Reduction of Renal Calcium Reabsorption in Man by Consumption of Dietary Protein

LINDSAY H. ALLEN, RICHARD S. BARTLETT
AND GEOFFREY D. BLOCK
Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06268

ABSTRACT This experiment was designed to test whether protein consumption reduces the amount of filtered calcium reabsorbed by the kidney. Nine subjects were each fed meals containing 18 g protein and 54 g protein. The intake of energy, sodium, calcium, phosphorus, magnesium and zinc was similar in the two meals. For 4 hours after the meal, measurements were made of serum calcium (total and filterable), serum creatinine, and urinary calcium, creatinine, zinc and nitrogen. Calcium reabsorption was calculated in five clearance periods, as (filterable calcium × GFR) minus urinary calcium. Urinary calcium, zinc and nitrogen were significantly higher between 2 and 4 hours after consumption of the high protein meal. Protein level did not affect urine pH or volume, serum total or filterable calcium, or GFR. The percentage reabsorption of filtered calcium was significantly lower 0.5 hours after the high protein meal, so that at 2.5 hours, reabsorption was 98.0% compared to 98.7% after the lower protein meal. We conclude that protein consumption reduces the amount of calcium reabsorbed by the kidney. J. Nutr. 109: 1345-1350, 1979.

INDEXING KEY WORDS dietary protein urinary zinc kidney calcium reabsorption

The excretion of urinary calcium is directly correlated with the protein content of the diet (1, 2). In a recent publication we presented data to show that the consumption of high protein diets does not affect intestinal absorption of calcium, but that the ability of the kidney to reabsorb calcium is probably impaired (3). The purpose of the present study was to further examine the role of the kidney in protein-induced hypercalciuria.

METHODS

Subjects were six male and three female adults, aged from 26 to 48 years. On two separate occasions, they were asked to consume an adequate protein meal, containing 18 g protein, or a high protein meal containing 54 g protein. The renal reabsorption of calcium was followed for 4 hours after the consumption of each meal. The order in which the meals were consumed was randomized among subjects. No more than three subjects were tested on the same day. Because the time elapsed between the two meals ranged from 3 to 9 days, any carry-over effects were assumed to be negligible. The protein source was cottage cheese (1% fat). After wetashing (4), the sodium, calcium, and zinc content of the cheese was analyzed by atomic absorption spectrophotometry, and...
phosphorus with an autoanalyzer using an adaptation of the phosphomolybdic reduction method (5). A liquid formula diet was ingested with the cheese so that the intake of energy, lactose, sodium, calcium, phosphorus, magnesium and zinc was the same at both levels of protein intake. A total of approximately 504 kcal, 12 g lactose, 1.97 g sodium, 400 mg calcium, 642 mg phosphorus, 67 mg magnesium and 2.1 mg zinc was consumed in each meal.

Subjects fasted after 2000 hours on the previous day. On waking, each subject emptied his bladder and discarded the urine, recorded the time of urination, then drank approximately 250 ml water. At 0845 hours, fasting serum and urine samples were obtained. The bladder was emptied as completely as possible at each urination, and the urine volume recorded. At 0900 hours the meal was consumed. Approximately 200 ml water were ingested immediately after consumption of the meal and every 30 minutes for the next 4 hours. Serum samples were obtained at 0.5, 1.5, 2.5 and 3.5 hours after the meal, and urine samples every 30 minutes for 4 hours. This procedure provided five clearance periods in each of which calcium reabsorption could be determined.

Serum was analyzed for total calcium,\(^6\) filterable calcium (6) and creatinine with an autoanalyzer. Urinary calcium and zinc were measured by atomic absorption spectrophotometry, and urinary creatinine with an autoanalyzer.\(^7\) Urinary nitrogen was determined by the Kjeldahl procedure (7).

Filtered calcium was calculated as the product of glomerular filtration rate (GFR, obtained from measurement of serum and urinary creatinine in each clearance period), and serum filterable calcium. Calcium reabsorption was obtained by subtraction of urine calcium from filtered calcium for each clearance period.

Data were analyzed by the paired Student’s t-test and two variable linear regression analysis (8). The level of significance based on these tests is indicated in the figures.

RESULTS

The rate of urine production started to increase soon after the consumption of either meal, from 0.84 ml/minute by 0.5 hours, and 2.17 ml/minute by 1 hour. For the next 3 hours, urine output ranged between 2.28 and 5.93 ml/minute. When a comparison was made between the two protein levels, there was no significant difference in urine volume at any point during the 4 hours. The average urine pH ranged between 5.8 and 6.4 for the 4 hours of the experiment, and was not significantly affected by the level of dietary protein.

The excretion of urinary calcium, zinc and nitrogen after consumption of the two diets is shown in figure 1. The fastest rate of calcium excretion occurred 1.5 hours after the adequate protein meal, and 2 hours after the high protein meal. When the two diets were compared, the rate of calcium excretion was significantly greater within 2 hours after the high protein meal, and remained so during the next 2 hours. During the 4 hours of the experiment, individual subjects excreted from 0 to 320% (average 73%) more calcium after the high protein compared to the adequate protein meal. Three of the subjects showed very little calciuric response to protein, while the others showed a much greater response. When data from individual subjects were compared, the magnitude of the increase in urine calcium was not correlated with the excretion of urine calcium at 0 hours, or with the total volume of urine excreted over 4 hours.

The pattern of zinc excretion was similar to that of calcium. Between 1.5 and 4.0 hours, zinc excretion was significantly greater when the high protein meal was consumed. Urinary nitrogen peaked around 2 hours after either meal. Nitrogen excretion was significantly higher at 2.0, 3.5 and 4.0 hours after the 54 g protein meal compared to the 18 g protein meal.

Neither total serum calcium nor filterable calcium (fig. 2) was affected by the level of dietary protein. Glomerular filtration

---

\(^{6}\) Atomic Absorption Spectrophotometer Model 303; Perkin-Elmer, Norwalk, Connecticut.

\(^{7}\) AutoAnalyzer II Continuous-Flow Analytical Instrument; Technicon Instruments Corp., Tarrytown, New York.


rate was increased over fasting values by the consumption of either meal, but was not significantly affected by the level of dietary protein (fig. 3). Filtered calcium increased during the first 0.5 hour after either meal, due to the increase in GFR.
Fig. 2 Filterable calcium and serum calcium during 4 hours after consumption of meals containing 18 g protein (●-●) or 54 g protein (□-□). Numbers at the top of the figure refer to the difference (Δ) and the standard error of the difference (SEΔ) between the response to the two diets.

Fig. 3 Glomerular filtration rate and percentage reabsorption of filtered calcium, during 4 hours after consumption of meals containing 18 g protein (●-●) or 54 g protein (□-□). Numbers at the top of the figure refer to the difference (Δ) and the standard error of the difference (SEΔ) between the response to the two diets. Differences are significant at * = P < 0.05, ** = P < 0.01 or *** = P < 0.001.
during that time. However, since differences in dietary protein level did not affect GFR or filterable calcium, they did not cause a change in the amount of calcium filtered.

The percentage of filtered calcium which was reabsorbed is shown in figure 3. The percentage reabsorbed was significantly decreased within 0.5 hour after consumption of the high protein meal, and remained so during the next 3.5 hours.

**DISCUSSION**

From the data presented in this paper, we conclude that consumption of dietary protein causes a reduction in the amount of filtered calcium reabsorbed by the kidney. From this and our previous study (3), we can now state that protein-induced calciuria occurs as the result of altered renal handling of calcium, rather than through an increase in intestinal calcium absorption or an initial effect on bone calcium turnover.

Margen et al. (1) found that dietary protein caused an increase in urine calcium within 24 hours, but no previous attempt has been made to follow urine calcium immediately after consumption of a single meal containing variable amounts of protein. In the present experiment, we found that the level of dietary protein affected urine calcium excretion within 2 hours. This observation is important, since meal-to-meal variation in protein intake is normally considerable; consumption of several consecutive meals containing the same level of protein is not required for urine calcium to change.

In longer-term experiments, the calciuric effect of dietary protein was independent of the level of dietary calcium (1). In a preliminary phase of the present experiment, the 54 g protein meal without added calcium (i.e. 60 mg calcium per meal) was fed on three occasions to one subject. The level of urine calcium excretion was very similar to that after consumption of the 54 g protein meal containing 400 mg calcium. It would appear, therefore, that when protein is consumed there is an increased excretion of urinary calcium, the magnitude of which depends directly on the level of protein rather than calcium in the diet.

In contrast to its effect on urine calcium, dietary protein does not influence the rate of excretion of phosphorus, sodium, potassium, or magnesium (1, 3). In the present study, we measured urinary zinc, and found that dietary protein level directly affects the excretion of zinc in a similar pattern to calcium. Whether protein consumption also reduces the renal reabsorption of zinc remains to be investigated.

It is well established that long-term consumption of high protein diets will increase GFR. The kidneys of animals fed high protein diets are larger, show hypertrophy and hyperplasia of glomeruli, tubules and interstitial cells (9), and have increased activities of amino acid catabolizing and gluconeogenic enzymes (10, 11). In our previous research with human subjects, we found GFR to be significantly increased within 30 days of consuming 225 g protein per day compared to 75 g protein per day (3). However, in the present short-term experiment, increasing protein intake produced no significant effect on total or filterable serum calcium, or GFR. In fact,
GFR was actually lower in three of the subjects when they consumed the higher protein meal. Kim and Linkswiler* found that consumption of a high protein diet (142 g/day) caused an increase in filtered calcium compared to a 47 g protein diet. This was due to the slight elevation of GFR caused by consuming the high protein diet for 10 days. The fractional reabsorption of calcium was reduced by the high protein diet from 98.4 to 97.4%. Due to the increase in GFR, these authors were unable to determine the relative contributions of increased filtered calcium and reduced calcium reabsorption to the calciuria. In contrast, filtered calcium was not increased in our short-term study, so that the calciuria must be attributed to decreased renal reabsorption.

Within our nine subjects, the increase in urine calcium over 4 hours ranged from 0 to 320% when the 54 g protein and 18 g protein diets were compared. When meals with a similar nutrient composition were fed four times per day to six young men, calcium excretion was from 130 to 210% higher after the high protein diet was consumed. When considering the potential harmful effects on bone calcium of long-term consumption of high protein diets, the magnitude of the calciuric response of an individual may well be more important than the average daily intake of protein.

ACKNOWLEDGMENTS

The authors wish to thank Julius Tlustohowicz and Thylan Nguyen for their technical assistance.

LITERATURE CITED
