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Short Communications

Effect of casein phosphopeptides on utilization of calcium in minipigs and vitamin-D-deficient rats

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Introduction

Several investigators have reported that phosphopeptides increase the intestinal absorption of calcium under short term conditions (5, 11, 13). Phosphopeptides are phosphoserine-rich sequences that are released during the in vitro (10, 11) or in vivo (6, 9) digestion of casein. We explored whether the absorption of calcium and thereby the mineralization of bone could be elevated in vivo by phosphopeptides during long-term nutrition studies. Further, we wondered whether such a stimulating effect is more obvious in vitamin-D-depleted animals, as reported by Mykkänen and Wasserman (11).

Methods

In Study 1, weanling piglets were fed on a semipurified diet for 12 weeks. The diet is described in detail elsewhere (3) and was modified as follows: the diet contained only 15 wt% protein, either as casein or whey protein concentrate, 0.3 % calcium, and 25 wt% sucrose instead of corn starch. Whey protein lacks such phosphoserine-rich sequences and, therefore, has no phosphopeptide-releasing potential.

In Study 2, weanling rats were fed a semipurified diet for 7 weeks. The diet contained 20 wt% protein, either as casein, whey protein concentrate, or whey protein concentrate plus a phosphopeptide preparation and 0.8 % calcium. Further components were: (g/100 g) soy oil, 4; cellulose, 3; minerals and vitamins, 6; and corn starch ad 100. (The phosphopeptide preparation was kindly provided by Sopharga, Ceully, France, and prepared according to the technical procedure described in a U.S. patent (2).) The rats were vitamin-D-depleted; they were bred from mothers which got a diet free of vitamin D. The animals were kept in a room

with artificial, UV-free light. Balance periods were performed in pigs in weeks 9–10, and in rats in week 5. At the end of both studies the animals were sacrificed. Femora were taken for analyses.

Results and discussion

There were no significant differences in content of calcium and hydroxylapatite or bending moment of the femur between casein- or whey-protein-fed piglets (Table 1). No significant difference in calcium balance was observed (data not shown). This demonstrates that casein-derived phosphopeptides did not raise the utilization of dietary calcium.

In vitamin-D-depleted rats the diets containing the phosphopeptide preparation or casein did not increase the apparent absorption or retention of calcium in comparison to whey protein (Table 2).

Other than what could be expected from the results reported earlier (5, 6, 10, 11, 13), we did not observe that phosphopeptides or casein-generating phosphopeptides elevate the bioavailability of calcium or change parameters of bone performance. This observation was made in two animal species: in pigs consuming diets with high (data not shown) or low calcium content, and in vitamin-D-replete (data not shown) and -depleted rats.

The calcium-absorption-stimulating effect of phosphopeptides had been observed by other investigators who used the ligated loop technique (7, 13) or in vitro methods (11).

However, in our controlled nutritional study no effect on calcium absorption and retention, or bone mineralization and stability was observed. We conclude that phosphopeptides do not contribute significantly to the utilization of calcium. Recent in vitro (4, 8) and in vivo studies (1) support this finding. Moreover, our results are in full agreement with Pointillart (12), who reported no calcium-absorption-stimulating effect of phosphopeptides in domestic pigs. One has to keep in mind, however, that

Table 1. Effect of diets containing casein or whey protein concentrate and 0.3 % calcium on parameters of femora.

Parameter	Casein		Whey protein	
	$\bar{x}$	SEM	$\bar{x}$	SEM
Wet weight (g)	40.7	1.9	42.0	5.9 ns[e])
Ash in DM[a]) (%)	57.4	1.3	56.6	1.4 ns
Calcium (mg/g wet)[b])	179.2	4.9	174.7	4.6 ns
Bone density[c]) (g hydroxylapatite/cm^3)	1.01	0.01	0.98	0.02 ns
Bending moment[d]) (kN)	1.05	0.07	0.93	0.06 ns

Values are means ± SEM of eight growing miniature pigs weighing 15–20 kg. [a]) = dry matter, [b]) calcium was measured by atom absorption spectrometry, [c]) bone density was measured radiographically according to Wolschendorf and Vanselow (14), [d]) bending moment was measured by a universal testing machine kindly provided by Beiersdorf, Hamburg, FRG, [e]) ns = $p > 0.05$.

Table 2. Effect of phosphopeptides (PP) on apparent calcium absorption, calcium retention, and plasma calcium in growing, vitamin-D-depleted rats.

	Casein	Whey protein	Whey protein + PP
	$\bar{x}$ (SEM)	$\bar{x}$ (SEM)	$\bar{x}$ (SEM)
Apparent Ca absorption[a] (%)	43.0 (1.7)	44.3 (1.2)	44.9 (1.8)
Ca retention[a] (mmol/5 d)	4.53 (0.24)	4.87 (0.22)	5.10 (0.35)
Plasma Ca[b]	2.57 (0.15)	2.57 (0.13)	2.70 (0.15)
Plasma $25(OH)D_3$[b][c]	n.d.	n.d.	n.d.

Values are means ± SEM for seven animals: [a]) of a 5-day balance period, [b]) at week 7, [c]) $25(OH)D_3$ = Cholecalcidiol, n.d. = not detectable (< 7 nmol/l). Cr_2O_3 was included as non absorbable marker in the diet. There were no significant differences between diets of any parameter.

in our study a rather crude mixture of phosphopeptides was used. Therefore, it cannot be excluded that specific phosphopeptides may enhance calcium absorption and retention.

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References

1. Brommage R, Berrocal R, Juillerat MA, Jost R (1990) Influence of casein phosphopeptides on intestinal calcium absorption. Abstr 23. Int Dairy Congress, Oct 7–12, 1990, Montreal, Canada
2. Brule G, Roger L, Fauquant J, Piot M (1982) Phosphopeptides from casein-based material. United States Patent No 4, 361 587, Nov 30
3. Hagemeister H, Scholz-Ahrens KE, Schulte-Coerne H, Barth CA (1990) Plasma amino acids and cholesterol following consumption of dietary casein or soy protein in minipigs. J Nutr 120, in press
4. Juillerat MA, Berrocal R, Getaz F, Jost R, Arnaud M (1990) In vivo and in vitro stability of casein phosphopeptides during intestinal transit. Abstr 23. Int Dairy Congress, Oct 7–12, 1990, Montreal, Canada
5. Lee YS, Noguchi T, Naito H (1979) An enhanced intestinal absorption of calcium in the rat directly attributed to dietary casein. Agric Biol Chem 43 (9):2009–2011
6. Lee YS, Noguchi T, Naito H (1980) Phosphopeptides and soluble calcium in the small intestine of rats given a casein diet. Br J Nutr 43:457–467
7. Lee YS, Noguchi T, Naito H (1983) Intestinal absorption of calcium in rats given diets containing casein or amino acid mixture: the role of casein phosphopeptides. Br J Nutr 49:67–76
8. Li Y, Tomé D, Desjeux JF (1989) Indirect effect of casein phosphopeptides on calcium absorption in rat ileum in vitro. Reprod Nutr Develop 29:227–233

9. Meisel H, Frister H (1988) Isolation and chemical characterization of a phosphopeptide from in vivo digests of casein. In: Barth CA, Schlimme E (eds) Milk proteins: Nutritional, clinical, functional and technological aspects. Steinkopff Verlag, Darmstadt, pp 150–154
10. Mellander O (1950) The physiological importance of the casein phosphopeptide calcium salts. II. Peroral calcium dosage of infants. Acta Soc Med Upsal 55:247–255
11. Mykkänen HM, Wasserman RH (1980) Enhanced absorption of calcium by casein phosphopeptides in rachitic and normal chicks. J Nutr 110:2141–2148
12. Pointillart A, Guéguen L (1989) Absence d'effect de l'incorporation d'un phosphopeptide du lait sur l'utilisation du calcium et du phosphore chez le jeune porc. Reprod Nutr Dev 29:477–486
13. Sato R, Noguchi T, Naito H (1986) Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. J Nutr Sci Vitaminol 32:67–76
14. Wolschendorf K, Vanselow K (1988) Erreichbare Genauigkeit bei der röntgendensitometrischen Mineralgehaltbestimmung des kompakten Knochens. In: Heuck FHW, Keck E (eds) Fortschritte der Osteologie in Diagnostik und Therapie. Springer-Verlag, Berlin Heidelberg, pp 399–403

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