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The effect of casein phosphopeptides on calcium utilization in young ovariectomized rats

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Einfluß von Kasein-Phosphopeptiden auf die Kalziumverwertung bei jungen ovarektomierten Ratten

Summary: The effect of casein phosphopeptides (CPPs) on Ca utilization in ovariectomized (OVX) rats was studied. A mixture of CPPs corresponding to the amino acid sequences 1–25 and 1–28 in the β-casein was isolated from the tryptic digest of β-casein (βCPP).

After being fed a low Ca diet for 30 days, OVX rats were fed experimental diets of which the Ca level was 0.1 %, 0.3 % or 0.5 % with or without 0.15 % βCPP for 28 days. During days 1–3 of the Ca refeeding period, rats fed βCPP with 0.5 % Ca showed a higher Ca absorption than control rats not supplemented with βCPP.

During days 7–9 and 26–28, there were no significant differences in Ca and P balances between the βCPP group and the control group for each dietary Ca level. Femoral Ca and P contents from rats fed βCPP tended to be higher than those from control rats. These results suggest that βCPP supplementation could have an effect on Ca absorption at a certain degree of Ca deficiency.

Zusammenfassung: Gegenstand der Untersuchung war der Einfluß von Kasein-Phosphopeptiden (CPPs) auf die Kalziumverwertung bei ovarektomierten Ratten (OVX). Aus dem tryptischen Extrakt von β-Kasein (βCPP) wurde eine den Aminosäuresequenzen 1–25 und 1–28 im β-Kasein entsprechende CPP-Mischung isoliert. Nach Füttern mit einer Ca-armen Diät über 30 Tage wurden die OVX-Ratten 28 Tage mit Versuchsdiäten mit Ca-Niveaus von 0,1 %, 0,3 % bzw. 0,5 % sowie mit bzw. ohne 0,15 % βCPP gefüttert. An den Tagen 1–3 der Ca-Ergänzungsperiode zeigten Ratten, an die βCPP mit 0,5 % Ca verfüttert wurde, eine höhere Ca-Absorption als ohne βCPP-Zusatz ernährte Kontrollratten. An den Tagen 7–9 und 26–28 war zwischen der βCPP-Gruppe und der Kontrollgruppe für das jeweilige Ca-Niveau kein sinnvoller Unterschied im Ca- und P-Haushalt feststellbar. Die femoralen Ca- und P-Gehalte von mit βCPP gefütterten Ratten tendierten zu höheren Werten als bei den Kontrollratten. Diese Ergebnisse deuten darauf hin, daß die βCPP-Ergänzungen bei einem gewissen Grad von Ca-Mangel die Ca-Absorption beeinflussen könnten.

Key words: Casein phosphopeptide – Ca utilization – bone – ovariectomy

Schlüsselwörter: Kasein-Phosphopeptide – Ca-Verwertung – Knochen – Ovarektomie

Abbreviations:

CPP	casein phosphopeptide
OVX	ovariectomized
FER	feed efficiency ratio

Introduction

Casein phosphopeptides (CPPs) are phosphoserine-rich sequences that are derived from the in vitro (17) or in vivo (14, 19, 20) digestion of bovine casein. CPP enhanced the absorption of Ca from a ligated loop of rat small intestine by inhibiting the precipitation of Ca phosphate (12). The Ca-absorption-stimulating effect of CPP has also been reported by using another in vitro technique (18). Tryptic hydrolysis of whole casein produced a mixture of phosphorylated peptides (3, 9), but it is not yet clear whether individual CPPs would have the same effect on Ca utilization or not. Other investigators have recently shown that CPP did not stimulate intestinal Ca absorption in vivo (4, 10). No effect of CPP on bone mineralization in growing pigs was observed (23, 27). However, the effects of CPP on Ca absorption in vivo could vary due to the degree of Ca requirement or the physiological state of the animals. Ovariectomized (OVX) rats are known as an animal model for postmenopausal osteoporosis, and ovariectomy combined with Ca deprivation severely reduced the bone mass in rats (8). The present study was undertaken to determine whether purified dietary phosphopeptides would improve the Ca utilization in young OVX rats with low body Ca and diminished bone mineralization.

Materials and Methods

Preparation of CPP

β-casein was fractionated from bovine casein by means of urea solutions (7). β-casein solution was incubated with TPCK bovine trypsin (SIGMA Chemical Co.) at an enzyme to protein ratio of 1:1000 at 37 °C for 24 h. During the incubation time the pH of reaction mixture was maintained at 8.0 by the occasional addition of 1N NaOH. After the incubation the pH was adjusted to 4.6 by 1N HCl. The supernatant was obtained by centrifugation. The Ca content was adjusted to 20mM by addition of 1M $CaCl_2$. Mixed with equal volume of ethanol, the precipitate was removed by centrifugation, washed with 50 % and then 100 % ethanol, and dried (βCPP). βCPP was roughly estimated to be a mixture of peptides corresponding to the amino acid sequences 1–25 and 1–28 in the β-casein by amino acid analysis (Table 1). This was also confirmed by the HPLC pattern of βCPP showing two major peaks (Fig. 1). Ninety percent of total amino acids in βCPP were derived from the amino acid residues of β-casein (1–28).

Diets

Diet composition is shown in Table 2. Calcium carbonate was used as a Ca source in the diets. Since the level of βCPP was low (0.15 %), the diets were not balanced for N, P or Ca. The P level was 0.3 % for all experimental diets. Egg albumin was used as a protein source to avoid phosphopeptides from casein digest.

Animals

Forty-two 5-week-old Sprague Dawley female rats were housed in individual stainless steel cages in a temperature-controlled (23 ± 2 °C) room with 50±5 % humidity and a 12-h light-dark cycle, and they were fed a low-Ca diet (Ca, 0.01 %; P, 0.3 %).

On the second day of feeding, rats were ovariectomized under diethyl ether anesthesia. After 31 days of feeding, animals were separated into six groups of seven rats, according to similar mean body weight. The six groups were fed one of the experimental diets and deionized water ad libitum for 28 days. Rats were put into individual metabolic cages for collecting fecal and urine samples during days 27–30 of Ca deprivation, and during days 1–3, 7–9, and 26–28 of the experimental diet feeding period. Body

Table 1. Amino acid composition of CPP

		β-casein[2]	
	βCPP[1]	(1–25)	(1–28)
		mg/gN	
Asx	351	295	510
Thr	239	266	230
Ser	780	1172	1014
Glu	2288	2297	1986
Gly	147	167	145
Val	443	522	452
Ile	487	585	506
Leu	725	877	759
Lys	94	0	282
Arg	514	776	672
Pro	252	257	222
Others	121	0	0
		%	
N[3]	11.3	14.0	14.6
P[4]	3.2	3.5	3.9
Ca[4]	3.6	0	0

[1] Lyophilized βCPP was hydrolyzed with 12N HCl at 110° C for 22 h and analyzed with an amino acid analyzer (Hitachi-835).
[2] Values are calculated from amino acid sequences of β-casein.
[3] Total N was measured by micro-Kjeldahl method.
[4] Inorganic P and Ca were determined by the method described in the text.

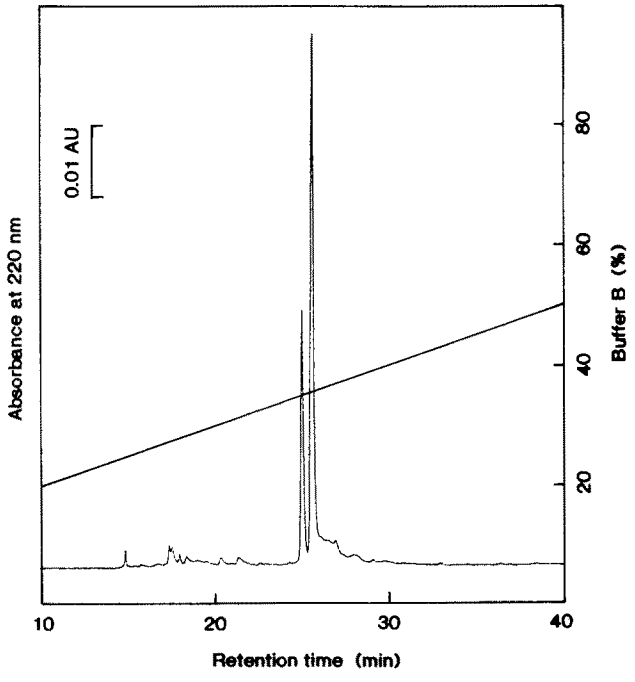

Fig. 1. A chromatogram of βCPP. Conditions: 4.6 × 150 mm Asahipak ODP-50 column; 0.8 mL/min of linear gradient from 0.1 % TFA solution (buffer A) to acetonitrile with 0.1 % TFA/buffer A (90 %, v/v) (buffer B); UV detector (220 nm); room temperature.

Table 2. Composition of diet

Ingredient	Low Ca	0.1 % Ca		0.3 % Ca		0.5 % Ca	
		Control	βCPP	Control	βCPP	Control	βCPP
				g			
Glucose monohydrate[1]	652	650	648.5	645	643.5	640	638.5
Egg albumin[2]	180	180	180	180	180	180	180
Cottonseed oil[3]	100	100	100	100	100	100	100
Cellulose[4]	30	30	30	30	30	30	30
Vitamin mix[5]	1	1	1	1	1	1	1
Choline chloride[6]	2	2	2	2	2	2	2
Ca-free mineral mix[7]	34.8	34.8	34.8	34.8	34.8	34.8	34.8
$CaCO_3$[3]	0.2	2.2	2.2	7.2	7.2	12.2	12.2
βCPP[8]			1.5		1.5		1.5

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[2] Q.P. Corp., Tokyo, Japan.
[3] Kanto Chemical Co., Inc., Tokyo, Japan.
[4] Toyo Roshi Co., Ltd., Tokyo, Japan.
[5] Made up in glucose monohydrate, supplying (mg/kg diet): thiamin · HCl, 5; riboflavin, 0.5; pyridoxine, 5; D-calcium pantothenate, 28; nicotinamide, 20; inositol, 200; folic acid, 0.2; cyanocobalamin, 0.02; biotin, 0.1.
[6] Tokyo Chemical Industry Co., Ltd., Tokyo, Japan.
[7] Made up in glucose monohydrate, supplying (mg/kg diet): potassium chloride, 11540; sodium chloride, 4180; magnesium sulfate, 3580; ferrous sulfate heptahydrate, 644; copper sulfate pentahydrate, 15.6; sodium fluoride, 22.6; cobalt clorided hexahydrate, 0.8; potassium iodate, 2.0; manganese sulfate pentahydrate, 12; zinc sulfate heptahydrate, 88; ammonium molybdate tetrahydrate, 1; potassium dihydrogen phosphate, 6500; dipotassium hydrogenphosphate, 8300.
[8] Phosphopeptides isolated from tryptic β-casein digest.
[9] The animals were fed, three times a week, a mixture of fat-soluble vitamins in 0.1 ml cottonseed oil that supplied 70 μg of β-carotene, 105 μg of 2-methy-1,4-naphtoquinone, 875 μg of α-tocopherol, and 525 I.U. of vitamin D_3 weekly.

weight and feed intake were recorded three times a week. After a 28-day experimental diet feeding period, the animals were fasted overnight and anesthetized by ether, and blood samples were taken from the aorta ventralis to determine plasma Ca and P concentrations. The right and left femurs were excised and the surrounding flesh was removed.

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 1N of nitric acid for Ca analysis by an atomic absorption spectrophotometer (Shimadzu, AA-640-12). Phosphorus was determined by a spectrophotometric method (1). Urinary Ca and P contents were directly determined as described above. Plasma Ca and inorganic P were determined by an autoanalyzer (Hitachi, 736).

Statistics

All data were analyzed by two-way analysis of variance (ANOVA) to determine main effects (dietary Ca level and βCPP supplementation). Beacuse no interaction (dietary Ca level × βCPP supplementation) was observed, individual means were compared by Tukeys q-test ($p < 0.05$) for each dietary Ca level if ANOVA showed a significant effect.

Results

Weight gain, feed intake and FER

The effect of dietary Ca level on weight gain, feed intake and feed efficiency ratio (FER) was significant (Table 3). Dietary βCPP supplementation did not affect these values significantly, although there was a trend for rats fed βCPP to weigh more than control rats for each dietary Ca level.

Table 3. Body weight gain, feed intake and feed efficiency ratio (FER)[1]

	0.1 % Ca		0.3 % Ca		0.5 % Ca	
	Control	βCPP	Control	βCPP	Control	βCPP
Body weight gain (g)	26.0 ± 3.6^{a}	27.1 ± 2.8^{ac}	39.4 ± 3.9^{ab}	41.7 ± 2.5^{b}	41.1 ± 3.9^{bc}	46.6 ± 3.7^{b}
Feed intake (g/day)	10.9 ± 0.4	11.3 ± 0.3	12.1 ± 0.6	12.3 ± 0.3	12.7 ± 0.9	12.7 ± 0.4
FER[2] (%)	8.4 ± 1.0^{a}	8.6 ± 0.8^{a}	11.6 ± 0.8^{ab}	12.1 ± 0.6^{ab}	11.8 ± 1.3^{ab}	13.0 ± 0.7^{b}

Two way ANOVA[3]

	Main effect	
	Dietary Ca level	βCPP supplementation
Body weight gain	**	NS
Feed intake	*	NS
FER	**	NS

[1] Values are means ± S.E. Means in the same row not sharing a common superscript letter are significantly different by Tukey's q-test ($p < 0.05$).
[2] FER (%) = (Body weight gain/feed intake) × 100.
[3] ** and *, significant effect ($p < 0.01$ and $p < 0.05$); NS, not significant ($p < 0.05$).

Mineral balance

During days 1–3, βCPP supplementation affected Ca absorption: animals fed βCPP with dietary Ca of 0.5 % gave a significantly higher Ca absorption than the control group (Table 4). A significantly higher dietary Ca intake during days 1–3, 66.9 ± 4.3 mg/day for the βCPP group vs. 52.7 ± 4.7 mg/day for the control group, was reflected partly in this high Ca absorption. No differences in Ca absorption or balance were observed between βCPP groups and control groups for the other balance test periods. During days 1–3, Ca digestibilities were more than 95 % in all groups, but the values from rats fed 0.3 % or 0.5 % Ca decreased on day 26–28. Likewise, decreases in Ca absorption for rats fed 0.5 % Ca were observed during days 26–28. This suggests that animals required more dietary Ca than was provided by the 0.5 % Ca diet for weeks 1–2 of Ca refeeding.

Although the effect of βCPP supplementation on P absorption was significant during days 1–3, digestibility or balance was not significantly affected (Table 5). During days 1–3, the increase in dietary P intake for rats fed βCPP with 0.5 % Ca was reflected in the P absorption and balance as was observed in Ca absorption.

Although addition of βCPP to diets increased their P contents by 1.6 %, no differences in dietary P intake due to the augmented P in diets were observed in any of the groups. Dietary Ca level had a significant effect on P balance during days 1–3 and 7–9. P balance for rats fed 0.5 % Ca or 0.3 % Ca decreased during days 26–28 compared with

Table 4. Changes in Ca balance[1]

		0.1 % Ca		0.3 % Ca		0.5 % Ca	
		Control	βCPP	Control	βCPP	Control	βCPP
Ca depletion, days 27–30							
Absorption[2]	(mg/day)	0.9 ± 0.1	0.9 ± 0.2	1.1 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Digestibility[3]	(%)	76.1 ± 6.8	71.6 ± 9.9	85.8 ± 3.0	81.5 ± 4.6	79.6 ± 4.5	73.3 ± 8.4
Balance[4]	(mg/day)	0.8 ± 0.1	0.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
Ca refeeding, days 1–3							
Absorption	(mg/day)	10.4 ± 0.8^{a}	11.3 ± 0.7^{a}	32.7 ± 1.7^{b}	35.0 ± 2.0^{b}	50.6 ± 4.3^{c}	61.8 ± 3.4^{d}
Digestibility	(%)	97.3 ± 0.4^{a}	96.5 ± 0.8^{ab}	97.1 ± 0.5^{ab}	96.6 ± 0.5^{ab}	96.1 ± 0.5^{ab}	94.9 ± 0.7^{b}
Balance	(mg/day)	10.3 ± 0.8^{a}	11.2 ± 0.7^{a}	32.6 ± 1.7^{b}	33.4 ± 1.9^{b}	49.7 ± 4.1^{c}	54.0 ± 5.4^{c}
Ca refeeding, days 7–9							
Absorption	(mg/day)	10.3 ± 0.4^{a}	10.7 ± 0.4^{a}	33.0 ± 1.5^{b}	32.8 ± 1.4^{b}	52.1 ± 4.2^{c}	50.0 ± 2.2^{c}
Digestibility	(%)	95.1 ± 1.7^{a}	94.3 ± 0.6^{a}	96.6 ± 0.5^{a}	94.2 ± 0.8^{a}	83.5 ± 3.1^{b}	82.7 ± 2.7^{b}
Balance	(mg/day)	10.1 ± 0.3^{a}	10.5 ± 0.4^{a}	32.7 ± 1.5^{b}	32.4 ± 1.4^{b}	50.2 ± 4.3^{c}	48.8 ± 2.1^{c}
Ca refeeding, days 26–28							
Absorption	(mg/day)	13.3 ± 0.6^{a}	12.1 ± 1.1^{a}	29.9 ± 0.8^{b}	27.6 ± 1.6^{b}	29.2 ± 4.9^{b}	34.3 ± 2.0^{b}
Digestibility	(%)	93.2 ± 2.6^{a}	93.9 ± 1.4^{a}	79.4 ± 2.4^{ab}	67.5 ± 3.5^{b}	37.3 ± 5.4^{c}	47.6 ± 4.7^{c}
Balance	(mg/day)	13.0 ± 0.5^{a}	11.8 ± 1.1^{a}	29.5 ± 0.8^{b}	26.9 ± 1.5^{b}	27.8 ± 5.1^{b}	33.2 ± 1.8^{b}

Two-way ANOVA[5]

	Absorption		Digestibility		Balance	
	A	B	A	B	A	B
Ca depletion, days 27–30	NS	NS	NS	NS	NS	NS
Ca refeeding, days 1–3	**	*	*	NS	**	NS
days 7–9	**	NS	**	NS	**	NS
days 26–28	**	NS	**	NS	**	NS

[1] Values are means ± S.E. Means in the same row not sharing a common superscript letter are significantly different by Tukey's q-test ($p < 0.05$).
[2] Absorption = intake-fecal excretion.
[3] Digestibility (%) = (absorption/intake) × 100.
[4] Balance = absorption-urinary excretion.
[5] A, dietary Ca level; B, βCPP supplementation; ** and *, significant effect ($p < 0.01$ and $p < 0.05$); NS, not significant.

the values during days 1–3, whereas P balance for rats fed 0.1 % Ca increased during days 26–28. This indicates that the P balance was associated with Ca utilization.

Dietary Ca level and βCPP supplementation significantly affected plasma Ca content: plasma Ca increased with increasing dietary Ca level, and the values for rats fed βCPP were slightly lower than those for control rats (data are not shown). There were not significant differences in plasma P content among the groups.

Femur mineral analyses

The level of dietary Ca and the βCPP supplementation affected Ca and P contents of the femur (Table 6). Femoral Ca and P for the group fed βCPP with 0.5 % dietary Ca were higher than for any of the other groups.

Table 5. Changes in P balance

		0.1 % Ca		0.3 % Ca		0.5 % Ca	
		Control	βCPP	Control	βCPP	Control	βCPP
Ca depletion, days 27–30							
Absorption	(mg/day)	30.4 ± 2.8	34.1 ± 2.3	34.2 ± 1.1	34.2 ± 2.3	33.3 ± 2.2	30.9 ± 1.2
Digestibility	(%)	90.3 ± 4.7	93.3 ± 1.0	94.7 ± 1.2	91.8 ± 1.5	90.2 ± 2.5	90.5 ± 2.4
Balance	(mg/day)	8.9 ± 1.8	8.5 ± 1.5	8.4 ± 1.0	9.3 ± 1.7	7.8 ± 1.9	5.8 ± 1.1
Ca refeeding, days 1–3							
Absorption	(mg/day)	32.4 ± 2.7	36.6 ± 2.0	34.0 ± 1.8	35.4 ± 2.3	31.7 ± 2.8	39.5 ± 2.5
Digestibility	(%)	95.5 ± 1.0	97.0 ± 0.3	95.9 ± 0.6	95.1 ± 1.3	95.6 ± 0.9	93.9 ± 1.1
Balance	(mg/day)	13.9 ± 2.7^{a}	16.3 ± 0.9^{a}	21.7 ± 1.1^{ab}	20.9 ± 1.8^{ab}	23.6 ± 3.2^{ab}	29.0 ± 3.5^{b}
Ca refeeding, days 7–9							
Absorption	(mg/day)	31.8 ± 1.1	33.2 ± 1.3	34.1 ± 1.8	34.2 ± 1.6	35.9 ± 2.4	33.9 ± 1.6
Digestibility	(%)	92.9 ± 1.6	92.5 ± 0.7	94.8 ± 1.2	93.2 ± 0.9	91.7 ± 1.7	89.0 ± 2.9
Balance	(mg/day)	7.4 ± 1.5^{a}	8.3 ± 0.9^{a}	18.6 ± 1.3^{b}	17.4 ± 1.4^{b}	26.0 ± 2.8^{c}	25.7 ± 1.6^{c}
Ca refeeding, days 26–28							
Absorption	(mg/day)	42.7 ± 1.5^{a}	42.6 ± 2.1^{a}	35.2 ± 1.9^{ab}	36.1 ± 1.7^{ab}	34.5 ± 3.1^{ab}	33.8 ± 2.1^{b}
Digestibility	(%)	94.6 ± 0.9^{a}	94.2 ± 1.1^{a}	88.4 ± 1.5^{a}	84.1 ± 2.5^{a}	70.8 ± 4.5^{b}	73.1 ± 4.1^{b}
Balance	(mg/day)	15.9 ± 1.7	19.9 ± 2.6	19.2 ± 1.8	17.4 ± 2.4	21.3 ± 2.9	21.8 ± 1.1

	Two-way ANOVA[5]					
	Absorption		Digestibility		Balance	
	A	B	A	B	A	B
Ca depletion, days 27–30	NS	NS	NS	NS	NS	NS
Ca refeeding, days 1–3	NS	*	NS	NS	**	NS
days 7–9	NS	NS	**	NS	**	NS
days 26–28	**	NS	**	NS	NS	NS

[1] Values are means ± S.E. Means in the same row not sharing a common superscript letter are significantly different by Tukey's q-test ($p < 0.05$).
[2] Absorption = intake-fecal excretion.
[3] Digestibility (%) = (absorption/intake) × 100.
[4] Balance = absorption-urinary excretion.
[5] A, dietary Ca level; B, βCPP supplementation; ** and *, significant effect ($p < 0.01$ and $p < 0.05$); NS, not significant.

Discussion

The present data suggest the possibility that dietary supplementation of βCPP could improve the Ca utilization in OVX rats. It would appear that the higher femoral mineral content in rats fed with βCPP might have resulted from better Ca and P balances over the 4-week Ca-refeeding period. Although the higher diet intake observed during days 1–3 partly reflected the mineral balance in the rats fed with βCPP, it is suggested that the better mineral balances throughout the Ca-refeeding period were responsible for the effect of βCPP.

OVX rats are characterized by low circulating blood levels of 1,25-dihydroxyvitamin D_3 (21), so that the major part of Ca absorption could depend, not on active transport, but on passive diffusion from the lumen. CPPs could enhance this passive diffusion by inhibiting the precipitation of Ca phosphate (13) and increasing soluble Ca (11), without

Table 6. Femur mineral analyses[1)]

	0.1 % Ca		0.3 % Ca		0.5 % Ca	
	Control	βCPP	Control	βCPP	Control	βCPP
Dry weight (mg)	325.3 ± 16.6^{a}	317.1 ± 13.2^{a}	415.6 ± 10.0^{b}	412.6 ± 10.1^{b}	410.9 ± 13.0^{b}	437.6 ± 7.8^{b}
Ca (mg/g)	156.5 ± 3.7^{a}	159.5 ± 2.6^{a}	180.5 ± 2.8^{b}	189.2 ± 4.2^{bc}	189.2 ± 3.6^{bc}	200.8 ± 7.6^{c}
P (mg/g)	74.6 ± 2.1^{a}	81.0 ± 2.1^{ab}	87.5 ± 1.8^{bc}	92.6 ± 1.4^{c}	92.8 ± 2.1^{c}	93.1 ± 2.3^{c}

Two way ANOVA[2)]

	Main effect	
	Dietary Ca level	βCPP supplementation
Dry weight	**	NS
Ca	*	*
P	**	*

[1)] Values are means ± S.E. Means in the same row not sharing a common superscript letter are significantly different by Tukey's q-test ($p < 0.05$).

[2)] ** and *, significant effect ($p < 0.01$ and $p < 0.05$); NS, not significant ($p < 0.05$).

depending on vitamin D (18). However, no significant increase in Ca absorption in the rats fed with βCPP was observed, except during days 1–3 with a dietary Ca level of 0.5 %, which is in agreement with previous observations (23, 27). This suggests that the effect of βCPP on Ca absorption could be observed only at a certain degree of Ca deficiency. Moreover, an adequate level of dietary Ca of more than 0.5 % seems to have been needed to show the effects of βCPP, and this should be further studied. With regard to bone mineralization, a difference in the bone status of the animals used might account for the statistically significant effects of βCPP in this study. Ovariectomy causes bone to become more susceptible to the resorptive actions of the parathyroid hormone (5, 22) due to diminished calcitonin, and Ca stimulates calcitonin secretion in OVX rats (6). Therefore, the effect of augmented Ca absorption by βCPP supplementation on bone mineralization may have been observed in the OVX rats. To confirm the effects of βCPP on bone mineralization, long-term feeding studies should be conducted on aged OVX rats, because the animals used in this study were relatively young for use as a model for postmenopausal bone loss (30).

Since αs_1- and αs_2-casein contain more phosphoseryl residues in the molecules, CPPs of various sizes and sequences are produced from a tryptic digest when compared with those from β-casein (16). A nonapeptide fragment corresponding to sequence 66–74 in αs_1-casein has been isolated as a major CPP from the jejunal chyme of minipigs fed on a 15 % casein diet (15). Although the βCPP used in this study was a little different from those produced from β- casein in vivo, it had the same core region consisting of consecutively bound phosphoserine (26). The present results support those of the previous study, in which a purified phosphopeptide, β-casein (1–25), enhanced Ca absorption from the ligated loop of rat small intestine and augmented the deposition of Ca in the femur (25). Contrary to this, supplementation with synthetic phosphoseryl peptide $[Ser(P)]_3$-Glu (28) or semipurified CPP (31) had little effect on the absorption or on the Ca content of the femur in rats. This discrepancy might be the result of a difference in the type of CPP or in the experimental animals, as already mentioned. However, it is

noteworthy that the dietary level of βCPP (0.15 %) in this study was substantially lower than that of purified CPP (3 %) or synthetic phosphopeptide (1.2 %) in those studies. The phosphates in CPPs are essential for the effect of CPP on Ca absorption (24). Moreover, the importance of neighboring amino acid residues for the physicochemical properties of phosphoseryl residues and for their cation-binding ability has been reported (2). However, neither a quantitative nor qualitative determination of the individual CPPs produced by in vivo or in vitro digestion of whole casein has been fully accomplished; therefore, their relative effects on Ca absorption are not known.

Although CPPs have resistance to proteolytic enzymes (17), further studies are necessary to observe changes in the digestion and absorption of CPP prepared in vitro when fed orally.

These results indicate that future studies may contribute evidence that establishes the possibility of applying dietary CPP to Ca deficiency in premature or very low birthweight infants, or to people suffering from osteoporosis (29).

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