

Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status¹⁻³

Adelia C Bovell-Benjamin, Fernando E Viteri, and Lindsay H Allen

ABSTRACT

Background: There is a need to determine whether iron absorption from iron amino acid chelates is protected from inhibition by dietary phytate and regulated normally by iron status.

Objective: The objective of this study was to compare iron absorption from ferrous sulfate, ferrous bisglycinate, and ferric trisglycinate in whole-maize meal; to determine whether iron from ferrous bisglycinate and ferrous sulfate exchanges in the intestinal pool; and to assess iron absorption from ferrous bisglycinate and ferric trisglycinate over a range of iron statuses.

Design: In study 1A, 10 iron-sufficient men consumed ferrous sulfate-fortified whole-maize meal porridge equilibrated with ⁵⁹Fe-sulfate on day 1 and ⁵⁵Fe-bisglycinate on day 2. In study 1B, these volunteers consumed ferrous sulfate-fortified porridge equilibrated with ⁵⁹Fe-sulfate and ⁵⁵Fe-bisglycinate simultaneously. In studies 2A and 2B, iron absorption from 3 mg Fe as ⁵⁹Fe-ascorbate, ⁵⁵Fe-bisglycinate, or ⁵⁹Fe-trisglycinate in water and in porridge was compared in 23 subjects with a range of iron statuses. Iron absorption was determined from blood radioactivity on day 16.

Results: In study 1A, geometric mean iron absorption from ferrous bisglycinate was 6.0% (range: 2.6–13.6%), 4 times higher than that from ferrous sulfate (1.7%; range: 1.0–3.3%; *P* < 0.05). In study 1B, absorption from neither source was different from that in study 1A. In studies 2A and 2B, absorption from all sources was strongly inversely related to serum ferritin, with geometric means of 32.5% (iron ascorbate), 9.1% (bisglycinate), and 15.3% (trisglycinate). Iron from ferric trisglycinate was poorly absorbed (2.3%; range: 0.5–9.2%) from maize.

Conclusion: In whole-maize meal, iron from ferrous bisglycinate is better absorbed than is iron from ferrous sulfate and does not exchange with iron from maize or ferrous sulfate in the intestinal pool. Absorption of iron from bisglycinate and trisglycinate is regulated normally by iron status. *Am J Clin Nutr* 2000;71:1563–9.

KEY WORDS Iron bioavailability, iron absorption, ferrous bisglycinate, ferric trisglycinate, iron amino acid chelates, iron fortification, iron status, maize, phytate, serum ferritin

INTRODUCTION

Iron deficiency remains the most prevalent nutrient deficiency in the United States and the rest of the world (1, 2). Iron deficiency is especially prevalent in developing countries, where diets

are based on cereals or legumes. Iron fortification of these staples is a potentially feasible approach to preventing iron deficiency and anemia, but absorption of iron from soluble and poorly soluble iron salts added as fortificants is impaired by the phytate in the cereals and legumes to the same extent as is absorption of the native iron (3). Iron fortification of whole-maize meal is especially problematic because of the high phytate content of whole-maize meal (1 mg/g maize meal), which inhibits iron absorption, and the high polyunsaturated fat content (60% of total fatty acids), which is readily oxidized by soluble iron (4, 5).

Iron amino acid chelates (FeAAs) are potentially useful iron fortificants for high-phytate foods. Ferrous bisglycinate and ferric trisglycinate have one molecular equivalent of Fe²⁺ or Fe³⁺ and 2 or 3 molecular equivalents of glycine, respectively. Ferrous bisglycinate and ferric trisglycinate are reportedly effective in treating iron deficiency anemia (6). It is theorized that the chelates prevent iron from binding to inhibitors in food or precipitating as insoluble ferric hydroxide in the pH of the small intestine (6). However, there is little published information on iron absorption from chelates in the presence of inhibitors.

Fairweather-Tait et al (7) found iron from iron-glycine chelate to be more readily utilized than iron from ferrous sulfate when it was fed to rats. Weanling rats fed iron-fortified casein-based formulas absorbed more iron from ferric glycinate than from ferrous sulfate (8). Olivares et al (9) used a double-isotopic method to measure iron absorption by women from cow milk fortified with ferrous bisglycinate and concluded that, although the milk inhibited the bioavailability of iron from bisglycinate, this form of iron was 2–2.5 times more bioavailable than was the iron from ferrous sulfate added to the milk (9, 10).

Excessive iron absorption can cause a variety of diseases in humans, including hepatic cirrhosis and diabetes mellitus (11). Although the mechanism has not been completely elucidated, it is well known that iron absorption is regulated by iron status and

¹From the Program in International Nutrition, the Department of Nutrition, University of California, Davis, and the Department of Nutritional Sciences, University of California, Berkeley.

²Supported in part by Albion Laboratories, Clearfield, UT.

³Reprints not available. Address correspondence to LH Allen, Program in International Nutrition, Department of Nutrition, University of California, Davis, CA 95616. E-mail: lhallen@ucdavis.edu.

Received October 19, 1999.

Accepted for publication November 10, 1999.

is considerably lower in persons with normal or high iron stores (12). However, it is possible that FeAACs can be absorbed intact, bypassing the mucosal cell mechanisms that regulate iron absorption. If this were the case, FeAACs would be unsafe as long-term fortificants in humans. The regulation of absorption from FeAACs has not been investigated adequately.

The present experiments were designed to 1) compare the bioavailability of iron from ferrous sulfate and bisglycinate added to whole-maize meal porridge high in phytate and determine whether there is any exchange of iron from bisglycinate with the intestinal iron pool (study 1), 2) determine whether iron absorption from a ferrous sulfate reference dose, ferrous bisglycinate, and ferric trisglycinate in water is regulated by iron status in iron-sufficient and iron-deficient women (study 2), and 3) evaluate the bioavailability of iron from ferric trisglycinate consumed in whole-maize meal porridge (study 2).

SUBJECTS AND METHODS

Subjects

Ten men from the city of Davis and the student body of the University of California, Davis, volunteered to participate in the study. None of the subjects was iron deficient or anemic as defined by World Health Organization criteria (13). All subjects were apparently healthy and had no history of disorders known to affect gastrointestinal absorption of iron. Any use of iron or vitamin supplements was discontinued before the first test meal and for the remainder of the study.

Approval for the study was obtained from the Human Subjects Review Committees and the Radiation Use Committees of the University of California at Davis and at Berkeley. All subjects provided written, informed consent. The maximum radiation exposure, even when total absorption of all the isotopes was assumed, fell well below the University's permissible annual dose [0.025 Sv (2500 mrem)/y] and the federal regulatory limit [0.05 (5000 mrem)/y].

Meal preparation and administration

Whole-maize meal (Jamaica Grains & Cereals Ltd, Kingston, Jamaica) was fortified with 0.011 mg Fe/g as either ferrous sulfate or ferrous bisglycinate (Albion Laboratories, Clearfield, UT). The total iron content of the meal was 1.863 mg/serving. ^{59}Fe as ferric chloride in 0.1 mol HCl/L (Amersham Life Sciences, Piscataway, NJ) was prepared for labeling the ferrous sulfate-fortified maize. To label the bisglycinate-fortified maize, bisglycinate was intrinsically labeled with ^{55}Fe as the chloride in 0.1 mol HCl/L. The bisglycinate was prepared by adding glycine slowly to the ^{55}Fe solution and reducing the iron to the ferrous state. A trace amount of the unlabeled bisglycinate and distilled water were then added to the solution and heated covered at 50°C for 2 h according to the procedure described by the manufacturers. Radioiron solutions prepared initially were used throughout the study.

The maize was prepared as a porridge similar to that typically used for feeding infants and young children in Jamaica by adding 87.5 g whole-maize meal to 625 mL boiling water, stirring until a cooked porridge of uniform consistency was obtained, and adding 50 g dry nonfat milk, 125 g margarine, and 37 g Jamaican brown sugar. ^{59}Fe [10 mL of 0.1 mol HCl containing 55.5 kBq (1.5 μCi)] and ^{55}Fe [10 mL of 0.1 mol HCl containing 111 kBq (3.0 μCi)] radioiron solutions were added to the different por-

tions of porridge with a pipette, mixed thoroughly, and allowed to equilibrate overnight in a refrigerator.

Immediately before being served, the labeled porridge was again mixed thoroughly and reheated in a microwave oven. After the subjects fasted overnight, they consumed the porridges for breakfast between 0700 and 1000 under supervision. After consuming each meal, the subjects rinsed their bowls with deionized water twice and consumed this water. Only water was permitted to be ingested for 4 h afterward.

Study 1: bioavailability of iron from ferrous bisglycinate and ferrous sulfate added to whole-maize meal porridge

Study 1A

Study 1A compared the bioavailability of iron from ferrous sulfate and from ferrous bisglycinate added to whole-maize meal porridge. After blood was drawn for measurement of baseline serum ferritin (immunoradiometric assay; Diagnostic Products Corporation, Los Angeles), hemoglobin (Hemocue Inc, Mission Viejo, CA), and red blood cell radioactivity, subjects consumed porridge labeled with ^{59}Fe -sulfate on day 1 and with ^{55}Fe -bisglycinate on day 2. A fasting blood sample was obtained 14 d later (day 16) for analysis of incorporated red blood cell radioactivity by the method of Viteri and Kohaut (14). Measurements of ^{59}Fe and ^{55}Fe incorporated into red blood cells were performed in triplicate 10-mL specimens of whole blood. Absorption of iron was calculated from blood volume; the latter was estimated from the sex, weight, and height of each subject. Red blood cell incorporation of absorbed radioactivity was assumed to be 85% (14).

Study 1B

The purpose of study 1B was to determine whether iron from ferrous bisglycinate exchanged in the intestinal pool with iron from ferrous sulfate consumed in the same meal. On day 16, after the fasting blood sample had been taken, the same subjects consumed 55.5 kBq ^{59}Fe -sulfate and 111 kBq ^{55}Fe -bisglycinate mixed together in the porridge made from maize fortified with 0.011 mg Fe dry wt/g as ferrous sulfate and equilibrated overnight as indicated previously. A final blood sample was drawn 14 d later (day 31) for analysis of red blood cell radioactivity, as described above.

Study 2: regulation by iron status of absorption of iron from ferrous sulfate, ferrous bisglycinate, and ferric trisglycinate in water and absorption of trisglycinate iron from whole-maize meal porridge

To attract participants with a wide range of iron statuses, recruitment posters that targeted women who suspected they were anemic, who were vegetarians, or who had heavy menstrual periods were posted at the University of California, Davis. As a result, 33 female faculty, staff, and students volunteered to be participants. They were informed that their acceptability as participants depended on their serum ferritin concentrations. The goal was to recruit 15 women with serum ferritin concentrations <12 $\mu\text{g/L}$ and 15 with serum ferritin concentrations >12 $\mu\text{g/L}$. A fasting blood sample was drawn for measurement of serum ferritin, hemoglobin, and hematocrit. Ten women were excluded from the study: 3 could no longer participate because of work- and family-related emergencies, 1 lost contact, and 6 were not required because there was an excess of women with serum ferritin concentrations <12 $\mu\text{g/L}$. Because pregnancy was an

TABLE 1Characteristics of male subjects in studies 1A and 1B and the percentage iron absorption from ferrous sulfate and bisglycinate in whole-maize porridge¹

Subject no.	Age	Weight	Hemoglobin	Ferritin	Iron absorption			
					Study 1A		Study 1B	
					FeSO ₄	Bisglycinate	FeSO ₄	Bisglycinate
	<i>y</i>	<i>kg</i>	<i>g/L</i>	<i>μg/L</i>	%			
3	22	80.4	151	14.2	3.3	13.6	3.5	22.3
11	30	48.2	156	36.7	1.5	8.3	0.8	4.0
12	26	74.9	153	55.3	1.0	2.6	0.9	4.5
2	26	82.6	153	58.7	1.8	10.2	3.0	18.2
6	25	75.0	153	93.1	1.2	4.8	0.4	3.9
7	19	66.0	128	117.3	1.1	4.4	0.9	7.9
8	30	87.1	152	137.0	1.6	10.8	1.8	12.2
4	28	94.2	145	150.7	1.8	8.2	1.1	6.7
13	27	109.0	160	255.7	1.1	2.6	0.4	2.8
14	28	98.3	151	398.0	1.5	3.9	0.6	4.3
\bar{x}	26.1	81.6	150.2	92.4 ²	1.7 ²	6.0 ^{2,3}	1.0 ²	6.8 ^{2,3}
SD	3.4	17.2	8.7	—	—	—	—	—
+1 SD	—	—	—	244.0	2.7	10.8	2.2	13.8
-1 SD	—	—	—	36.8	1.0	3.3	0.5	3.4

¹In study 1A, ferrous sulfate and bisglycinate were given separately to subjects on days 1 and 2; in study 1B, the same subjects consumed ferrous sulfate and bisglycinate in the same meal.

²Geometric mean.

³Significantly different from ferrous sulfate, $P < 0.05$ (repeated-measures ANOVA).

exclusion criterion for safety reasons, all women were given a urine pregnancy test (Wampole Laboratories, Cranbury, NJ). All subjects were apparently healthy and had no history of disorders known to affect iron absorption. Vitamin and iron supplements were discontinued before and throughout the study.

Preparation of labeled solutions

A reference dose of ferrous sulfate was prepared to provide each subject with 30 mg ascorbic acid and 3 mg Fe as ferrous sulfate. Radioiron solutions of ferrous bisglycinate and ferric trisglycinate were prepared with 10 mL distilled water. The ⁵⁹Fe-sulfate and the bisglycinate intrinsically labeled with ⁵⁵Fe were prepared as described for study 1. A suspension of trisglycinate intrinsically labeled with ⁵⁹Fe or ⁵⁵Fe that provided 3 mg Fe/subject as trisglycinate was prepared by adding glycine slowly to the ⁵⁹FeCl₃ or ⁵⁵FeCl₃ solution. The unlabeled trisglycinate and distilled water were then added to these solutions, which were heated covered at 50°C for 2 h according to the procedures described by the manufacturers. The label was confirmed by counting the supernate and insoluble trisglycinate precipitate in the solution that was administered to the subjects; the insoluble fraction was labeled with the isotope, with essentially no isotope in the soluble supernate, as was intended.

Study 2A

After blood was drawn for measurement of baseline radioactivity, serum ferritin, and hemoglobin, the subjects drank the solution containing either 55.5 kBq ⁵⁹FeSO₄ or 111 kBq ⁵⁵Fe-bisglycinate. Each subject received a dose of the reference solution (A) and the bisglycinate in water (B) on 2 consecutive mornings in the sequence AB to half of the subjects and BA to the other half, as described by Hallberg (15). The reference dose and the bisglycinate solution were consumed at breakfast time under supervision, between 0700 and 1000 after an overnight fast. On each occasion after drinking the solutions, the subjects rinsed their containers

twice with deionized water and drank this water. Only water was allowed to be ingested for 4 h afterward. A fasting blood sample was then taken 2 wk later (day 16) for analysis of incorporated red blood cell radioactivity. The methods used to measure iron absorption were similar to those described for study 1.

Study 2B

On day 16, after the same subjects gave a fasting blood sample, and on day 17, the subjects were randomly assigned to consume either 55.5 kBq ⁵⁹Fe-trisglycinate in water or 111 kBq ⁵⁵Fe-trisglycinate equilibrated overnight with whole-maize meal porridge. The porridge was fortified with 0.011 mg Fe as trisglycinate/g maize. A final fasting blood sample was drawn 14 d later (day 31) for measurement of radioactivity incorporated into erythrocytes.

Statistical analysis

Absorption data were log transformed because of the highly skewed distribution of iron absorption measurements observed in humans (16) and were analyzed with repeated-measures analysis of variance (ANOVA) and Pearson's correlation coefficients. Post hoc comparisons were made by using Tukey's test. Statistical analyses were performed by using SAS software (SAS Institute Inc, Cary, NC).

RESULTS

Study 1

The pertinent characteristics of the 10 men participating in this study, who ranged in age from 19 to 30 y, are shown in **Table 1**. None of these subjects was iron deficient; all had serum ferritin concentrations ≥ 14.0 $\mu\text{g/L}$ and hemoglobin concentrations between 128 and 160 g/L. The percentage of iron absorbed from radiolabeled ferrous sulfate and bisglycinate administered with whole-maize meal porridge in 2 different meals or in the same

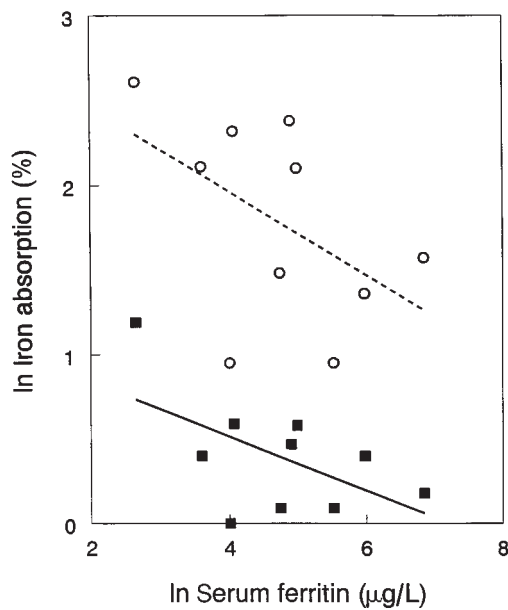


FIGURE 1. Relation between ln serum ferritin and percentage iron absorption from ferrous sulfate (■; $r = -0.62$, $P < 0.04$) and ferrous bisglycinate (○; $r = -0.66$, $P < 0.05$) consumed in whole-maize meal porridge.

meal is also shown in Table 1. It is evident that, in whole-maize meal porridge, iron from bisglycinate was absorbed significantly better than was iron from ferrous sulfate and that the percentage absorbed from each source was unaffected by the presence of the other source of iron. For studies 1A and 1B combined, the average iron absorption from bisglycinate was 4.7 times greater than that from ferrous sulfate (6.4 and 1.3%, respectively; $P < 0.05$).

Figure 1 is a scattergram of the percentage iron absorption from both fortificants separately and the natural logarithm (ln) of plasma ferritin. The linear correlation coefficients between the ln of baseline plasma ferritin and the ln of iron absorption from both iron fortificants were similar and significantly different from zero. However, the linear regression equations were very different; percentage iron absorption was $22.6 - 3.4 \ln$ ferritin for bisglycinate and $4.2 - 0.5 \ln$ ferritin for ferrous sulfate. The negative slopes in relation to plasma ferritin, reaching the lowest iron absorption values of 2.6% and 0.4% for bisglycinate and ferrous sulfate, respectively, strongly suggest that iron absorption from both fortificants was effectively down-regulated by iron reserves. There was an almost-perfect correlation between the percentage of iron absorbed from bisglycinate and ferrous sulfate administered with whole-maize meal porridge ($r = 0.99$, $P < 0.001$), indicating that subjects who absorbed the lowest or the highest amount of iron from ferrous sulfate also absorbed the lowest or the highest amount of iron from bisglycinate (**Figure 2**). The correlations between ln percentage iron absorption from both fortificants and ln plasma ferritin were also similar, but the correlation coefficients from only the ferrous sulfate ($r = -0.534$) or the bisglycinate ($r = -0.589$) data, as expected, were lower than when both forms were considered together. Importantly, the equations had similar regression coefficients.

Study 2

The characteristics of the women participating in study 2 and the percentages of iron absorbed from radiolabeled ferrous

ascorbate, bisglycinate and triglycinate in water, and triglycinate administered with whole-maize meal porridge are shown in **Table 2**. The women had a wide range of iron statuses, as was intended. Although 23 women were enrolled, complete data were obtained and analyzed for only 21 women. One woman withdrew from the study for personal reasons at the end of study 2A; data for the other woman were discarded because of technical problems in the measurement of erythrocyte radioactivity.

Mean iron absorption was highest from the ferrous ascorbate reference dose (geometric mean: 32.5%) and lowest from bisglycinate in water (9.1%). In contrast with the results of study 1, when the iron isotopes were fed with maize, iron absorption from ferrous sulfate was better than that from bisglycinate when the ferrous sulfate was consumed as a reference dose. The differences between the 3 sources of iron (ferrous ascorbate, ferrous bisglycinate, and ferric triglycinate) were significant. Again, the percentage iron absorption from ferrous ascorbate and bisglycinate under these conditions was significantly correlated ($r = 0.76$), as was that from ferrous ascorbate and ferric triglycinate ($r = 0.61$) and from bisglycinate and triglycinate ($r = 0.71$). As is evident from Table 2, the lowest absorption efficiencies were 4.2, 1.4, and 1.9 and the highest were 78.7, 38.0, and 59.5 for ferrous ascorbate, ferrous bisglycinate, and ferric triglycinate, respectively. These data support the effective down-regulation of iron absorption from all 3 sources of iron as iron reserves increase. This down-regulation was apparent from the highly significant negative correlations between ln iron absorption from ferrous ascorbate and bisglycinate and ln serum ferritin (**Figure 3**).

Administration of triglycinate in a porridge of whole-maize meal resulted in a significant fall in iron absorption to 2.3% compared with 15.3% when the triglycinate was administered in water (Table 2). **Figure 4** is a scattergram with regression lines and shows the relation between ln serum ferritin and ln iron absorption from triglycinate administered in 2 ways: with water

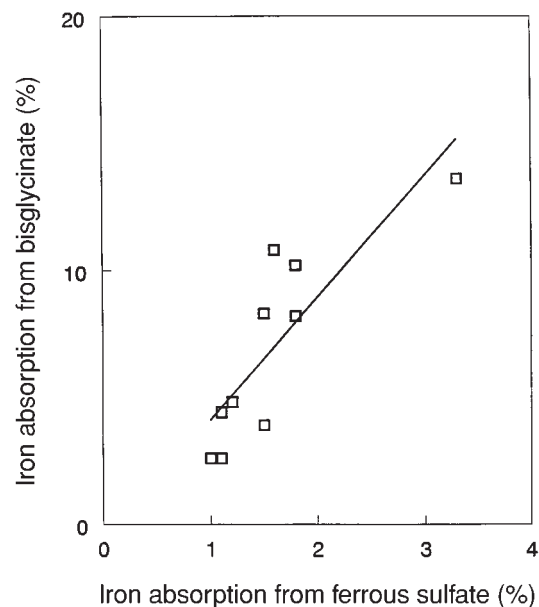


FIGURE 2. Correlation between iron absorption from ferrous sulfate and ferrous bisglycinate consumed in whole-maize meal porridge.

TABLE 2

Characteristics of female subjects in studies 2A and 2B and the iron absorption percentage from ferrous sulfate, ferrous bisglycinate, and ferric trisglycinate in water and ferrous trisglycinate in whole-maize porridge¹

Subject no.	Age	Weight	Hemoglobin	Ferritin	Iron absorption			
					Study 2A		Study 2B	
					FeSO ₄	Bisglycinate in water	Trisglycinate in water	Trisglycinate in maize
	<i>y</i>	<i>kg</i>	<i>g/L</i>	<i>μg/L</i>			%	
4	20	59.0	122	1.0	64.6	38.0	31.5	4.4
13	18	60.9	128	1.0	78.7	30.0	25.2	5.8
3	19	67.7	129	4.5	47.6	6.3	14.7	3.0
20	21	59.5	119	4.5	12.4	2.5	2.3	0.5
14	24	59.2	125	5.0	67.9	35.5	54.9	3.4
38	20	53.2	134	5.6	48.7	14.8	23.1	4.6
18	23	78.8	137	6.3	29.4	14.0	26.1	2.8
2	19	69.0	123	6.6	74.0	25.6	34.8	6.6
23	21	70.5	139	6.8	30.5	13.4	27.0	2.0
15	18	84.5	125	7.1	71.6	32.9	16.3	1.4
21	48	75.0	132	7.1	28.1	18.9	29.1	3.3
22	21	54.5	121	7.1	29.9	2.6	14.2	1.6
5	21	58.2	120	7.9	64.0	19.6	40.3	4.9
12	25	49.2	121	10.4	39.2	6.2	21.0	2.0
35	34	72.7	127	10.5	19.1	3.4	9.6	1.2
24	19	76.9	117	11.7	56.0	13.4	20.2	9.2
9	28	54.5	124	15.8	15.8	1.7	7.6	1.0
8	18	57.7	136	25.1	34.0	13.2	9.2	4.8
19	30	54.5	143	25.1	6.8	9.7	25.3	1.6
27	45	64.4	118	39.8	33.6	9.4	2.6	0.5
29	18	58.7	123	63.1	4.2	1.4	1.9	0.6
\bar{x}	24.3	63.7	126.8	8.2 ²	32.5 ^{a,2}	9.1 ^{c,2}	15.3 ^{b,2}	2.3 ^{d,2}
SD	8.5	9.7	7.4	—	—	—	—	—
+1 SD	—	—	—	22.2	71.8	30.9	39.3	5.4
-1 SD	—	—	—	3.0	14.7	2.6	6.0	1.0

¹In study 2A, subjects consumed ferrous sulfate (with ascorbic acid) and bisglycinate separately in water on 2 different days; in study 2B, the same subjects consumed ferric trisglycinate in water and trisglycinate in maize on 2 different days. Means with different superscript letters are significantly different, $P < 0.05$ (repeated-measures ANOVA and Tukey's test).

²Geometric mean.

and with whole-maize meal porridge. The regression equations were as follows: percentage iron absorption = $34.19 - 6.371 \ln$ serum ferritin ($r = -0.475$) and $4.84 - 0.827 \ln$ serum ferritin ($r = -0.362$), respectively. It is important to note from the regression equations that estimated mean \ln iron absorption from the 3 sources was virtually 0% when serum ferritin was between 40 and 56 $\mu\text{g/L}$.

DISCUSSION

The results of study 1 showed clearly that, after whole-maize meal porridge was equilibrated overnight with ferrous bisglycinate or ferrous sulfate, singly or combined, the proportion of absorbed iron was higher from the chelate than from the ferrous sulfate. The absorption of the latter form of iron added to various plant food sources was shown previously to represent that of the nonheme-iron pool (3, 4). It can therefore be stated with confidence that the process of absorption of iron from ferrous bisglycinate in whole-maize meal porridge differs from that of the nonheme iron pool and that its absorption is most probably higher because it is protected from the inhibitory factors present in the porridge. A second finding of study 1 was that the absorption of the iron in bisglycinate ingested with the whole-maize meal porridge was effec-

tively down-regulated, as was that of ferrous sulfate, by the iron reserves of the individual expressed as the serum ferritin concentration. These findings extend those of Olivares et al (9), who reported an inverse relation ($r = -0.60$, $P < 0.03$) between serum ferritin concentration and absorption of iron from bisglycinate in iron-replete subjects that was similar to the correlation we found ($r = -0.61$, $P < 0.03$).

In study 2, the relative characteristics of iron absorption from both a reference dose of ferrous ascorbate and bisglycinate in water were investigated. Compared with iron absorption when the compounds were administered with whole-maize meal porridge, absorption from ferrous sulfate was ≈ 19 times higher (32.5% compared with 1.7%), whereas absorption from bisglycinate was only slightly higher (9.1% compared with 6%) (studies 1 and 2, respectively). Given that ferritin concentrations were different between the 2 studies (Tables 1 and 2), we calculated the estimated mean absorption for different serum ferritin values on the basis of the linear regressions depicted in Figures 1 and 3. Mean adjusted absorption values showed that iron absorption from the reference dose was significantly higher (a 20-fold increase for a serum ferritin concentration of 20 $\mu\text{g/L}$) than absorption from ferrous sulfate fed in whole-maize meal porridge. In contrast, iron absorption from bisglycinate in water did not differ significantly from that of

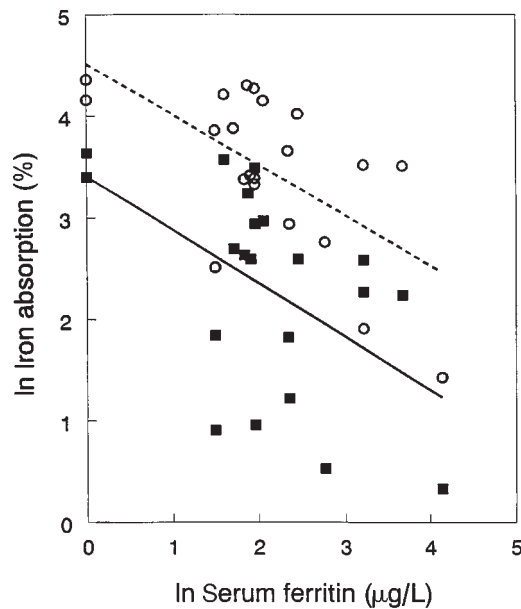


FIGURE 3. Relation between ln serum ferritin and percentage iron absorption from a reference dose of ferrous ascorbate (■; $r = -0.61$, $P < 0.03$) and ferrous bisglycinate (○; $r = -0.78$, $P < 0.001$) in water.

bisglycinate in maize and remained consistently one-third of that from ferrous ascorbate at all serum ferritin concentrations. Fox et al (17) found no difference between the bioavailability of iron from bisglycinate and that from ferrous sulfate when these were added to a high-phytate cereal weaning food fed to young infants. However, the ferrous sulfate was mixed with ascorbic acid so that the actual comparison was between ferrous ascorbate (which has better iron bioavailability from high phytate foods than does ferrous sulfate) and bisglycinate.

It is important to note that, in study 2, the regression equation for percentage iron absorption from bisglycinate compared with serum ferritin, in water, was almost identical to that reported by Olivares et al (9). On the other hand, the difference between iron absorption from bisglycinate with ascorbic acid in water (35%) and in milk (8%) reported by those authors was much greater than the small difference in its absorption in water compared with maize in the present study. Absorption of iron from bisglycinate is more depressed by milk than by maize porridge. When the volunteers in the present study consumed ^{55}Fe -bisglycinate and ^{59}Fe -sulfate mixed together in the porridge, absorption of the labeled irons was not affected, showing that there is no mixing of the labels in the intestinal pool. Had the iron from bisglycinate exchanged with the iron in that pool, both kinds of iron would have been equally absorbed. We do not understand the process by which iron from bisglycinate is absorbed in humans, but the results obtained in these experiments suggest that it differs from that for iron from ferrous sulfate at the intraluminal level or in the mechanisms involved in the entry of iron into the intestinal cell. However, once it is located intracellularly, the iron from bisglycinate most probably enters the common cellular pool because it is effectively regulated by the iron status of the subjects, as is the iron from ferrous ascorbate and ferrous sulfate. The effect of ascorbic acid on absorption of iron from bisglycinate became evident in the case of milk, suggesting that the

reducing potential of ascorbic acid has a greater effect on the interaction of bisglycinate with some milk component than it does directly on iron from bisglycinate. This interpretation of the effect of ascorbic acid is based also on the small difference observed between absorption of iron from bisglycinate from whole-maize meal porridge and from an ascorbic acid solution. Bisglycinate seems to be effective in protecting its iron from the intraluminal inhibitory effect of phytate in maize.

Trisglycinate produces very different iron absorption values from those for ferrous sulfate and bisglycinate discussed above. Trisglycinate is a ferric chelate of glycine that is reportedly quite insoluble except at very low pH: 87% at pH 2–3 and 5% at pH 7 according to the manufacturers. When trisglycinate was administered with whole-maize meal porridge it was very poorly absorbed and the negative slope between ln percentage iron absorbed and ln serum ferritin was the flattest of all. On the other hand, iron absorption from trisglycinate in water was significantly higher than when the trisglycinate was fed in whole-maize meal porridge and when bisglycinate was administered in water. These results suggest that iron is released from trisglycinate in the intestinal lumen and enters the intestinal iron pool as free iron. This iron would be well absorbed from water but poorly absorbed in the presence of the phytates in maize. Why the iron would be released is not clear, but one possible explanation is hydrolysis of the trisglycinate by tripeptidases on the mucosal side of the enterocyte (18).

In conclusion, iron from ferrous bisglycinate is better absorbed than is that from ferrous sulfate or maize in the presence of absorption inhibitors in whole-maize meal porridge and appears not to mix with the nonheme-iron pool before its intracellular incorporation into the common intracellular iron pool. The results suggest that, once iron from bisglycinate is located intracellularly, its absorption is regulated in the same manner

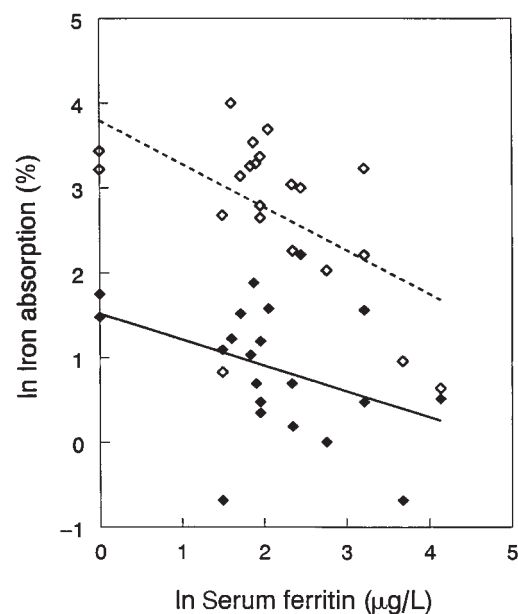



FIGURE 4. Relation between ln serum ferritin and percentage iron absorption from ferric trisglycinate consumed in water (◇; $r = -0.68$, $P < 0.01$) and ferric trisglycinate consumed in whole-maize meal porridge (◆; $r = -0.53$, $P < 0.01$).

as is iron from ferrous sulfate that labels the intracellular iron pool. Thus, ferrous bisglycinate is probably an effective and safe source of iron and is particularly useful in diets that are rich in phytates. We reported elsewhere that although bisglycinate promotes rancidity in whole-maize meal (19), porridge made from bisglycinate-fortified whole-maize meal with or without butylated hydroxyanisole is acceptable to infants, toddlers, and their parents (20). We were unable to predict the behavior of bisglycinate in the presence of other inhibitors of iron absorption but this seems to vary (eg, from milk compared with whole-maize meal porridge). It appears that ferric trisglycinate is not a useful iron fortificant for maize in humans. Finally, although the mechanisms by which FeAAs are absorbed by humans remain unclear, the results of this study suggest that these chelates are handled differently from food nonheme iron, possibly intraluminally or during the initial steps of iron absorption by the intestinal cell. These mechanisms need to be studied in the future. 

We acknowledge Bouakham Chanthapaseuth and Joanne Graham for their assistance in conducting the studies and Albion Laboratories for partially funding the project.

REFERENCES

- World Health Organization. Malnutrition. The global picture. Geneva: World Health Organization, 2000.
- West CE. Strategies to control nutritional anemia. *Am J Clin Nutr* 1996;64:789–90.
- Allen LH, Ahluwalia N. Improving iron status through diet. The application of knowledge concerning dietary iron bioavailability in human populations. Arlington, VA: John Snow, Inc/OMNI, 1997. Available from Most at www.mostproject.org.
- Cook JD, Layrisse M, Martínez-Torres C, Walker R, Monsen ER, Finch CA. Food iron absorption measured by an extrinsic tag. *J Clin Invest* 1972;51:805–15.
- Galliard T. Rancidity in cereal products. In: Allen JC, Hamilton RJ, eds. *Rancidity in foods*. New York: Elsevier Science Publishing Co, 1989:141–5.
- Piñeda O, Ashmead HD, Perez JM, Lemus CP. Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. *J Appl Nutr* 1994;46:2–13.
- Fairweather-Tait SJ, Fox TE, Wharf SG, Ghani NA. A preliminary study of the bioavailability of iron- and zinc-glycinate. *Food Addit Contam* 1992;9:97–01.
- Langini S, Carbone N, Galdi M, et al. Ferric glycinate iron bioavailability for rats as determined by extrinsic radioisotopic labeling of infant formulas. *Nutr Rep Int* 1988;38:729–35.
- Olivares M, Pizarro F, Pineda O, Name J, Hertrampf E, Walter T. Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in humans. *J Nutr* 1997;127:1407–11.
- Stekel A, Olivares M, Pizarro F, Chadud P, Lopez I, Amar M. Absorption of fortification iron from milk formulas in infants. *Am J Clin Nutr* 1986;43:917–22.
- Beutler E. How little we know about the absorption of iron. *Am J Clin Nutr* 1997;66:419–20.
- Hallberg L, Hultén L, Gramatkovski E. Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? *Am J Clin Nutr* 1997;66:347–56.
- International Nutritional Anemia Consultative Group. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Washington, DC: INACG, 1998.
- Viteri FE, Kohaut BA. Improvement of the Eakins and Brown method for measuring ⁵⁹Fe and ⁵⁵Fe in blood and other iron-containing materials by liquid scintillation counting preparation using microwave digestion and ion-exchange column purification of iron. *Anal Biochem* 1997;244:116–23.
- Hallberg L. Food iron absorption. In: Cook JD, ed. *Iron*. London: Churchill Livingstone, 1980:116–33.
- Taylor P, Martínez-Torres C, Leets I, et al. Relationship among iron absorption, percent saturation of plasma transferrin and serum ferritin concentration in humans. *J Nutr* 1988;118:1110–5.
- Fox TE, Eagles J, Fairweather-Tait SJ. Bioavailability of iron glycine as a fortificant in infant foods. *Am J Clin Nutr* 1998;67:664–8.
- Ugolev AM, Timofeeva NM, Smirnova LF, et al. Membrane and intracellular hydrolysis of peptides: differentiation, role and interrelations. In: Elliot K, O'Connor M, eds. *Peptide transport and hydrolysis (CIBA Symposium)*. Amsterdam: Elsevier, 1977:221.
- Bovell-Benjamin AC, Allen LH, Frankel EN, Guinard J-X. Sensory quality and lipid oxidation of maize porridge as affected by iron amino acid chelates and EDTA. *J Food Sci* 1999;64:371–6.
- Bovell-Benjamin AC, Allen LH, Guinard J-X. Toddler's acceptance of whole-maize meal porridge fortified with ferrous bisglycinate. *Food Qual Pref* 1999;10:123–8.