Erythorbic acid is a potent enhancer of nonheme-iron absorption

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ABSTRACT

Background: Erythorbic acid, a stereoisomer of ascorbic acid with similar physicochemical properties, is widely used as an antioxidant in processed foods.

Objectives: The aims of the present study were to evaluate the effect of erythorbic acid on iron absorption from ferrous sulfate at molar ratios of 2:1 and 4:1 (relative to iron) and to compare the effect of erythorbic acid directly with that of ascorbic acid at a molar ratio of 4:1.

Design: Iron absorption from iron-fortified cereal was measured in 10 women on the basis of erythrocyte incorporation of stable iron isotopes ($^{57}$Fe or $^{58}$Fe) 14 d after administration. Each woman consumed 4 ferrous-sulfate-fortified test meals (containing 5 mg Fe/meal) with or without added erythorbic or ascorbic acid. The data were evaluated by use of paired $t$ tests, and the results are presented as geometric means.

Results: Iron absorption from the test meal without any added enhancer was 4.1%. The addition of erythorbic acid (at molar ratios of 2:1 and 4:1 relative to iron) increased iron absorption 2.6-fold (10.8%; $P < 0.0001$) and 4.6-fold (18.8%; $P < 0.0001$), respectively. The addition of ascorbic acid (molar ratio of 4:1) increased iron absorption 2.9-fold (11.7%; $P = 0.0004$). At a molar ratio of 4:1, erythorbic acid was 1.6-fold ($P = 0.0002$) as potent an enhancer of iron absorption as was ascorbic acid.

Conclusion: Although erythorbic acid is a potent enhancer of iron absorption, its lack of antiscorbutic activity limits its usefulness in iron-fortification programs. However, it may play a major role in enhancing iron bioavailability from mixed diets that include foods preserved with erythorbic acid. Am J Clin Nutr 2004;79:99–102.

KEY WORDS Erythorbic acid, ascorbic acid, iron, absorption, humans, stable isotopes

INTRODUCTION

Ascorbic acid is a potent enhancer of nonheme-iron absorption, increasing the absorption of native food iron and of iron fortificants, which dissolve in the gastric juice and enter the common iron pool (1). The enhancing effect appears to be due to both the reducing power and the chelating action of ascorbic acid (2). Ascorbic acid has been shown to at least partially overcome the negative effects of all major inhibitors of iron absorption, as recently reviewed by Hurrell (1). This vitamin is therefore an important factor for enhancing nonheme-iron absorption, and information about its intake is important when evaluating iron bioavailability from diets.

Ascorbic acid is commonly added to iron-fortified foods to ensure adequate iron absorption but is also frequently used as an antioxidant in industrially produced foods. Erythorbic acid (synonyms: isoascorbic acid, d-araboascorbic acid) is a stereoisomer of ascorbic acid that differs from ascorbic acid only in the relative position of the hydrogen and hydroxyl groups on the fifth carbon atom in the molecule. Because of its strong reducing properties, erythorbic acid has similar technologic applications to ascorbic acid as a water-soluble antioxidant (3) and is widely used as an additive in processed foods (4).

However, the antiscorbutic activity of erythorbic acid is limited and has been reported to be only one-twentieth of that of ascorbic acid in guinea pigs (5). In humans, neither the antiscorbutic activity of erythorbic acid nor its physiologic activity as an antioxidant has been investigated. Erythorbic acid may, however, have an ascorbic acid–sparring effect: Sauberlich et al (6) reported that intake of erythorbic acid together with ascorbic acid increases plasma ascorbic acid values to a greater extent than does ascorbic acid alone.

Because ascorbic and erythorbic acids have similar physical and chemical properties (3), it was speculated that erythorbic acid will also enhance iron absorption. However, only 2 studies have attempted to evaluate the effect of erythorbic acid on iron absorption. Greger et al (7) reported no effect of erythorbic acid on iron absorption in healthy men on the basis of results from 5-d balance studies, and Lee et al (8) reported no effect of erythorbic acid on iron absorption in rats. Although both studies reported a lack of effect, it is important to stress that the chemical balance technique is not a sensitive method for evaluating iron absorption and that rats are not an appropriate animal model to use to predict human iron absorption because the effect of ascorbic acid on iron absorption in rats and humans is not comparable (9, 10). Conclusive data on the effect (or lack of effect) are important, because it has been estimated that the average US diet may provide substantial amounts of erythorbic acid (up to 200 mg/d) from processed foods (11).

The aims of the present study were to investigate whether erythorbic acid influences iron absorption in a dose-dependent...
manner and to evaluate the effect of erythorbic acid compared with that of ascorbic acid. Erythrocyte incorporation of stable isotopes 14 d after administration was used to measure iron absorption in healthy women from a cereal-based test meal fortified with ferrous sulfate.

SUBJECTS AND METHODS

Subjects

Ten apparently healthy women (aged 20–26 y; body weight range: 50–60 kg) were recruited from the student population of the Swiss Federal Institute of Technology, Zurich, and the University of Zurich. Exclusion criteria included pregnancy or lactation and known gastrointestinal or metabolic disorders. No medication (except oral contraceptives) or vitamin and mineral supplements were allowed during the study. Women who regularly took vitamin and mineral supplements discontinued taking them 2 wk before the start of the study.

The study protocol was reviewed and approved by the Ethical Committee at the Swiss Federal Institute of Technology, Zurich. The subjects were informed orally and in writing about the aims and procedures of the study, and written informed consent was obtained from all study subjects.

Study design

Iron absorption was measured on the basis of erythrocyte incorporation of stable-isotope-labeled iron 14–15 d after the intake of labeled test meals. Ferrous sulfate, labeled with \(^{57}\)Fe or \(^{58}\)Fe, was added to the different test meals as described below. A crossover study design was used in which each woman acted as her own control. Iron absorption from 4 separate test meals was measured in each woman. The first 2 test meals (meals 1 and 2) were fed on days 1 and 2, and a second pair of labeled test meals (meals 3 and 4) was fed on days 17 and 18. Within each pair of test meals, the order of administration was randomized. All test meals were fed under standardized conditions after the women had fasted overnight ending between 0700 and 0900. No intake of food or fluids was allowed for 3 h after intake of the test meals. Blood samples were drawn on days 0, 16, and 32. Body weight and body height were measured on day 0.

Test meals

The test meals consisted of 50 g roller-dried, wheat-based infant cereal (Nestlé PTC, Orbe, Switzerland) fed with reconstituted milk [8 g milk powder (Sano Lait; Coop Schweiz, Basel, Switzerland) and 75 mL deionized water]. The infant cereal was made from 79.7% wheat flour, partially hydrolyzed; 10% sucrose; 4% honey; 3% palm oil; 0.3% calcium carbonate; and 3% water. Except for calcium, no minerals or vitamins were added. Each test meal contained 5 mg added Fe: 4 mg Fe as \(^{58}\)FeSO\(_4\) plus 1 mg Fe as FeSO\(_4\) of natural isotopic composition or 5 mg Fe as \(^{57}\)FeSO\(_4\). Deionized water (200 g) was served as a drink and was consumed by all women with all test meals. Erythorbic acid (Merck KGaA, Darmstadt, Germany) was added to test meals 2 and 3 at a molar ratio of erythorbic acid to added iron of 2:1 (31.5 mg erythorbic acid, meal 2) and 4:1 (63 mg erythorbic acid, meal 3). Ascorbic acid (Merck KGaA) was added to meal 4 at a molar ratio of ascorbic acid to added iron of 4:1 (63 mg ascorbic acid).

Stable-isotope labels

\(^{57}\)Fe and \(^{58}\)Fe ferrous sulfate were prepared from isotopically enriched elemental iron (Chemgas, Boulogne, France) by dissolution in sulfuric acid and dilution to the appropriate concentration.

Erythorbic acid and ascorbic acid doses

Aqueous solutions of food-grade erythorbic acid and ascorbic acid were prepared freshly on the day of administration and were added to the test meals at the time of serving.

Quantification of iron isotopes in labeled iron fortificants

Isotope dilution mass spectrometry was used to precisely determine the concentration of \(^{57}\)Fe and \(^{58}\)Fe stable isotopes in the ferrous sulfate solutions. An accurately measured amount of iron of natural isotopic composition was added to aliquots taken from the prepared solutions of labeled iron fortificants. The iron standard was prepared gravimetrically from an isotopic reference material (IRMM-014; EU Institute of Reference Materials, Geel, Belgium). Isotopic analysis was performed by using negative thermal ionization mass spectrometry (12). Iron concentrations in each labeled iron fortificant solution were calculated on the basis of the shift in iron isotopic abundances, the determined isotopic abundances of the isotope labels, and the natural iron isotopic abundances (13).

Iron-status measurements

Venous blood samples (7 mL) were drawn in EDTA-treated tubes the day before the first labeled test meal was administered, on day 16, and on day 32. Hemoglobin concentrations were measured in all blood samples. Plasma ferritin was measured in blood samples drawn on day 0 only, and the incorporation of \(^{57}\)Fe and \(^{58}\)Fe into red blood cells was determined in the blood samples drawn on days 16 and 32. Blood samples were portioned into aliquots for the analysis of hemoglobin, and plasma was separated, portioned into aliquots, and frozen for later analysis of ferritin. Hemoglobin was measured by the cyanmethemoglobin method (Sigma kit 525; Sigma Diagnostics, St Louis), and plasma ferritin was measured by enzyme-linked immunosorbent assay (Spectro Ferritin MT; Ramco Laboratories, Houston). Commercial quality-control materials (Dia HT 16-1, 2, 3; DiaMed, Cressier sur Morat, Switzerland, and Ramco Laboratories) were analyzed in parallel.

Quantification of iron isotope in blood

Each isotopically enriched blood sample was analyzed in duplicate for its iron isotopic composition as previously described by Davidson et al (14). The blood samples were mineralized by microwave digestion by using a mixture of nitric acid and hydrogen peroxide. The iron was separated from the matrix by anion-exchange chromatography and a solvent-extraction step into diethyl ether. The isotopic analyses were performed by negative thermal ionization mass spectrometry (12).

Calculation of iron absorption

The amounts of \(^{57}\)Fe and \(^{58}\)Fe isotope labels in blood 14 d after test meal administrations were calculated on the basis of the shift in iron isotope ratios and the amount of iron circulating in the body. The calculations were based on the principles
of isotope dilution and took into account that the iron isotope labels were not monoisotopic (13). Circulating iron was calculated on the basis of blood volume and hemoglobin concentration (15). Blood volume calculations were based on height and weight according to Brown et al (16). For calculations of fractional absorption, 80% incorporation of the absorbed iron into red blood cells was assumed (17).

**Food analysis**

Samples of cereal and milk powder were analyzed for iron and calcium by electrothermal-flame atomic absorption spectrophotometry (SpectrAA 400; Varian, Mulgrave, Australia) after mineralization by microwave digestion (MLS-Ethos plus; Mikrowellen-Labor-System, Leutkirch, Switzerland) in a nitric acid–hydrogen peroxide mixture with the use of a standard addition technique to minimize matrix effects. Phytic acid in the cereal was determined by a modification of the Makower (18) method in which cerium replaced iron in the precipitation step.

**Statistics**

Fractional iron absorption values are presented as geometric means (−1 SD, +1 SD). Student’s paired *t* test was used to compare absorption data within the study. Absorption values were logarithmically transformed before statistical analysis (EXCEL 2002; Microsoft Corporation, Redmond, WA). *P* values < 0.05 after Bonferroni adjustment, ie, 0.05/6, are referred to as statistically significant.

**RESULTS**

None of the women had a hemoglobin concentration <120 g/L. However, 4 women had low iron stores, as indicated by low plasma ferritin values (<12 μg/L).

The test meals contained 0.6 mg native Fe (1.1 mg Fe/100 g cereal, 0.15 mg Fe/100 g milk powder), 167 mg Ca (148 mg Ca/100 g cereal, 115 mg Ca/100 g milk powder), and 84 mg phytic acid (168 mg phytic acid/100 g cereal). The ascorbic acid content of the cereal and milk powder was not measured because it was assumed to be negligible.

Geometric mean iron absorption from the test meal fortified with ferrous sulfate was 4.1% (Table 1). The addition of ascorbic acid at a molar ratio of 4:1 (relative to added iron) increased iron absorption 2.9-fold (geometric mean: 11.7%; *P* = 0.0004). The addition of erythorbic acid (at molar ratios of 2:1 and 4:1 relative to added iron) increased iron absorption 2.6- and 4.6-fold, respectively (geometric means: 10.8% and 18.8%, respectively, *P* < 0.0001). Iron absorption was significantly enhanced by increasing the molar ratio of erythorbic acid from 2:1 to 4:1 (*P* = 0.01). Iron absorption from the test meal with added erythorbic acid (molar ratio of 4:1 relative to added iron) was 1.6-fold higher (*P* = 0.0002) than that from the test meal fortified with ascorbic acid at the same molar ratio.

**DISCUSSION**

The results of the present study clearly show that erythorbic acid is a potent enhancer of iron absorption from ferrous sulfate. The dose-dependent effect observed with erythorbic acid is also consistent with the previously reported dose-dependent effect of ascorbic acid (19, 20). The enhancing effect of erythorbic acid on iron absorption was not an unexpected finding because erythorbic and ascorbic acids have similar physicochemical properties. However, our results indicate that the enhancing effect of erythorbic acid on iron absorption from ferrous sulfate exceeds that of ascorbic acid. This finding may be explained by the different absorption kinetics of the 2 acids. Earlier studies in humans and guinea pigs showed that absorption of erythorbic acid is slower than that of ascorbic acid (11, 21), potentially resulting in a prolonged presence of erythorbic acid in the duodenum. In addition, because erythorbic acid is reported to oxidize more rapidly than ascorbic acid (22), it may reduce ferric iron to ferrous iron more efficiently, thus increasing the amount of readily absorbable ferrous iron in the duodenum. However, the rapid oxidation unfortunately also means that erythorbic acid is somewhat less stable than is ascorbic acid during food processing (23). One of the major problems with the use of ascorbic acid as an enhancer of iron absorption is its susceptibility to losses during food storage and food preparation (24). Although erythorbic acid has not been evaluated in this context, these problems would not be expected to be less for erythorbic acid.

Nonetheless, erythorbic acid is widely consumed as an additive in processed foods and could be expected to positively influence iron absorption when such foods are included in the diet. It is approved as a food additive in most North American and Latin American countries, as well as in most Asian, Oceanian, and Central European countries (25). Its advantage over ascorbic acid is its lower price (R Frères, personal communication, 2002). In the United States, erythorbic acid is widely used as a food additive in many processed food items, includ-

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**Table 1**

<table>
<thead>
<tr>
<th>Test meal and molar ratio (erythorbic or ascorbic acid to added iron)</th>
<th>Iron absorption</th>
<th>Iron absorption range</th>
<th>Absorption ratio (relative to meal 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>4.1 (1.7, 9.5)²</td>
<td>1.0–14.3</td>
<td>—</td>
</tr>
<tr>
<td>Erythorbic acid (2:1)</td>
<td>10.8 (3.9, 30.2)</td>
<td>1.5–43.0</td>
<td>2.6 (1.8, 4.0)</td>
</tr>
<tr>
<td>Erythorbic acid (4:1)</td>
<td>18.8 (7.3, 48.7)</td>
<td>3.4–60.1</td>
<td>4.6 (2.9, 7.3)</td>
</tr>
<tr>
<td>Ascorbic acid (4:1)</td>
<td>11.7 (3.8, 36.1)</td>
<td>1.9–47.1</td>
<td>2.9 (1.6, 5.3)</td>
</tr>
</tbody>
</table>

¹ *n = 10. Plasma ferritin geometric x (−1 SD, +1 SD): 13.0 (6.0, 28.3) μg/L. Mean values with different superscript letters are significantly different P < 0.05 after Bonferroni adjustment. ² Geometric mean (−1 SD, +1 SD).
ing baked goods, fruit and water ices, meat and meat products, vegetables and vegetable juices, candy, and nonalcoholic beverages (4). It has been estimated that the average diet can provide as much as 200 mg erythorbic acid/d (11), which would be expected to markedly influence dietary nonheme-iron absorption. Consequently, erythorbic acid could have long-term effects on iron status. The results of the present study suggest that dietary intake of erythorbic acid should be taken into account when estimating dietary iron bioavailability (26).

The influence of erythorbic acid on iron absorption would be expected to be of lesser importance in the European Union and in Switzerland, because legislation in these countries restricts the use of erythorbic acid to semipreserved and preserved meat, fish, and crustacean products (27, 28). Consequently, in Western European countries, intake of erythorbic acid would be associated with animal tissue and would therefore be expected to have a limited influence on dietary nonheme-iron absorption, because the promotive effects of ascorbic acid and animal tissue on nonheme-iron absorption are not additive when consumed in the same meal (29, 30).

In conclusion, the results of the present study show that erythorbic acid is a potent enhancer of iron absorption from ferrous sulfate. Intake of erythorbic acid can therefore be an important factor enhancing dietary iron bioavailability in population groups consuming foods preserved with this antioxidant. Erythorbic acid’s lack of antiscorbutic activity, however, limits its usefulness for food fortification programs.

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MCF, LD, and RFH contributed to the study design. MCF and CZ were responsible for the implementation of the study. CZ was responsible for the preparation of the stable-isotope labels and the analytic work. The statistical analysis was done by MCF and CZ. The manuscript was written by MCF and LD and was edited by CZ and RFH. None of the authors had any conflicts of interest.

REFERENCES