Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 31:258–268 (1992)

Bioavailabilities of calcium, phosphorus and magnesium from whey mineral complex in growing male rats

H. Tsuchita[1], T. Kuwata[1], C. Sakamaki[2], K. Kuwano[2], S. Shinoda[3], and T. Yoshida[4]

[1] Central Research Institute, Meiji Milk Products Co., Ltd.
[2] Tokyo Kasei Gakuin Junior College
[3] Tachikawa College of Tokyo
[4] Kagawa Nutrition University, Tokyo, Japan

Die biologische Verfügbarkeit von Calcium, Phosphor und Magnesium aus dem Mineralkomplex der Molke in heranwachsenden männlichen Ratten

Summary: The bioavailabilities of Ca, P and Mg from whey mineral complex (WMC) were studied from the viewpoint of the mineral balance and bone properties in growing male rats and compared with bovine bone ash (BBA) and $CaCO_3$.

Ca source showed no significant effect on feed intake, body weight gain or feed efficiency ($p < 0.05$). When the dietary Ca level was 0.3 %, the dry weight of the femur in rats fed WMC was significantly higher than that in rats fed BBA. The femur P content of rats fed 0.3 % Ca as WMC was significantly higher than that of rats fed BBA or $CaCo_3$. The breaking energy of the femur from rats fed WMC was significantly higher than that from rats fed BBA at a Ca level of 0.2 % or 0.3 %. There was the same tendency in bone densities as was observed in breaking properties. There was no significant difference in quantitative values for Ca balance among three groups of rats, whereas those rats fed WMC had a significantly higher P retention than other groups. Ca bioavailability from WMC and the effect on the utilization of P and Mg were discussed.

Zusammenfassung: Die biologische Verfügbarkeit von Ca, P und Mg aus dem Mineralkomplex der Molke (WMC) wurde in bezug auf die Mineralbilanz und Knochenbeschaffenheit an wachsenden männlichen Ratten untersucht und mit Rinderknochenasche (BBA) und $CaCO_3$ verglichen.

Der Einfluß der Ca-Quelle auf die Futteraufnahme, Körpergewichtszunahme und Futterverwertung war nicht signifikant ($p > 0{,}05$). Bei einer Zulage von 0,3 % Ca in Form von WMC war das Trockengewicht der Rattenfemora erheblich höher als bei den mit BBA gefütterten. Die Fe-

Abbreviations

WMC, whey mineral complex; BBA, bovine bone ash; FER, feed efficiency ratio; ANOVA, analysis of varience

mora von Ratten, welche 0,3 % Ca in Form von WMC erhielten, speicherten signifikant mehr P als die der mit BBA bzw. $CaCO_3$ gefütterten, der Energieaufwand bis zum Femurbruch der mit WMC gefütterten Ratten war bedeutend höher als bei den Ratten, die mit 0,2 bzw. 0,3 % Ca als BBA gefüttert wurden. Die bezüglich der Bruchfestigkeit festgestellte Tendenz traf auch auf die Knochendichte zu. Unter den drei Rattengruppen wurden keine bedeutenden quantitativen Unterschiede in der Ca-Bilanz festgestellt; bei den mit WMC gefütterten Ratten zeigte sich jedoch eine signifikant höhere P-Retention als bei den anderen Gruppen. Die Konsequenzen für die biologische Verfügbarkeit von Ca aus WMC und deren Auswirkungen auf die Nutzung von P und Mg werden besprochen.

Key words: Whey minerals – Ca availability – P utilization – bone

Schlüsselwörter: Molkenminerale – Ca-Verfügbarkeit – P-Nutzung – Knochen

Introduction

It is a well-known fact that an adequate intake of Ca is important, not only during the growing years of childhood and adolescence, but also during adult years. Evidence is growing in support of the hypothesis that increased Ca intake reduces the risk of osteoporosis (16, 30), hypertension (24, 39), and cancer (4, 27, 38).

The average Ca intake of the Japanese, 550.7 mg/day (26), is below the RDA for Japan. There seems to be a risk that the Ca intake of many individuals is inadequate. The data also indicates that dairy products provided 24 %; fruits and vegetables, 20 %; meats, poultry, eggs and fish, 20 %; grains, legumes and soy, 21 % of actual Ca intake. Since dairy products are high in Ca and show good bioavailability in human (31, 32) as well as in experimental animals (7, 41), increased consumption is recommended for Ca supplementation. However, there are many persons who do not ingest milk or milk products due to a milk intolerance, lactase deficiency (33), or compliance. As a part of whey utilization, methods have been developed to recover the minerals from acid whey for mineral supplementation (22). Flynn et al. (15) reported the high bioavailability of Ca and Zn from whey mineral complex (Ca, 9.5 %; P, 5.5 %; lactose, 47.0 %; protein, 26.0 %) in rats by slope ratio assay. However, the mineral bioavailability from whey mineral complex (WMC) might vary due to the mineral form and the components derived from milk.

In this study, the relative bioavailability of Ca in WMC, which contained more Ca and less protein than that reported by Flynn et al. (15), and the effects on the utilization of P and Mg were studied from the viewpoint of mineral balance and bone properties in growing male rats.

Materials and Methods

Ca sources and diets

Whey mineral complex (WMC) was prepared from acid whey. The precipitate, which was formed by neutralizing acid whey with alkali solution, was directly concentrated by microfiltration, and then spray dried. The composition of WMC was: moisture, 5.9 %; ash, 50.2 %; protein, 2.6 %; fat, 0.2 %; Ca, 17.0 %; P, 8.4 %; Mg, 0.3 % Na, 1.3 %; K, 1.4 %; Cl, 2.2 %. A lactose content of 41.1 % was esti-

mated by subtracting the percentage of moisture, ash, protein, and fat from 100. Bovine bone ash (BBA) was obtained from Naruto Chemicals Co. Ltd. (Tokushima, Japan) as a reference for natural mineral mixture and Ca carbonate was used as a standard. The composition of BBA was: Ca, 38.6 %; P, 17.4 %; Mg, 0.6 %; Na, 0.9 %; K, 0.01 %. Diet composition is shown in Table 1. Diets contained 0.1 %, 0.2 % or 0.3 % Ca as WMC, BBA, or $CaCO_3$. The P level of all diets was 0.4 % as Na_2HPO_4 and WMC and/or BBA. Lactose was added to the diets to yield 0.72 % which was contained in a WMC diet of 0.3 % Ca level. The contents of Ca, P, and Mg in each diet were analyzed (Table 1). Whey protein isolate (BIPRO, Le Sueur Isolates, Minnesota, USA) was used as a protein source in each experimental diet.

Table 1. Composition and analysis of diets

	Diet[1]								
	WMC			BBA			$CaCo_3$		
	Ca level (%)								
Item	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
Ingredient	g/kg								
Whey protein isolate[2]	150	150	150	150	150	150	150	150	150
Corn starch[3]	668	668	668	668	668	668	668	668	668
Corn oil[4]	50	50	50	50	50	50	50	50	50
Cellulose[5]	50	50	50	50	50	50	50	50	50
Vitamin mix[6]	10	10	10	10	10	10	10	10	10
Choline bitartrate[7]	2	2	2	2	2	2	2	2	2
Mineral mix[8]	30	30	30	30	30	30	30	30	30
Sucrose[9]	13.2	12.0	20.7	13.9	13.4	12.8	11.9	9.4	6.9
Lactose[9]	4.8	2.4	0	7.2	7.2	7.2	7.2	7.2	7.2
WMC	5.9	11.8	17.7						
BBA				2.6	5.2	7.8			
$CaCO_3$[9]							2.5	5.0	7.5
Na_2HPO_4[7]	16.1	13.8	1.6	16.3	14.2	12.2	18.4	18.4	18.4
Analyzed	mg/100g								
Ca	164	252	366	162	280	369	179	276	372
P	451	468	469	440	451	440	423	483	411
Mg	58	59	58	57	60	60	59	62	59

[1] Abbreviations used: WMC, whey mineral complex; BBA, bovine bone ash.
[2] Le Sueur Isolates, Le Sueur, MN, USA.
[3] Japan Maize Products Co., Ltd., Tokyo, Japan.
[4] Tokyo Oil and Fats Co., Ltd., Tokyo, Japan.
[5] Toyo Roshi Co., Ltd., Tokyo, Japan.
[6] AIN-76™ vitamin mixture (3), Nitchiku Medical Industries, Ltd., Yokohama, Japan.
[7] Nacalai Tesque, Inc., Kyoto, Japan.
[8] Modified from AIN-76™ mineral mix for rats (16), supplying (mg/kg diet): sodium chloride, 2590; potassium citrate monohydrate, 7700; potassium sulfate, 1820; 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.
[9] Kanto Chemical Co., Inc., Tokyo, Japan.

Animals

Forty-five Sprague Dawley male weanling rats (Japan SLC. Inc., Shizuoka, Japan) were housed in individual aluminium cages in a temperature-controlled (23 ± 2 °C) room with 50 ± 10 % humidity and a 12-h light-dark cycle. The rats were separated into nine groups of five animals, according to similar mean body weight, 52 g. The nine 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 day 21, the three groups of animals fed diets supplemented with 0.3 % Ca were put into individual metabolic cages. Fecal and urine samples were collected from day 22 to day 25, then weighed and frozen for subsequent determination of mineral contents. 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.

Breaking properties of bone and bone densities

The right and left femurs were excised and the surrounding flesh was removed. The breaking properties of femurs were determined using a stress-strain measuring apparatus, Dynagraph (Iio Electric, Tokyo) according to the method described by Ezawa et al. (13). The shaft of each femur was mounted on two supports 10 mm apart; the plunger which was programmed to move at 100 mm/min applied force (50 kg, maximum load) to the femur midway between the two supports. The breaking force and breaking energy were recorded. The fractured femurs were extracted in a Soxhlet apparatus with diethyl ether. The femurs were removed from the Soxhlet, 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. For a quantitative measurement of the change in a roentgenographic bone image, the films were exposed in a standardized condition. The film-to-target distance was 0.6 m; the settings were 36 kV and 6 mAs. The films were processed using an automatic developing processor (Konishiroku, New QX1200). The scanning of films was performed with a Konica micro-densitometer PDS-15 (34).

Analysis

The fecal samples were freeze-dried, weighed, and wet-ashed with nitric acid: perchloric acid (4 : 1) in Uniseal decomposition vessels (Uniseal Decomposition Vessels Ltd., Haifa, Israel) under high pressure at 130 °C. The dried and defatted femurs and experimental diets were also wet-ashed as described above. The urine samples were ashed overnight in a muffle furnace at 600 °C. The ashed samples were then dissolved in 0.1 N of nitric acid and diluted with deionized water for Ca and Mg analyses by an atomic absorption spectrophotometer (Shimadzu, AA-640-13). The P content was determined by a spectrophotometric method (2). Plasma Ca, inorganic P, and Mg were determined by an autoanalyzer (Hitachi, 736). &&zfe2-1,6

Statistics

All data except for mineral balance were analyzed by two-way analysis of variance (ANOVA) to determine main effects (Ca source and Ca level) and interaction (Ca source x Ca level). When significant or high F ratios were found, individual means were compared by adopting the least significant difference test for each dietary Ca level ($p < 0.05$).

Results

Feed intake, weight gain and FER

The type of Ca source did not affect feed intake or weight gain (Table 2). The effect of the Ca level on weight gain and feed intake was significant ($p < 0.05$); the values of those animals fed WMC increased as the Ca level in the diet increased (Table 2). Neither the level of dietary Ca nor the type of dietary Ca source affected FER.

Table 2. Body weight gain, feed intake and feed efficiency ratio (FER) of rats fed WMC, BBA or $CaCO_3$ over a period of 4 weeks[1]

	Ca level (%)			Two-way ANOVA[3]		
Ca source	0.1	0.2	0.3	A	B	A x B
Feed intake (g)						
WMC[2]	417.4 ± 10.2	444.7 ± 10.3	461.3 ± 16.8			
BBA	436.3 ± 7.7	452.2 ± 18.3	439.6 ± 12.3	NS	*	NS
$CaCO_3$	393.6 ± 21.9	443.7 ± 16.8	427.9 ± 9.6			
Body weight gain (g)						
WMC	161.0 ± 6.5	172.7 ± 7.6	188.2 ± 6.0			
BBA	168.2 ± 4.0	178.5 ± 7.2	172.3 ± 7.0	NS	*	NS
$CaCO_3$	154.2 ± 8.7	169.4 ± 7.6	170.6 ± 7.2			
FER[4] (%)						
WMC	38.5 ± 0.9	38.8 ± 1.3	40.8 ± 0.3			
BBA	38.6 ± 0.6	39.5 ± 0.2	39.1 ± 0.7	NS	NS	NS
$CaCO_3$	39.3 ± 0.1	38.2 ± 0.5	39.8 ± 0.1			

[1] Values are means ± S.E.
[2] Abbreviations used: WMC, whey mineral complex; BBA, bovine bone ash; NS, not significant.
[3] A indicates Ca source and B indicates Ca level. * indicates a significant effect ($p < 0.05$).
[4] FER = (Body weight gain/feed intake) x 100.

Mineral balance

There were no significant differences in quantitative values for Ca metabolism among three groups of rats, however, the apparent digestibility and retention coefficient of Ca in rats fed WMC were slightly higher than those in rats of the other groups (Table 3). There were few differences in apparent digestibility of P among the groups.

Table 3. Balance of Ca, P and Mg in rats fed diets containing 0.3 % Ca[1]

	Ca source		
	WMC[2]	BBA	$CaCO_3$
Ca			
Intake (mg/day)	67.4 ± 3.2	63.3 ± 2.6	64.9 ± 2.4
Fecal excretion (mg/day)	8.6 ± 0.9	12.6 ± 2.3	10.3 ± 1.5
Apparent digestibility[3] (%)	87.3 ± 0.9	80.2 ± 3.6	84.2 ± 2.4
Urinary excretion (mg/day)	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.1
Retention coefficient[4] (%)	99.4 ± 0.0	99.1 ± 0.3	99.4 ± 0.0
P			
Intake (mg/day)	86.3 ± 4.1^{a}	75.6 ± 3.1^{b}	71.7 ± 2.6^{b}
Fecal excretion (mg/day)	9.2 ± 1.3^{a}	7.9 ± 1.5^{ab}	5.1 ± 1.2^{b}
Apparent digestibility (%)	89.4 ± 1.1	89.5 ± 2.1	92.9 ± 1.8
Urinary excretion (mg/day)	33.8 ± 2.0^{a}	47.6 ± 0.4^{b}	43.8 ± 0.5^{ab}
Retention coefficient (%)	56.2 ± 1.6^{a}	29.5 ± 6.5^{b}	33.9 ± 7.2^{b}
Mg			
Intake (mg/day)	10.6 ± 0.5	10.2 ± 0.4	10.4 ± 0.4
Fecal excretion (mg/day)	2.8 ± 0.2	2.8 ± 0.4	2.6 ± 0.1
Apparent digestibility (%)	73.3 ± 1.1	72.3 ± 3.5	74.7 ± 0.9
Urinary excretion (mg/day)	0.3 ± 0.1^{a}	0.7 ± 0.1^{b}	0.4 ± 0.1^{ab}
Retention coefficient (%)	95.9 ± 0.6^{a}	90.8 ± 0.9^{b}	94.2 ± 0.8^{ab}

[1] Values are means ± S.E. Means in the same row not sharing a common superscript letter are significantly different by the least significant difference test ($p < 0.05$).
[2] Abbreviations used: WMC, whey mineral complex; BBA, bovine bone ash.
[3] Apparent digestibility (%) = (absorption/intake) x 100.
Absorption = intake-fecal excretion.
[4] Retention coefficient (%) = (retention/absorption) x 100.
Retention = absorption-urinary excretion.

However, the urinary excretion of P in rats fed WMC was significantly lower; they had a significantly higher P retention coefficient than the other groups. No significant differences in the parameters of Mg balance were found among the groups except that urinary excretion in rats fed BBA was significantly higher than in the WMC diet group.

Breaking properties of bone and bone densities

The effects of Ca source and level on the breaking force and breaking energy of the femur were significant (Table 4). The breaking energy of femurs from rats fed 0.2 or 0.3 % Ca as WMC was significantly higher than from rats fed BBA, and slightly higher than those fed $CaCO_3$. There was the same tendency in bone densities as was observed in breaking properties. The bone density of the humerus from rats fed WMC was significantly higher than that from rats fed BBA when the dietary Ca level was 0.3 % (Table 4).

Table 4. Breaking force, breaking energy and density of bone[1]

	Ca level (%)			Two-way ANOVA[3]		
Ca source	0.1	0.2	0.3	A	B	A×B
Breaking force of femur (× 10^7 dyn)						
WMC[2]	3.2 ± 0.2	8.0 ± 0.4	10.3 ± 0.3			
BBA	3.2 ± 0.2	6.3 ± 0.4	8.6 ± 0.5	*	*	NS
$CaCO_3$	3.2 ± 0.2	7.7 ± 0.8	9.5 ± 0.6			
Breaking energy of femur (× 10^5 erg/cm^3)						
WMC	4.1 ± 0.5	10.0 ± 0.8^a	13.8 ± 0.8^a			
BBA	4.1 ± 0.2	5.3 ± 0.9^b	9.6 ± 0.9^b	*	*	NS
$CaCO_3$	3.9 ± 0.4	7.9 ± 1.4^{ab}	12.9 ± 1.3^{ab}			
Bone density of humerus						
WMC	67.4 ± 3.4	71.8 ± 0.8	85.6 ± 1.5^a			
BBA	70.8 ± 1.5	76.8 ± 0.7	76.4 ± 2.6^b	NS	*	*
$CaCO_3$	65.0 ± 3.0	73.2 ± 2.9	84.6 ± 0.9^a			
Bone density of ulna						
WMC	67.0 ± 2.9	74.4 ± 1.0	85.0 ± 1.9			
BBA	69.0 ± 3.2	73.8 ± 0.9	76.2 ± 2.5	NS	*	NS
$CaCO_3$	67.4 ± 1.2	74.4 ± 2.1	79.6 ± 3.0			

[1] Values are means ± S.E. Means not sharing a common superscript letter within a column are significantly different by the least significant difference test ($p < 0.05$).
[2] Abbreviations used: WMC, whey mineral complex; BBA, bovine bone ash; NS, not significant.
[3] A indicates Ca source and B indicates Ca level.
* indicates a significant effect ($p < 0.05$).

Table 5. Femur mineral analyses from rats fed WMC, BBA or $CaCO_3$ over a period of 4 weeks[1]

	Ca level (%)			Two-way ANOVA[3]		
Ca source	0.1	0.2	0.3	A	B	A×B
Fat-free dry weight (mg)						
WMC[2]	394.2 ± 12.9	497.4 ± 10.6	599.2 ± 16.9^a			
BBA	408.7 ± 14.9	479.1 ± 26.7	517.3 ± 11.0^b	NS	*	NS
$CaCO_3$	385.9 ± 14.2	489.5 ± 30.6	551.9 ± 21.3^{ab}			
Ca (mg)						
WMC	83.3 ± 3.6	126.3 ± 2.2	150.3 ± 5.3			
BBA	83.2 ± 2.8	111.2 ± 6.0	131.8 ± 3.7	NS	*	NS
$CaCO_3$	80.5 ± 5.3	121.8 ± 12.8	142.1 ± 8.8			
P (mg)						
WMC	44.9 ± 2.2	66.2 ± 2.0	79.9 ± 3.2^a			
BBA	42.0 ± 0.6	60.5 ± 3.1	68.3 ± 2.5^b	*	*	NS
$CaCO_3$	39.5 ± 2.4	59.3 ± 4.2	67.9 ± 2.3^b			
Mg (mg)						
WMC	1.5 ± 0.1	2.3 ± 0.2	2.3 ± 0.2			
BBA	1.3 ± 0.1	2.1 ± 0.2	2.2 ± 0.1	NS	*	NS
$CaCO_3$	1.3 ± 0.1	2.2 ± 0.4	2.2 ± 0.2			
Ca/P ratio						
WMC	1.86 ± 0.03	1.92 ± 0.08	1.88 ± 0.02			
BBA	1.98 ± 0.07	1.84 ± 0.01	1.93 ± 0.03	*	NS	NS
$CaCO_3$	2.04 ± 0.09	2.04 ± 0.09	2.09 ± 0.11			

[1] Values are means ± S.E. Means not sharing a common superscript letter within a column are significantly different by the least significant difference test ($p < 0.05$).
[2] Abbreviations used: WMC, whey mineral complex; BBA, bovine bone ash; NS, not significant.
[3] A indicates Ca source and B indicates Ca level.
* indicates a significant effect ($p < 0.05$).

Femur mineral analyses

The level of dietary Ca affected the dry weight of the femur and its mineral content (Table 5). The effect of the Ca source on the dry weight of the femur was not significant. However, when the Ca level was 0.3 % the value in rats fed WMC was significantly higher than that in rats fed BBA, and slightly higher than that in the $CaCO_3$ diet group. Rats fed WMC had a significantly higher femur P content than rats fed BBA or $CaCO_3$ at the dietary Ca level of 0.3 %. Ca/P ratio depended on the type of Ca source; the value was higher in rats fed $CaCO_3$ than in rats fed WMC or BBA at each dietary Ca level.

Plasma mineral analyses

Each plasma mineral concentration was affected by the level of dietary Ca (Table 6). There were significant differences in plasma P concentrations among the animal groups when the dietary Ca levels were 0.2 and 0.3 %.

Table 6. Plasma mineral analyses from rats fed WMC, BBA or $CaCO_3$ over a period of 4 weeks[1]

	Ca level (%)			Two-way ANOVA[3]		
Ca source	0.1	0.2	0.3	A	B	A×B
Ca (mg/100mL)						
WMC[2]	10.72 ± 0.07	11.02 ± 0.11	10.94 ± 0.17			
BBA	10.88 ± 0.20	11.10 ± 0.11	11.16 ± 0.15	NS	*	NS
$CaCO_3$	10.40 ± 0.08	11.06 ± 0.14	11.02 ± 0.14			
Pi (mg/100mL)						
WMC	7.90 ± 0.13	8.32 ± 0.10[a]	6.84 ± 0.04[a]			
BBA	7.54 ± 0.14	7.82 ± 0.11[b]	7.40 ± 0.14[b]	NS	*	*
$CaCO_3$	7.48 ± 0.33	8.10 ± 0.13[ab]	7.58 ± 0.16[b]			
Mg (mg/100mL)						
WMC	1.76 ± 0.07	1.76 ± 0.07	1.54 ± 0.08			
BBA	1.82 ± 0.10	1.68 ± 0.09	1.60 ± 0.10	NS	*	NS
$CaCO_3$	1.90 ± 0.09	1.54 ± 0.09	1.66 ± 0.08			

[1] Values are means ± S.E. Means not sharing a common superscript letter within a column are significantly different by the least significant difference test ($p < 0.05$).

[2] Abbreviations used: WMC, whey mineral complex; BBA, bovine bone ash; Pi, inorganic phosphorus; NS, not significant.

[3] A indicates Ca source and B indicates Ca level.

* indicates a significant effect ($p < 0.05$).

Discussions

Ca bioavailability

Many factors surrounding natural Ca sources affect Ca bioavailability. To discuss the influence of the physical form of Ca on bioavailability, some of these factors were controlled in this study. Protein (9, 11), vitamin D (1, 28) and lactose (25, 5), all of which affect Ca absorption, were equal in all experimental diets. Although

WMC and BBA contained small amounts of Mg, there were few differences in Mg content among the experimental diets (Table 1).

In typical milk, about two-thirds of the Ca is colloidal and the rest is diffusible. Colloidal Ca is partly incorporated in the micellar Ca phosphate and partly bound in a more direct manner to casein (17). Depending on the preparation method of WMC, most of the Ca in WMC could be colloidal Ca phosphate. Also, a small proportion of colloidal Ca that was bound to the whey protein or freed from casein might be contained therein. Ca phosphate in WMC was amorphous, whereas that in BBA was crystalline hydroxyapatite as demonstrated by an analysis of x-ray diffraction spectrometry (the data are not shown). Thus there seem to be some differences in in vitro or in vivo solubility among the Ca sources tested in this study. It appears likely that soluble Ca in vivo is needed for absorption, the significance of in vitro solubility has not been established (8, 40). The result for Ca balance (Table 3) did not clearly show differences in Ca absorption that could be attributed to the form of Ca. However, the availability of Ca from WMC, as judged by bone properties (Tables 4 and 5), was higher than that from the other Ca sources, especially BBA when dietary Ca level was 0.2 % or 0.3 %.

Effect of WMC on the utilization of P and Mg

In a mineral balance study, variations of P content in experimental diets partially reflected P intake. However, a higher P retention in rats fed WMC was ascribed, not to an excessive P intake, but to a lower urinary P excretion. Previous studies have shown that increasing dietary P increased urinary and fecal P excretion (18, 19, 35) and decreased plasma Ca (37) in rats; therefore, P metabolism in rats fed WMC could have been affected by other factors contained in WMC. Although hormones and hormone-like substances identified in milk (23) or whey (12) could have been transferred to WMC, their effects on P metabolism have not, as yet, been reported. Recently, Igarashi et al. (21) reported that whey Ca (Ca, 26.4 %; P, 14.6 %; lactose, 0.64 %; protein, 8.3 %) increased the strength of the femur in ovariectomized rats, and they suggested the possible existence of some bioactive substances in whey Ca.

The high retention of P for rats fed WMC reflected the dry weight and P content of the femur (Table 5). Howe and Beecher (20) reported that the dry weight of the femur and its P concentration were greater when 0.8 % rather than 0.35 % P was fed to young rats, and that high P intakes could be detrimental to the bone flexibility of growing rats. Likewise, in young female rats, a low Ca, high P diet decreased bone density in all bones investigated (6). However, the present data for bone properties from rats fed WMC showed more cause for optimism than those from rats fed the other Ca sources, especially BBA. This suggests that a relatively higher P retention for rats fed WMC increased bone mineralization (14, 29) or decreased bone resorption (10).

No significant differences were found in femur Mg content and Mg balance between rats fed WMC and $CaCO_3$. Although Mg could influence bone metabolism as reviewed by Schwartz (36), there seems to be no relationship between Mg balance and bone properties as a result of this study.

It is suggested from the results of studies on bone properties and mineral balance that WMC is superior as a natural source of minerals.

This study was carried out with an adequate dietary P level, 0.4 %. Additional studies are needed to determine the effect of WMC on P metabolism and bone mineralization at lower or excessive dietary P levels.

Acknowledgements

The authors wish to thank Professor I. Ezawa, Japan Women's University, for measurements on femur breaking properties and Dr. T. Inomata, Azabu University, for bone-density analyses.

References

1. Allen LH (1982) Calcium bioavailability and absorption: A review. Am J Clin Nutr 35:783–808
2. Allen RJL (1940) The estimation of phosphorus. Biochem J 34:858–865
3. American Institute of Nutrition (1977) Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. J Nutr 107:1340–1348
4. Appleton GVN, Davies PW, Bristol JB, Williamson FCN (1987) Inhibition of intestinal carcinogenesis by dietary supplementation with calcium. Br J Surg 74:523–525
5. Armbrecht HJ (1987) Age and the effects of lactose on calcium and phosphorus uptake by rat small intestine. Nutr Res 7:1169–1177
6. Bauer KD, Griminger P (1983) Long-term effects of activity and of calcium and phosphorus intake on bones and kidneys of female rats. J Nutr 113:2111–2121
7. Buchowski MS, Miller DD (1990) Calcium bioavailability from ripening cheddar cheese. J Food Sci 55 (5):1293–1295, 1364
8. Buchowski MS, Sowizral KC, Lengemann FW, Campen DV, Miller DD (1989) A comparison of intrinsic and extrinsic tracer methods for estimating calcium bioavailability to rats from dairy foods. J Nutr 119:228–234
9. Calvo MS, Bell RR, Forbes RM (1982) Effect of protein-induced calciuria on calcium metabolism and bone status in adult rats. J Nutr 112:1401–1413
10. Cuisinier-Gleizes P, George A, Guiliano C, Mathieu H (1971) Stimulation of bone resorption by phosphate deprivation in the rat. Isr J Med Sci 7:355
11. Danielson AD, Kaup SM, Holt DL, Peo ER, Lewis AJ (1988) The interaction between protein, calcium and phosphorus levels on performance and bone integrity in male rats. Nutr Rep Internat 38:897–903
12. Derouiche AF, Legrand C, Bour JM, Capiaumont J, Gelot MA, Dousset B, Belleville F, Nabet P, Linden G (1990) Biochemical aspects of a whey fraction capable of promoting hybridoma proliferation. Comparison with fetal calf serum. Lait 70:313–324
13. Ezawa I, Okada R, Nozaki Y, Ogata E (1979) Breaking properties and ash contents of the femur of growing rats fed a low calcium diet. J Jpn Soc Nutr Food Sci 32:329–335
14. Feinblatt J, Belanger LF, Rasmussen H (1970) Effect of phosphate infusion on bone metabolism and parathyroid hormone action. Am J Physiol 218:1624–1631
15. Flynn A, McKenna L, Kearney RD, Connolly JF (1988) Bioavailability of calcium and zinc in a whey mineral complex. Irish J Food Sci Technol 12:41–51
16. Fujita T, Fukase M, Miyamoto H, Matsumoto T, Ohue T (1990) Increase of bone mineral density by calcium supplement with oyster shell electrolysate. Bone and Mineral 11:85–91
17. Holt C (1985) The milk salts: Their secretion, concentrations and physical chemistry. In: Fox PF (ed) Development in dairy chemistry-3, Elsevier Applied Science Publishers Ltd. England, pp 143–181
18. Howe JC, Beecher GR (1981) Effect of dietary protein and phosphorus levels on calcium and phosphorus metabolism of the young, fast growing rat. J Nutr 111:708–720
19. Howe JC, Beecher GR (1981) Effect of dietary protein and phosphorus levels on calcium and phosphorus metabolism of adult rats. Nutr Rep Internat 24:919–929
20. Howe JC, Beecher GR (1983) Dietary protein and phosphorus: Effect on calcium and phosphorus metabolism in bone, blood and muscle of the rat. J Nutr 113:2185–2195

21. Igarashi T, Ezawa I, Ogata E (1990) Effects of whey calcium on bone metabolism in ovariectomized osteoporosis model rats. J Jpn Soc Nutr Food Sci 43:437–443
22. Kearney RD, Connolly JF (1984) Nutritional potential of milk salts. Irish J Medical Sci 154:164–165
23. Koldovsky O, Thornburg W (1987) Hormones in milk. J Pediatric Gastroenterology Nutr 6:172–196
24. McCarron DA, Morris CD (1985) Blood pressure response to oral calcium in persons with mild to moderate hypertension. Annals Intern Med 103:825–831
25. Miller SC, Miller MM, Omura TH (1988) Dietary lactose improves endochondral growth and bone development and mineralization in rats fed a vitamin D-deficient diet. J Nutr 118:72–77
26. Ministry of Health and Welfare (1990) National Nutritional Survey, 1988. In: Report of national nutritive conditions. Daiichi Shuppan Co., Ltd., Tokyo, pp 61–89
27. Newmark HL, Wargovich MJ, Bruce WR (1984) Colon cancer and dietary fat, phosphate and calcium: A hypothesis. J Natl Cancer Inst 72:1323–1325
28. Norman AW (1990) Intestinal calcium absorption: a vitamin D-hormone-mediated adaptive response. Am J Clin Nutr 51:290–300
29. Pechet MM, Bobadilla E, Carroll EL, Hesse RH (1967) Regulation of bone resorption and formation. Am J Med 43:696–710
30. Polley KJ, Nordin BEC, Baghurst PP, Walker CJ, Chatterton BE (1987) Effect of calcium supplementation on forearm bone mineral content in postmenopausal women: A prospective, sequential controlled trial. J Nutr 117:1929–1935
31. Recker RR, Heaney RP (1985) The effect of milk supplementation on calcium metabolism, bone metabolism and calcium balance. Am J Clin Nutr 41:254–263
32. Recker RR, Bammi A, Barger-Lux MJ, Heaney RP (1988) Calcium absorbability from milk product, an imitation milk, and calcium carbonate. Am J Clin Nutr 47:93–95
33. Rosensweig NS (1969) Adult human milk intolerance and intestinal lactase deficiency: A review. J Dairy Sci 52:585–587
34. Saito H, Nakamury R (1974) Roentgenographic measurement of bone density. J Jpn Orthop Ass 48:479–486
35. Schoenmakers ACM, Hoitinga JR, Lemmens AG, Beynen AC (1988) Influence on dietary phosphorus restriction on calcium and phosphorus metabolism in rats. Internat J Vit Nutr Res 59:200–206
36. Schwartz R (1988) Magnesium status, bone. In: Hemphill DD (ed) Trace substances in environmental health. 22. Proceedings of the University of Missouri's 22nd Annual Conference on trace substances in environmental health, St. Louis, Missouri. Columbia, MO: University of Missouri, 143–152
37. Sie T, Draper HH, Bell RR (1974) Hypocalcemia, hyperparathyroidism and bone resorption in rats induced by dietary phosphate. J Nutr 104:1195–1201
38. Sorensen AW, Slattery ML, Ford MH (1988) Calcium and colon cancer: A review. Nutr Cancer 11:135–145
39. Strazzullo P, Siani A, Guglielmi S, CiCarlo A, Galletti F, Cirillo M, Mancini M (1986) Controlled trial of long-term oral calcium supplementation in essential hypertension. Hypertension 8:1084–1088
40. Wien EM, Schwartz R (1983) Composition of in vitro and in vivo measurements of dietary Ca exchangeability and bioavailability. J Nutr 113:388–393
41. Wong NP, LaCroix DE (1980) Biological availability of calcium in dairy products. Nutr Rep Internat 21:673–680

Received April 7, 1992
accepted September 3, 1992

Authors' address:

Hiroshi Tsuchita, Central Research Institute, Meiji Milk Products Co., Ltd.,
1-21-3 Sakaecho, Higashimurayamashi, 189, Japan