 min after blood sampling with a selective ion analyzer (ISE Ca⁺⁺/pH Analyzer 634; Ciba Corning Diagnostics, Halstead, United Kingdom). The ionized calcium concentration was adjusted to pH 7.4. Serum total calcium, phosphate, magnesium, and urinary calcium, phosphate, and magnesium concentrations were measured by routine laboratory methods. The concentration of intact PTH (iPTH) was analyzed with the Allegro intact PTH kit from Nichols Institute (San Juan Capistrano, CA). The intraassay CV for iPTH was 1% and the interassay CV was 3.7%.

Statistical analyses

The data are expressed as means ± SEMs. After testing for normality, logarithmic transformations were used when necessary. Analysis of variance (ANOVA) was used to test differences in biochemical responses among the six study sessions. If the sphericity assumption was not met, Huynh-Feldt adjusted P values were used. If a significant difference was found with repeated-measures ANOVA, contrast analysis was used to compare the response curves of study sessions with the control session. Analyses were made with BMDP statistical software for VAX/VMS mimicomputer (18). The results are presented as two panels in the figures only for readability.

RESULTS

The analysis of the response curves showed a significant difference in ionized calcium concentration (Figure 1) among the study sessions (P = 0.003). The concentration of ionized calcium increased significantly after ingesting cheese (P = 0.004) and calcium salt (P = 0.05) and tended to increase after milk (P = 0.1), whereas no difference was found between the spinach and sesame seed sessions compared with the control session. No differences were found in the serum total calcium concentration among the study sessions. The response curves of the serum phosphate concentrations were affected (P = 0.0001) by the foodstuffs. Compared with the control session, a significant increase was found in serum phosphate concentration (Figure 2) after the cheese and milk sessions (P = 0.004, P = 0.02, respectively). Sesame seeds and spinach did not affect the serum phosphate concentration. No differences in serum magnesium concentrations were found among the study sessions.

The iPTH secretion (Figure 3) was affected by the test substances (P = 0.05). The decrement in PTH secretion after ingesting calcium salt (P = 0.007) and cheese (P = 0.002) were significant compared with the control session. The other foodstuffs did not significantly affect PTH secretion.

Urinary calcium excretion, which in the short term reflects calcium absorption (Figure 4), was significantly affected by some of the tested foodstuffs (P = 0.00005). The urinary excretion of calcium with cheese was 141% (P = 0.001), with milk was 107% (P = 0.004), and with calcium salt was 75% (P = 0.02) above that of the control session. No significant changes were found in urinary calcium excretion after the ingestion of sesame seeds or spinach. Although there was a
trend for the urinary excretion of phosphate to increase after cheese and milk, the changes were not significant. Urinary magnesium excretion was affected by the tested foodstuffs ($P = 0.02$), being above that of the control session with calcium salt ($P = 0.04$) and cheese ($P = .04$).

**DISCUSSION**

To our knowledge, this is the first study in which the acute metabolic effects of calcium-rich foodstuffs on calcium metabolism have been investigated. The response in ionized calcium concentration differed among the foodstuffs tested. Calcium salt increased the serum ionized calcium concentration, as was shown earlier (9, 19). There was a remarkable difference among the foods studied. The ingestion of fermented cheese increased the serum ionized calcium concentration, but the effect of milk on serum ionized calcium concentration was not significant. One factor affecting the response in ionized calcium concentration after a calcium load is the amount of calcium absorbed (8). Milk calcium has been shown to be absorbed easily in human experiments using extrinsically radiolabeled milk (20). To our knowledge, the bioavailability of calcium from fermented cheese has not been investigated, but the bioavailability of calcium from fresh cheese (rennet-precipitated casein) has been reported to be equal to that of milk (20). Some studies have reported that lactose enhances calcium absorption (13), but this finding has not been supported by others (21). Although we did not measure calcium absorption, our results do not support the finding that lactose enhances calcium absorption because the increase in ionized calcium after the ingestion of cheese, which contains minimal amounts of lactose, was more pronounced than after milk. It may be that the long bacterial fermentation of cheese affects calcium absorbability. Another possibility is that the greater fat content of cheese slowed gastric emptying and thus enabled more calcium to be absorbed.
It has been shown that serum ionized calcium declines rapidly after a phosphate load (15, 22). The phosphate content was almost equal in the portions of milk, cheese, and sesame seeds. The amount of phosphate in the foods was small and it seems unlikely that it could have affected the serum ionized calcium concentrations to a large extent, although serum phosphate rose significantly after ingestion of cheese and milk.

Spinach and sesame seeds did not affect the serum ionized calcium concentration. The bioavailability of calcium from spinach is poor as shown in a calcium absorbability experiment (17). The bioavailability of calcium from sesame seeds is also poor, as indicated by a rat experiment with the tibia calcium content used as an indicator of bioavailability (23). These facts explain the low response in serum ionized calcium concentration after ingestion of these two foodstuffs.

The serum PTH concentration decreases when serum ionized calcium concentration increases, which in turn is affected in a dose-dependent manner by the amount of calcium that is absorbed from the intestine (8, 9). Of the foodstuffs studied, only fermented cheese decreased the PTH concentration significantly, which is in line with the response in the serum ionized calcium concentration. The average decrease in serum PTH after the milk session was ~20% compared with the control session, which may have been significant with a larger number of subjects. This response is small and was reached after a 400-mg dose of calcium, which, in an everyday situation, would be considered large when milk is the source. A normal dose would be a glass of milk (0.2 L) corresponding to ~240 mg Ca, which would give an even smaller PTH response. Some other nutrients and factors could directly affect PTH secretion. Phosphate increases PTH secretion (14, 15) and magnesium decreases (16) it. This finding could not explain the different response of PTH to milk and cheese, however, because the amount of phosphate was similar in these foodstuffs, and the calculated amount of magnesium in the portions was < 40 mg and did not affect the serum magnesium concentration.

In a controlled situation there is a strong correlation between the acute intake of calcium and urinary calcium excretion (24). In our study, urinary calcium excretion did not increase after ingestion of sesame seeds and spinach, which confirms the low absorption of calcium from these products. Urinary calcium excretion after the ingestion of milk and cheese was equal to that after calcium salt, which indicates that calcium was absorbed equally from these.

Why was calcium excretion affected by both milk and cheese, whereas the ionized calcium and PTH responses were not affected by milk? The reason for this is unknown. Serum phosphate changed in the same manner after the ingestion of both foodstuffs. The meals themselves could not have influenced the biochemical response because the same meals were served at all study sessions and at the control session.

Can recommendations be given for foodstuffs that are especially good for bone health on the basis of the results in this experiment? The results of our study indicate that the metabolic effect is different among different foodstuffs. Calcium bioavailability in spinach and sesame seeds seems to be poor. However, grinding could increase the bioavailability of calcium in sesame seeds, but this must be confirmed by more studies. Although calcium in milk has been shown to be absorbed as well as calcium carbonate in absorbability studies.
(20), our results indicate that fermented cheese could be a better dietary source of calcium than milk when the metabolic effects of the foodstuffs are considered. The effect of other dietary sources of calcium on calcium metabolism and on bone metabolism need to be evaluated. This study again shows that the bioavailability of calcium from different sources should be discussed and action should be taken to include information on bioavailability in the databanks used. Bioavailability is important in studies in which the effect of dietary calcium on bone metabolism and its role in the prevention of osteoporosis are investigated.

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