Urinary Calcium and Calcium Balance in Young Women Affected by High Protein Diet of Soy Protein Isolate and Adding Sulfur-Containing Amino Acids and/or Potassium

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Summary The effects of sulfur-containing amino acids (SAA) and potassium (K) on urinary excretion and retention of calcium (Ca) of 27 young Japanese women were studied. A basal diet low in protein level (50 g per day) was fortified by meat or soy protein isolate (SPI) to a protein level of 100 g per day, and effects of addition of apple to these high protein diets, and addition of SAA and/or potassium (K) to the high SPI diet, especially on urinary Ca excretion, were studied. The addition of meat which increased protein intake to 100 g caused the increase in apparent absorption and urinary excretion of Ca with increased excretion of urinary sulfur (S), phosphate, ammonia, and titratable acids (TA), whereas addition of SPI did not. The addition of apple to high meat diet decreased absorption and urinary excretion of Ca. Urinary Ca, S, K, ammonia, and TA excretion increased by the addition of SAA to high SPI diet in a manner similar to the meat diet. Consequently, SAA-supplemented diet had a significantly negative effect on Ca retention. In SPI + SAA, K diet period, urinary K excretion markedly increased, and increments in urinary Ca, ammonia, and TA excretion were reversed. These changes observed in SPI + SAA, K diet period were similar to those by adding apple to meat diet without any effect on Ca absorption. The results suggest that the hypercalciuria induced by high meat diet is mainly caused by high content of SAA and may be reversed by the ingestion of K-rich foodstuffs, and soy protein does not induce hypercalciuria because of it contains less SAA than animal protein.

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Results of earlier studies indicated that an increase in protein intake caused increases in urinary calcium and apparent absorption of calcium (1-4), and the prevailing opinion has been that a high protein intake has a beneficial effect on calcium retention by causing an increase in calcium absorption.

In recent years, Linkswiler and her co-workers reported that the substantial increase in urinary calcium was not accompanied by a comparable increase in absorption, and calcium retention was not improved as the protein intake was increased under strict conditions of examination (5-10). From the data of their subjects given high and low protein diets of mixed protein source, they suggested that the hypercalciuria caused by the high protein intake was due primarily to the decrease in fractional tubular reabsorption of calcium related to the increased renal acid excretion. Furthermore, participation of sulfur-containing amino acids (SAA) in the hypercalciuria of high protein feeding in adult rats was reported by Whiting et al. (11).

This study was undertaken to elucidate the effects of SAA on the calciuric response to a high intake of protein by feeding soy protein, containing less SAA than animal protein, as the protein source with the supplementation of SAA or not. The effect of increased intake of potassium on calcium retention was also investigated.

**EXPERIMENTAL**

**Subjects.** Twenty-seven female students aged 19 to 24 years participated in three series of experiments: seven for experiment 1, six for experiment 2, and fourteen for experiment 3. The characteristics of the subjects are shown in Table 1. During the experiments they lived in a metabolic unit of Kagawa Nutrition College and continued their usual activities. In experiment 3 the subjects were requested to walk as part of their daily living to present inactivity caused by residing on the college campus. The subjects ate all foods and drinks given them and drank deionized water ad libitum.

**Experimental design.** Figure 1 shows the experimental design. In experiment 1, after 5 days of basal diet period (protein intake 55 g/day), four of the seven subjects were given the high protein diets (100 g protein/day) containing 340 g of meat (meat diet) and the others 52 g of soy protein isolate (SPI diet) for 9 days. Immediately after a break period (i.e., 50-60 g protein/day), they were given the other experimental diet for each subject. In experiment 2, after the 5-day basal diet period, three of the six subjects were given 160 g of apple added to the high protein diet containing meat (meat + apple diet), and the others apple added to high SPI diet (SPI + apple diet) for 9 days. Then, immediately after a break period they were...
Table 1. Characteristics of the subjects.

| Experiment | Mean Age | Mean Height | Mean Weight | Energy intake (kcal/kg) |
|------------|----------|-------------|-------------|------------------------|
|            | Subj. No.| (yr)        | (cm)        | Basal | Meat | SPI |
| 1          | 7        | 20.0        | 161.0       | 35.6 | 38.3 | 36.7 |
|            | SD       | 1.9         | 6.7         | 5.2  | 4.5  | 4.6  |
| 2          | 6        | 20.7        | 158.3       | 38.1 | 38.4 | 37.4 |
|            | SD       | 2.1         | 3.7         | 4.2  | 3.7  | 3.1  |
| 3          | 14       | 21.4        | 159.6       | 35.1 | 35.8 | 35.4 |
|            | SD       | 1.5         | 5.7         | 4.4  | 3.6  | 4.4  |

Given the other experimental diet of meat+apple or SPI+apple for each subject. In experiment 3, the 14 subjects were firstly given the SPI high protein diet for 8 days. Immediately after a break period, they were continuously given the experimental diets containing SPI with 2.0 g of SAA (SPI+SAA diet) and then adding 3.0 g of potassium bicarbonate to the SPI+SAA diet (SPI+SAA,K diet), each for a 7-day period, respectively. The order of feeding of the two diets was changed in half of the subjects.

An example of the composition of the experimental diets is shown in Table 2. During SPI+SAA and/or SPI+SAA,K periods in experiment 3, 1.0 g of l-
methionine, 1.0 g of L-cystine, and/or 3.0 g of potassium bicarbonate (1.17 g as potassium), taken in three equally divided doses for each meal, were ingested along with the diet. Energy supply from the diets was modified by the substitution of a certain amount of sugar, soft drinks, and rice when meat, SPI, or apple was added. Approximate maintenance energy intake was determined individually by detailed analysis of the foods consumed before the experiment. When the body weight of a subject decreased or increased distinctly during the experimental period, a certain amount of sugar, soft drinks, and rice were added to or subtracted from the diet. These procedures were in accord with the declaration of Helsinki (1964).

**Sample collection and analyses.** Twenty-four hour urine and feces of the last 4 days of each experimental period were collected. Urinary pH, titratable acid (12), excretion of calcium, phosphate, sulfur (13), potassium, ammonia, and creatinine were measured. As a fecal marker, 0.5 g of carmine was given before breakfast on the first day and the day after the collection period. Fecal calcium, phosphate, and sulfate excretion and dietary calcium contents were analyzed.

*J. Nutr. Sci. Vitaminol.*

### Table 2. Composition of the experimental diets (g/day).

| Expt. diet       | Experiments 1 and 2 | Experiment 3 |
|------------------|---------------------|--------------|
|                  | Basal | Meat | SPI | Meat + Apple | SPI + Apple | SPI | SPI + SAA | SPI + SAA, K |
| Milk products    | 243   | 243  | 243 | 243          | 243         | 233 | 233       | 233          |
| Egg              | 33    | 33   | 43  | 33           | 43          | 43  | 43        | 43           |
| Fishes           | 43    | 43   | 43  | 43           | 43          | 43  | 43        | 43           |
| Pork             | 33    | 193  | 33  | 193          | 33          | 33  | 33        | 33           |
| Chicken          | 25    | 105  | 25  | 105          | 25          | 25  | 25        | 25           |
| Soybeans         | 23    | 23   | 23  | 23           | 23          | 25  | 25        | 25           |
| SPI*             | —     | —    | 52  | —            | 52          | 52  | 52        | 52           |
| Vegetables, rich in carotene | 88   | 88   | 88  | 88           | 88          | 58  | 58        | 58           |
| Other vegetables | 91    | 96   | 91  | 96           | 91          | 117 | 117       | 117          |
| Potatoes         | 40    | 40   | 40  | 40           | 40          | 40  | 40        | 40           |
| Fruits           | 61    | 61   | 68  | 221          | 228         | 65  | 65        | 65           |
| Rice             | 158   | 108  | 78  | 98           | 78          | 55  | 55        | 55           |
| Breads           | 61    | 61   | 61  | 61           | 61          | 61  | 61        | 61           |
| Sugar            | 31    | 18   | 24  | 8            | 27          | 34  | 34        | 34           |
| Fats and oils    | 11    | 11   | 25  | 11           | 25          | 20  | 20        | 20           |
| Soft drinks*     | 420   | 210  | 210 | 210          | —           | 200 | 200       | 200          |
| SAA*            | —     | —    | —   | —            | —           | 2.0 | 2.0       | 2.0          |
| KHCO₃            | —     | —    | —   | —            | —           | 3.0 | —         | —            |

*a* Soy protein isolate (Fujipro R; Fuji Oil Co., Osaka, Japan). *b* Carbonated beverages containing sugar but not fruit juice. *c* Sulfur-containing amino acids; 1.0 g of methionine and 1.0 g of cystine.
Table 3. Nutrient content in the experimental diets.

| Expt. diet  | Experiments 1 and 2 | Experiment 3 |
|-------------|---------------------|--------------|
|             | Basal | Meat | SPI | Meat + Apple | SPI + Apple | SPI | SPI + SAA | SPI + SAA, K |
| Protein (g)a| 55.1  | 101.0 | 99.2 | 101.2 | 98.9 | 101.5 | 101.7 | 101.6 |
| SAA (g)b   | 1.82  | 3.72  | 3.03 | 3.71  | 3.03 | 3.36  | 5.35  | 5.35  |
| Ca (mg)b   | 543   | 544   | 609  | 549   | 615  | 610   | 605   | 604   |
| P (mg)a    | 1,047 | 1,399 | 1,386 | 1,404 | 1,396 | 1,272 | 1,273 | 1,268 |
| P/Ca       | 1.9   | 2.6   | 2.3  | 2.6   | 2.3  | 2.1   | 2.1   | 2.1   |
| K (g)b     | 2.14  | 2.83  | 2.09 | 3.00  | 2.26 | 0.97  | 0.98  | 2.14  |
| Fe (mg)b   | 8.4   | 11.0  | 8.8  | 11.3  | 9.0  | 9.1   | 9.0   | 9.2   |
| Fats (%)b  | 30.3  | 29.0  | 30.3 | 27.5  | 28.9 | 23.1  | 23.0  | 24.1  |
| Crude fiber (g)b | 5.8 | 5.5 | 5.1 | 11.7 | 11.3 | 5.8 | 5.7 | 5.8 |
| Vitamin A (IU)b | 2,253 | 2,278 | 2,338 | 2,278 | 2,339 | 2,663 | 2,599 | 2,688 |
| Vitamin B1 (mg)b | 1.19 | 2.97 | 2.81 | 2.95  | 2.80 | 0.85 | 0.92 | 0.95 |
| Vitamin B2 (mg)b | 1.05 | 1.60 | 1.53 | 1.53  | 1.60 | 1.13 | 1.13 | 1.14 |
| Vitamin C (mg)b | 62.8 | 66.1 | 66.3 | 70.9  | 71.1 | 57.6 | 62.7 | 68.2 |

*a Measured on lyophilized samples. b Calculated from the Food Composition Table.

Calcium was measured by flame atomic absorption spectrometry (Hitachi, model 170-5A) by addition of the strontium, potassium was by flame photometry (Tokyo Koden, model ANA-10BL), and urinary pH was measured with a pH meter (Toa, model HM-18ET). Urinary phosphate, ammonia, and creatinine were measured by the method of Fiske-Subbarow, by phenol-hypochlorite reaction and by Jaffé's reaction, respectively.

Statistical methods. The significance of difference of the results was tested by the paired Student's t test for the data in each experiment of 1, 2, and 3 or the Student's t test for the data of other experiments in comparing them to each other.

RESULTS

Table 3 shows the nutrient contents of the experimental diets. Protein intake in high protein diet periods was about 100 g, which was twice that of basal diet or break period in all three experiments. SAA content increased in the high protein diet and was higher in meat diet than SPI diet. Potassium content increased by the addition of apple to meat or SPI diet. In experiment 3, potassium and/or SAA contents of the experimental diets were markedly increased by the supplement of SAA and/or potassium bicarbonate.

Intake, urinary, and fecal excretions, apparent absorption of calcium, and calcium balance are summarized in Table 4. Meat diet increased urinary calcium excretion significantly and slightly improved (not statistically significant) the
Table 4. Intake, urinary and fecal excretions, apparent absorption and balance of calcium.*

| Experiment 1 | Experiment 2 | Experiment 3 |
|--------------|--------------|--------------|
|              | Basal        | Meat         | SPI          | Basal        | Meat + Apple | SPI + Apple | SPI          | SPI + SAA | SPI + SAA, K |
| Ca intake (mg/day) | 543 ± 3<sup>b</sup>  | 544 ± 4<sup>e,d</sup> | 609 ± 4<sup>b,c,d</sup> | 543 ± 2<sup>b</sup> | 548 ± 1<sup>b,c,d</sup> | 615 ± 2<sup>b,c,d</sup> | 610 ± 3 | 605 ± 6 | 604 ± 6 |
| Urinary Ca (mg/day)  | 145 ± 48<sup>b</sup> | 174 ± 41<sup>b,c</sup> | 143 ± 43<sup>c</sup> | 153 ± 65 | 154 ± 80<sup>c</sup> | 136 ± 70<sup>c</sup> | 116 ± 38<sup>b</sup> | 150 ± 47<sup>b,c</sup> | 122 ± 41<sup>c</sup> |
| Fecal Ca (mg/day)  | 456 ± 56 | 407 ± 97<sup>c,e</sup> | 511 ± 63<sup>c</sup> | 460 ± 178 | 509 ± 202 | 513 ± 139 | 477 ± 84 | 497 ± 93<sup>e</sup> | 505 ± 71 |
| Absorption (%)  | 16 ± 10 | 25 ± 18 | 16 ± 10 | 15 ± 33 | 7.2 ± 37 | 16 ± 23 | 22 ± 14 | 18 ± 15 | 16 ± 12 |
| Balance (mg/day)  | −58 ± 37 | −37 ± 62 | −45 ± 30<sup>e</sup> | −70 ± 149 | −114 ± 172 | −34 ± 108 | 25 ± 7<sup>7,b,e</sup> | −40 ± 80<sup>b</sup> | −23 ± 66<sup>b</sup> |

*Means ± SD for seven (experiment 1), six (experiment 2), and fourteen (experiment 3) subjects. <sup>b</sup>Significantly different from the value for basal diet period in experiments 1 and 2 or for SPI diet period in experiment 3 (p<0.05). <sup>c</sup>Significantly different between the values for meat diet period and SPI diet period in experiment 1, for meat+apple period and SPI+apple period in experiment 2 or for SPI+SAA period and SPI+SAA,K period in experiment 3 (p<0.05). <sup>d</sup>Significantly different between the values for meat diet period and meat+apple period or the values for SPI diet period and SPI+apple period (p<0.05). <sup>e</sup>Significantly different between the values for meat diet period and SPI+SAA diet period or for SPI diet periods in experiments 1 and 3 (p<0.05).
apparent absorption of calcium compared with those of basal diet period. In SPI diet period, calcium absorption and urinary excretion of calcium were as low as those of basal period. In meat + apple diet period, fecal calcium excretion increased, calcium absorption decreased and urinary calcium excretion decreased to the level of basal period. Addition of apple to high SPI diet did not affect calcium absorption, urinary or fecal excretion of calcium. SAA-supplemented diet had a significant negative effect on calcium retention, caused by a significant increase in urinary calcium, but there was no change in fecal excretion or absorption of calcium. In SPI+SAA,K diet period, urinary calcium decreased significantly but no change in fecal excretion or absorption of calcium was observed.

Figure 2 shows the change in urinary pH and excretion of calcium, sulfur, potassium, ammonia, titratable acids, and creatinine. Urinary creatinine increased in meat and meat+apple diet periods. The extents of increment were much as those observed in high meat diets we reported elsewhere (14). In other experimental periods, urinary creatinine excretion was constant in subjects in all three experiments. Meat diet increased urinary titratable acids, phosphate, sulfur, potassium, and ammonia excretion compared to basal diet. SPI diet increased the excretion of phosphate and sulfur but not titratable acids, potassium or ammonia excretion. Apple increased potassium excretion when added to meat diet. Supplementation of SAA to SPI diet decreased urinary pH, increased the excretion of titratable acids, phosphate, sulfur, potassium, and ammonia in a manner similar to meat diet. In SPI+SAA,K diet period urinary potassium excretion increased markedly, and increased excretion of ammonia and titratable acids by the supplement of SAA was reversed. These results observed in SPI+SAA,K diet period were similar to those in meat + apple diet period.

DISCUSSION

Many investigators observed that the hypercalciuria caused by the high protein intake was due to the decrease in fractional tubular reabsorption of calcium and to the increase in glomerular filtration rate (8, 9, 15–17). Hegsted and Linkswiler (9) reported that these changes were exhibited long term, i.e., over the 60-day experimental period.

It was also demonstrated that high protein diets produced an increase in total renal acid, ammonium, and sulfate excretions whereas urinary sodium excretion decreased in human subjects (17–19). Zemel et al. (20) showed that SAA added to the low protein diet also caused urinary calcium to increased. The catabolism of SAA is the major source of endogenous acid production. Acid stress directly inhibits the renal tubular reabsorption of calcium and high levels of dietary protein also impair renal calcium reabsorption. Schuette et al. (19) mentioned that at low calcium intakes, protein or SAA-induced increases in urinary calcium resulted in increased bone reabsorption indicated by increases in urinary hydroxyproline excretion. Furthermore, Allen et al. (21) pointed out that the consumption of high
Fig. 2. Means (±SD) of urinary pH, titratable acidity (TA), sulfur, and of phosphate, potassium, ammonia, and creatinine excretion. *p<0.05, **p<0.01.
calcium diets was unlikely to prevent the negative calcium balance and probable bone loss induced by the consumption of high protein diets.

In the present study, daily intake of calcium was about 0.6 g and was almost constant throughout the series of experiments. In the first experiment, the high protein diet of added meat caused a hypercalciuria with slight improvement of calcium absorption, increased excretion of urinary acid and other constituents whereas SPI high protein diet did not. In the third experiment, addition of SAA to SPI high protein diet also increased urinary loss of calcium and other components except phosphorus without any improvement of absorption. The results showed that increased intake of SAA in meat induced hypercalciuria mainly by impairment of renal calcium reabsorption.

When the phosphorus intake was raised, decrease in urinary calcium excretion and improved calcium balance at the high protein intake have been observed (10, 19, 20, 22, 23). Phosphate supplement achieving P/Ca ratio from twice to five times diminished hypercalciuria in these experiments. The investigators suggest a decrease in parathyroid hormone-mediated bone reabsorption by phosphate supplement.

In the present study, phosphorus intake and P/Ca ratio were held rather constant at 1.0 g to 1.4 g and 1.9 to 2.6, respectively. So, phosphorus did not contribute mainly to decrease of urinary calcium observed in meat+apple diet and SPI+SAA,K diet periods compared with meat diet and SPI+SAA diet periods, respectively. It is more likely that high potassium content in these diets caused increased calcium retention. Lutz (18) observed that sodium bicarbonate ingestion alkalized the urine and reversed the increase in urinary calcium associated with the higher protein intake. Increased intake of potassium-rich foods would alleviate acid stress and consequently reduce the inhibition of the renal tubular reabsorption of calcium.

Fecal calcium excretion significantly decreased in meat diet period caused by decrease in fecal dry weight compared to basal diet period. Addition of apple reversed the decreased of fecal calcium excretion and resulted in deterioration of calcium retention. Kelsay et al. (24) also reported a significantly lower calcium balance on the high fiber diet from fruits and vegetables than on the low fiber diet. In the present study, addition of SPI increased the concentration of calcium in dry feces significantly and daily fecal excretion of calcium, although this was not statistically significant, compared with low protein diet. Addition of apple to SPI diet caused further effect on the fecal excretion of calcium, although this was not statistically significant, compared with low protein diet. Addition of vegetable protein on calcium absorption and retention should be elucidated in detail in the future.

In experiments 1 and 2, the mean of calcium balance was negative in all groups given different dietary treatment in spite of the intake being as much as the calcium allowance recommended by the Ministry of Health and Welfare (Japan) (26).
subjects complained about their inactivity in the metabolic unit and on the college campus during the experimental period compared to the time before the experimental period. Then, in experiment 3 we requested the subjects to walk in their daily living, and to report daily the time of standing and the amount of activities measured by pedometer. As a result, the subjects became somewhat active; they stood daily for about 5 h and the mean pedometer counts were about 10,000 in a day. Thus, improvement in calcium retention of the subjects in SPI diet period in experiment 3 compared to those in experiments 1 is assumed to be related to their daily activity, although calcium intake or other dietary conditions are similar in both experimental periods. On the other hand, Kitano et al. (27) reported there was no significant change in urinary calcium excretion and calcium balance when their Japanese male subjects exercised on a bicycle ergometer for two 1-h periods daily. The effect of exercise or activities on calcium metabolism is to be elucidated in further study.

Large coefficients of variation of urinary and fecal calcium excretions were observed under the strict dietary condition in the present experiments. Those as large as in our subjects were also found in the papers by other investigators (9, 15, 18, 23, 25). It might be partly caused by the difference in endogenous factors, for example hormonal condition, and the activities of individual subjects. The effects of these factors on calcium metabolism should be elucidated in the future.

From the present results and the facts discussed above, we conclude that SPI has not any hypercalciuria effect observed in the case of meat as the intake of high protein, because of lower content of SAA, and potassium diminishes a detrimental effect caused by high meat diet. Some kinds of dietary fiber and daily activity of the individual are likely to affect the absorption and retention of calcium. However, in the present study the contribution of dietary contents of calcium and phosphate to improve calcium retention has not been clarified. The effect of high protein diets on calcium nutrure over a lifetime, in older males and females and adolescent boys and girls should be elucidated in the future. Studies on effective ways to decrease urinary calcium concentration are needed to lower not only the incidence of osteoporosis but also the risk of calcium stone-formation in the urinary tract (28).

REFERENCES

1) Sherman, H. C. (1920): Calcium requirement of maintenance in man. J. Biol. Chem., 44, 21–27.
2) Hawks, J. E., Bray, M. M., Wilde, M. O., and Dya, M. (1942): The interrelationship of calcium, phosphorus and nitrogen in the metabolism of pre-school children. J. Nutr., 24, 283–288.
3) McCance, R. A., Widdowson, E. M., and Lehmann, H. (1942): The effect of protein intake on the absorption of calcium and magnesium. J. Biochem., 36, 681–691.
4) Knapp, E. L. (1957): Factors influencing the urinary excretion of calcium. I. In normal persons. Biochem. J., 38, 117–121.
5) Johnson, N. E., Alcantala, E. N., and Linkswiler, H. M. (1970): Effect of level of protein intake on urinary and fecal calcium and calcium retention of young adult males. J.

J. Nutr. Sci. Vitaminol.
HYPERCALCIURIA BY S-AMINO ACIDS

Nutr., 100, 1425–1430.
6) Walker, R. M., and Linkswiler, H. M. (1972): Calcium retention in the adult human males as affected by protein intake. J. Nutr., 102, 1297–1302.
7) Anand, C. R., and Linkswiler, H. M. (1974): Effect of protein intake on calcium balance of young men given 500 mg calcium daily. J. Nutr., 104, 695–700.
8) Kim, Y., and Linkswiler, H. M. (1974): Effect of level of protein intake on calcium metabolism and on parathyroid and renal function in the adult human male. J. Nutr., 109, 1399–1404.
9) Hegsted, M., and Linkswiler, H. M. (1981): Long-term effects of level of protein intake on calcium metabolism in young adult women. J. Nutr., 111, 244–251.
10) Hegsted, M., Schuette, S. A., Zemel, M. B., and Linkswiler, H. M. (1981): Urinary calcium balance in young men as affected by level of protein and phosphorus intake. J. Nutr., 111, 553–562.
11) Whiting, S. J., and Draper, H. H. (1980): The role of sulfate in the calciuria of high protein diets in adult rats. J. Nutr., 110, 212–222.
12) Lemann, J., Lennon, E. J., and Brock, J. (1966): A potential error in the measurement of urinary titratable acid. J. Lab. Clin. Med., 67, 906–913.
13) Jackson, S. G., and McCandless, E. L. (1978): Simple, rapid, turbidometric determination of inorganic sulfate and/or protein. Anal. Biochem., 90, 802–808.
14) Kaneko, K., Amagai, S., and Koike, G. (1976): Effect of meat or protein intake on daily creatinine excretion in urine. J. Jpn. Soc. Food Nutr., 36, 341–345.
15) Chu, J. Y., Margen, S., and Costa, S. M. (1975): Studies in calcium metabolism. II. Effects of low calcium and variable protein intake on human calcium metabolism. Am. J. Clin. Nutr., 28, 1028–1035.
16) Allen, L. H., Bartlett, R. S., and Block, G. D. (1979): Reduction of renal calcium absorption in man by consumption of dietary protein. J. Nutr., 109, 1345–1350.
17) Schuette, S. A., Zemel, M. B., and Linkswiler, H. M. (1980): Studies on the mechanism of protein-induced hypercalciuria in older men and women. J. Nutr., 110, 305–315.
18) Lutz, J. (1984): Calcium balance and acid-base status of women as affected by increased protein intake and by sodium bicarbonate ingestion. Am. J. Clin. Nutr., 39, 281–288.
19) Schuette, S. A., Hegsted, M., Zemel, M. B., and Linkswiler, H. M. (1981): Renal acid, urinary cyclic AMP, and hydroxyproline excretion as affected by level of protein, sulfur amino acid, and phosphorus intake. J. Nutr., 111, 2106–2116.
20) Zemel, M. B., Schuette, S. A., Hegsted, M., and Linkswiler, H. M. (1981): Role of sulfur-containing amino acids in protein-induced hypercalciuria in men. J. Nutr., 111, 545–552.
21) Allen, L. H., Oddoye, E. A., and Margen, S. (1979): Protein-induced hypercalciuria: a longer term study. Am. J. Clin. Nutr., 32, 741–749.
22) Zemel, M. B., and Linkswiler, H. M. (1981): Calcium metabolism in the young adult male as affected by level and form of phosphorus intake and level of calcium. J. Nutr., 111, 315–324.
23) Schuette, S. A., and Linkswiler, H. M. (1982): Effects on Ca and P metabolism in humans by adding meat, meat plus milk, or purified proteins plus Ca and P to a low protein diet. J. Nutr., 112, 338–349.
24) Kelsay, J. L., Behall, K. M., and Prather, E. S. (1979): Effect of fiber from fruits and vegetables on metabolic responses of human subjects. I. Calcium, magnesium, iron, and silicon balances. Am. J. Clin. Nutr., 32, 1876–1880.
25) Dokkum, W. V., Wesstra, A., Luyken, R., and Hermus, R. J. J. (1986): The effects of
a high-animal and a high-vegetable-protein diet on mineral balance and bowel function by young men. *Br. J. Nutr.*, 56, 341–348.

26) Committee on Nutrition (Japan) (1979): Dietary calcium allowances, *in* Recommended Dietary Allowances for Japanese, Ministry of Health and Welfare, Tokyo.

27) Kitano, T., Esashi, T., and Azami, S. (1988): Effect of protein intake on mineral (calcium, magnesium, and phosphorus) balance in Japanese males. *J. Nutr. Sci. Vitaminol.*, 34, 387–398.

28) Robertson, W. G., Heyburn, P. J., Peacock, M., Hanes, F. A., and Swaminathan, R. (1979): The effect of high animal protein intake on the risk of calcium stone-formation in the urinary tract. *Clin. Sci.*, 57, 285–288.