British Journal of Nutrition (1992), **68**, 271–282

Bioavailability of magnesium and calcium from cow's milk and soya-bean beverage in rats

BY ELIZABETH J. BRINK[1], PIETER R. DEKKER[1], EMERENTIA C. H. VAN BERESTEIJN[1]* AND ANTON C. BEYNEN[2,3]

[1] *Department of Nutrition, Netherlands Institute for Dairy Research, PO Box 20, 6710 BA Ede, The Netherlands*

[2] *Department of Laboratory Animal Science, State University, PO Box 80.166, 3508 TD Utrecht, The Netherlands*

[3] *Department of Human Nutrition, Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands*

(*Received 11 September 1990 – Accepted 5 July 1991*)

The milk components lactose and casein enhance the apparent absorption of magnesium and possibly also of calcium, whereas phytate, which occurs in soya-bean products, has an inhibitory effect. This implies that soya-bean beverage *v*. cow's milk could lower bioavailability of Mg and Ca. This hypothesis was tested in two experiments with growing rats. Feeding soya-bean beverage *v*. cow's milk consistently lowered body-weight gain, enhanced bone turnover, as measured by increased plasma alkaline phosphatase (*EC* 3.1.3.1) activity and increased urinary hydroxyproline excretion, and decreased Mg and Ca concentrations in the femur. Because the mineral compositions of the soya-bean beverage and the cow's milk were different, the intake of Mg was higher in rats fed on soya-bean beverage, whereas that of Ca was higher in rats fed on cow's milk. Supplementation of the soya-bean beverage either with phosphorus and Ca or with P, Ca and methionine, to concentrations identical to those in milk, restored growth and bone mineralization. When using diets carefully balanced for Mg, Ca, P, sodium, potassium and methionine, soya-bean beverage *v*. cow's milk in the diets decreased apparent absorption and urinary excretion of Mg and Ca. Hydrolysis of lactose in milk decreased absorption and urinary excretion of Mg; it did not significantly affect Ca absorption but lowered urinary Ca excretion. The present study shows that soya-bean beverage *v*. milk depresses Mg and Ca bioavailability, as would be predicted on the basis of reported effects of their purified components.

Cow's milk: Soya-bean beverage: Mineral bioavailability: Rats

Constituents of cow's milk products, when compared with those of soya-bean products, increase the apparent absorption of magnesium and possibly also of calcium. Casein *v*. soya-bean protein improves Mg absorption (Brink *et al.* 1991) but does not affect Ca absorption (Van der Meer *et al.* 1988). Lactose, a constituent of milk, stimulates the absorption of Mg (Andrieux & Sacquet, 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989; Brink *et al.* 1991). The effect of lactose on Ca absorption is controversial with improved Ca absorption (Andrieux & Sacquet, 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989) and no effect (Greger *et al.* 1987; Sheikh *et al.* 1987; Recker *et al.* 1988) being reported. Phytate, which is present in soya-bean products in significant amounts (Reddy *et al.* 1982), suppresses the absorption of Mg and Ca (Nwokolo & Bragg, 1977; Davies, 1979; Lonnerdal *et al.* 1989; Brink *et al.* 1991).

* For reprints.

Table 1. *Analysed composition of the cow's milk (M), lactase (EC 3.2.1.23)-treated cow's milk (L) and soya-bean beverage (S)*

	M	L	S
Energetic value (kJ/kg)*	2180	2180	2180
Nitrogen (g/kg)	5·3	5·3	6·1
Fat (g/kg)	23	23	26
Lactose (g/kg)	45	9	0
Other carbohydrates (g/kg)	0	36	39
Phytate (mmol/kg)	nd	nd	2·6
Magnesium (mmol/kg)	4·9	4·9	11·5
Calcium (mmol/kg)	30	30	5
Phosphorus (mmol/kg)	31	31	23
Potassium (mmol/kg)	40	40	47
Sodium (mmol/kg)	21	21	28
Water (mol/kg)	64	64	64

nd, not determined.
* Calculated on the basis of chemical analysis; energetic values used (kJ/g): protein 16·8, carbohydrates 16·8, fat 37·8. For conversion of nitrogen into protein concentrations a factor of 6·35 was used for milk and lactase-treated milk, and a factor of 5·91 was used for soya-bean beverage.

Based on the effects of constituents of milk products *v.* those of soya-bean products, it follows that substitution of soya-bean beverage for cow's milk in the diet lowers bioavailability, especially apparent absorption, of Mg and Ca. However, it could be suggested that the constituents do not exhibit the anticipated effects when present in the matrix of the intact products. In order to test this suggestion we fed cow's milk and a soya-bean beverage to young growing rats and determined mineral bioavailability. The effect of lactose in intact milk was ascertained by comparing intact and lactase-treated milk. The soya-bean beverage used was deficient in methionine and Ca but contained more Mg than cow's milk. To find out whether differences in mineral absorption from the intact products could be caused by differences in concentrations of these nutrients, we also used diets that were balanced for methionine, Ca and Mg. Mg and Ca bioavailability was assessed by measurement of their apparent absorption, urinary excretion and concentrations in plasma and femur. Plasma alkaline phosphatase (*EC* 3.1.3.1) activity and urinary hydroxyproline excretion were determined as indices of bone turnover.

MATERIALS AND METHODS

General procedures

Male Wistar (Cpb:WU) rats, aged 4 weeks, were housed individually in polycarbonate-topped metabolism cages with stainless-steel-wire bottoms (3140 mm^2 × 120 mm) in a room with controlled temperature (20–22°), relative humidity (50–60%) and lighting (light, 06.00–18.00 hours). For 4 weeks the animals were fed on the experimental diets containing cow's milk, lactase-treated milk or soya-bean beverage. Diets and demineralized water were freely available.

Sterilized cow's milk was prepared in The Netherlands Institute for Dairy Research. Lactase-treated milk was obtained by addition of 200 mg lactase (*EC* 3.2.1.23; Maxilact-a 40·000; Gist Brocades, Delft) to 1 litre of the sterilized milk and incubation for 24 h at 10°. This led to hydrolysis of 80% of the lactose. The soya-bean beverage was obtained from Protevit, Fijnaart. Table 1 shows the analysed composition of the cow's milk, lactase-treated milk and soya-bean beverage. The milk, lactase-treated milk and soya-bean beverage were fed in the form of diets shown in Tables 2 and 3. Basically, either a protein-free mixture without minerals or a protein-rich mixture with minerals was added to make a porridge. The experimental diets were freshly prepared twice weekly.

Feed consumption was recorded daily and body-weights were measured weekly. During the last 4 d of the experimental period, faeces and urine of each animal were collected. At the end of the experimental period, between 08.30 and 12.30 hours, fasting blood samples were drawn by orbital puncture under light diethyl ether anaesthesia and immediately followed by decapitation. Femora were removed.

For analysis of minerals, diets and faeces were freeze-dried, homogenized and wet-ashed with nitric acid and perchloric acid. Femora were cleaned of adhering tissue, defatted in diethyl ether for 50 h and weighed in air as well as under water for volume measurement. Fat-free femora were dried for 16 h at 105° and dry-ashed in a muffle furnace for 16 h at 550°. After appropriate dilution, Mg and Ca were analysed by atomic absorption spectrophotometry (Perkin Elmer 1100; Bodenseewerk Perkin Elmer & Co. GmbH, Germany). Samples of the diets were also analysed for sodium and potassium by atomic emission spectrophotometry and for phosphorus by the Fiske–Subbarow method (Fiske & Subbarow, 1924). Nitrogen in the diets was analysed by the macro-Kjeldahl method (International Dairy Federation, 1986) and lactose by high-performance liquid chromatography (Brons & Olieman, 1983). Methionine was determined according to the method described by Moore (1963). The phytate content of the base products and the complete diets was analysed as described by Slump *et al.* (1987).

Bioavailability of Mg and Ca was evaluated by determination of their apparent intestinal absorption, urinary excretion and contents in plasma and femur. Bone turnover was assessed by measurement of plasma alkaline phosphatase and urinary hydroxyproline using commercial kits purchased from Boehringer, Mannheim, Germany and Hypronosticon, Organon Teknica, Oss respectively. Apparent intestinal absorption was calculated as mineral intake minus faecal excretion, and was expressed as such and as percentage of intake.

Expt 1

This experiment was designed to compare bioavailability of Mg and Ca from cow's milk, lactase-treated cow's milk and soya-bean beverage. These products were compared in either a protein–mineral-free or protein–mineral-rich (Mg-free) diet. The use of two dietary backgrounds was anticipated to provide clues as to whether the differential effects of the cow's milk and soya-bean beverage become less pronounced when incorporated into more nutritionally adequate diets. Since we were especially interested in Mg bioavailability from intact cow's milk and soya-bean beverage, no Mg was added to the protein–mineral-rich dietary background.

Forty-two rats, with a mean body-weight of 83 g, were randomly divided into six groups of equal size and were fed on diets containing either milk, lactase-treated milk or soya-bean beverage. To 2800 g of these products, which corresponded to 300 g dry matter, 700 g of either a protein–mineral-free or protein–mineral-rich ingredient mixture was added as indicated in detail in Table 2. In essence, the protein–mineral-free mixture consisted of rice flour (Molenaar kindermeel; Milupa, Amersfoort) supplemented with cellulose, iron and vitamins. The protein–mineral-rich mixture contained rice flour, cellulose, ovalbumin (NIVE-WRC, Amersfoort), mineral and vitamin premix. The protein–mineral-rich mixture did not contain Mg.

Table 2. *Expt 1. Composition of the diets containing cow's milk (M), lactase (EC 3.2.1.23)-treated cow's milk (L) or soya-bean beverage (S)*

	Protein–mineral-free background			Protein–mineral-rich background		
Experimental diets...	M	L	S	M	L	S
Ingredients (g dry wt)						
Milk*	300	–	–	300	–	–
Lactase-treated milk*	–	300	–	–	300	–
Soya-bean beverage*	–	–	300	–	–	300
Protein–mineral-free mix†	700	700	700	–	–	–
Protein–mineral-rich mix‡	–	–		700	700	700
Chemical analysis (mmol/kg dry wt)						
Nitrogen	1543	1536	1729	2286	2264	2464
Phytate	nd	nd	6·8	nd	nd	7·0
Lactose	339	35	0	348	35	0
Magnesium	20·6	20·6	37·0	24·7	24·7	41·2
Calcium	92·5	92·5	15·0	135·0	135·0	67·5
Phosphorus	109·7	109·7	90·3	154·8	154·8	135·5
Sodium	60·9	60·9	82·6	156·5	156·5	178·3
Potassium	169·2	169·2	189·7	130·8	130·8	151·3

nd, not determined.

* Used as such: to 2800 g of these products, which corresponded with 300 g dry matter, 700 g of either the protein mineral-free or protein–mineral-rich mixture was added.

† Consisted of the following (g/kg dry diet): rice flour 620·91, cellulose 57, sunflower oil 8, vitamin premix 10, ferrous sulphate 0·09, chromium trioxide 4. The composition of the vitamin premix (AIN-76) was as described by the American Institute of Nutrition (1977).

‡ Consisted of the following components (g/kg dry diet): ovalbumin 105, cellulose 56, rice flour 396·2, palm oil 105, mineral premix 24·5, vitamin premix 9·1, choline chloride 1·4, chromium trioxide 2·8. The mineral premix consisted of the following (mg): $CaCO_3$ 1926, $CaHPO_4.2H_2O$ 4667, KH_2PO_4, 3418, $FeSO_4.7H_2O$ 176, $MnSO_4.H_2O$ 167, $ZnSO_4.7H_2O$ 98, $CuSO_4.5H_2O$ 78, KIO_3 0·3, $Na_2SeO_3.5H_2O$ 0·3, $CrK(SO_4)_2.12H_2O$ 13, dextrose 13 956·4. The composition of the vitamin premix (AIN-76) was as described by the American Institute of Nutrition (1977).

Expt 2

The aim of this experiment was to compare mineral availability from the different diets under conditions of equal growth and mineral intake by the rats. The soya-bean beverage is deficient in P, Ca and methionine; thus, these nutrients were added, either alone or in combination, to concentrations identical to those in cow's milk. The soya-bean beverage contained more Mg, Na and K than cow's milk. In order to balance the diets for these components as well, they were added to cow's milk to concentrations identical to those in the soya-bean beverage. The composition of the diets used in Expt 2 is shown in Table 3. All diets contained the protein–mineral-free mixture. Forty-two rats, with a mean body-weight of 79 g, were randomly divided into six dietary groups of equal size and fed on the diets.

Statistics

A priori defined contrasts were evaluated using Bonferroni's test for multiple comparisons (Fleiss, 1986). The level of statistical significance adjusted for multiple comparisons was preset at $P < 0.05$. The following contrasts were tested. Expt 1: lactase-treated cow's milk *v.* cow's milk against both dietary backgrounds; soya-bean beverage *v.* cow's milk against both dietary backgrounds; protein–mineral-rich diets *v.* protein–mineral-free diets with

Table 3. *Expt 2. Composition of the diets containing cow's milk (M) or soya-bean beverage (S)*

Experimental diets with protein–mineral-free background...	M		S			
Supplements*...	None	Mg+Na+K	None	Met	P+Ca	P+Ca+Met
Ingredients (g dry wt)						
Milk*	300	300	–	–	–	–
Soya-bean beverage*	–	–	300	300	300	300
DL-methionine (Met)	–	–	–	0·95	–	0·95
$CaHPO_4.2H_2O$	–	–	–	–	4·4	4·4
$CaCO_3$	–	–	–	–	6·6	6·6
$MgCO_3$		1·2	–	–	–	–
$NaHCO_3$	–	1·8	–	–	–	–
$KHCO_3$	–	5·8	–	–	–	–
Protein–mineral-free mix†	700	700	700	700	700	700
Chemical analysis (mmol/kg dry wt)						
Nitrogen	1678	1664	1793	1771	1764	1786
Met	27·5	27·5	22·1	28·1	20·8	28·2
Phytate	nd	nd	6·8	6·8	6·9	7·0
Magnesium	20·6	34·6	37·0	36·8	36·9	37·1
Calcium	100·0	100·0	12·5	12·7	102·5	103·8
Phosphorus	106·5	106·1	80·6	77·4	104·8	105·2
Sodium	52·2	82·6	82·6	81·5	80·3	80·3
Potassium	125·6	164·1	164·8	163·7	163·9	164·2

nd, not determined.

* Used as such: to 2800 g of these products, which corresponded with 300 g dry matter, 700 g of either the protein-mineral-free or protein–mineral-rich mixture was added.

† Consisted of the following (g/kg dry diet): rice flour 620·91, cellulose 57, sunflower oil 8, vitamin premix 10, ferrous sulphate $FeSO_4$ 0·09, chromium trioxide 4. The composition of the vitamin premix (AIN^{-76}) was as described by the American Institute of Nutrition (1977).

identical product. Expt 2: non-supplemented soya-bean beverage *v.* non-supplemented cow's milk; supplemented cow's milk *v.* non-supplemented cow's milk; soya-bean beverage supplemented with P, Ca and methionine *v.* supplemented cow's milk; soya-bean beverage supplemented with methionine *v.* non-supplemented soya-bean beverage; soya-bean beverage supplemented with P and Ca *v.* non-supplemented soya-bean beverage; soya-bean beverage supplemented with P, Ca and methionine *v.* non-supplemented soya-bean beverage; soya-bean beverage supplemented with P, Ca and methionine *v.* soya-bean beverage supplemented with methionine; soya-bean beverage supplemented with P, Ca and methionine *v.* soya-bean beverage supplemented with P and Ca. Only those contrasts that reached statistical significance are indicated in Tables 4–7.

RESULTS

Expt 1

Body-weight gain and feed intake

Body-weight gain and feed intake of rats fed on soya-bean beverage against the protein–mineral-free background were significantly decreased when compared with rats fed

Table 4. *Expt 1. Body-weight gain, feed intake and magnesium and calcium balance of rats given diets containing cow's milk (M), lactase* (EC *3.2.1.23*)*-treated cow's milk (L) or soya-bean beverage (S) in a protein–mineral-free or protein–mineral-rich background**

(Values are expressed as group means for seven rats per dietary group)

	Protein–mineral-free background			Protein–mineral-rich background			
Experimental diets...	M Mean	L Mean	S Mean	M Mean	L Mean	S Mean	SED
Body-wt gain (g/28 d)	137	144	88[b]	140	150	148[g]	6
Feed intake (g/28 d)†	455	478	391[b]	397[e]	421[f]	407	8
Mg balance							
Mg intake (μmol/d)	358	366	556[b]	325	329	551[d]	22
Faecal Mg excretion (μmol/d)	90	144[a]	226[b]	70	95[c]	222[d]	14
Mg absorption (μmol/d)	267	226[a]	329[b]	255	235[c]	329[d]	9
Mg absorption (%)	75	62[a]	59[b]	79	71[c,f]	60[d]	1
Urinary Mg excretion (μmol/d)	185	136[a]	206	177	152	222[d]	8
Plasma Mg (mmol/l)	0·82	0·82	0·90[b]	0·86	0·83	0·88	0·01
Ca balance							
Ca intake (μmol/d)	1525	1575	255[b]	1950[e]	1973[f]	937[d,g]	94
Faecal Ca excretion (μmol/d)	351	419	20[b]	810[e]	788[f]	44[d,g]	37
Ca absorption (μmol/d)	1174	1156	205[b]	1139	1185	893[d,g]	80
Ca absorption (%)	77	73	94[b]	59[e]	60[f]	95[d]	2
Urinary Ca excretion (μmol/d)	33	9[a]	3[b]	26	17	4[d]	2
Plasma Ca (mmol/l)	2·90	2·93	2·85	2·93	2·95	2·91	0·01

a–g, *a priori* selected contrasts which are significantly different ($P < 0·05$): a, L *v.* M in the protein–mineral-free background; b, S *v.* M in the protein–mineral-free background; c, L *v.* M in the protein–mineral-rich background; d, S *v.* M in the protein–mineral-rich background; e, M in the protein–mineral-rich background *v.* M in the protein–mineral-free background; f, L in the protein–mineral-rich background *v.* L in the protein–mineral-free background; g, S in the protein–mineral-rich background *v.* S in the protein–mineral-free background.

SED, standard error of difference.

* For details of procedures, see pp. 272–275.

† On a dry weight basis.

on cow's milk (Table 4). There were no significant differences between groups fed on the diets containing the protein–mineral-rich mixture. Replacement of the protein–mineral-free mixture by the protein–mineral-rich mixture in the diet with soya-bean beverage significantly increased body-weight gain. Such replacement did not influence body-weight gain when the diet contained either milk or lactase-treated milk, while it significantly decreased feed intake.

Mg balance

Irrespective of the composition of the dietary background, which in either case did not contain added Mg, Mg intake was significantly increased in rats fed on soya-bean beverage (Table 4). This was associated with an increased absolute Mg absorption and urinary and faecal Mg excretion, while percentage Mg absorption was decreased. Lactase-treated *v.* normal cow's milk significantly decreased intestinal Mg absorption and lowered urinary Mg excretion. Mg concentration in plasma was increased by soya-bean beverage *v.* cow's milk against a protein–mineral-free background.

Table 5. *Expt 1. Bone variables in rats given diets containing cow's milk (M), lactase* (EC *3.2.1.23)-treated cow's milk (L) or soya-bean beverage (S) in protein–mineral-free or protein–mineral-rich background**

(Values are expressed as group means for seven rats per dietary group)

	Protein–mineral-free background			Protein–mineral-rich background			
Experimental diets...	M	L	S	M	L	S	
Variable	Mean	Mean	Mean	Mean	Mean	Mean	SED
Plasma alkaline phosphatase (*EC* 3.1.3.1) activity (U/l)	91	96	119[a]	110	97	84[b,c]	4
Urinary hydroxyproline excretion (μmol/d)	7·6	8·0	9·4[a]	8·0	8·3	9·0[b]	0·3
Femur magnesium (mmol/cm³)	0·16	0·15	0·11[a]	0·16	0·16	0·14[b,c]	0·006
Femur calcium (mmol/cm³)	4·85	4·68	2·72[a]	4·84	4·67	4·20[b,c]	0·16

a–c, *a priori* selected contrasts which are significantly different ($P < 0·05$): a, S *v.* M in the protein–mineral-free background; b, S *v.* M in the protein–mineral-rich background; c, S in the protein–mineral-rich background *v.* S in the protein–mineral-free background.

SED, standard error of difference.

* For details of procedures, see pp. 272–275.

Ca balance

Ca intake was significantly lower in rats fed on soya-bean beverage instead of cow's milk, which was associated with decreased absolute Ca absorption, decreased urinary and faecal Ca excretion and an increased percentage Ca absorption (Table 4). Absolute Ca absorption in rats fed on soya-bean beverage was significantly higher with the diets containing the protein–mineral-rich mixture instead of the protein–mineral-free mixture. Hydrolysis of lactose in milk did not significantly affect Ca absorption, but it decreased urinary Ca excretion. The latter effect just failed to reach statistical significance in rats fed on diets with the protein–mineral-rich background. Plasma concentrations of Ca were not significantly influenced by the experimental diets.

Bone metabolism

When the rats were fed on the diets containing the protein–mineral-free mixture, urinary hydroxyproline excretion and plasma alkaline phosphatase activity were significantly increased by soya-bean beverage *v.* cow's milk (Table 5). When soya-bean beverage and cow's milk were compared against a protein–mineral-rich (Mg-free) background, urinary hydroxyproline excretion was still increased, but plasma alkaline phosphatase activity was significantly decreased in rats fed on soya-bean beverage. Mg and Ca concentrations in femur were significantly lower in rats fed on the soya-bean beverage instead of cow's milk, irrespective of the background composition of the diet. Hydrolysis of lactose in milk did not significantly affect bone variables.

Expt 2

Body-weight gain and feed intake

Body-weight gain was significantly lower in rats fed on non-supplemented soya-bean beverage than in rats given non-supplemented milk (Table 6). Growth retardation in the

Table 6. *Expt 2. Body weight gain, feed intake and magnesium and calcium balance of rats given diets containing cow's milk (M) or soya-bean beverage (S) in a protein–mineral-free background either with or without supplements**

(Values are expressed as group means for seven rats per dietary group)

Experimental diets...	M		S				
Supplements...	None Mean	Mg+Na +K Mean	None Mean	Met Mean	P+Ca Mean	P+Ca +Met Mean	SED
Body-wt gain (g/28 d)	172	165	138[b]	140	158	165[e]	4
Feed intake (g/28 d)†	447	446	446	428	472	467	8
Mg balance							
Mg intake (μmol/d)	356	625[a]	623[b]	623	705	661	25
Faecal Mg excretion (μmol/d)	98	228[a]	245[b]	258	384[d]	355[c,e,f]	22
Mg absorption (μmol/d)	258	397[a]	378[b]	364	321[d]	305[c,e,f]	12
Mg absorption (%)	72	65[a]	61[b]	59	46[d]	46[c,e,f]	3
Urinary Mg excretion (μmol/d)	133	271[a]	252[b]	239	146[d]	199[c]	15
Plasma Mg (mmol/l)	0·88	0·88	0·98	0·97	0·91	0·91	0·03
Ca balance							
Ca intake (μmol/d)	1900	2035	213[b]	210	2104[b]	1973[e,f]	130
Faecal Ca excretion (μmol/d)	500	610	8[b]	7	826[d]	753[c,e,f]	71
Ca absorption (μmol/d)	1400	1424	205[b]	203	1278[d]	1220[c,e,f]	98
Ca absorption (%)	74	70	96[b]	97	61[d]	62[c,e,f]	3
Urinary Ca excretion (μmol/d)	70·3	67·5	3·3[b]	3·4	37·1[d]	31·3[c,e,f]	7
Plasma Ca (mmol/l)	2·85	2·84	2·74[b]	2·70	2·80	2·84[f]	0·01

a–f, *a priori* selected contrasts which are significantly different ($P < 0{\cdot}05$): a, M supplemented with Mg, Na and K *v*. M without supplements; b, S without supplements *v*. M without supplements; c, S supplemented with P, Ca and methionine *v*. M supplemented with Mg, Na and K; d, S supplemented with P and Ca *v*. S without supplements; e, S supplemented with P, Ca and Met *v*. S without supplements; f, S supplemented with P, Ca and Met *v*. S supplemented with Met.

SED, standard error of difference.

* For details of procedures, see pp. 272–276.

† On a dry weight basis.

rats fed on soya-bean beverage had disappeared after supplementation of the diet with either P plus Ca or with the combination of P, Ca and methionine. Feed intakes of rats fed on the experimental diets were not significantly different.

Mg balance

Intake, absolute absorption, and urinary and faecal excretion of Mg were significantly higher in rats fed on non-supplemented soya-bean beverage compared with milk (Table 6). Percentage Mg absorption was decreased in rats fed on soya-bean beverage. Supplementation of soya-bean beverage with methionine alone did not affect Mg balance. Adding P plus Ca to the soya-bean beverage diet caused a significant increase in faecal Mg excretion and reduced urinary excretion as well as intestinal absorption. Further supplementation with methionine did not significantly influence urinary and faecal excretion of Mg. The addition of Mg, Na and K to the cow's milk diet caused an increased intake of Mg, associated with increased urinary and faecal excretion, and absolute absorption of Mg. As a consequence, percentage Mg absorption was decreased. Although Mg intake did not differ between the rats fed on the diet containing either soya-bean beverage supplemented with P, Ca and methionine or milk supplemented with Mg, Na and

Table 7. *Expt. 2. Bone variables in rats given diets containing cow's milk (M) or soya-bean beverage (S) in protein–mineral-free background either with or without supplements**

(Values are expressed as group means for seven rats per dietary group)

Experimental diets...	M		S				
Supplements...	None	Mg+Na +K	None	Met	P+Ca	P+Ca +Met	
Variable	Mean	Mean	Mean	Mean	Mean	Mean	SED
Plasma alkaline phosphatase (*EC* 3.1.3.1) activity (U/l)	77	83	117[a]	103	60[c]	58[b,d,e]	5
Urinary hydroxyproline excretion (μmol/d)	8·1	8·3	13·4[a]	13·8	8·2[c]	9·2[d,e]	0·5
Femur magnesium (mmol/cm³)	0·16	0·16	0·09[a]	0·08	0·15[c]	0·15[d,e]	0·007
Femur calcium (mmol/cm³)	4·78	4·83	2·45[a]	2·18	4·68[c]	4·65[d,e]	0·25

a–e, *a priori* selected contrasts which are significantly different ($P < 0·05$): a, S without supplements *v.* M without supplements; b, S supplemented with P, Ca and methionine *v.* M supplemented with Mg, Na and K; c, S supplemented with P and Ca *v.* S without supplements; d, S supplemented with P, Ca and Met *v.* S without supplements; e, S supplemented with P, Ca and Met *v.* S supplemented with Met.

SED, standard error of difference.

* For details of procedures, see pp. 272–276.

K, the former showed significantly lower absolute and percentage Mg absorption. Plasma Mg concentrations were not significantly affected by the dietary treatments.

Ca balance

Intake, absolute absorption and urinary excretion of Ca were significantly lower in rats fed on non-supplemented soya-bean beverage than in rats fed on non-supplemented milk (Table 6). Percentage Ca absorption was significantly higher in rats fed on soya-bean beverage. Ca balance was not affected after supplementation of the soya-bean beverage with methionine. The addition of P plus Ca or the combination of P, Ca and methionine caused similar increases in Ca intake, absolute absorption and urinary and faecal excretion of Ca, while percentage Ca absorption was similarly reduced. Fortification of milk with Mg, Na and K did not significantly affect Ca balance. Although Ca intake was not different between the two groups, percentage and absolute Ca absorption in rats fed on the soya-bean-beverage diet supplemented with P, Ca and methionine were significantly lower than in rats fed on the cow's milk diet either without or with added Mg, Na and K. Plasma Ca concentrations were decreased in rats fed on non-supplemented soya-bean beverage in comparison with those fed on non-supplemented milk.

Bone metabolism

Urinary hydroxyproline excretion and plasma alkaline phosphatase activity were significantly increased in rats fed on non-supplemented soya-bean beverage compared with cow's milk (Table 7). The addition of methionine to the soya-bean-beverage diet did not affect these variables, but addition of either P plus Ca or the combination of P, Ca and methionine decreased both variables significantly. In rats fed on the soya-bean-beverage diet supplemented with P, Ca and methionine, plasma alkaline phosphatase activity was significantly lower than in rats fed on the cow's milk diet supplemented with Mg, Na and

K. However, urinary hydroxyproline excretion did not differ significantly between the two dietary groups.

Mg and Ca concentrations in the femur were significantly lower in rats fed on non-supplemented soya-bean beverage than in rats fed on cow's milk. Femur Mg and Ca were not significantly affected by the addition of methionine to the soya-bean beverage diet. Supplementation of the soya-bean beverage diet with either P plus Ca or P, Ca and methionine significantly increased Mg and Ca concentrations in the femur. The addition of Mg, Na and K to milk did not affect bone variables. Mg and Ca concentrations in the femur were similar in rats fed on either soya-bean beverage supplemented with P, Ca and methionine or cow's milk supplemented with Mg, Na and K.

DISCUSSION

When soya-bean beverage and cow's milk were incorporated into diets containing a protein–mineral-free mixture, soya-bean beverage compared with cow's milk consistently depressed body-weight gain and caused decreased femur concentrations of Mg and Ca in both experiments. Bone turnover in rats given soya-bean beverage was increased, as indicated by increased plasma alkaline phosphatase activity (indicator of bone formation) and increased urinary hydroxyproline excretion (indicator of bone resorption). These effects of soya-bean beverage were most probably caused by the low concentration of Ca in this product (Table 1) as they were reversed after the addition of Ca (Tables 5 and 7). Ca deficiency in young rats is known to cause growth retardation and increased bone turnover (Irving, 1973).

Rats fed on soya-bean beverage supplemented with P, Ca and methionine had lower plasma alkaline phosphatase activities than rats fed on cow's milk supplemented with Mg, Na, and K (Table 7). This most probably relates to the protein source. With the use of carefully balanced diets, soya-bean protein isolate decreased plasma alkaline phosphatase activities in rats when compared with casein (E. J. Brink, P. R. Dekker, E. C. H. van Beresteijn and A. C. Beynen, unpublished results). However, the effect of protein on plasma alkaline phosphatase was not associated with decreased Mg and Ca concentrations in the femur (Table 7).

Under conditions of equal growth rates and the feeding of diets with identical Mg and Ca concentrations, intestinal absorption and urinary excretion of Mg and Ca were significantly higher in rats fed on cow's milk than in those given soya-bean beverage (Table 6). This was not reflected in a difference in femur concentrations of Mg and Ca (Table 7). Apparently, the absorbed amounts of these minerals in rats fed on soya-bean beverage sufficed to sustain normal bone mineralization. In rats fed on soya-bean beverage diets not supplemented with P plus Ca, absolute Ca absorption did not suffice (Tables 6 and 7).

Hydrolysis of lactose in milk significantly decreased Mg absorption, but did not affect Ca absorption (Table 4). This agrees with the fact that lactose *v.* other carbohydrates increases Mg absorption (Andrieux & Sacquet, 1983; Schaafsma *et al.* 1988; Greger *et al.* 1989; Behling & Greger, 1990). The effect of lactose on Ca absorption is controversial. Lactose has been reported to enhance Ca absorption (Fournier *et al.* 1971; Leichter & Tolensky, 1975; Armbrecht & Wassermann, 1976; Favus & Angeid-Backmann, 1984). However, no effect of lactose has also been reported (Greger *et al.* 1987; Sheikh *et al.* 1987; Recker *et al.* 1988; Behling & Greger, 1990). The reason why lactose stimulates Mg absorption, but perhaps not that of Ca, is not known.

Apart from the absence of lactose in soya-bean beverage, the presence of phytate may also explain why soya-bean beverage *v.* cow's milk lowers mineral absorption. Phytate decreases Mg and Ca absorption (Nwokolo & Bragg, 1977; Davies, 1979; Lonnerdal *et al.*

1989). Addition of phytate to diets with casein, to a concentration identical to that in diets containing soya-bean protein, significantly decreased apparent absorption of Mg (Brink *et al.* 1991) and Ca (E. J. Brink, P. R. Dekker, E. C. H. van Beresteijn and A. C. Beynen, unpublished results) in rats to a level seen after feeding soya-bean protein. Thus, phytate in soya-bean protein appears responsible for the reduction in Mg and Ca absorption induced by soya-bean protein when compared with casein.

In conclusion, the results of the present study indicate that lactose and phytate, when present in the matrix of intact cow's milk and soya-bean beverage respectively, still exert their influence on the bioavailability of Mg and Ca. Soya-bean beverage *v.* cow's milk lowers apparent absorption of Mg and Ca.

E. J. B. was supported by the European Community (grant no. 1150/86-33.07). The authors wish to thank Maria Peters of the Laboratory Animals Center, Wageningen Agricultural University, for biotechnical assistance.

REFERENCES

American Institute of Nutrition (1977). Report of the American Institute of Nutrition *ad hoc* committee on standards for nutritional studies. *Journal of Nutrition* **117**, 1340–1348.

Andrieux, C. & Sacquet, E. (1983). Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Reproduction Nutrition Développement* **23**, 258–271.

Armbrecht, H. J. & Wasserman, R. H. (1976). Enhancement of Ca^{++} uptake by lactose in the rat small intestine. *Journal of Nutrition* **106**, 1265–1271.

Behling, A. R. & Greger, J. L. (1990). Importance of lactose in yogurt for mineral utilization. *Journal of Agricultural and Food Chemistry* **38**, 200–204.

Brink, E. J., Dekker, P. R., van Beresteijn, E. C. H. & Beynen, A. C. (1991). Inhibitory effect of dietary soybean protein versus casein on magnesium absorption in rats. *Journal of Nutrition* **121**: 1374–1381.

Brons, C. & Olieman, C. (1983). Study of high performance liquid chromatographic separation of reducing sugars applied to the determination of lactose in milk. *Journal of Chromatography* **259**, 79–86.

Davies, N. T. (1979). Anti-nutrient factors affecting mineral utilization. *Proceedings of the Nutrition Society* **38**, 121–126.

Favus, M. J. & Angeid-Backmann, E. (1984). Effects of lactose on calcium absorption and secretion by rat ileum. *American Journal of Physiology* **246**, G281–G285.

Fiske, C. H. & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**, 375–400.

Fleiss, J. L. (1986). *The Design and Analysis of Clinical Experiments*, pp. 103–107. New York: John Wiley and Sons.

Fournier, P., Dupuis, Y. & Fournier, A. (1971). Effect of lactose on the absorption of alkaline earth metals and intestinal lactase activity. *Israel Journal of Medical Sciences* **7**, 389–391.

Greger, J. L., Gutkowski, C. M. & Khazen, R. R. (1989). Interactions of lactose with calcium, magnesium and zinc in rats. *Journal of Nutrition* **119**, 1691–1697.

Greger, J. L., Krzykowski, C. E., Khazen, R. R. & Krashoc, C. (1987). Mineral utilization by rats fed commercially available calcium supplements or milk. *Journal of Nutrition* **117**, 717–724.

International Dairy Federation (1986). Determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. International IDF standard no. 20A. Brussels: International Dairy Federation.

Irving, J. T. (1973). *Calcium and Phosphorus Metabolism*. New York: Academic Press.

Leichter, J. & Tolensky, A. F. (1975). Effect of dietary lactose on the absorption of protein, fat and calcium in the postweanling rat. *American Journal of Clinical Nutrition* **28**, 238–241.

Lonnerdal, B., Sandberg, A. S., Sandstrom, B. & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *Journal of Nutrition* **119**, 211–214.

Moore, S. (1963). On the determination of cystine as cysteic acid. *Journal of Biological Chemistry* **238**, 235–237.

Nwokolo, E. N. & Bragg, D. B. (1977). Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. *Canadian Journal of Animal Science* **57**, 475–477.

Recker, R. R., Bammi, A., Barger-Lux, J. & Heaney, R. (1988). Calcium absorbability from milk products, an imitation milk and calcium carbonate. *American Journal of Clinical Nutrition* **47**, 93–95.

Reddy, N. R., Sathe, S. K. & Salunkhe, P. K. (1982). Phytates in legumes and cereals. *Advances in Food Research* **28**, 1–92.

Schaafsma, G., Dekker, P. R. & de Waard, H. (1988). Nutritional aspects of yogurt. 2. Bioavailability of essential minerals and trace elements. *Netherlands Milk and Dairy Journal* **42**, 135–146.

Sheikh, M. S., Santa Ana, C. A., Nicar, M. J., Schiller, L. R. & Fordtran, J. S. (1987). Gastrointestinal absorption of calcium salts. *New England Journal of Medicine* **317**, 532–536.
Slump, P., Bos, K. D. & Wolters, M. G. E. (1987). Improved determination of phytate by HPLC. *Euro Food Chemistry* IV. *Rapid Analysis in Food Processing and Food Control*, vol. 1, pp. 151–155. Loen, Norway: Norwegian Food Research Institute.
Van der Meer, R., de Vries, H. T. & van Tintelen, G. (1988). The phosphorylation state of casein and the species-dependency of its hypercholesterolaemic effect. *British Journal of Nutrition* **59**, 467–473.

Printed in Great Britain