

Influence of Phosvitin and Calcium Gluconate Concentration on Permeation and Intestinal Absorption of Calcium Ions

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Abstract The effect of egg yolk phosvitin on the permeation and absorption of calcium was investigated *in vitro* in relation to calcium gluconate concentration. Obtained results indicate that phosvitin significantly reduces the intestinal calcium absorption from 1 and 10 mM of calcium gluconate solution. It is associated with the formation of the complex of Ca (II) ions with phosvitin. The process of calcium permeation increases under phosvitin influence when calcium gluconate concentrations rise up to 10 mM. At a higher concentration of calcium gluconate (20 mM), no effect of phosvitin was seen on permeation of calcium ions.

Keywords Phosvitin · Calcium gluconate · Permeability · Intestinal absorption · *In vitro*

Introduction

Calcium is an essential element involved in electrolytic balance and regulatory mechanisms in the organism [1, 2]. Insufficient calcium intake is a recognized risk of osteoporosis. Calcium absorption ranges from 10–75%, depending on the age and physiological status of individuals.

Increasing the effectiveness of calcium absorption by means of substances that positively influence its absorption are active areas of interest. Some of the substances that have been found

to increase the intestinal calcium absorption from the gastrointestinal tract include vitamin D, magnesium, casein, inulin and other resistant sugars, and some amino acids [3].

The same effect could be demonstrated by phosvitin phosphopeptides [3, 4]. Phosvitin is the most active peptide from egg yolk and comprises about 7% of yolk dry matter [5]. In egg yolk, phosvitin naturally forms calcium aggregates created by phosphocalcic bridges [6].

Phosvitin is an acidic protein rich in phosphate groups bound mainly by serine. In acidic solutions with low ionic strength, phosvitin has a good potential for metal chelating. It is able to form complexes with divalent ions such as Ca^{2+} , Mg^{2+} , Co^{2+} , Mn^{2+} , and with Fe^{3+} [6, 7].

It has been suggested that phosvitin phosphate anions enhance calcium absorption and bioavailability. In some studies, phosvitin phosphopeptides were found to have a high calcium-binding ability. Moreover, these phosphopeptides inhibit formation of insoluble calcium salts and improve Ca(II) absorption in the small intestine. Phosvitin peptides added to diets at a concentration of 0.125–0.5% (1.25–5.0 mg/g) significantly increase Ca absorption and accumulation in bones [4, 8, 9].

In this study we determined the influence of phosvitin on permeation of calcium ions in relation to three different concentrations of calcium gluconate from a simulated stomach environment to an acceptor medium that imitated the ileum section of the GI tract.

Materials and Methods

Substances

Phosvitin from egg yolk—phosphoglycoprotein ($M=34$ kDa) containing 8–10% (w/w) phosphorus was purchased from

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Sigma-Aldrich Chemie, Buchs. Calcium gluconate $\text{Ca}(\text{C}_6\text{H}_{11}\text{O}_7)_2 \times \text{H}_2\text{O}$ ($M=448.4$ g/mol, 8% calcium content) was purchased from Pharma Cosmetics, Poland. All substances were analytically pure and compatible with quality standards and certificates.

Small Intestine

The study was carried out on 15 specimens of small intestine that were collected from 6-month-old pigs weighing 100 ± 2 kg. The intestine specimens weighed on average 290 ± 10 mg. Once removed from the carcass and dissected, the intestines were washed with 0.9% NaCl solution to an absorbance value with an extinction coefficient $\varepsilon < 0.02$ at 278 nm, and then quickly frozen at -20°C until needed for the experiments [13].

Physiological Fluids

Physiological fluids mimicking the natural conditions of the stomach and ileum: artificial gastric juice at pH 1.3 and artificial intestinal fluid at pH 7.5, respectively [11, 12].

Research Model

The study was carried out in a standard Franz diffusion cell with two 2-ml identical chambers fitted with input and output channels [14]. One side acted as the donor (D) chamber and the other as the acceptor (A). A disk cut from the small intestine tissue was used to separate compartments D and A, which were kept at the same level as required in the “side-by-side” method. Chamber D (simulating stomach) was filled with 2 ml of artificial gastric juice (pH 1.3) in which calcium gluconate at different concentrations (40, 400, or 800 mg/l) were dissolved (control groups) or 20 $\mu\text{g/l}$ of phosvitin were additionally added. Chamber A (ileum) was filled with 2 ml of the artificial intestinal fluid (pH 7.5). The lowest concentration was used as model of a calcium-deficient state.

The experiment was carried out with a stable chamber oscillation at a rate of 50 rpm. After 0, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, and 5.0 h, the entire content of chamber A was collected and replaced with 2.0 ml of fresh fluid. At the end of each experiment, the content of both chambers was collected and the amount of Ca(II) that permeated through the intestine tissue was calculated from the difference $D - A$, where D is the total amount of Ca(II) in milligrams per liter that remains in chamber D and A is the total amount of Ca(II), also in milligrams per liter that permeated through the intestine after 5 h.

Calcium Analysis

A validated spectrophotometric method was used for the determination of calcium (II) ions using a “Calcium O-CPC Kit” from Pointe Scientific [15]. The absorbance was measured at $\lambda=570$ nm in 1.0-cm glass cuvettes with a UV-VIS “Marcel-Media” spectrophotometer (France). The photometric accuracy of the spectrophotometer was ± 0.005 A. The empirical regression equation $y=1.06x+0.41$ was used to establish the relationship between $[\text{Ca}^{2+}]$ and the absorbance. The significance of the equation was $R^2=0.994$.

Statistical Analysis

The data are given as means \pm standard deviation, with $n=10$ for all groups. The permeability and absorption process of Ca(II) ions depends on the concentration of calcium gluconate in the control groups and were used to compare the results of runs where phosvitin was also added. The Student's *t* test was used to establish statistical significance, set at $p < 0.05$. The software packages Excel (Microsoft) and Statistic for Windows 5.1 (StatSoft Inc.) were used for all calculations.

Results and Discussion

Calcium homeostasis maintains the Ca(II) concentration within physiological limits in the organism. The balance between permeation and intestinal calcium absorption is one of the Ca(II) homeostasis mechanisms. The amount of absorbed calcium depends on many factors that may stimulate or inhibit the absorption process [1–3, 16]. Some researchers claim that calcium is a threshold nutrient, meaning that the relationship between Ca intake and its level in the body occurs just between a certain range and higher intakes do not result in higher calcium accumulation in bones [10, 17].

After 5 h in the calcium-deficient model (1 mM calcium gluconate), 11 mg/l (27%) Ca(II) was lost from D but

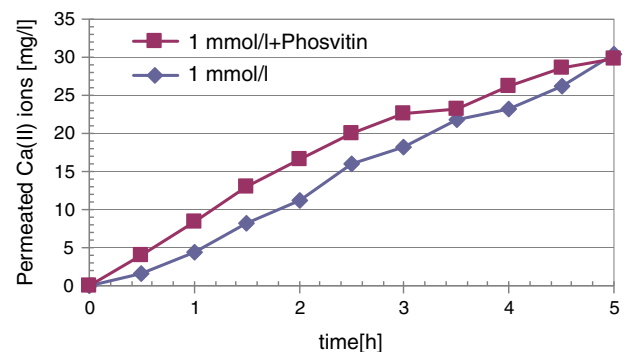


Fig. 1 Influence of phosvitin on permeation of Ca(II) ions at 1 mM calcium gluconate concentration during 5 h

Table 1 Influence of phosvitin and calcium gluconate concentration on permeation and absorption of Ca(II) ions through small intestine after 5 h

Concentration of calcium gluconate		Donor (D)				Acceptor (A) Ca(II) permeated		Ca(II) absorbed (D–A)	
mM	mg/l	Ca(II) loss		Residual Ca(II)		(mg/l)	(%)	(mg/l)	(%)
		(mg/l)	(%)	(mg/l)	(%)				
1	40.0	10.5	26.2	29.5±2.2	73.9	27.9±1.2	69.8	−17.4±1.5	−43.6
1+phosvitin	40.0	19.6	49.1	20.4±3.1	50.9	↓27.4±1.9	68.5	↓−7.8±0.9*	−19.5
10	400	327.6	81.9	72.4±3.8	18.1	30.0±1.6	7.5	297.6±1.9	74.4
10+phosvitin	400	251.6	62.9	148.4±3.2*	37.1	↑54.4±0.9*	13.6	↓197.2±3.5*	49.3
20	800	445.4	55.7	354.6±2.7	44.3	83.8±0.9	10.5	361.6±3.3	45.2
20+phosvitin	800	428.0	53.5	372.0±4.2	46.5	↑72.0±5.2	9.0	↓356.0±2.9	44.5

* $p < 0.05$, statistically significant in relation to control groups at the same gluconate concentration

28 mg/l (70%) reached chamber A. The 17 mg/l (44%) difference must have been supplied by the tissue separating these chambers. When phosvitin is also added, the loss of Ca(II) from D increased to 20 mg/l (50%), while 27 mg/l (68%) passed on to A, suggesting that phosvitin inhibited the mobilization from the intestine tissue, supplying only 20% of Ca(II) ions in hypocalcemia. The influence of phosvitin on permeation of Ca(II) from 1 mM calcium gluconate solution is shown in Fig. 1.

Phosvitin did not change the amount of Ca(II) passing through but caused a twofold decrease of its absorption. The permeation and absorption data in relation to calcium gluconate concentration are given in Table 1.

Increasing the concentration of Ca(II) ions to 10 mM, almost the same amount of Ca(II) ions (30 mg/l, 75%) permeated through small intestine while 298 mg/l were absorbed by the small intestine tissue. This process is significantly changed by phosvitin, which causes a twofold increase of Ca(II) permeation, Fig. 2.

At an even higher concentration of calcium gluconate (20 mM), 84 mg/l (11%) of Ca(II) ions passed from D to A and 362 mg/l were absorbed. Addition of phosvitin did not change Ca(II) permeation or absorption at this concentration. The influence of phosvitin on the dynamics of Ca(II)

permeation from 20 mM calcium gluconate solution is given in Fig. 3.

Recent findings indicate that egg yolk phosvitin added to diet significantly enhances Ca absorption and accumulation in bones of rats [4]. Phosvitin contains 300 ppm of bound Ca(II) and in its presence over 400 ppm out of 1,000 ppm added protein is soluble, compared to 150 ppm without phosvitin [4].

The results from this study suggest that egg yolk phosvitin decreases the Ca absorption at calcium-deficient condition. At 10 mM calcium gluconate phosvitin significantly increases Ca(II) permeation ($p < 0.05$). These results are in agreement with previous reports about the decrease of absorption of calcium, magnesium, and iron caused by phosvitin [18].

Conclusions

The obtained results suggest that in a calcium deficiency state, phosvitin might inhibit mobilization of calcium from the small intestine. Egg yolk phosvitin significantly decreases Ca(II) absorption from 1 and 10 mM Ca gluconate

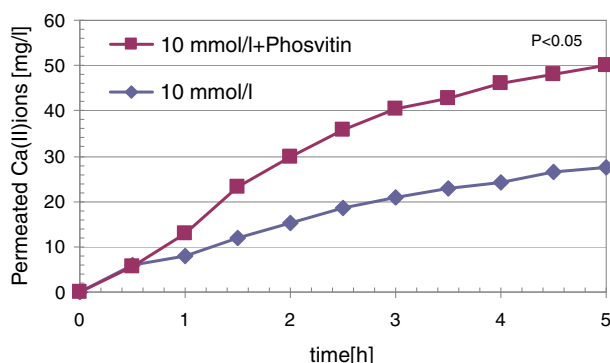


Fig. 2 Influence of phosvitin on permeation of Ca(II) ions at 10 mM calcium gluconate concentration during 5 h

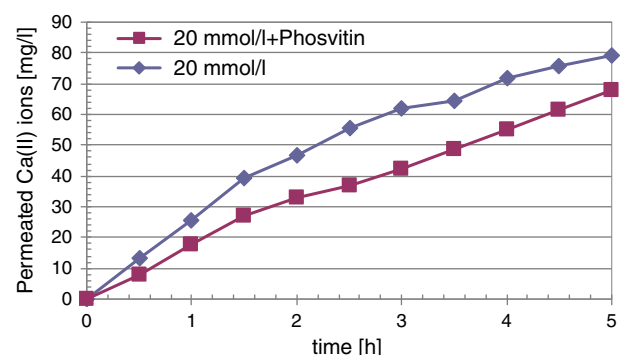


Fig. 3 Influence of phosvitin on Ca(II) ions permeation at 20 mM calcium gluconate concentration during 5 h

solutions and substantially increases its permeation from the 10 mmol/l Ca gluconate solution.

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References

1. Straub DA (2007) Calcium supplementation in clinical practice: a review of forms, doses, and indications. *Nutr Clin Pract* 3:286–296
2. Bronner F (2009) Recent developments in intestinal calcium absorption. *Nutr Rev* 67:109–113
3. Dolińska B, Mikulska A, Ryszka F (2008) Promotory wchłaniania wapnia. [Factors enhancing calcium's absorption]. *Ann Acad Med Siles* 1:89–96
4. Choi J, Jung Ch (2005) Effectiveness of phosvitin peptides on enhancing bioavailability of calcium and its accumulation in bones. *Food Chem* 93:577–583
5. Dolińska B, Woźniak D, Ryszka F (2009) Białka żółtka jaja kurzego, właściwości i zastosowanie. *Farm Przegl Nauk* 6:19–22
6. Belhomme C, David-Briand E, Guerin-Dubiard C (2008) Phosvitin–calcium aggregation and organization at the air–water interface. *Colloids and Surfaces B: Biointerfaces* 63:12–20
7. Catellani O, Guerin-Dubiard C, David-Briand E (2004) Influence of physicochemical conditions and technological treatments on the iron binding capacity of egg yolk phosvitin. *Food Chem* 85:569–577
8. Jiang Bo, Mine J (2000) Preparation of novel functional oligo-phosphopeptides from hen egg yolk phosvitin. *J Agric Food Chem* 48:90–994
9. Jiang Bo, Mine J (2001) Phosphopeptides derived from hen egg yolk phosvitin: effect of molecular size on the calcium-binding properties. *Biosci Biotechnol Biochem* 65:1187–1190
10. Dolińska B, Mikulska A, Ryszka F (2008) Skuteczność preparatów wapnia w profilaktyce jego niedoborów [Calcium preparation effectiveness in prevention of acalcerosis]. *Farm Przegl Nauk* 7–8:5–8
11. Dolińska B, Mikulska A, Caban A, Cieślak A, Ryszka F (2011) A model for calcium permeation into small intestine. *Biol Trace Elem Res* 140:95–102
12. Dolińska B, Mikulska A, Ostróżka-Cieślak A, Ryszka F (2011) The influence of condition on permeation of Ca(II) ions from solutions of selected calcium's salts through model membrane. *Biol Trace Elem Res* 142:456–464
13. Toledo-Pereyra LH, Lopez-Neblina F, Toledo A (2010) Organ freezing. In: Toledo-Pereyra LH (ed) *Organ preservation for transplantation third edition*. (/books/lu/id/2258/). Landes Bioscience, Austin
14. Franz TJ (1975) Percutaneous absorption and the relevance of in vitro data. *J Invest Derm* 64:190–195
15. Nowatzke W, Woolf E (2007) Best practices during bioanalytical method validation for the characterization of assay reagents and the evaluation of analyte stability in assay standards, quality controls, and study samples. *AAPS J* 9:E117–E122
16. Hoenderop J, Nilius B, Bindels R (2005) Calcium absorption across epithelia. *Physiol Rev* 85:373–422
17. Heaney RP (2002) The importance of calcium intake for lifelong skeletal health. *Calcif Tissue Int* 70:70–73
18. Ishikawa SI, Tamaki S, Arihara K (2007) Egg yolk protein and egg yolk phosvitin inhibit calcium, magnesium, and iron absorptions in rats. *J Food Sci* 6:412–419