Balances of Calcium, Magnesium and Phosphorus in Japanese Young Adults

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Summary This study was conducted to estimate the requirements of calcium (Ca), magnesium (Mg) and phosphorus (P) in Japanese young adults. From 1986 to 2000, 109 volunteers (23 males, 86 females), ranging from 18 to 28 y old, took part in mineral balance studies after written informed consent was obtained. The duration of the study periods ranged from 5 to 12 d, with 2–4 d of adaptation. Foodstuffs used in each study were selected from those commercially available. The minerals present in diet, feces, urine and sweat were measured by atomic absorption spectrophotometer (Ca, Mg) or spectrophotometer (P). The dietary intakes of Ca, Mg and P ranged from 4.83–23.58, 2.44–7.83 and 13.46–45.69 mg/kg BW/d, respectively. Dietary intake (Intake) of Ca was positively correlated to apparent absorption (A.A.) (r²=0.425), which was also correlated with urine excretion (Urine) (r²=0.327) and balance (Bal) (r²=0.382). Intake of Ca was slightly but significantly correlated with Bal (r²=0.036, p=0.048). The mean value and upper limit of the 95% confidence interval for the regression equation between Intake and Bal when balance is equal to zero (Mean and upper limit) for Ca were 11.752 and 12.555 mg/kg BW/d, respectively. Intake of Mg was positively correlated to A.A. (r²=0.451), which was also correlated with Urine (r²=0.306, p=0.048). The mean value and upper limit of the 95% confidence interval for the regression equation between Intake and Bal when balance is equal to zero (Mean and upper limit) for Mg were 11.752 and 12.555 mg/kg BW/d, respectively. Intake of P was positively correlated to A.A. (r²=0.959), which was also correlated with both Urine (r²=0.908) and Bal (r²=0.135). Intake of P was slightly but significantly correlated with Bal (r²=0.103, p=0.0013). Mean and upper limits for P were 22.584 and 24.059 mg/kg BW/d, respectively. Intakes of Mg and P correlated negatively with their respective A.A. rates (%) (r²=0.120 for Mg, r²=0.109 for P). However, there was not much of a correlation for Ca. Balance of Ca was positively correlated with that of Mg (r²=0.541), but not with that of P.

Key Words human, calcium, magnesium, phosphorus, balance

SUBJECTS AND METHODS

From 1986 to 2000, 109 volunteers (23 males, 86 females), ranging from 18 to 28 y old, took part in mineral balance studies after written informed consent was obtained. The ethical committee, established by the...
Table 1. Subjects and dietary intake of energy and nutrients.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Sex</th>
<th>Sub n</th>
<th>Duration (d)</th>
<th>Energy (kcal/d)</th>
<th>Protein (g/d)</th>
<th>Fat (% of energy)</th>
<th>Intake of minerals</th>
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<td></td>
<td></td>
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<td>8</td>
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<td>294</td>
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<tr>
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<td>65</td>
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<tr>
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<tr>
<td>8</td>
<td>m</td>
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<td>89</td>
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<tr>
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<td>5</td>
<td>3,250</td>
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<td>719</td>
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</table>

Total: 109

* Mg (180 mg/d) was added to the diet as magnesium oxide (MgO).
** Low-calcium study.
# Mineral lost during exercise was estimated (n=49).

National Institute of Health and Nutrition in 1990, approved the studies. All studies were carried out in the Humanities Ward of the National Institute of Health and Nutrition.

The duration of the balance study periods ranged from 5 to 12 d, with 2–4 d of adaptation period. Body temperature and weight in the morning were measured throughout the experiment in all subjects. However, menstrual cycle for female subjects was not taken into consideration in these studies.

In each study, the same quantity of the diet, which varied in each experiment, was supplied to each of the subjects during the balance period without consideration of body weight.

However, small changes to the diet were carried out during the adaptation period so as to ensure consumption of all the food supplied.

The subjects ingested a coloring marker for their feces (Carmine 0.5 g: Merk KgaA, Germany) just before breakfast in the morning at the beginning and the end of the balance period. In one study (No. 8, Table 1), magnesium oxide was added to the diet (5). In six studies (Nos. 4–7, 9, and 10, Table 1) (n=49), sweat from the arm during exercise on a bicycle ergometer (intensity: 1–1.5 kp, velocity: 50–60 rpm, duration: 30–60 min/trial, frequency: once or twice a day, room temperature: 22–29°C, humidity: 40–65% RH) was collected to estimate element loss through sweat (Table 1).

The foodstuffs used in each study were selected from those commercially available. Some foodstuffs were avoided because of a heterogeneous content of nutrients revealed through chemical measurements taken before the studies. Both processed and nonperishable foodstuffs were purchased at the same time from the same lot before the experiments so as to ensure the same content of nutrients. Fresh foodstuffs were obtained from the same district by way of the same market.

Dietary menus were designed by a registered dietician so as to meet dietary allowances in Japan (8), except for the low calcium studies (Nos. 1 and 2, Table 1) for which food composition tables were used (9).

All foodstuffs were washed with ion-free water (passed through an ion-exchange resin) if necessary, weighed, cooked separately, and distributed uniformly to dishes for the subjects and diet sample(s).

The subjects were required to consume all of the diet. They were allowed no other food, but could drink as much ion-free water as they wanted. The weight of the water consumed was measured and recorded.

Duplicated diet samples were obtained throughout the experiments and kept in a refrigerator for 1 d until blended.

For blending, refrigerated diet samples were weighed and put into a mixer (MX150S, National, Japan). An adequate volume of the ion-free water was added, and then the mixture was gradually homogenized for about 30 min using a slide trans (RIKO-SLIDETRANS RSA-5, Tokyo-Rikosha, Japan) attached to the mixer.

The homogenized diet samples were prepared in triplicate and weighed in polypropylene bottles. Five milliliters of nitric acid (UGR grade, Kanto Chemical Co., Inc., Japan) was then added and the mixture was kept at room temperature until digestion.

Digestion was undertaken in hard glass beakers (Pyrex, Iwaki Glass, Japan) on a hot plate at temperatures below 140°C, using nitric acid and hydrogen peroxide (for trace analysis, Wako Pure Chemical Industries Ltd., Japan). The interior of the bottle was washed by the nitric acid that was added to the sample in the beakers.

After digestion, an adequate volume of pure water
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(Milli-Q, Millipore, Japan) was added to the samples, which were then put on a hot plate at temperatures below 90°C for one night to measure the phosphorus content. Then 0.5 N nitric acid was added to attain a fixed volume. Calcium (Ca) and magnesium (Mg) were measured by an atomic absorption spectrophotometer (AAS, Varian AA-5, Australia) after the mixture was diluted to an appropriate concentration with 0.5 N nitric acid. When measuring Ca, a final concentration of 2,500 ppm of strontium (strontium chloride hexahydrate, for AAS analysis, Wako Pure Chemical Industries, Ltd.) was added to the samples to avoid interference due to P. The height of the burner was adjusted to get the same absorption factor for the same Ca concentration of different pH values, thereby avoiding interference due to pH. Phosphorus was measured using a colorimeter (Molybdenum blue method) (7, 10).

Fecal specimens were collected throughout the experiment and were separated into those originating from the diet during the balance periods based on the ingested coloring marker appearing in the feces. Fecal samples were measured in the same way as the diet samples. Urine samples were directly diluted by 0.5 N nitric acid, and measured in the same way as the other samples.

Arm sweat during exercise was collected after cleaning the skin surface of one side of the hand and arm with pure water and ion-free gauze treated with ethylene-diamine-tetraacetic acid and diammonium salt (EDTA: Wako Pure Chemical Industries, Ltd.), covering the whole arm with a long polyethylene bag wrapped with tape. The collected sweat was filtered with a 0.10 μm pore size Teflon filter (Fluoro pore, Sumitomo Electric Industries, Ltd., Japan) and ethanol (for trace analysis, Wako Pure Chemical Industries, Ltd.) to remove any solids. Sweat volume was calculated by the weight loss during exercise as estimated by a balance (sensitivity 10 g) (7). Total sweat loss of minerals during exercise throughout the balance period was divided by the days of the balance period, and estimated as the sweat loss (mg/kg BW/d).

In order to correct various characteristics of the subjects, such as sex, weight and other traits, all data were expressed as mg/kg BW/d.

Using these data, the apparent absorption and balance of these minerals were calculated, and regression equations were computed for Ca, Mg and P. Statistics were obtained by StatView-J5.0.

Some indicators in this paper are defined as follows:

- Apparent absorption = (Intake) - (Fecal output) mg/kg BW/d
- Apparent absorption (%) = [(Intake) - (Fecal output)]/ (Intake) × 100 (%)
- Balance = (Intake) - ([Fecal output] + [Urine output] + [Sweat loss]) mg/kg BW/d

*Only when sweat loss during exercise was estimated.

RESULTS

In one study, phosphorus was not determined, so the number of balance studies for P was 97. The concentration of P in sweat was too low to measure.

The dietary intake of Ca, Mg and P ranged from 294–1,131, 154–379 and 807–2,198 mg/d, respectively (Table 1). The weight ratio of Ca/Mg (g/g) in the diets ranged from 1.56 to 4.39. The highest value of Ca/Mg in the diet was obtained in the Mg restriction experiment. Intakes of these three minerals were positively correlated with each other (Fig. 1).

In terms of body weight, the dietary intake of Ca, Mg and P ranged from 4.83–23.58, 2.44–7.83 and 13.46–45.69 mg/kg BW/d, respectively. The relationships between dietary intake, apparent absorption, urine output and balance for Ca, Mg and P are shown in Figs. 2–4.

Intake of Ca was positively correlated with apparent absorption (A.A.) (r²=0.425), which was also correlated with both urine output (Urine) (r²=0.327) and balance (Bal) (r²=0.382). Intake of Ca was slightly but significantly correlated with Bal (r²=0.036, p=0.048). The mean value and upper limit of the 95% confidence interval for the regression equation between Intake and Bal when balance is equal to zero were 11.752 and 12.555 mg/kg BW/d, respectively.

Intake of Mg was positively correlated with A.A. (r²=0.451), which was also correlated with both urine output (Urine) (r²=0.486) and balance (Bal) (r²=0.349). However, the Intake of Mg was not correlated with Bal. Using the above regression equations (Intake vs. A.A. and A.A. vs. Bal), the mean value of Mg intake when balance is equal to zero was indirectly calculated to be 4.395 mg/kg BW/d.

Intake of P was positively correlated with A.A. (r²=0.959), which was also correlated with both Urine...
Fig. 2. Dietary intake, apparent absorption, urine output and balance of calcium (Ca) (mg/kg BW/d) \((n=109)\). Mean value and upper limit of 95% confidence interval of dietary intake at balance=0 are 11.752 and 12.555 mg/kg BW/d, respectively.

Fig. 3. Dietary intake, apparent absorption, urine output and balance of magnesium (Mg) (mg/kg BW/d) \((n=109)\). Dietary intake of Mg was not correlated with Mg balance, although Mg absorption was positively correlated with both balance and dietary intake of Mg. Using relations between the intake vs. apparent absorption and apparent absorption vs. balance, the mean value of dietary intake of Mg at balance=0 was calculated to be 4.395 mg/kg BW/d \((=1.484\times1.787+1.743)\).

Fig. 4. Dietary intake, apparent absorption, urine output and balance of phosphorus (P) (mg/kg BW/d) \((n=97)\). Mean value and upper limit of 95% confidence interval of dietary intake at balance=0 are 22.584 and 24.059 mg/kg BW/d, respectively.
(r²=0.908) and Bal (r²=0.135). Intake of P was slightly but significantly correlated with Bal (r²=0.103, p=0.0013). The mean value and upper limit of the 95% confidence interval for the regression equation between Intake and Bal when balance is equal to zero were 22.584 and 24.059 mg/kg BW/d, respectively.

The relationships between dietary intake and A.A. rate (%) of the minerals are shown in Fig. 5. Intake of Mg and P correlated negatively with their respective A.A. rates (%) (r²=0.120 for Mg, r²=0.109 for P). However, this correlation was not observed for Ca.

Correlation coefficient matrixes for each item are shown in Table 2. The sweat concentrations of P were below the limits of measurement. The sweat loss of Mg ranged from 0.00 to 0.06 (0.01±0.01, mean±SD) mg/kg BW/d, and contributed little to the Mg balance. However, that of Ca ranged from 0.04 to 0.19 (0.10±0.04, mean±SD) mg/kg BW/d, and contributed slightly to the Ca balance. The relationships between the balances of Ca, Mg and P are shown in Fig. 6. The balance for Ca is positively correlated with that for Mg, but not with that for P.

**DISCUSSION**

This is the first paper to give the relations between dietary intake and balance of Ca, Mg and P in Japanese young adults based on the experimental data of 109 subjects.

Eighty-six out of 109 subjects were females. Adding to this, male subjects ingested a high-energy diet (No. 11, Table 1), and high- or low-Mg diets (No. 8, Table 1). These heterogeneous experimental conditions may affect the results of this analysis. However, these data are important in giving a wide range of dietary intake of Ca, Mg and P. In this paper, results of balances studied were expressed as divided by the body weight (kg). This treatment can be partly compensated for sex difference.

**Relationships between dietary intakes and balances of Ca, Mg and P**

There were weak but significant correlations between dietary intake and balance for Ca and P, but not for Mg. The dietary intake of Ca was well correlated with apparent absorption (r²=0.425). The apparent absorption was also well correlated with urine excretion (r²=0.382). These correlations resulted in a weak but significant positive correlation between the intake and balance of Ca (r²=0.036). The mean value and upper limit of the 95% confidence interval of the dietary intake of Ca when the balance of Ca is equal to zero were 11.752 and 12.555 mg/kg BW/d, respectively. This value is
somewhat higher than the recent Japanese RDA of Ca (10 mg/kg BW/d) (8).

On the other hand, the dietary intake of Ca explained only 3.6% of the balance of Ca. Other factors affecting the apparent absorption and urinary excretion of Ca, such as physical exercise (13–15), energy intake (15, 16), physical and mental stress (15, 17) and sodium intake (7), are thought to be more important in keeping the Ca balance positive than Ca intake itself. There seems to be no correlation between dietary intake and the apparent absorption ratio (%) of Ca (Fig. 5). This also suggested that the intake of Ca was not the main factor in regulating the intestinal absorption of Ca.

The dietary intake of Mg was well correlated with apparent absorption ($r^2=0.451$). The apparent absorption was also well correlated with urine excretion ($r^2=0.486$). However, this did not result in a significant correlation between the intake and balance of Mg ($r^2=0.018$). Dietary intake of Mg was not correlated with Mg balance, although apparent absorption of Mg was positively correlated with both the balance and dietary intake of Mg. Using relationships between both the intake vs. apparent absorption, and apparent absorption vs. balance, the mean value of the dietary intake of Mg when the balance is equal to zero was calculated to be 4.395 mg/kg BW/d. This value is close to that obtained from the non-significant regression equation between the intake and balance of Mg (4.548 mg/kg BW/d). Assuming the equation is of some value, the upper limit of the 95% confidence interval of the dietary intake of Mg when the balance of Mg is zero was 4.791 mg/kg BW/d. In a previous study without low-calcium experiments, reported in another paper (6), the intake value determined to keep the Mg balance at zero was 4.713 mg/kg BW/d, a little bit higher than in this report. However, both values are very near the recent Japanese RDA of 4.5 mg/kg BW/d (8).

On the other hand, the dietary intake of Mg explained only 1.8% of the balance of Mg. As with Ca, other factors affecting the apparent absorption and urinary excretion of Mg, such as physical exercise (13–
energy intake (15, 16) and physical and mental stress (15, 17), are thought to be more important for keeping the Mg balance positive than Mg intake itself. There seems to be some correlation between dietary intake and the apparent absorption ratio (%) of Mg ($r^2=0.120$) (Fig. 5). This also suggests that the intake of Mg is still one of the factors regulating the intestinal absorption of Mg. Furthermore, the balances of Ca and Mg were positively correlated ($r^2=0.541$) (Fig. 6), suggesting that the balances of the two minerals may be regulated in part by the same mechanism(s).

The dietary intake of P was well correlated with apparent absorption ($r^2=0.959$). The apparent absorption was also well correlated with urinary excretion ($r^2=0.908$). These correlations resulted in a weak but significant positive correlation between the intake and balance of P ($r^2=0.103$). The mean value and upper limit of the 95% confidence interval of the dietary intake of P when the balance of P is equal to zero were 22.584 and 24.059 mg/kg BW/d, respectively. This value is rather higher than the recent Japanese RDA of 700 mg/d (8).

Dietary intake of P explained only 10.3% of the balance of P. In the case of P, the apparent absorption was strongly correlated with both dietary intake and urine excretion, which suggests that regulation of intestinal absorption is weak. However, the regulation depends partly on dietary intake and 60–80% of ingested P is absorbed (Fig. 5). Although the intake of P was well correlated with the intakes of Ca and Mg, the balance of P was not correlated with the balances of Ca and Mg (Fig. 6). The mechanisms of P regulation seem to be separate, at least in part, from those of Ca and Mg.

In conclusion, within the ranges of this study the balances of Ca, Mg and P were strongly affected by factors other than the intake of the minerals themselves.

Acknowledgments

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REFERENCES