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An inositol phosphate as a calcium absorption enhancer in rats

Xin Shen, Connie M. Weaver, Anna Kempa-Steczko, Berdine R. Martin, Brian Q. Phillippy,* and Robert P. Heaney†

*Department of Foods and Nutrition, Purdue University, West Lafayette, IN USA; *USDA-ARS, New Orleans, LA USA; and †Creighton University, Omaha, NE USA*

Certain inositol phosphate breakdown compounds have been shown to chelate and increase the solubility of minerals. A rat model using femur uptake of ^{45}Ca was tested for its ability to screen the effect of inositol phosphytate breakdown products on enhancing calcium absorption. Phytate was shown to inhibit calcium absorption in a dose-related manner. A significant enhancing effect of 1,2,3,6-tetraphosphate on calcium absorption was found at the highest level studied ($P < 0.05$), which suggests that this compound may be a candidate for enhancing calcium absorption. (J. Nutr. Biochem. 9:298–301, 1998) © *Elsevier Science Inc. 1998*

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Introduction

The presence of dietary phytate [inositol hexakisphosphate ($InsP_6$)] has been associated with inhibition of mineral absorption including calcium, zinc, iron, and magnesium.[1–5] This inhibition occurs through formation of insoluble phytate-mineral complexes that are less available for absorption in the intestinal tract.[6] Formation of these complexes are pH dependent and chelation strength increases with increasing atomic number of the mineral moving from the alkaline earth metals through transition metals in the periodic table. Thus, calcium is less strongly bound by phytate than iron or zinc, but it is more abundant in the gastrointestinal tract.

Phytate is the major form of phosphorus in plant seeds. Some hydrolysis products of phytate, including inositol pentakisphosphate ($InsP_5$), inositol tetrakisphosphate ($InsP_4$), and lower inositol phosphates ($InsP_3$ and $InsP_2$), are the result of phytate dephosphorylation by endogenous phytase, such as occurs in wheat bran,[7] by exogenous phytase or phosphatase from microbial or fungal sources such as enzymatic pretreatment of feed[8] or during fermentation processes of food.[9,10] However, few studies have examined the effect of these lower inositol phosphates on mineral absorption. Lonnerdal et al.[11] reported that although $InsP_6$ strongly inhibits calcium absorption, there was no significant difference in nonabsorbed ^{45}Ca in cecum of colon of rats fed $InsP_3$, $InsP_4$, or $InsP_5$. These phytate intermediates were prepared nonenzymatically and were not identified. Recently, several inositol phosphate compounds prepared by enzymatic hydrolysis of sodium phytate using wheat phytase were fully characterized and tested for the ability of their inositol phosphate iron chelates to catalyze oxidative reactions.[12] Phytate breakdown products with a 1,2,3-triphosphate grouping increased solubility of iron. These compounds are potential candidates for enhancing cation absorption. Thus, the purpose of this study was to screen $Ins(1,2,3,6)P_4$, $Ins(1,2,5,6)P_4$, and $Ins(1,2,3,5,6)P_5$ as potential candidates for calcium absorption enhancers.

Materials and methods

Materials

Calcium ascorbate, sodium phytate, and wheat phytase were purchased from Sigma Chemical Co. (St. Louis, MO); Bio-Rad AG1-X8 200-400 mesh resin was purchased from Bio-Rad Laboratories (Hercules, CA); high specific activity $^{45}CaCl_2$ was purchased from Amersham (Arlington Heights, IL); and male Sprague-Dawley rats were purchased from Harlan Industries (Indianapolis, IN).

Address correspondence to Dr. Connie M. Weaver, Department of Foods and Nutrition, Purdue University, 1264 Stone Hall, West Lafayette, IN 47907-1264 USA.
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Preparation of Ins(1,2,3,6)P_4, Ins(1,2,5,6)P_4, and Ins(1,2,3,5,6)P_5

Inositol phosphates were prepared by an enzymatic method developed by Phillippy et al.[13,14] Briefly, the procedure involved the following steps. Sodium phytate 2 g was dissolved in 500 ml H_2O, titrated to pH 5 with 0.1 N HCl, adjusted to 1 L with H_2O, and hydrolyzed with 400 mg wheat phytase for 5 hours at 50°C. The reaction was stopped by titrating to pH 2. The broth was filtered through Whatman Number 5 filter paper. The sample (1 L) was loaded on a 3.8 by 18 by 26 cm Bio-Rad AG 1-X8 200-400-mesh anion-exchange resin column at 4 ml/min and eluted as 100 20-ml fractions with a gradient of 0 to 1 N HCl. Peaks were identified by mixing 1 ml 0.1% $Fe(NO_3)_3$ in 2% $HClO_4$ and 2 ml H_2O with 50 μl from each fraction. Absorbence was measured at 290 nm and the corresponding fractions of Ins(1,2,3,6)P_4, Ins(1,2,5,6)P_4, and Ins(1,2,3,5,6)P_5 were collected and titrated to pH 5 with NaOH, lyophilized, dissolved in 2 ml H_2O, adjusted to pH 7 with 5 N NaOH, and stored at −20°C. The resulting inositol phosphates were verified by nuclear magnetic resonance (NMR) spectroscopy.

Rat feeding protocol

All animal protocols were approved by the Purdue Animal Care and Use Committee. Male Sprague-Dawley (weighing 100–125 g or 120–140 g) rats were fed the American Institute of Nutrition (AIN) semipurified diet[15] for 7 days before administration of radiotracers. Rats were individually housed in stainless steel cages with a controlled 12-hour reversed light-dark cycle. Rats were fasted 12 hours before receiving the test solution by gavage. The test solutions (1 ml for each rat) contained 0.625 mmol (25 mg) calcium as calcium ascorbate and 6 μCi ^{45}Ca as $^{45}CaCl_2$ plus inositol phosphates as described below. Approximately 4 hours following administration of the test meals, rats were returned to the semipurified diet.

Initially, the model was tested by confirming that an inhibitory response of $InsP_6$ on calcium absorption could be assessed. The rats used in this study weighed between 110 and 125 g. Rats were divided into three groups of six rats each: one control group, which received no phytate, and two $InsP_6$ groups, which received 30 μmol or 90 μmol phytic acid as sodium phytate.

In the second study, inositol phytate breakdown products were screened for their ability to enhance calcium absorption. Rats weighing from 120 to 140 g were used. Larger rats were used after the first study to facilitate administration by gavage. In the first experiment, four groups of eight rats each were used: a control group without inositol phosphates and three $InsP_4$ groups with 8.3 μmol, 16.6 μmol, or 24.8 μmol Ins(1,2,3,6)P_4, respectively. In the second experiment, four groups of ten rats each were studied: a control group without inositol phosphates, an Ins(1,2,5,6)P_4 at 24.8 μmol group, and two Ins(1,2,3,5,6)P_5 groups at 16.6 μmol or 24.8 μmol.

To obtain fractional calcium absorption, an extra group of rats in each experiment received intraperitoneal (IP) injections containing 6 μCi ^{45}Ca per 0.3 ml isotonic saline.

Analysis

The rats were sacrificed with carbon dioxide 24 hours postdosing and left femurs were removed. Femurs were dissolved in 3 ml concentrated HNO_3 and brought up to a volume of 25 ml with deionized water. Aliquots (1 ml) of the solutions were diluted with 15 ml liquid scintillation cocktail for determination of ^{45}Ca with a Beckman LS 1800 scintillation counter (Beckman Instruments). Absorption of ^{45}Ca from test meals was calculated from the amount of ^{45}Ca in the left femur of each rat receiving an oral dose compared to that in the left femur of rats receiving IP injections (assumes 100% absorption):

$$\% \text{ Absorption} = \frac{\%\ ^{45}\text{Ca oral dose/femur}}{\%\ ^{45}\text{Ca IP dose/femur}} \times 100$$

Means of the various experiment groups were compared using analysis of variance (ANOVA; Dunnet.[16])

Table 1 Phytate effect on ^{45}Ca absorption in rats

Group	^{45}Ca absorption (% of IP injection) mean ± SD[1]
control	55.5 ± 8.8—a
$InsP_6$ (20 mg or 30.0 μmol)	47.9 ± 7.1—a
$InsP_6$ (60 mg or 90.1 μmol)	42.4 ± 10.7—b

[1]Different letters indicate means are significantly different at $P < 0.05$.
IP, intraperitoneal; $InsP_6$, inositol hexakisphosphate.

Results

The dose effect of $InsP_6$ on calcium absorption in rats is shown in *Table 1*. Although the level of $InsP_6$ was inversely related to ^{45}Ca absorption, only the higher level of phytate used achieved significance at $P < 0.05$ compared with the control.

The effect of inositol phosphates with fewer phosphate groups on calcium absorption is shown in *Table 2*. Ins(1,2,3,6)P_4 increased calcium absorption but significance ($P < 0.05$) was not achieved until the highest level (24.8 mmol). At the levels tested, there was no effect of Ins(1,2,5,6)P_4 or Ins(1,2,5,6)P_5 on calcium absorption.

Discussion

Prior to screening inositol phosphates for enhancers of calcium absorption, the rat model was tested for its ability to detect an inhibitory effect of $InsP_6$ on calcium absorption. Previously, we have shown that phytate rich foods inhibit calcium absorption using this model,[4] but purified phytate

Table 2 The effect of inositol phosphates on ^{45}Ca absorption in rats

Group	^{45}Ca absorption (% of IP injection) mean ± SD
Experiment 1	
Control	26.2 ± 2.9
Ins(1,2,3,6)P_4 (8.3 μmol or 5.6 mg)	29.0 ± 4.1
Ins(1,2,3,6)P_4 (16.6 μmol or 11.2 mg)	28.0 ± 5.1
Ins(1,2,3,6)P_4 (24.8 μmol or 16.8 mg)	30.7 ± 2.0[1]
Experiment 2	
Control	23.0 ± 2.0
Ins(1,2,5,6)P_4 (24.8 μmol)	22.5 ± 1.5
Ins(1,2,3,5,6)P_5 (16.6 μmol)	22.6 ± 1.5
Ins(1,2,3,5,6)P_5 (24.8 μmol)	23.2 ± 2.9

[1]Means are significantly different from the control within an experiment at $P < 0.05$.
IP, intraperitoneal; $InsP_4$, inositol tetrakisphosphate; $InsP_5$, inositol pentakisphosphate.

has not been tested using this model. The model was able to show an inhibitory effect of $InsP_6$ on calcium absorption, but achieved significance only at the highest level tested.

Both total phytate content and the molar ratio of phytate:calcium have been suggested as predictors of calcium absorption.[1,2,17,18] The molar ratios of phytate:calcium corresponding to 20 mg and 60 mg phytic acid used in this study were 0.05 and 0.15, respectively. Bullock et al.[19] concluded that calcium solubility was at a minimum when the initial molar ratio of phytate:calcium was 0.2 at pH 7 irrespective of the initial calcium concentration. Only the highest level of phytate used in the present study approached this ratio. However, there is not general agreement about the phytate:calcium ratio that affects calcium absorption. In a review of this concept, Weaver et al.[20] concluded that the total phytate content of food may be more important than the molar ratio. On the other hand, many reports measure all of the inositol phosphates as if they were phytate. This makes the phytate:calcium ratio or total phytate content less meaningful and difficult to compare. We expected that a 30 μmol phytate load (the lowest level studied) would inhibit ^{45}Ca absorption from a 25 mg calcium load in rats because we previously had found that 0.5 wheat bran, which contains 30 μmol phytate (20 mg as phytic acid), was inhibitory to calcium absorption.[21] Lignan purified from the wheat bran showed no inhibitory effect on calcium absorption. Perhaps several constituents in combination are responsible for the inhibition of calcium absorption in wheat bran.

When phosphate groups are removed from phytate, inositol phosphates become more soluble and the mineral-binding strength of phytate becomes progressively lower.[22,23] Dephosphorylation of phytate by processing such as occurs during fermentation and leavening by yeast improves calcium absorption.[2,3] $Ins(1,2,3,6)P_4$, $Ins(1,2,5,6)P_4$, and $Ins(1,2,3,5,6)P_5$ are three of the predominant products produced by phytase from wheat and rice bran.[24,25] Lonnerdal et al.[11] reported that $InsP_4$ and $InsP_5$ did not inhibit calcium absorption, but no enhancer of calcium absorption has been previously identified among the inositol phosphates. $Ins(1,2,3,6)P_4$ enhanced calcium absorption by almost 17%. If the magnitude of enhancement could be transferred from rats to humans on a load of one third of the daily requirement in each meal (the approach used in this rat model), then at least 50 mg extra calcium would be absorbed at each meal in humans. This is the equivalent of raising the calcium content of the meal by 163 mg. Perhaps even higher levels of enhancement could be achieved with more of the enhancer.

Although the effect of higher levels of $Ins(1,2,5,6)P_4$ and $Ins(1,2,3,5,6)P_5$ on calcium absorption is not known, they were not effective in enhancing calcium absorption at the same level as $Ins(1,2,3,6)P_4$. This may be due to the lack of the 1,2,3, grouping or the higher level of phosphorylation for these respective compounds. Interestingly, $Ins(1,2,3,6)P_4$ is more potent than $Ins(1,2,5,6)P_4$ as a second messenger.[26] Perhaps inositol phosphates increase membrane permeability by opening channels when binding to receptors to facilitate absorption. $InsP_3$ receptors have been reported in the gastrointestinal tract.[27]

Few other calcium absorption enhancers have been identified; these include some amino acids and caseinphosphopeptides. Purified $Ins(1,2,3,6)P_4$ added to foods has the potential for enhancing calcium absorption beyond that observed in the absence of inhibitors.

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