Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans\textsuperscript{1–3}

Torsten Bohn, Lena Davidsson, Thomas Walczyk, and Richard F Hurrell

ABSTRACT
Background: Phytic acid has been reported to impair the absorption of minerals and trace elements such as calcium, zinc, and iron in humans. However, limited information is available on the effect of phytic acid on magnesium absorption.

Objective: The objective was to evaluate the effect of phytic acid on fractional apparent magnesium absorption in humans.

Design: Two stable-isotope studies were performed with 8–9 healthy adults per study. Test meals were based on 200 g phytic acid–free wheat bread; test meals with and without added phytic acid were served on days 1 and 3 according to a crossover design. Phytic acid was added in amounts similar to those naturally present in whole-meal (1.49 mmol) and in brown bread (0.75 mmol). Each test meal was labeled with 0.7 mmol \textsuperscript{25}Mg or 1.1 mmol \textsuperscript{26}Mg. The total magnesium content was standardized to 3.6 mmol in all test meals. Apparent magnesium absorption was based on fecal monitoring.

Results: The addition of phytic acid lowered fractional apparent magnesium absorption from 32.5 \pm 6.9\% (no added phytic acid) to 13.0 \pm 6.9\% (1.49 mmol added phytic acid; \textit{P} < 0.0005) and from 32.2 \pm 12.0\% (no added phytic acid) to 24.0 \pm 12.9\% (0.75 mmol added phytic acid; \textit{P} < 0.01). The inhibiting effect of phytic acid was dose dependent (\textit{P} < 0.005).

Conclusion: The results show that fractional magnesium absorption from white-wheat bread is significantly impaired by the addition of phytic acid, in a dose-dependent manner, at amounts similar to those naturally present in whole-meal and brown bread. \textit{Am J Clin Nutr} 2004;79:418–23.

KEY WORDS Magnesium absorption, phytic acid, wheat bread, stable isotopes, fecal monitoring

INTRODUCTION
Phytic acid, myo-inositol hexakisphosphate, is widely distributed in nature because it is the major storage form of phosphorus in cereals, legumes, and oil seeds (1). It is typically found in the outer (aleuron) layers of cereal grains and in the endosperm of legumes and oil seeds. For example, cereal products such as bran and whole-meal bread are rich in phytic acid (2). The daily intake of phytic acid has been estimated to be \approx 200–800 mg in industrialized countries and \approx 2 g in developing countries (3).

Although an inhibiting effect of phytic acid on mineral and trace element absorption has been reported for iron, zinc, calcium, and manganese (4–10), information on the effect of phytic acid on magnesium absorption is limited. Negative magnesium balances were reported to correlate with dietary phytic acid intake, on the basis of observations in 2 human subjects (11), and magnesium absorption was shown to be significantly impaired when phytic acid was added to white-wheat bread (12). In addition, chemical balance studies have indicated significantly increased magnesium absorption after dephytinization of bran muffins (13). However, the dose-dependent effect of phytic acid on magnesium absorption has not been evaluated with the use of isotopic techniques in humans.

The aim of the present study was to evaluate the effect of phytic acid on magnesium absorption in adult humans. Test meals were based on phytic acid–free white-wheat bread; phytic acid was added to simulate the native content of phytic acid in whole-meal and brown-wheat bread. Fractional apparent magnesium absorption was evaluated with a stable-isotope technique based on extrinsic labeling of the meals and fecal monitoring of the excreted labels.

SUBJECTS AND METHODS

Subjects
Twenty (10 women and 10 men) apparently healthy free-living adults were recruited. Exclusion criteria included pregnancy and lactation. No medication, except for oral contraceptives, was allowed during the study. Intake of mineral and vitamin supplements was not permitted 2 wk before the start of and during the study. All subjects were informed about the aims and the procedures of the study orally and in writing, and written informed consent was obtained from all participants. The participants were instructed not to change their dietary habits or lifestyle during the study. The study protocol was reviewed and approved by the Ethical Committee at the Swiss Federal Institute of Technology, Zurich.

Isotopic labels
Highly enriched \textsuperscript{25}MgO (1.04 \pm 0.01\% \textsuperscript{24}Mg, 98.73 \pm 0.01\% \textsuperscript{25}Mg, and 0.23 \pm 0.01\% \textsuperscript{26}Mg) and \textsuperscript{26}MgO (0.39 \pm 0.01\% \textsuperscript{24}Mg, 99.61 \pm 0.01\% \textsuperscript{26}Mg, and 0.00 \pm 0.01\% \textsuperscript{25}Mg).

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2 Supported by the Swiss Federal Institute of Technology (grant 41-2701.5).

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As 26 MgO) were dissolved in 10 mL of 4 mol HCl/L and diluted to 100 mL with water. Solid NaHCO$_3$ (Merck, Darmstadt, Germany) was added to adjust the solution to a pH of 6. Concentrations of the 25 Mg and 26 Mg isotope labels in solution were determined by isotope dilution mass spectrometry against a 26 Mg internal standard of natural isotopic composition (Titrisol; Merck). Unless otherwise specified, all chemicals were commercial grade, and acids were further purified by surface distillation.

Test meals

All test meals were based on 200 g phytic acid–free white-wheat bread. Bread rolls were prepared by mixing 1 kg white-wheat flour (Migros, Zurich, Switzerland) with water (600 g), salt (10 g), sugar (32 g), and dry yeast (15 g). The dough was left to ferment for 5 h at room temperature. The bread rolls were baked for 15 min at 200 °C. Individual servings were weighed and stored frozen (−25 °C) until served. The magnesium content of all test meals was standardized by adding a solution of MgCl$_2$ (Merck) to the white-wheat bread before serving. Phytic acid in its dodecasodium form (Sigma, Buchs, Switzerland) was dissolved in water (73.5 mmol/mL), and aliquots of phytic acid and stable-isotope labels in solution were pipetted onto the wheat bread 1 h before administration. The rare earth elements ytterbium and europium (in chloride form; Aldrich, Buchs, Switzerland) were added to 600 mL water, which was served as a drink.

Preparation of fecal pools and mineralization

Each individual stool sample was freeze-dried (Modulyo; Edwards, North Bergen, NJ) and ground to a powder in a mortar. All stool samples, beginning with the first fecal sample dyed by the first brilliant blue marker until, but not including, the stools dyed by the second marker, were included in the fecal pool. After undergoing a drying step in a drying chamber (Binder, Tuttingen, Germany) at 20 h at 65 °C to standardize humidity, followed by cooling at room temperature (4–h), all individual stool samples were weighed and milled (mill with 1-mm pores, ZM1; Retsch, Haan, Germany), starting with the first (most enriched) samples. Milled fecal material was transferred back into its original container

**TABLE 1**

<table>
<thead>
<tr>
<th>Study 1 (n = 9)</th>
<th>Study 2 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Magnesium (mmol)</strong></td>
<td><strong>Magnesium (mmol)</strong></td>
</tr>
<tr>
<td>Test meal A</td>
<td>Test meal B</td>
</tr>
<tr>
<td>3.63 ± 0.04*</td>
<td>3.64 ± 0.05</td>
</tr>
<tr>
<td><strong>Stable-isotope label (mmol)</strong></td>
<td><strong>Stable-isotope label (mmol)</strong></td>
</tr>
<tr>
<td>25Mg</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>26Mg</td>
<td>—</td>
</tr>
<tr>
<td><strong>Phytic acid (mmol)</strong></td>
<td><strong>Phytic acid (mmol)</strong></td>
</tr>
<tr>
<td>Test meal A</td>
<td>Test meal B</td>
</tr>
<tr>
<td>1.488 ± 0.022</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Phytic acid:magnesium (molar ratio)</strong></td>
<td><strong>Phytic acid:magnesium (molar ratio)</strong></td>
</tr>
<tr>
<td>Test meal A</td>
<td>Test meal B</td>
</tr>
<tr>
<td>0.41</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Fecal marker (nmol)</strong></td>
<td><strong>Fecal marker (nmol)</strong></td>
</tr>
<tr>
<td>Test meal A</td>
<td>Test meal B</td>
</tr>
<tr>
<td>Ytterbium</td>
<td>31.38 ± 0.58</td>
</tr>
<tr>
<td>Europium</td>
<td>—</td>
</tr>
</tbody>
</table>

* Test meals A and B were based on 200 g phytic acid–free white-wheat bread and 600 mL water served as a drink. Both meals, in each study, were served as 2 identical portions at breakfast and lunch on days 1 and 3. ND, not detectable; the limit of detection was <0.5 μmol/100 g.

0.11 ± 0.01% 25Mg, and 99.50 ± 0.01% 26Mg) labels were purchased from Chemotrade (Düsseldorf, Germany). The enriched 25Mg label (28 mmol as 25MgO) and 26Mg label (43 mmol as 26MgO) were dissolved in 10 mL of 4 mol HCl/L and diluted to 100 mL with water. Solid NaHCO$_3$ (Merck, Darmstadt, Germany) was added to adjust the solution to a pH of 6. Concentrations of the 25Mg and 26Mg isotope labels in solution were determined by isotope dilution mass spectrometry against a commercial magnesium standard of natural isotopic composition (Titrisol; Merck). Unless otherwise specified, all chemicals were of analytic grade, and acids were further purified by surface distillation. Only 18 MΩ water (Milli Q Water System; Millipore, Zurich, Switzerland) was used for the laboratory analyses and test meal preparation.

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tainer, dried again for 20 h at 65 °C, cooled for 4 h at room temperature, and reweighed. All milled stool samples included in a single pool were combined in a 2-L polyethylene container (Semadeni) and mixed for 90 min with a rotator (UG 70/20; Micro Motor, Basel, Switzerland). Aliquots of freeze-dried pooled stool samples (1.0–1.6 g), freeze-dried wheat bread (0.25 g), and plasma (1 g), were mineralized in a microwave digestion system (MLS 1200; MLS GmbH, Leutkirch, Germany) in a mixture of 14 mol HNO₃/L and 8.8 mol H₂O₂/L (Merck). All samples were mineralized in duplicate.

**Separation of magnesium**

Magnesium was separated from the mineralized stool samples by cation-exchange chromatography with a strongly acidic ion-exchange resin (AG 50W X-8, 200–400 mesh; Bio-Rad, Hercules, CA). Aliquots containing ≈30 μmol Mg were evaporated to dryness, redissolved in 1 mL of 0.7 mol HCl/L, and transferred onto the top of a column (1-cm inner diameter; Bio-Rad) filled with the ion-exchange resin to a height of 7 cm. The column was rinsed with 56 mL of 0.7 mol HCl/L, followed by 24 mL of 0.9 mol HCl/L to elute sodium and potassium. Magnesium was eluted with 12 mL of 1.4 mol HCl/L. The solution was evaporated to dryness and redissolved in 50 μL water. Magnesium recovery, evaluated with a diluted magnesium standard solution (Titrisol; Merck), was found to be 94.8 ± 1.8% (n = 10). Resins were regenerated with 30 mL of 6 mol HCl/L and replaced after the fifth run. Only acid-washed polytetrafluoroethylene and polyethylene laboratory ware were used during sample processing. Aliquots of the ²⁵Mg isotope label were processed in parallel with each batch for blank monitoring, from ion-exchange chromatography onward. Sample contamination due to natural magnesium was 10.8 ± 7.0 nmol (n = 9) for combined sample preparation and filament loading, which was <0.4% of the amount of magnesium separated.

**Isotopic analysis by thermal ionization mass spectrometry**

About 20 nmol Mg, separated from fecal samples, was loaded onto the metal surface of the evaporation filament of a double-rhenium filament ion source. Magnesium was coated with 5–10 μg silica gel 100, 0.8 μmol boric acid, and 30 nmol Al as AlCl₃ (all chemicals were from Merck). Compounds were loaded in aqueous solution and dried electrothermally at 0.8 A after each step. Finally, the evaporation filament was heated to dull red heat (1.6 A) for 30 s. The ionization filament remained unloaded. Isotope ratios were determined with a single-focusing magnetic sector field instrument (MAT 262; Finnigan MAT, Bremen, Germany) equipped with a Faraday cup multicolonlector device for simultaneous ion-beam detection. The evaporation filament was heated to 1230 °C according to a standardized procedure. The ionization filament was heated gradually to 1250–1350 °C until a stable Mg⁺ ion beam of 1–2 × 10⁻¹⁵ A was obtained. Each measurement consisted of 30 consecutive isotope ratio measurements. Repeatability (5 independent analyses) was ±0.2% (relative SD) for the ²⁵Mg/²⁴Mg isotope ratio and ±0.4% for the ²⁶Mg/²⁴Mg isotope ratio. A standard reference material (standard reference material 980; National Institute of Standards and Technology, Gaithersburg, MD) was analyzed in parallel. The results (0.12631 ± 0.00009 for the ²⁵Mg/²⁴Mg isotope ratio and 0.13876 ± 0.00059 for the ²⁶Mg/²⁴Mg isotope ratio) agreed with isotope ratios from the International Union of Pure and Applied Chemistry: 0.12663 ± 0.00013 and 0.13932 ± 0.00026, respectively (14).

**Magnesium analysis by atomic absorption spectroscopy**

Quantitative magnesium analysis of the mineralized and diluted samples of plasma, bread, and fecal material was performed by flame atomic absorption spectroscopy (SpectraAA 400; Varian, Mulgrave, Australia). Plasma samples were measured by external calibration with the use of a commercial magnesium standard (Titrisol; Merck). All other samples were measured by an internal calibration technique (standard addition) to minimize matrix effects. In addition, all measured solutions contained La(NO₃)₃ at 5000 mg La/L to suppress precipitation of magnesium salts. Certified reference materials—Seronorm Trace Elements Serum (Nycomed, Oslo) and wheat flour 1567a (National Bureau of Standards, Gaithersburg, MD)—were analyzed in parallel. Ytterbium and europium were measured by electrothermal atomic absorption spectroscopy by external calibration with the use of a standard solution containing ytterbium and europium (Titrisol; Merck). Mineralized fecal samples were diluted with 1 mol HCl/L, and aliquots (10 μL) were injected into pyrolytically coated graphite tubes. Heating procedures and absorption wavelengths were used according to the manufacturer (15).

**Phytic acid analysis**

Samples of bread rolls were freeze-dried and ground in a mortar. Phytic acid was extracted from a 1-g aliquot with 0.5 mol HCl/L. The extract was purified by anion-exchange chromatography, evaporated to dryness, and redissolved in water before analysis with the use of reversed-phase HPLC (16).

**Calculations**

Molar amounts and ratios of the ²⁵Mg and ²⁶Mg isotope labels in the samples were calculated on the basis of double-isotope dilution principles (17, 18). Fractional apparent magnesium absorption was calculated on the basis of the dose (μmol) of the stable isotope label of magnesium administered (D₀) and the amount of the label excreted in feces (F₀).

\[
AA(\%) = \frac{D₀ - F₀D₀}{D₀} \times 100
\]

The recovery of the rare earth elements ytterbium and europium was used to evaluate the completeness of the stool collections.

**Statistics**

Calculations were made by using commercial software: EXCEL 97 (Microsoft, Chicago) and SPSS 10.0 (SPSS Inc, Chicago). Results are presented as arithmetic means ± SDs. The normal distribution of magnesium absorption data was verified with the use of the Kolmogorov-Smirnoff test. Homogeneity between groups was tested by Levene’s test. Paired Student’s t test (two-tailed) was used to compare magnesium absorption from the 2 different test meals within each study. The dose effect of phytic acid on magnesium absorption was evaluated with the use of an unpaired Student’s t test based on absorption ratios (with/without added phytic acid). In addition, a linear mixed model was used to evaluate the dose effect of phytic acid on magnesium absorption. This model included magnesium absorption as the dependent factor and meal (with or without added phytic acid) and study (1.49 or 0.75 mmol added phytic acid) as
fixed factors. Subject, as a random factor, was nested within study. \( P \) values < 0.05 were considered statistically significant.

**RESULTS**

**Subjects and test meals**

The ages and body mass indexes (in kg/m\(^2\)) of the subjects were 27 ± 12 y and 22.1 ± 3.8, respectively, in study 1 (\( n = 9 \)) and 24 ± 2 y and 21.7 ± 1.0 in study 2 (\( n = 8 \)). Mean plasma magnesium concentrations were 0.77 (range: 0.67–0.85) mmol/L and 0.84 (range: 0.77–0.90) mmol/L in studies 1 and 2, respectively. Two subjects had slightly lower magnesium concentrations (0.67 and 0.73 mmol/L) than the reported normal range (0.75–0.90 mmol/L) (19).

The native content of phytic acid in the wheat bread was below the detection limit (<0.5 \( \mu \text{mol/100 g}; \ n = 3 \)). The native content of magnesium in the wheat bread was 0.96 ± 0.03 mmol/100 g (\( n = 3 \)). The magnesium and phytic acid contents of the labeled test meals are presented in Table 1.

**Magnesium absorption**

Addition of 1.49 mmol phytic acid to 200 g phytic acid–free bread inhibited apparent magnesium absorption significantly: 32.5 ± 6.9% (test meal A) compared with 13.0 ± 6.9% (test meal B) (\( P < 0.0005 \), Figure 1). One subject was excluded from the evaluation because of a low recovery of ytterbium (<85%). The exclusion criteria were based on the estimated relatively high combined uncertainty of the recovery of rare earth elements in fecal material and on previous studies that used rare earth elements as fecal markers (20, 21). For all other subjects, mean ytterbium recovery was 99.5% (range: 86.4–107.1%) and mean europium recovery was 101.7% (range: 89.5–116.3%). Addition of 0.75 mmol phytic acid to 200 g bread inhibited magnesium absorption significantly: 32.2 ± 12.0% (test meal A) compared with 24.0 ± 12.9% (test meal B) (\( P < 0.01 \), Figure 2). Two subjects were excluded from the evaluation because of low ytterbium recovery. For all other subjects, mean ytterbium recovery was 105.4% (range: 97.5–115.6%) and europium recovery was 96.9% (range: 90.2–120.6%). The inhibitory effect of phytic acid on magnesium absorption was dose dependent (\( P < 0.005 \), unpaired Student’s \( t \) test). On the basis of a linear mixed model, a statistically significant effect of meal (with or without added phytic acid; \( P < 0.001 \)) on magnesium absorption was observed, but no significant effect of study was observed (1.49 or 0.75 mmol added phytic acid). The study-by-meal interaction was statistically significant (\( P < 0.005 \)), which indicated that magnesium absorption was significantly influenced by the amount of phytic acid added to the meal.

The mean loss of fecal material during stool pool preparation, determined by weighing the pools before and after milling, was 1.9 ± 1.1%. The measured isotopic enrichment of the stool pools was 5.2 ± 1.9% (\(^{24}\text{Mg}/^{25}\text{Mg}\)) and 8.4 ± 2.7% (\(^{24}\text{Mg}/^{26}\text{Mg}\)) on the basis of differences in the measured isotope ratios of fecal pools and natural isotope ratios of a standard (Titrisol; Merck) divided by the measured isotope ratio of the standard.

**DISCUSSION**

Mean fractional apparent magnesium absorption was ≈60% lower when phytic acid was added to phytic acid–free white-wheat bread at an amount similar to that in whole-meat wheat bread (1.49 mmol/200 g) and ≈25% lower when added at an amount similar to that in brown bread (0.75 mmol/200 g). The inhibiting effect of phytic acid was dose dependent (\( P < 0.005 \)). Although this is the first time that the inhibition of magnesium absorption by phytic acid was observed with single meals, such an effect was indicated by chemical balance studies in humans that evaluated phytic acid added to white bread (12) and dephytinized bran (13). In these earlier studies, a somewhat more limited inhibitory effect of phytic acid on fractional apparent magnesium absorption was found at molar ratios of phytic acid to magnesium that were similar to those used in the present study, i.e., absorption of 13% and 38% at molar ratios of 0.2 and 0.4,
respectively. However, it is important to stress that the magnitude of the inhibitory effect might have been influenced by the control diets, which were not completely free of phytic acid, or by adaption to decreased dietary magnesium bioavailability.

As with iron, zinc, and calcium, it is assumed that magnesium–phytic acid or protein–magnesium–phytic acid complexes are formed in the intestine, which are insoluble at a pH > 6 (22–24) and thus are not absorbable. However, the stability of the magnesium–phytic acid complex is weaker than phytic acid complexes with iron, copper, and zinc (25, 26). It is also important to stress that endogenous losses of magnesium represent a significant fraction of total magnesium losses and that phytic acid can be expected to form complexes with both food magnesium and endogenous magnesium in the gastrointestinal tract. However, on the basis of rat studies, it is not certain whether the reabsorption of endogenous magnesium is inhibited by phytic acid (27, 28).

In the present study, all test meals were based on phytic acid–free wheat bread and differed only by whether phytic acid was added. We chose not to dephytinize whole-meal and brown bread so as to avoid potential differences between test meals because of differences in ingredients, food-preparation methods, or both. The phytic acid content and molar ratios of phytic acid to magnesium in the test meals were similar to those reported for whole meal and brown bread. In whole-meal bread, the phytic acid content is reported to be in the range 0.7–1.6 mmol/100 g, and the molar ratio is between 0.2 and 0.5; in brown bread, the corresponding values are <0.1–0.4 mmol/100 g, and the molar ratio is <0.1–0.3 (2). The addition of phytic acid would seem a useful approach to simulate native phytic acid because phytic acid complexes are largely soluble at a pH of 2–3, as in the stomach, and mineral binding is weak (23, 24). Thus, under these conditions, an exchange of minerals bound to phytic acid can be expected to occur. This approach was used previously in human studies, for example, to evaluate the effect of phytic acid on iron absorption (29). The magnitude of the inhibitory effect of added phytic acid on iron absorption in the study by Hallberg et al (29) was similar to that reported for native phytic acid by Hurrell et al (30).

Our data indicate that fractional magnesium absorption from whole-meal and brown bread is significantly inhibited compared with that from phytic acid–free white-wheat bread. However, the absolute amounts of magnesium absorbed from whole-meal and brown bread can be expected to be higher than those from white bread because of the 2–3-fold higher magnesium content (31), unless other components in whole-meal and brown bread (such as dietary fiber, minerals, and trace elements) influence magnesium absorption or modify to a great extent the effect of phytic acid on magnesium absorption. In conclusion, the results of the present study indicate that fractional magnesium absorption from white-wheat bread is significantly inhibited by phytic acid, in a dose-dependent manner, when it is added at amounts similar to those naturally present in whole-meal and brown bread.

All authors contributed to the study design. TB was responsible for the data collection and data analysis. TW was partly responsible for the data analysis. The manuscript was prepared by TB and LD and revised by TW and RFH. None of the authors reported any conflict of interest.

REFERENCES


