Comparison of the Effects of Whey Mineral Complexes on Bone Metabolism in Male Growing Rats

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Summary The effects of whey mineral complexes (WMC1 and WMC2) on bone metabolism were studied in male growing rats. The contents of Ca, P, and protein in WMC1 were 18.2, 8.4, 2.3%, respectively, whereas those of WMC2 were 26.4, 14.6, and 10.5%, respectively. WMCs were added to diets as a sole source of Ca: the levels of dietary Ca were 0.3 and 0.6%. CaCO3 was used as a reference. There was no difference in body weight gain and quantitative values for Ca balance among the groups at the same level of dietary Ca. Rats fed WMC2 had a higher femoral Ca and bone density of humerus. The bone properties in rats fed WMC1 were not as high as those in rats fed WMC2. The P absorption and absorption rate were affected significantly by the type of dietary Ca source as well as the levels of dietary Ca. The percent of tubular reabsorption of P of rats fed WMC1 or WMC2 had a tendency to be higher than that of rats fed CaCO3 at each dietary Ca level. The results of urinary cAMP excretion showed that the parathyroid hormone function in rats fed WMC2 was relatively lower. The differences in minerals and other constituents between WMC1 and WMC2 are discussed from the viewpoint of bone metabolism.

Key Words whey mineral complex, calcium absorption, phosphorus absorption, bone density, bone minerals, urinary cyclic AMP, urinary hydroxyproline

As a part of whey utilization, methods have been developed to recover the minerals from acid whey for mineral supplementation (1). Although inorganic phosphorus and calcium are the major components of minerals recovered, lactose, protein, or other milk components could be transferred from whey to the mineral preparation. Therefore, the composition of whey mineral preparation could vary due to the preparation method. Rats fed whey mineral complex showed high availability of Ca and Zn (2). Whether the mineral components of other substances are responsible for the high mineral utilization remains unclear (3).

In this study, chemical components of two whey mineral complexes prepared
by separate methods were compared and their effects on mineral utilization were studied.

MATERIALS AND METHODS

Mineral sources and diets. Two whey mineral complexes (WMC) were tested: WMC1 and WMC2. WMC was prepared from the permeate of ultrafiltrated acid whey (molecular weight cutoff, 2,000–3,000). The precipitate, which was formed by neutralizing with alkali solution, was directly concentrated by microfiltration (0.45 μm), and then spray-dried (WMC1). WMC2 was prepared by washing the precipitate with water and then spray-dried. The Ca and P contents of WMC2 were greater than those of WMC1, whereas the Na, K, and Cl in WMC2 were relatively low (Table 1). Protein profiles of the WMCs are shown in Fig. 1. Amino acid compositions of total nitrogenous compounds and non-nitrogenous compounds were analyzed (Fig. 2).

Diet composition is shown in Table 2. Diets contained 0.3 or 0.6% Ca as WMC1, WMC2, or CaCO$_3$ as a standard. The P level of all diets was 0.3%. Lactose was added to the diets to yield 0.8% which was contained in the 0.6% Ca level WMC1 diet. The contents of Ca, P, and Mg in each diet were analyzed (Table 2).

| Table 1. Chemical composition of whey mineral complex (WMC). |
|-------------------------------------------------------------|
| WMC1 (%) | WMC2 (%) |
|----------|----------|
| Moisture$^1$ | 5.9 | 9.0 |
| Protein$^2$ | 2.30 | 10.46 |
| NPN$^3$ | 0.23 | 0.22 |
| Fat$^4$ | 0.2 | 0.2 |
| Lactose$^5$ | 26.00 | 0.33 |
| Ash$^6$ | 50.2 | 73.6 |
| Ca$^7$ | 18.2 | 26.4 |
| P$^8$ | 8.4 | 14.6 |
| Na$^9$ | 1,300 | 234 |
| K$^9$ | 1,380 | 140 |
| Cl$^9$ | 2,220 | 142 |
| Mg$^7$ | 334 | 447 |
| Urea N$^{10}$ | 98.8 | 12.6 |

$^1$Weight loss after heating at 98°C for 5h. $^2$Kjeldahl N × 6.38. $^3$Non-protein nitrogen: Kjeldahl N of 12% trichloroacetic acid-soluble fraction. $^4$Rose-Gottlieb method. $^5$Enzymatic assay using a measuring kit (Boehringer Mannheim Yamano-uchi Co., Ltd., Japan). $^6$Ash weight after ignition at 550°C overnight. $^7$Atomic absorption spectrophotometry (Shimadzu, AA-640-13). $^8$Spectrophotometric method (4). $^9$Coulometric method using a chloride counter (Hiranuma Sangyo, CL-6L). $^{10}$Urease-indophenol method using a measuring kit (Wako Pure Chemical Ind. Ltd., Osaka, Japan).

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Fig. 1. SDS-PAGE patterns (15% acrylamide gel) of proteins from WMCs. Lane: 1, standard mixture of bovine serum albumin, β-lactoglobulin, and α-lactalbumin; 2, whey protein isolate; 3, whey mineral complex 1 (WMC1); 4, WMC 2. Molecular weight markers from top to bottom are myosin, β-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme, and aprotinin. Protein loads, 3 μg. Protein bands were stained with Coomassie brilliant blue R.

Fig. 2. Amino acid composition of total nitrogenous compounds and non-protein nitrogen (NPN) fraction in WMC1 and WMC2. Samples were digested with 1N HCl at 110°C for 22 h and analyzed by an amino acid analyzer (Hitachi-835).
Table 2. Composition and analysis of diets.

| Ingredient | 1 | 2 | 3 | 4 | 5 | 6 |
|------------|---|---|---|---|---|---|
| Casein¹    | 150 | 150 | 150 | 150 | 150 | 150 |
| Corn starch² | 467.0 | 458.6 | 471.2 | 451.3 | 470.1 | 454.6 |
| Glucose monohydrate³ | 200 | 200 | 200 | 200 | 200 | 200 |
| Soybean oil⁴  | 50 | 50 | 50 | 50 | 50 | 50 |
| Cellulose⁵   | 50 | 50 | 50 | 50 | 50 | 50 |
| Vitamin mix⁶  | 10 | 10 | 10 | 10 | 10 | 10 |
| L-Methionine⁷ | 2.3 | 2.3 | 2.3 | 2.3 | 2.3 | 2.3 |
| Choline bitartrate⁸ | 2 | 2 | 2 | 2 | 2 | 2 |
| Mineral mix⁹  | 35 | 35 | 35 | 35 | 35 | 35 |
| Lactose¹⁰    | 8 | 8 | 16 | 8 | 16 |
| KH₂PO₄¹¹     | 4.2 | 0.9 | 3.9 | 0.2 | 15.1 | 15.1 |
| KH₃PO₄¹¹     | 5.4 | 1.0 | 5.0 | 0.2 | — | — |
| Potassium citrate | — | 8.0 | 0.8 | 9.4 | — | — |
| WMC1¹¹      | 16.1 | 32.2 | | | | |
| WMC2        | 11.8 | 23.6 | | | | |
| CaCO₃⁸       | 7.5 | 15.0 | | | | |

| Analyzed | 1 | 2 | 3 | 4 | 5 | 6 |
|----------|---|---|---|---|---|---|
| Ca       | 276 | 601 | 302 | 609 | 278 | 638 |
| P        | 442 | 436 | 448 | 457 | 418 | 436 |
| Mg       | 59 | 62 | 58 | 64 | 58 | 60 |

¹Oriental Yeast Co., Ltd., Tokyo, Japan. ²Japan Maize Products Co., Ltd., Tokyo, Japan. ³Sanmatsu Ind. Co., Tokyo, Japan. ⁴Japan Oil and Fats Co., Ltd., Tokyo, Japan. ⁵Toyo Roshi Co., Ltd., Tokyo, Japan. ⁶AIN-76 vitamin mixture (5). ⁷Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. ⁸Wako Pure Chemical Ind. Ltd., Osaka, Japan. ⁹Modified from AIN-76 mineral mixture for rats (5), supplying (mg/kg diet): sodium chloride, 2,590; potassium citrate monohydrate, 7,700; potassium sulfate, 1,820; magnesium oxide, 840; manganese carbonate, 122.5; ferric citrate, 210; zinc carbonate, 56; cupric carbonate, 10.5; potassium iodate, 0.35; sodium selenite, 0.35; chromium potassium sulfate, 19.25. ¹⁰Kanto Chemical Co., Inc., Tokyo, Japan. ¹¹WMC, whey mineral complex.

Animals. Forty-eight Sprague-Dawley male weanling rats (Japan SLC, Inc., Shizuoka, Japan) were housed in individual aluminum metabolic cages in a temperature-controlled (23±2°C) room with 50±10% humidity and a 12-h light-dark cycle. After a 5-day adaptation period in which all rats were fed a stock diet (CE-7, Clea Japan, Inc., Tokyo, Japan) with dietary Ca of 1.08% and P of 1.03%, the rats were separated into six groups of eight animals having similar mean body weight of 78 g. The six groups were fed one of the experimental diets and deionized water ad libitum for 28 days. Body weight and feed intake were recorded three times a week. On days 3, 10, 17, and 25, urine samples were collected for determination of hydroxyproline and cyclic AMP. For a mineral balance study,

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fecal and urine samples were collected from day 22 to day 24. After a 28-day feeding period, the animals were fasted overnight and anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight), and blood samples were taken from the aorta ventralis to determine plasma mineral concentrations.

**Bone densities.** The right and left femurs were excised and the surrounding flesh was removed. The femurs were dried in an oven at 100°C for 2 h and then weighed. The left foreleg was excised and the surrounding skin was removed. The bone densities of the humerus and ulna were obtained by microphotodensitometry of the roentgenographic images (6). For quantitative measurement of the change in a roentgenographic bone image, the films were exposed under standardized conditions. The film-to-target distance was 0.6 m; the settings were 36 kV and 6 mAs. The X-ray source was a high-voltage X-ray generator (Toshiba, KXO-15). The films were processed using an automatic developing processor (Konishiroku, New QX1200). The films were scanned with a Konica micro-densitometer PDS-15.

**Analysis.** The femurs and the fecal samples were dried, weighed, and ashed overnight in a muffle furnace at 600°C. The ashed samples were then dissolved in 1 N of nitric acid for Ca analysis by an atomic absorption spectrophotometer (Shimadzu, AA-640-13). Phosphorus was determined by a spectrophotometric method (4). Urinary Ca and Mg contents were directly determined as described above. Plasma Ca, inorganic P, Na, K, Cl, Mg, and alkaline phosphatase (ALP) were determined by an autoanalyzer (Hitachi, 736). Urinary hydroxyproline was estimated colorimetrically (7). Cyclic AMP was measured by competitive binding assay using a cyclic AMP[3H] assay system (Amersham Japan Co. Ltd., Japan). Creatinine was determined colorimetrically using a measuring kit (Wako Pure Chemical Ind. Ltd., Japan).

**Statistics.** All data were analyzed by one-way analysis of variance (ANOVA). When significant F ratios were found, individual means were compared by Tukey’s q-test (p < 0.05). Two-way ANOVA was also adopted to determine main effects (Ca source and Ca level) and interaction (Ca source × Ca level).

**RESULTS**

**Feed intake, weight gain, and FER**

Neither the type of dietary Ca source nor the level of dietary Ca affected feed intake and weight gain (Table 3). Feed efficiency ratios (FERs) from animals fed 0.6% Ca diets were slightly lower than those fed 0.3% Ca diets.

**Mineral balances**

There was no significant difference in quantitative values for Ca balance among the three groups of rats fed the same level of dietary Ca (Table 4). The Ca absorption rate in rats fed the 0.6% Ca diets were lower than those in rats fed the
Table 3. Body weight gain, feed intake, and feed efficiency ratio (FER).\(^1\)

|                     | Dietary Ca level and Ca source | Two-way ANOVA\(^1\) |
|---------------------|-------------------------------|---------------------|
|                     | 0.3\% Ca                     | 0.6\% Ca           | A  | B  | A ×B |
|                     | WMC1\(^2\)  | WMC2  | CaCO\(_3\) | WMC1  | WMC2  | CaCO\(_3\) |     |     |      |
| Feed intake (g/day) | 18.2±0.5  | 9.0±0.6 | 18.2±0.5 | 18.5±0.4  | 18.4±0.5 | 18.7±0.4 | NS | NS | NS   |
| Body weight gain (g/day) | 6.7±0.2  | 7.1±0.3 | 6.7±0.2 | 6.5±0.2  | 6.5±0.2 | 6.9±0.2 | NS | NS | NS   |
| FER\(^4\) (%)      | 36.5±0.7  | 37.4±0.7 | 37.0±0.5 | 35.3±0.8  | 35.3±0.6 | 36.6±0.5 | NS | *  | NS   |

\(^1\)Values are M±SE. \(^2\)WMC, whey mineral complex. \(^3\)A, dietary Ca source; B, dietary Ca level; *, significant effect (p < 0.05); NS, not significant. \(^4\)FER = (body weight gain/feed intake) × 100.
Table 4. Mineral balance and tubular reabsorption of P (%TRP).  

|                | Dietary Ca level and Ca source | 0.3% Ca | 0.6% Ca |
|----------------|--------------------------------|---------|---------|
|                |                                | WMC1    | WMC2    | CaCO₃   | WMC1    | WMC2    | CaCO₃   |
| Ca             | Absorption (mg/day)            | 53.8 ± 1.4 | 55.8 ± 1.0 | 55.3 ± 2.3 | 58.3 ± 2.3 | 54.2 ± 2.1 | 59.5 ± 2.3 |
|                | Absorption rate (%)            | 79.6 ± 1.8ᵃ | 78.8 ± 2.5ᵃ | 83.9 ± 1.7ᵃ | 44.0 ± 1.5ᵇ | 40.8 ± 1.8ᵇ | 42.0 ± 1.1ᵇ |
|                | Retention (mg/day)             | 53.7 ± 1.4 | 55.7 ± 1.0 | 55.2 ± 2.3 | 58.1 ± 2.3 | 54.1 ± 2.1 | 59.3 ± 2.3 |
| P              | Absorption (mg/day)            | 88.8 ± 2.1ᵃ | 91.9 ± 2.2ᵃ | 92.7 ± 3.2ᵃ | 56.6 ± 1.6ᵇ | 51.3 ± 2.1ᵇᶜ | 67.6 ± 2.5ᵈ |
|                | Absorption rate (%)            | 87.5 ± 0.9ᵃ | 86.3 ± 1.2ᵃ | 93.0 ± 0.6ᵇ | 56.8 ± 1.0ᶜ | 51.6 ± 2.3ᵈ | 63.8 ± 1.0ᶜ |
|                | Retention (mg/day)             | 64.6 ± 2.4ᵃ | 66.4 ± 2.4ᵃ | 57.4 ± 3.4ᶜ | 47.6 ± 1.8ᵇ | 45.5 ± 2.6ᵇ | 49.7 ± 1.6ᶜ |
|                | %TRP (%)                       | 64.9 ± 4.8ᵃ | 78.4 ± 2.4ᵃ | 59.2 ± 5.6ᵃ | 91.4 ± 1.1ᵇ | 92.5 ± 1.1ᵇ | 80.1 ± 2.8ᵇ |
| Mg             | Absorption (mg/day)            | 6.6 ± 0.3ᵃ | 6.6 ± 0.6ᶜ | 7.0 ± 0.7ᵃ | 5.3 ± 0.3ᵃᵇ | 4.3 ± 0.5ᵇ | 5.5 ± 0.3ᵃᵇ |
|                | Absorption rate (%)            | 52.8 ± 2.3ᵃᶜ | 50.3 ± 4.1ᵃᶜ | 62.8 ± 4.5ᵃ | 39.4 ± 2.3ᶜ | 33.0 ± 5.3ᵇ | 47.2 ± 8.1ᶜ |
|                | Retention (mg/day)             | 6.0 ± 0.5 | 5.2 ± 0.8 | 5.4 ± 1.1 | 4.1 ± 0.5 | 3.4 ± 0.8 | 4.9 ± 0.5 |

Two-way ANOVA

|                | Absorption | Absorption rate | Retention | %TRP |
|----------------|------------|-----------------|-----------|------|
|                | A          | B               | A × B     | A    | B   | A × B |
| Ca             | NS         | NS              | NS        | NS   | NS  | NS    |
| P              | **         | **              | **        | NS   | **  | NS    |
| Mg             | NS         | **              | **        | **   | NS  | NS    |

¹ Values are M±SE. Means in the same row not sharing a common superscript letter are significantly different by Tukey's q-test (p < 0.05). ² WMC, whey mineral complex. ³ Absorption = intake - fecal excretion. ⁴ Absorption rate (%) = (absorption/intake) × 100. ⁵ Retention = absorption - urinary excretion. ⁶ %TRP = \( \frac{1 - (\text{urinary } P \times \text{serum creatinine})/(\text{serum } P \times \text{urinary creatinine})}{1} \) × 100. ⁷ A, dietary Ca source; B, dietary Ca level; ** and *, significant effect (p < 0.01 and p < 0.05); NS, not significant.
0.3% Ca diets. The P absorption and absorption rate were affected significantly by the levels of dietary Ca and the type of dietary Ca source (Table 4). Rats fed WMC1 or WMC2 showed a lower P absorption at the dietary Ca level of 0.6% and a lower P absorption rate at the dietary Ca level of 0.3% than rats fed CaCO3. However, the lower P absorption in rats fed WMC1 or WMC2 did not result in a lower P retention, because the urinary P excretions were significantly low. The percent of tubular reabsorption of P (%TRP) of rats fed WMC1 or WMC2 had a tendency to be higher than that of rats fed CaCO3 at each dietary Ca level. Mg absorption, absorption rate, and retention were affected significantly by the level of dietary Ca, and the absorption rate was also affected by the type of dietary Ca source (Table 4). Rats fed CaCO3 gave higher values than rats fed WMCs especially at the 0.6% dietary Ca level.

Femur mineral analyses and bone densities

There were no significant differences in the dry weight of femur among the experimental groups fed the same level of dietary Ca (Table 5). However, Ca content in the femur and the Ca/P ratio were significantly affected by the type of dietary Ca source. Rats fed WMC2 had the highest Ca content among the groups. The level of dietary Ca affected the P, Mg contents and Ca/P ratio of the femur (Table 5). The Ca/P ratio from rats fed WMC1 or WMC2 was significantly higher than that from rats fed CaCO3. The bone density of the humerus was affected by the level of dietary Ca and the type of dietary Ca source (Table 5). The bone density of the humerus from rats fed WMC2 at the 0.6% dietary Ca level was significantly higher than that from rats fed CaCO3. The bone density of the ulna was affected by the level of dietary Ca, but a significant difference was not observed among the groups.

Urinary hydroxyproline and cyclic AMP excretion

The urinary hydroxyproline and cAMP excretion of all the animals increased with their growth. However, when expressed per milligram of creatinine, those values decreased during the test period (Fig. 3). This might reflect the differences in dietary Ca and P levels after replacing the stock diet with the test diets. The change in urinary hydroxyproline excretion was similar among the three groups of rats fed the 0.6% Ca diets (Fig. 3). However, when rats were fed the 0.3% Ca diets, the WMC2 group tended to show a higher hydroxyproline excretion on days 3 and 10, suggesting high bone resorption. The parathyroid hormone (PTH) function monitored by the urinary cyclic AMP excretion was significantly affected (p < 0.05) by the level of dietary Ca on each day of measurement (Fig. 4), the rats fed WMC2 showing a lower value at each dietary Ca level.

Plasma mineral analyses

Two-way ANOVA shows that the type of dietary Ca source affected plasma Na and ALP activity (Table 6). The plasma Na concentration in rats fed CaCO3...
Table 5. Femur mineral analyses and bone density of humerus and ulna.1

|                  | Dietary Ca level and Ca source |                 |                 |                 |                 |                 |
|------------------|--------------------------------|----------------|----------------|----------------|----------------|----------------|
|                  | 0.3% Ca                        | 0.6% Ca        |                 |                 |                 |                 |
|                  | WMC1²                          | WMC2           | CaCO₃          | WMC1           | WMC2           | CaCO₃          |
| Femur            |                                |                |                |                |                |                |
| Dry weight (mg/100 g) | 137 ± 3ª       | 134 ± 2ª       | 136 ± 2ª       | 147 ± 2ª       | 143 ± 3ª       | 146 ± 3ª       |
| Ca³ (mg/g)       | 197 ± 3ªc       | 208 ± 2ªc      | 203 ± 3ªabc    | 201 ± 3ªabc    | 209 ± 3ªc      | 195 ± 2ªb      |
| P (mg/g)         | 100 ± 3ª        | 102 ± 1ª       | 106 ± 1ª       | 123 ± 2ª       | 123 ± 2ª       | 125 ± 2ªb      |
| Mg (mg/g)        | 4.0 ± 0.6       | 4.2 ± 0.8      | 4.1 ± 0.9      | 3.8 ± 1.3      | 4.1 ± 0.2      | 3.9 ± 0.4      |
| Ca/P ratio (× 100) | 196 ± 4ªb      | 204 ± 1ªb      | 191 ± 3ª       | 164 ± 4ª       | 170 ± 3ª       | 156 ± 3ªd      |
| Humerus          |                                |                |                |                |                |                |
| Bone density     | 44.5 ± 1.0ª     | 46.5 ± 1.0ªab  | 44.3 ± 0.7ªa   | 47.1 ± 0.6ªab  | 48.8 ± 0.5ªb   | 45.3 ± 1.1ªa   |
| Ulna             |                                |                |                |                |                |                |
| Bone density     | 49.8 ± 0.9       | 48.5 ± 0.8     | 46.4 ± 1.2     | 50.0 ± 1.0     | 50.4 ± 1.1     | 48.0 ± 1.0     |

Two-way ANOVA⁴

|                  | A  | B  | A × B |
|------------------|----|----|-------|
| Femur            |    |    |       |
| Dry weight       | NS | ** | NS    |
| Ca               | ***| NS | NS    |
| P                | NS | ** | NS    |
| Mg               | NS | *  | NS    |
| Ca/P ratio       | ***| ** | NS    |
| Humerus          |    |    |       |
| Bone density     | ** | ** | NS    |
| Ulna             |    |    |       |
| Bone density     | NS | *  | NS    |

1 Values are M ± SE. Means in the same row not sharing a common superscript letter are significantly different by Tukey’s q-test (p < 0.05). ²WMC, whey mineral complex. ³Weight in mg per gram of femoral dry weight. ⁴A, dietary Ca source; B, dietary Ca level; ** and *, significant effect (p < 0.01 and p < 0.05); NS, not significant.
was significantly higher than that in rats fed WMC1 at the 0.3% dietary Ca level. Plasma ALP activity in rats fed WMC2 was significantly greater than that in rats fed CaCO₃ at the 0.6% dietary Ca level. The plasma Ca and Mg concentrations were significantly affected by the level of dietary Ca.

**DISCUSSION**

*Mineral components.* It is well known that milk and milk products exhibit superior Ca bioavailability not only because of their high Ca content, but because of the effects of proper Ca/P ratio, lactose, and casein phosphopeptides (8). The WMCs used in this study had common components, but their compositions were different (Table 1). Therefore, although the levels of dietary Ca, P, and lactose were adjusted in this study, the influence of other components extracted from acid whey should be discussed. The data for bone properties suggests that WMC2 had a positive effect on bone formation compared with WMC1 or CaCO₃. The chemical form of Ca was similar in WMC1 and WMC2, because most of the Ca in WMCs
Effects of dietary Ca level and Ca source on urinary cyclic AMP excretion. Each point represents M±SE of eight animals. Means not sharing a common superscript letter at each day of measurement are significantly different (p<0.05).

could be colloidal Ca phosphate judging from their preparation method. The major differences in mineral components between WMC1 and WMC2 were the Na, K, and Cl contents. Since the Na and Cl contents in WMC1 were much higher, their dietary levels of WMC1 diets were augmented compared with those of CaCO₃ or WMC2 diets. However, an increase in urinary Ca excretion was not accompanied by the high dietary Na in this study. In changes of bone resorption from rats fed WMC1, no remarkable increases were observed compared with the other groups (Fig. 3). As for the increase in PTH function, rats fed WMC1 with 0.3% Ca gave slightly but not significantly higher values on days 17 and 25 (Fig. 4). Thus, the augmented Na ingestion (10) could have affected bone metabolism of rats fed WMC1, but to what extent this was simply due to Na is not clear.

By calculation, the amounts of augmented dietary Mg in WMC diets were similar in rats fed WMC1 and WMC2: 10% increase at 0.3% Ca diets and 20% at 0.6% Ca diets compared with that of control rats. However, there was little difference in Mg contents among the experimental diets as determined by analysis (Table 2). The Mg absorption rate of rats fed WMC1 or WMC2 had a tendency to be lower than that of control rats, but no significant difference in Mg retention was observed between the groups. Moreover, the femoral Mg was not affected by
Table 6. Plasma mineral analyses and alkaline phosphatase (ALP).

|                  | Dietary Ca level and Ca source |                  |                  |
|------------------|-------------------------------|------------------|------------------|
|                  | WMC1 2                         | WMC2             | CaCO₃            | WMC1 2                         | WMC2             | CaCO₃            |
| Ca (mg/liter)    | 103.5±0.8                      | 101.6±1.0        | 102.5±0.8        | 103.1±0.5                      | 105.3±1.1        | 104.4±0.8        |
| P₃ (mg/liter)    | 75.6±3.4                       | 81.5±2.5         | 74.9±3.2         | 78.0±2.7                       | 78.1±2.7         | 73.4±2.0         |
| Mg (mg/liter)    | 25.0±1.0ₕᵇ                     | 24.8±1.1ₕᵇ      | 25.8±1.5ₕ         | 22.4±1.0ₕᵇ                     | 23.5±0.9ₕᵇ      | 21.0±0.8ₕ         |
| Na (mEq/liter)   | 141.3±0.6ₖ                      | 141.5±0.3ₕᵇ     | 143.1±0.3ₖ        | 141.4±0.5ₕᵇ                     | 142.0±0.3ₕᵇ     | 142.4±0.6ₕ        |
| Cl (mEq/liter)   | 98.5±1.1                       | 100.8±0.8        | 100.1±0.9        | 100.0±0.9                       | 100.4±0.7        | 100.8±0.8        |
| K (mEq/liter)    | 4.2±0.2ₕ                        | 4.3±0.1          | 4.1±0.1          | 4.1±0.2ₕ                        | 4.0±0.1          | 4.0±0.2ₕ          |
| ALP (U)          | 26.1±1.2ₕᵇ                       | 25.4±0.9ₕᵇ     | 24.9±1.5ₕᵇ        | 24.8±1.6ₕᵇ                       | 29.4±2.0ₕᵇ     | 22.5±0.3ₕ         |

Two-way ANOVA³

|                  | A | B | A × B |
|------------------|---|---|-------|
| Ca               | NS| * | NS    |
| P₃              | NS| NS| NS    |
| Mg               | NS| **| NS    |
| Na               | **| NS| NS    |
| Cl               | NS| NS| NS    |
| K                | NS| NS| NS    |
| ALP              | * | NS| *     |

¹ Values are M±SE. Means in the same row not sharing a common superscript letter are significantly different by Tukey’s q-test (p < 0.05). ² WMC, whey mineral complex. ³ A, dietary Ca source; B, dietary Ca level; ** and *, significant effect (p < 0.01 and p < 0.05); NS, not significant.
EFFECTS OF WHEY MINERAL COMPLEXES ON BONE dietary Ca source nor dietary Ca level (Table 5). Therefore, although the influence of Mg on bone metabolism was reported (11), the differences in bone metabolism between the experimental groups were not explained by the Mg absorption or Mg content in the femur.

Nitrogenous compounds and lipids. The bone properties of rats fed WMC2 were improved compared with those of rats fed CaCO₃: The bone density of the humerus and Ca content in the femur of rats fed WMC2 at the 0.6% dietary Ca level was significantly higher than those from rats fed CaCO₃ (Table 5). The effect of WMC2 on bone density or femoral Ca content was comparable with the microdensitometric characteristics of the bone from rats fed whey calcium (3) of chemical composition similar to that of WMC2. However, there is a difference in the result of the Ca balance study: no increase in urinary Ca excretion of rats fed WMCs was observed. This could be due to the different animal models used, especially to that of the calcium status. The results of the urinary cyclic AMP excretion suggested a lower PTH function in WMC2 groups. In addition, high % TRPs observed with rats fed WMC2 could reflect the diminished effect of PTH on reabsorption of P at proximal renal tubuli (12). However, an increase of serum Ca that reduces PTH was not observed with rats fed WMCs (Table 6). Calcitonin inhibits the direct action of PTH upon the release of Ca from bone, but it also produces a phosphaturic effect (12). Therefore, it is suggested that other factors besides the presence of minerals could have been involved in bone metabolism from rats fed WMC2. Rats fed WMC1 also showed high %TRPs but the PTH function could have been affected adversely by the high Na ingestion as described above. With WMC1, the effect of substances other than Na on PTH function was not clear.

WMC2 had more protein than WMC1 (Table 1). SDS-PAGE patterns showed that the protein composition of WMC2 was similar to that of whey protein isolate, but different from that of WMC1 (Fig. 1). However, as for the influence of dietary protein on Ca absorption (13, 14), the contribution of milk whey proteins such as β-lactoglobulin or α-lactalbumin in WMCs to bone metabolism could be neglected because of the small quantities contained in the diets. With WMC2, a difference in amino acid composition between total nitrogenous compounds and non-protein nitrogen (NPN) fraction suggests the existence of peptides or amino acids that were not produced from the decomposition of major whey proteins (Fig. 2). On the other hand, with WMC1, the total amino acid composition was similar to that of NPN fraction. In addition, free amino acids or peptides in the NPN fraction of WMC1 were lower than those in WMC2: 386 mg/100 g for WMC1 and 1,770 mg/100 g for WMC2. The existence of biologically active peptides such as hormones and growth factors in milk have been reported (15), but their effects on bone metabolism in vivo or on PTH function are not well known. Whether the differences in the effects on bone metabolism between WMC1 and WMC2 could reflect the differences in nitrogenous compounds or not deserves further investigation.

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The lipid contents of WMCs were quite low and further analysis of the lipids was not carried out. Vitamin D has been known to be a hormone which regulates Ca and P homeostasis. In this study all the animals were fed the same and sufficient amount of vitamin D₃ (5), and vitamins D₂ and D₃ were not detected in WMCs (data are not shown). However, vitamin D metabolites have been reported in bovine milk (16). To determine these substances not only in WMCs, but also in blood samples from animals fed WMCs, is necessary to understand the effect of WMCs on bone metabolism.

Rats fed WMC2 showed excellent bone properties (Table 5), which suggests the usefulness as a nutritional supplement in human diets. Substances other than minerals transferred from acid whey to WMC2 seemed to have an effect on bone metabolism. Further research on nitrogenous compounds or lipids in WMCs would help improve their mineral bioavailabilities.

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