Effects of High Protein Diet and Sodium Bicarbonate Supplementation on Calcium Metabolism in Rats

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Summary This study was conducted to determine the effect of a high protein diet on calcium metabolism in rat. Wistar strain male rats (50 days old) were divided into 5 groups (day 0): control diet (18% casein); high protein diet (18% casein + 20% lactalbumin); high protein and 0.1% sodium bicarbonate diet; high protein and 0.2% sodium bicarbonate diet; and high protein and 0.4% sodium bicarbonate diet. On days 0, 1, 3, 5, 7, 9, urine samples were collected and, at the same time, feces were collected from half of the animals in each group. Urinary titratable acidity (TA-HCO₃⁻), ammonium ion (NH₄⁺), and net acid excretion (NAE) were measured as an index of acid-base balance in rat body. Urinary volume was rapidly increased and the increase of urinary volume continued throughout the study in rats fed the high protein diet. Urinary excretions of calcium and phosphorus were increased after day 3 and day 1, respectively, in rats fed the high protein diet. The high protein diet depressed calcium absorption and elevated phosphorus absorption from the digestive tract in rats fed the high protein diet. The high protein diet decreased TA-HCO₃⁻, which was closely correlated with the decrease of NAE. Sodium bicarbonate supplementation to the high protein diet had little effect on urinary calcium excretion and NAE. This study suggested that there was no relationship between metabolic acidosis and hypercalciuria in rats fed the high protein diet.

Key Words calcium metabolism, urinary net acid excretion, acid-base balance, high protein diet, sodium bicarbonate, rat

It has been reported that a high protein intake causes an increase in urinary calcium excretion in man (1, 2) and rat (3, 4). And some workers indicated that protein-induced hypercalciuria resulted from a decrease in fractional renal calcium reabsorption (5–7). However, the mechanism(s) by which a high protein diet acts on renal tubule is controversial.

Linkswiler and co-workers have suggested that a high protein intake causes...
mild chronic acidosis, which inhibits renal calcium reabsorption (5). Whiting and Draper have demonstrated that sulfate which is derived from sulfur-containing amino acids' catabolism make a complex with free calcium in the renal tubule ultrafiltrate and this complex decreases calcium reabsorption (6).

This study was conducted to determine whether protein-induced hypercalciuria was attributed to mild chronic acidosis or not. Urinary net acid excretion was used as an index of systemic acid-base balance in this study.

MATERIALS AND METHODS

Thirty Wistar strain male rats of 50 days old were used. They were housed in metabolic cages with the room temperature at 24°C and a 12-h light-dark cycle.

For 5 days before the study, they were fed control diet (C). Then, they were

Table 1. Composition and mineral content of experimental diets.

|            | C   | HPtn | HPtn + NaHCO₃^a |
|------------|-----|------|-----------------|
|            | (g/kg diet) |     |                 |
| Casein     | 180 | 180  | 180             |
| Lactalbumin|     | 201  | 201             |
| Dextrose   | 555 | 359  | 359             |
| Lard       | 150 | 150  | 150             |
| Corn Oil   | 50  | 50   | 50              |
| Cellulose  | 25  | 25   | 25              |
| Vitamin Mix^b | 6.0 | 6.0  | 6.0             |
| Mineral Mix^c | 14.7 | 14.7 | 10.7–13.7      |
| CaCO₃      | 12.1| 10.2 | 10.2            |
| Ca(H₂PO₄)₂H₂O| 6.8 | 3.8  | 3.8             |
| NaHCO₃     |     | —    | 1.0–4.0         |

|            | (mg/g diet) |     |                 |
|------------|-------------|-----|-----------------|
| Calcium    | 5.9         | 4.6 | 4.6             |
| Phosphorus | 2.0         | 2.5 | 2.5             |
| Magnesium  | 4.0         | 3.4 | 3.4             |
| Sodium     | 16.3        | 55.4| 55.4            |
| Potassium  | 4.3         | 7.8 | 7.8             |

^a C, HPtn, HPtn + NaHCO₃ represent control diet, high protein diet, and sodium bicarbonate supplementation to the high protein diet, respectively. ^b One gram of vitamin mixture contains 500 IU of vitamin A, 100 IU of vitamin D₃, 5 mg of vitamin E acetate, 5.2 mg of vitamin K, 1.2 mg of vitamin B₁, 4 mg of vitamin B₂, 0.8 mg of vitamin B₆, 4 mg of vitamin B₁₂, 30 mg of vitamin C, 20 μg of d-biotin, 200 μg of folic acid, 5 mg of Ca-pantothenate, 5 mg of p-aminobenzoic acid, 6 mg of nicotinic acid, 6 mg of inositol, and 200 mg of choline chloride. ^c Supplying (mg/kg diet): MgCO₃, 6,900; ZnCO₃, 96; FeSO₄·7H₂O, 124; CuSO₄·5H₂O, 20; MnSO₄·H₂O, 150; KI, 1.3; NaCl, 2,300; Na₂CO₃, 1,600; K₂CO₃, 3,530; Na₂SeO₃, 0.22.
divided into 5 groups: control diet (C); high protein diet (HPtn); high protein and 0.1% sodium bicarbonate diet (0.1%); high protein and 0.2% sodium bicarbonate diet (0.2%); high protein and 0.4% sodium bicarbonate diet (0.4%). The diet composition and mineral contents are shown in Table 1. Dietary composition was similar to that of Whiting and Draper (6), which extremely induced hypercalciuria. Each group was offered 20.0 g/day of diet individually and free access to distilled water.

Feed intake was recorded daily and body weight was measured at three-day intervals. Urine samples (24 h) were collected under liquid paraffin on days 0, 1, 3, 5, 7, 9. At the same time, feces were collected from half of the animals in each group. Urinary volume was recorded and urinary excretion rates of calcium and phosphorus were measured by atomic absorption spectrophotometry and the method of Gomori (8), respectively. After being wet-ashed, the contents of calcium and phosphorus in feed and fecal samples were analyzed by the same method. Magnesium in food samples was analyzed by atomic absorption spectrophotometry, sodium and potassium by flame emission spectrophotometry. To measure urinary net acid excretion, a urine sample was boiled with hydrochloric acid to remove all CO₂. After cooling at room temperature, the urine sample was titrated to pH 7.40 using Potentiometric Automatic Titrator (Kyoto Electronics, AT-118) (TA-HCO₃⁻). Formaldehyde (8%) solution was added to the urine sample titrated to

![Graph of daily body weight gain and feed conversion](image-url)
Table 2. Effects of the high protein diet and/or sodium bicarbonate supplementation on urinary volume and urinary excretion rates of calcium and phosphorus.

|       | C       | HPtn    | 0.1%    | 0.2%    | 0.4%    |
|-------|---------|---------|---------|---------|---------|
|       | Urinary volume (ml/day) |       |         |         |         |
| day 0 | 5.70    | 6.73    | 5.51    | 6.34    | 6.29    |
|       | ± 1.56  | ± 4.33  | ± 1.26  | ± 1.02  | ± 1.44  |
| day 1 | 5.77a   | 10.96b  | 10.68b  | 10.33b  | 10.95b  |
|       | ± 2.79  | ± 4.76  | ± 3.64  | ± 2.15  | ± 3.12  |
| day 3 | 5.00a   | 12.38b  | 11.49b  | 12.70b  | 12.16b  |
|       | ± 1.47  | ± 3.32  | ± 2.54  | ± 2.60  | ± 2.50  |
| day 5 | 6.27a   | 12.33b  | 11.49b  | 12.35b  | 12.50b  |
|       | ± 2.92  | ± 3.22  | ± 2.99  | ± 3.73  | ± 1.80  |
| day 7 | 6.13a   | 11.99b  | 11.09b  | 12.20b  | 11.67b  |
|       | ± 2.00  | ± 2.65  | ± 3.28  | ± 4.31  | ± 1.96  |
| day 9 | 6.62a   | 12.80b  | 12.38b  | 11.11b  | 11.69b  |
|       | ± 2.17  | ± 2.30  | ± 4.67  | ± 3.90  | ± 1.91  |
|       | Urinary calcium (mg/day) |       |         |         |         |
| day 0 | 0.54    | 0.59    | 0.43    | 0.55    | 0.53    |
|       | ± 0.26  | ± 0.19  | ± 0.09  | ± 0.16  | ± 0.30  |
| day 1 | 0.59    | 0.82    | 0.74    | 0.98    | 0.72    |
|       | ± 0.16  | ± 0.46  | ± 0.21  | ± 0.43  | ± 0.08  |
| day 3 | 0.65a   | 1.34b   | 1.15a,b  | 0.97a,b  | 0.77a   |
|       | ± 0.33  | ± 0.52  | ± 0.47  | ± 0.26  | ± 0.32  |
| day 5 | 0.52a   | 1.13b   | 1.30b   | 1.16b   | 0.95b   |
|       | ± 0.18  | ± 0.52  | ± 0.39  | ± 0.25  | ± 0.33  |
| day 7 | 0.81a   | 0.97a,b  | 1.26b   | 1.02a,b  | 0.93a,b  |
|       | ± 0.25  | ± 0.43  | ± 0.30  | ± 0.39  | ± 0.23  |
| day 9 | 0.66a   | 1.46b   | 1.28b   | 1.17a,b  | 1.09b   |
|       | ± 0.24  | ± 0.68  | ± 0.38  | ± 0.55  | ± 0.34  |
|       | Urinary phosphorus (mg/day) |       |         |         |         |
| day 0 | 7.84a,b  | 6.16a   | 9.31b   | 10.39a,b  | 9.10a,b  |
|       | ± 3.93  | ± 2.14  | ± 1.78  | ± 5.43  | ± 4.25  |
| day 1 | 7.02    | 9.05    | 10.06   | 11.29   | 13.18   |
|       | ± 3.40  | ± 4.11  | ± 6.25  | ± 3.60  | ± 6.03  |
| day 3 | 4.72a   | 14.96b  | 11.55b  | 12.74b  | 13.71b  |
|       | ± 2.74  | ± 2.88  | ± 6.27  | ± 5.67  | ± 6.58  |
| day 5 | 4.75a   | 9.15a,b  | 12.91b  | 10.51a,b  | 14.51b  |
|       | ± 2.27  | ± 7.78  | ± 7.50  | ± 6.75  | ± 6.72  |
| day 7 | 4.21a   | 15.08b  | 10.45a,b  | 8.61a,b  | 14.61b  |
|       | ± 1.97  | ± 2.97  | ± 7.77  | ± 7.48  | ± 6.58  |
| day 9 | 4.71a   | 11.82b  | 8.88a,b  | 10.28a,b  | 12.14a,b  |
|       | ± 2.54  | ± 6.49  | ± 6.49  | ± 7.67  | ± 8.33  |

All values are mean ± SD for 6 rats. a,bMeans within a row that do not have a common letter in their superscripts differ (p<0.05).
pH 7.40 using NaOH. The amount of NaOH is equal to NH₄⁺. Urinary net acid excretion (NAE) was estimated by TA-HCO₃⁻ plus NH₄⁺ (9, 10).

The effects of dietary treatment were statistically analyzed by Student’s t-test.

RESULTS

Body weight gains were slightly decreased in the HPtn group. Feed intakes were depressed in rats fed the high protein diet and feed conversions were insignificantly affected by the dietary treatment (Fig. 1).

Urinary volume and urinary excretion rate of calcium and phosphorus are shown in Table 2. Rats fed high protein diets rapidly exhibited the increase in urinary volume, and this continued throughout the study. Urinary calcium excretion on day 1 was unaffected by the dietary treatment, while on day 3 the high protein diet increased urinary calcium at the amount of 206% of the control. Hypercalciuria continued in rats fed the high protein diet. As was the case for urinary calcium, the high protein diet increased urinary phosphorus excretion after day 3. The degree

Table 3. Effects of the high protein diet and/or sodium bicarbonate supplementation on the calcium and phosphorus balance to the rate of intake on day 5, 7, 9.

|                  | C       | HPtn    | 0.1%    | 0.2%    | 0.4%    |
|------------------|---------|---------|---------|---------|---------|
| Calcium          |         |         |         |         |         |
| Intake           | 100     | 100     | 100     | 100     | 100     |
| Feces            | 54.3ᵃ   | 61.2ᵃᵇ  | 46.2ᵃᵇ  | 43.2ᵇ   | 49.4ᵃᵇ  |
| ± 10.7           | ± 25.1  | ± 16.4  | ± 6.9   | ± 9.4   |         |
| Urine            | 0.82ᵃ   | 1.93ᵇ   | 1.94ᵇ   | 1.86ᵇ   | 1.58ᵇ   |
| ± 0.39           | ± 0.90  | ± 0.66  | ± 0.37  | ± 0.51  |         |
| Absorbed         | 45.7ᵃ   | 38.8ᵃᵇ  | 53.8ᵃᵇ  | 56.8ᵇ   | 50.6ᵃᵇ  |
| ± 10.7           | ± 25.1  | ± 16.4  | ± 6.9   | ± 9.4   |         |
| Retained         | 44.9ᵃ   | 36.9ᵃᵇ  | 51.9ᵃᵇ  | 54.9ᵇ   | 48.4ᵃᵇ  |
| ± 10.9           | ± 25.2  | ± 16.6  | ± 7.1   | ± 9.5   |         |
| Phosphorus       |         |         |         |         |         |
| Intake           | 100     | 100     | 100     | 100     | 100     |
| Feces            | 39.9ᵃ   | 18.5ᵇ   | 18.6ᵇ   | 17.3ᵇ   | 21.0ᵇ   |
| ± 9.9            | ± 6.2   | ± 9.5   | ± 4.1   | ± 7.6   |         |
| Urine            | 11.7ᵃ   | 34.8ᵇᶜ  | 16.8ᵃᶜ  | 32.4ᵇᶜ  | 39.7ᵇ   |
| ± 6.8            | ± 24.7  | ± 15.6  | ± 21.5  | ± 26.6  |         |
| Absorbed         | 60.1ᵃ   | 81.5ᵇ   | 81.4ᵇ   | 82.7ᵇ   | 79.0ᵇ   |
| ± 9.9            | ± 6.2   | ± 9.5   | ± 4.1   | ± 7.6   |         |
| Retained         | 48.4ᵃ   | 46.7ᵃᵇᶜ | 66.6ᵇᶜ  | 50.4ᵇᶜ  | 37.3ᵃ   |
| ± 14.8           | ± 23.3  | ± 16.2  | ± 23.4  | ± 29.5  |         |

All values are means ± SD for 3 rats.ᵃᵇᶜ Means within a row that do not have a common letter in their superscripts differ (p<0.05).
Table 4. Effects of the high protein diet and/or sodium bicarbonate supplementation on net acid excretion (NAE).

|       | C     | HPtn | 0.1%   | 0.2%   | 0.4%   |
|-------|-------|------|--------|--------|--------|
| NAE (mEq/day) |
| day 0 | 0.37 ± 0.16 | 0.45 ± 0.41 | 0.41 ± 0.10 | 0.53 ± 0.37 | 0.30 ± 0.22 |
| day 1 | 0.35 ± 0.13 | -0.34b ± 0.22b | -0.22b ± 0.34 | -0.14b ± 0.11 | -0.29b ± 0.35 |
| day 3 | 0.22a ± 0.13 | -0.34b ± 0.10b | -0.10b ± 0.21 | -0.02b ± 0.16 | -0.08b ± 0.17 |
| day 5 | 0.16a ± 0.16 | -0.12ab ± 0.33 | 0.02ab ± 0.31 | 0.07ab ± 0.25 | -0.05b ± 0.14 |
| day 7 | 0.18a ± 0.18 | -0.27b ± 0.01ab | -0.01ab ± 0.13abc | -0.03abc ± 0.18 | -0.20b ± 0.20 |
| day 9 | 0.21a ± 0.08 | -0.24b ± 0.23 | 0.03ab ± 0.20 | 0.00ab ± 0.18 | -0.76ab ± 1.91 |

All values are means ± SD for 6 rats. a,b,c Means within a row that do not have a common letter in their superscripts differ (p<0.05).

Table 3 shows that the high protein diet did not increase phosphaturia but reduced NAE. Decreased TA-HCO₃⁻ in rats fed the high protein diet might be closely correlated with the reduction of NAE, although the high protein diet slightly increased urinary NH₄⁺ excretion. The decrease in TA-HCO₃⁻ was observed from day 1 in rats fed the high protein diet. Sodium bicarbonate supplementation to the high protein diet had little effect on excretion of TA-HCO₃⁻, NH₄⁺, and NAE.
Table 5. Effects of the high protein diet and/or sodium bicarbonate supplementation on urinary titratable acidity (TA-HCO$_3^-$) and ammonium ion (NH$_4^+$) excretion.

|       | C          | HPtn       | 0.1%      | 0.2%      | 0.4%      |
|-------|------------|------------|-----------|-----------|-----------|
|       | TA-HCO$_3^-$ (mEq/day) |            |           |           |           |
| day 0 | -0.11 ± 0.14 | -0.16 ± 0.11 | -0.11 ± 0.08 | -0.05 ± 0.19 | -0.18 ± 0.14 |
| day 1 | -0.10 ± 0.08 | -1.20 ± 0.72 | -1.13 ± 1.02 | -0.72 ± 0.15 | -0.87 ± 0.36 |
| day 3 | -0.28 ± 0.17 | -1.38 ± 0.74 | -1.34 ± 1.12 | -0.79 ± 0.27 | -1.29 ± 1.39 |
| day 5 | -0.33 ± 0.17 | -1.71 ± 1.20 | -1.38 ± 1.12 | -1.32 ± 1.27 | -1.07 ± 1.39 |
| day 7 | -0.60 ± 0.87 | -1.26 ± 0.67 | -1.48 ± 1.32 | -1.73 ± 1.34 | -1.11 ± 0.78 |
| day 9 | -0.47 ± 0.56 | -1.97 ± 1.50 | -1.50 ± 1.45 | -1.76 ± 1.62 | -1.56 ± 1.59 |

|       | NH$_4^+$ (mEq/day) |            |           |           |           |
| day 0 | 0.48 ± 0.10 | 0.61 ± 0.38 | 0.52 ± 0.15 | 0.57 ± 0.23 | 0.48 ± 0.15 |
| day 1 | 0.45 ± 0.17 | 0.88 ± 0.61 | 0.90 ± 0.82 | 0.58 ± 0.16 | 0.57 ± 0.16 |
| day 3 | 0.50 ± 0.19 | 1.04 ± 0.80 | 1.24 ± 1.00 | 0.77 ± 1.16 | 1.21 ± 1.35 |
| day 5 | 0.49 ± 0.14 | 1.59 ± 0.89 | 1.40 ± 1.05 | 1.00 ± 1.14 | 1.38 ± 1.35 |
| day 7 | 0.79 ± 0.92 | 0.99 ± 0.59 | 1.47 ± 1.18 | 1.86 ± 1.28 | 1.07 ± 0.79 |
| day 9 | 0.68 ± 0.52 | 1.67 ± 1.49 | 1.53 ± 1.53 | 1.75 ± 1.75 | 0.80 ± 0.80 |

All values are means ± SD for 6 rats. a,b Means within a row that do not have a common letter in their superscripts differ (p < 0.05).

DISCUSSION

The high protein diet in this study caused the increase in urinary calcium excretion. This effect was observed immediately after the feeding of high protein diet. And the degree of the hypercalciuria was the most prominent on day 3 in rats fed the high protein diet. Similar to this study, Whiting and Draper have shown that high protein diet caused a sharp increase in calcium excretion (6). But the extent of increase in urinary calcium excretion was larger in their study than in this study. Although the reason why the difference in the extent of hypercalciuria occurred was not clear, the difference might be attributable to the age of animals. Whiting and Draper used adult male rats; in contrast, young male rats were used.
in this study. The response of urinary calcium excretion to a high protein diet might be more sensitive in older rats than in younger ones.

It was observed that the high protein diet induced hypercalciuria; however, sodium bicarbonate supplementation to the high protein diet did not effect a decrease in urinary calcium excretion. Also, NAE, as an index of systemic acid-base balance, did not increase in rats fed the high protein diet throughout the study. This indicates that protein-induced hypercalciuria did not result from mild metabolic acidosis. Draper and co-workers showed that urinary pH was unaffected by high protein level ingestion and suggested that the high protein diet did not induce metabolic acidosis (11). Compared to men, rats may be capable of maintaining normal acid-base balance under conditions of increased acid production (12). In other words, hydrogen ions which derived from oxidation of excess amino acids might be within a physiological range (5).

It was observed in this experiment that the high protein diet increased urinary phosphorus excretion. Since parathyroid hormone (PTH) inhibits renal tubule reabsorption of phosphorus and stimulates urinary excretion of phosphorus (13), the increase in urinary phosphorus excretion cannot be attributed to a reduction in activity of PTH. Alternatively, renal tubule reabsorption of calcium and phosphorus might be directly inhibited under conditions of increased urea and ammonia productions, i.e., increased urea productions elevated osmotic diuresis, leading to the decrease in reabsorbable calcium and phosphorus. Elevated urinary volume and NH₄⁺ excretion appear to support this possibility.

It was shown, in man, with a high protein food intake, that hypercalciuria was not responsible for increased intestinal calcium absorption (14, 15). In this study, the high protein diet tended to reduce calcium absorption from the digestive tract of rats. There may be two possibilities with respect to the causes of reduced calcium absorption in rats fed the high protein diet.

Moyer et al. showed that a high protein diet tended to depress the body weight gain and the body weight was correlated with the mucosal weight in the duodenum and ileum. And they suggested that the reduced mucosal growth in the duodenum and ileum by the high protein diet led to the decrease in calcium absorption (16). Another possibility is the formation of calcium complex in the digestive tract. The strong binding of calcium to albumin (Kₐ = 10 liter/mol, pH = 7.40) (17) might diminish the concentration of free calcium in the digestive tract, leading to the depression of calcium absorption.

The high affinity of calcium with albumin might account for the increase in phosphorus absorption. In other words, free phosphorus level might be elevated as a result of calcium and albumin binding in the digestive tract. The effect of pH on binding of calcium to albumin is controversial in in vitro study (18). Sodium bicarbonate supplementation to the high protein diet improved the decreased calcium absorption in this study. Although rising pH was thought to weaken the affinity of calcium for albumin in the gut, further study is needed to clarify the effect of pH in the digestive tract on calcium absorption.

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