Effect of Protein Intake on Mineral (Calcium, Magnesium, and Phosphorus) Balance in Japanese Males

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Summary A 26-day balance study was conducted to examine the effect of a high protein diet on calcium, magnesium, and phosphorus utilization in six healthy males (age 20–22 years, body weight 54.0–64.4 kg, body height 165–173 cm). In addition, the effect of physical exercise on calcium, magnesium, and phosphorus balance was also examined. After a 2-day stabilization period, two levels of protein—control (1.0 g/kg of body weight) and high (2.0 g/kg of body weight) protein—diets were given for three 4-day periods at each protein level. During the last 4-day period of each protein level, subjects exercised on a bicycle ergometer for two 1-h periods daily at a load of 1.5 kp., 50 cyc./min. The high protein diet increased urinary calcium and caused a significant negative calcium balance. Magnesium balance tended to be negative in the control diet. There were no significant changes in urinary calcium, magnesium, and phosphorus and also in the calcium, magnesium, and phosphorus balance during physical exercise.

Key Words protein, calcium, magnesium, phosphorus, exercise, balance study

As early as 1920 Sherman found that the addition of meat to a low calcium diet caused an increase in urinary calcium in men (1). Later, other investigators made the same observation (2, 3). But McCance et al. reported that an increase in protein intake was accompanied by an increase in urinary calcium, and attributed this to an increase in absorption (4). Therefore, high protein intake was supposed to increase the calcium balance.

However, in recent years Linkswiler and her co-workers found that high protein intake was the cause of an increase in urinary calcium but did not find any
increases in absorption of calcium even under very strict conditions of examination, and suggested that over 100 g per day of protein intake disturbed calcium utilization (5). On the other hand, Japanese adults averaged 85.7 ± 19.5 g of protein intake according to the national survey of nutrition by the Ministry of Health and Welfare (6). Therefore, about 35% of Japanese adults had an intake of over 100 g of protein per day.

This paper reported the effects of increasing the protein from the Recommended Dietary Allowance (RDA) level (1.18 g/kg) to twice the RDA level on urinary and fecal calcium, phosphorus, and magnesium and on calcium, phosphorus, and magnesium balance in Japanese adult males.

MATERIALS AND METHODS

Subjects. The subjects were six male students who described themselves as being healthy. None were taking medication. Relevant information about the subjects is given in Table 1. The subjects lived in the metabolic unit of the National Institute of Nutrition during the experimental periods. Except for the dietary and other restrictions necessary to conduct the experiments, the subjects were free to engage in normal activities. The subjects clearly understood that they were to eat all foods given them and were not to eat any other foods or drink any other beverage except deionized water which was allowed ad libitum. The experimental protocol was approved by the committee of the National Institute of Nutrition on the use of humans as experimental subjects.

Study condition. Six subjects participated in the 26-day metabolic study. The study consisted of a 2-day stabilization period followed by six 4-day experimental periods. During the 2-day stabilization period all subjects were fed the high protein diet, and during the experimental periods the subjects were given the control and the high protein diets for three 4-day periods, respectively. The order of feeding level of protein to each subject is given in Table 2 and was such that at any given time three subjects were being fed the control diet, and three subjects were receiving the high protein diet. In experimental period III, the subjects were exercised on a bicycle.

Table 1. Subject characteristics.

| Subject (no) | Age (years) | Weight (kg) | Height (cm) |
|--------------|-------------|-------------|-------------|
| 1            | 22          | 65.7        | 173.1       |
| 2            | 21          | 61.0        | 169.2       |
| 3            | 21          | 64.4        | 165.0       |
| 4            | 21          | 54.0        | 166.5       |
| 5            | 20          | 61.2        | 166.5       |
| 6            | 21          | 63.3        | 167.1       |

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Table 2. Dietary protein supply program.

| Period | Subjects |
|--------|----------|
|        | 1  | 2  | 3  | 4  | 5  | 6  |
| Stabilization (2 days) | high | high | high | high | high | high |
| I 1–4 days | high |
| II 5–8 days | high |
| III 9–12 days* | high |
| I 13–16 days | control |
| II 17–20 days | control |
| III 21–24 days* | control |

*The subjects exercised on a bicycle ergometer for two 1-h periods daily during the experimental period at load of 1.5 kp., 50 cyc./min.

Table 3. Composition of basal formula diet (in the case of 50 kg body weight).

| Component                  | Control (g/day) | High protein (g/day) |
|----------------------------|-----------------|----------------------|
| Ground beef                | 95              | 240                  |
| Whole egg, spray-dried     | 10              | 20                   |
| Egg white, spray-dried     | 7.5             | 15                   |
| Skim milk                  | 15              | 45                   |
| Rice                       | 200             | 200                  |
| Soft flour                 | 15              | 15                   |
| Corn starch                | 35              | 10                   |
| Sugar                      | 40              | 10                   |
| Mashed potatoes, dried     | 15              | 15                   |
| Vegetable oil              | 37.5            | 15                   |
| Kidney beans               | 30              | 30                   |
| Green peas                 | 30              | 30                   |
| Sweet corn                 | 50              | 50                   |
| Pumpkin                    | 45              | 45                   |
| Tomato, canned             | 25              | 25                   |
| Spinach                    | 45              | 45                   |
| Carrot                     | 50              | 50                   |
| Onion                      | 25              | 25                   |
| Strawberry                 | 45              | 45                   |
| Fruit juice (orange)       | 100             | 100                  |
| Fruit juice (grapefruit)   | 100             | 100                  |
| Agar                       | 2.5             | 2.5                  |
| Calcium monophosphate      | 1.40            | 0                    |
ergometer for two 1-h periods daily at a load of 1.5 kp., 50 cyc./min.

**Diet.** The basal diet was the same for all subjects. The main protein source was ground beef. Both the control and the high protein diets consisted of ordinary foods as shown in Table 3. Daily intake of selected nutrients is shown in Table 4. The control diet followed the Recommended Dietary Allowance for Japanese adult men. The high protein diet provided twice the RDA level. Each subject was given nutrients which were calculated based on the body weight on the first day of each period.

**Sample collection.** Complete collections of urine and feces were made from all subjects during the entire study. Urine collections were made for each subject on a 24-h basis. Creatinine excretion was determined daily on the 24-h composites to estimate the completeness of the urine collections. Fecal collections were made on a 4-day period composite basis. Carmine markers were used to separate the fecal collections for each experimental period. The fecal markers were given at breakfast on the first day of the period. Feces were stored in a cooling box until the end of the collection period. Each 4-day fecal composite was homogenized completely in a blender. Blood was drawn from the antecubital vein before breakfast on the last day of each period.

**Analysis.** Food, fecal and urine samples were digested by wet ashing. Samples were digested with concentrated nitric acid and PCA mixture (1:1). The digests were made up to volume with 0.5 N HCl solution. Calcium and magnesium were measured by flame atomic absorption spectrometry (Varian Techtron AA-5) used in addition to the strontium method. Phosphorus was measured by the molybdenum method of Gomori.

### RESULTS

The effect of the level of protein intake on urinary calcium, apparent calcium absorption and calcium balance is shown in Table 5. Urinary calcium excretion of

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**Table 4. Nutrients given to subjects (per kg of body weight).**

|                 | Control       | High protein |
|-----------------|---------------|--------------|
| Protein (g)     | 1.0           | 2.0          |
| Energy (kcal)   | 40            | 40           |
| Fat             | 20–25% of energy |
| Proteinb (g)    | 1.18          | 2.17         |
| Calcium (mg)    | 12.4          | 13.5         |
| Phosphorus (mg) | 25.4          | 28.0         |
| Magnesium (mg)  | 3.67          | 5.05         |

* Calculated from food table.  † Calculated from nitrogen (N × 6.25).
An increase in protein intake caused the mean value of urinary calcium to increase significantly from $104 \pm 11$, $114 \pm 12$, and $118 \pm 14$ mg to $189 \pm 18$, $173 \pm 10$, and $169 \pm 13$ mg per day, respectively ($p < 0.01$). But exercise load did not increase the mean value of urinary calcium in either diet group. The mean value of calcium absorption did not change through the experimental period. The effect of the level of protein intake on calcium balance varied among the subjects. However, the mean value of calcium balance decreased significantly from $51.5 \pm 21.7$, $40.0 \pm 14.6$, and $49.0 \pm 19.9$ to $-10.3 \pm 23.5$, $-8.50 \pm 26.6$, and $-23.5 \pm 13.7$ mg per day, respectively ($p < 0.05$).
Table 5. Calcium balance.

| Period          | Intake  | Fecal output | Absorption | Urinary output | Retention |
|-----------------|---------|--------------|------------|----------------|-----------|
|                 |         | I (1–4 days) | II (5–8 days) | III (9–12 days) | I (1–4 days) | II (5–8 days) | III (9–12 days) | I (1–4 days) | II (5–8 days) | III (9–12 days) |
| Control (mg)    | 765 ± 21 | 609 ± 21 | 610 ± 12 | 600 ± 18 | 156 ± 29 | 154 ± 24 | 167 ± 26 | 104 ± 11 | 114 ± 12 | 118 ± 14 | 51.5 ± 21.7 | 40.0 ± 14.6 | 49.0 ± 19.9 |
| % of intake     | 97.9 ± 3.6 | 80.2 ± 2.7 | 78.5 ± 3.3 | 79.0 ± 2.8 | 686 ± 8 | 82.6 ± 1.5 | 21.0 ± 2.8 | 178 ± 27 | 165 ± 32 | 147 ± 16 | 6.50 ± 2.78 | 5.04 ± 1.87 | 6.23 ± 2.60 |
| High protein (mg) | 832 ± 22 | 656 ± 11 | 667 ± 10 | 686 ± 8 | 20.1 ± 3.6 | 19.8 ± 2.7 | 21.5 ± 3.0 | 149 ± 18 | 173 ± 10* | 169 ± 13** | -10.3 ± 23.5* | -8.50 ± 26.6* | -23.5 ± 13.7** |
| % of intake     | 97.9 ± 3.6 | 80.2 ± 2.7 | 78.5 ± 3.3 | 79.0 ± 2.8 | 686 ± 8 | 82.6 ± 1.5 | 21.0 ± 2.8 | 178 ± 27 | 165 ± 32 | 147 ± 16 | 6.50 ± 2.78 | 5.04 ± 1.87 | 6.23 ± 2.60 |

Mean ± SE. There was a statistically significant difference at $p < 0.01$ (**) and $p < 0.05$ (*) between control diet and high protein diet groups by paired $t$ test.
### Table 6. Magnesium balance.

| Period       | Intake         | Absorption | Retention     |
|--------------|----------------|------------|---------------|
|              | Control (mg)   | High protein (mg) | Control (mg)   | High protein (mg)   |
| I (1-4 days) | 218±6          | 300±9*     | 84.3±8.3      | 28.7±2.1*           |
| II (5-8 days)| 146±8          | 200±10*    | 87.9±3.8      | 40.1±1.7            |
| III (9-12 days)| 66.9±4.1     | 66.6±2.6   | 84.3±8.3      | 40.4±1.7            |
| I (1-4 days) | 139±7          | 209±9*     | 40.1±1.7      | 13.5±10.4*          |
| II (5-8 days)| 65.0±2.3       | 70.1±2.6   | 84.3±8.3      | 40.4±1.7            |
| III (9-12 days)| 63.9±3.1     | 68.5±5.2   | 13.5±10.4*    | 40.4±1.7            |
| % of intake  | 100±7*         | 100±7*     | 50±5.2        | 50±5.2              |

Mean ± SE. There was a statistically significant difference at p<0.05 (*) between control diet and high protein diet groups by paired t test.
Table 7. Phosphorus balance.

| Period          | Intake     | Fecal output | Absorption | Urinary output | Retention |
|-----------------|------------|--------------|------------|----------------|-----------|
|                 |            | I (1–4 days) | II (5–8 days) | III (9–12 days) | I (1–4 days) | II (5–8 days) | III (9–12 days) | I (1–4 days) | II (5–8 days) | III (9–12 days) |
| Control (mg)    | 1,510 ± 42 | 279 ± 16     | 289 ± 16   | 276 ± 13       | 1,230 ± 47 | 1,224 ± 47  | 1,233 ± 44     | 171 ± 74     | 47.2 ± 55.8  | 104 ± 51        |
| % of intake     |            | 18.5 ± 1.3   | 19.2 ± 1.2 | 18.4 ± 1.0     | 81.6 ± 1.3 | 80.8 ± 1.2 | 81.6 ± 1.0     | 11.0 ± 4.7   | 2.78 ± 3.68 | 6.53 ± 3.31    |
| High protein (mg) | 1,669 ± 48 | 307 ± 10     | 297 ± 10   | 321 ± 21       | 1,355 ± 42*| 1,370 ± 52*| 1,355 ± 56*    | 344 ± 57.7*  | 280 ± 73*   | 164 ± 40        |
| % of intake     |            | 18.5 ± 0.6   | 17.9 ± 0.9 | 19.3 ± 1.4     | 81.5 ± 0.6 | 82.1 ± 0.9 | 80.7 ± 1.4     | 20.6 ± 3.2*  | 16.7 ± 4.1* | 9.63 ± 2.26    |

Mean ± SE. There was a statistically significant difference at $p<0.05(*)$ between control diet and high protein diet groups by paired $t$ test.
Table 8. Effect of protein intake on serum total protein, albumin, and minerals.

|          | T. Protein (g/100 ml) | Albumin (g/100 ml) | Calcium (mg/100 ml) | Phosphorus (mg/100 ml) | Magnesium (mg/100 ml) |
|----------|-----------------------|--------------------|---------------------|------------------------|----------------------|
| Initial  | 7.69 ± 0.09           | 4.92 ± 0.04        | 10.1 ± 0.1          | 3.82 ± 0.23            | 1.98 ± 0.05          |
| Control diet period |
| I (1–4 days) | 7.38 ± 0.17          | 4.94 ± 0.07        | 10.2 ± 0.1          | 3.51 ± 0.20            | 2.01 ± 0.05          |
| II (5–8 days) | 7.49 ± 0.21          | 4.80 ± 0.10        | 10.2 ± 0.1          | 3.52 ± 0.22            | 2.05 ± 0.06          |
| III (9–12 days) | 7.48 ± 0.13         | 4.80 ± 0.08        | 10.3 ± 0.1          | 3.54 ± 0.15            | 2.02 ± 0.07          |
| High protein diet period |
| I (1–4 days) | 7.72 ± 0.08          | 4.82 ± 0.08        | 10.3 ± 0.1          | 3.54 ± 0.09            | 2.02 ± 0.04          |
| II (5–8 days) | 7.62 ± 0.12          | 4.94 ± 0.08        | 10.2 ± 0.1          | 3.48 ± 0.12            | 2.00 ± 0.03          |
| III (9–12 days) | 7.54 ± 0.09         | 4.87 ± 0.08        | 10.3 ± 0.1          | 3.34 ± 0.10            | 2.03 ± 0.05          |

Mean ± SE.

Magnesium balance results are shown in Table 6. Magnesium contents of the control and the high protein diets were 218 ± 6 mg and 300 ± 9 mg, respectively, and this difference was significant (p < 0.05). There were significant changes in the mean value of magnesium absorption between control and high protein diets. However, the rate of magnesium absorption did not change through the experimental period. The mean value of urinary magnesium was not affected by the level of protein intake. In the case of the control diet, magnesium balances showed negative values. There was no significant change in magnesium utilization during the exercise period.

Phosphorus balance results are shown in Table 7. Absorption and balance of phosphorus were significantly affected by the level of protein intake (p < 0.05). But urinary phosphorus was not affected by the level of protein intake. There was no significant change in phosphorus utilization during the exercise period.

Serum total protein, albumin, calcium, phosphorus, and magnesium levels are shown in Table 8. None of these were affected by the level of protein intake.

**DISCUSSION**

The data presented here agree with those of previous studies indicating that a high level of dietary protein causes increased urinary calcium excretion (7–9). The dramatic increases in urinary calcium which followed increases in protein intake were not accompanied by comparable increases of calcium absorption. However, McCance et al. indicated that the increase of urinary calcium by high protein intake was caused by the increased absorption of calcium (4). In recent years other investigators have suggested that high protein intake did not change the absorption of calcium but did increase the urinary calcium (10–12).

It would appear, therefore, that the increase in urine calcium is not due to increased intestinal absorption of calcium. We did not measure endogenous calcium
secretion in this experiment, but it seems unlikely that it reduced sufficiently to account for the increase in urinary calcium.

On the other hand, some investigators have suggested urinary calcium excretion was affected by exercise (13–15). In this study urinary calcium was not decreased by exercise at a given level of diet. Further study is needed to clarify whether or not there is a correlation between exercise load and urinary calcium.

Calcium balance was shown to be negative at the RDA level of calcium when protein intake was high. This observation is similar to the results reported elsewhere that a high protein intake over 100 g per day and calcium intake ranging from 500 to 800 mg clearly showed a negative calcium balance (16, 17). Spencer et al. suggested that high protein from purified protein sources effected the calcium balance, but that a high protein diet using usual protein sources did not affect the calcium balance (18, 19). Our study indicated that the calcium balance was negative when using usual protein sources such as egg white, whole egg, skim milk, etc. Marsh et al. reported that there were different findings in the bone density between omnivorous and vegetarian groups (20, 21). Other investigators observed that bone density of Eskimos was less than that of Caucasians (22). From these points of view, it appears that a high protein intake from usual protein sources is not available for calcium metabolism. But epidemiological observations are needed to clarify the relationship between calcium and protein intake in practical situations.

RDA of magnesium for Japanese is not clear (23). Magnesium intake of Japanese adults ranged from 200 to 300 mg per day according to the report of Kimura et al. (24). Goto presented similar data in which magnesium intake was about 250 mg per day in adult men (25). From the present data, magnesium balances were negative in the control diet containing 218 ± 18 mg per day (3.67 mg per kg of body weight), but were not negative in the high protein diet containing 300 ± 21 mg per day (5.05 mg per kg of body weight). Schuette et al. indicated that magnesium utilization was not affected by the level of protein intake (26). Mahalko et al. suggested that magnesium utilization was correlated to magnesium intake level more than to the level of protein intake (27). Nordin suggested that the magnesium requirement for adults was about 4.0 mg per kg of body weight (28). RDA of magnesium for adults in the U.S.A. and Canada was 5.0 mg and 4.3 mg per kg of body weight, respectively (29). From these surveys and experimental data, the present level of magnesium consumption may be too low for Japanese. However, experimental data are lacking concerning the magnesium balance in Japanese.

Phosphorus utilization was increased by the high protein diet. This observation was similar to the findings of other phosphorus balance studies (30, 31).

This study was designed in conformity to rule on Declaration of Helsinki.

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