IDPAS # 318

The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas[1,2]

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ABSTRACT The effect of varying concentrations of ascorbic acid on the absorption of iron from a soy-based infant milk formula containing 6 mg iron/100 g was examined in 64 adult Indian females using the extrinsic radioactive tag method. The corrected geometric mean absorption from the basic soy formula was only 1.8%. Addition of ascorbic acid in a concentration of 40 mg/100 g, did not significantly increase absorption (3.3%; $t = 1.8$, $p > 0.07$) but raising the concentration to 80 mg/100 g did so (6.9%; $t = 2.4$, $p < 0.02$). No further significant increase was noted when the concentration of ascorbic acid was increased to 160 mg/100 g (7.7%; $t = 0.4$, $p > 0.7$). The inhibitory effect of soy on iron absorption was further demonstrated by a direct comparison between the soy-based formula and a similar product based on cows' milk. The comparison was made at two concentrations of ascorbic acid. At 40 mg/100 g the geometric mean iron absorption from the soy formula was 2.4% compared with 5.3% from the milk formula ($t = 2.8$, $p < 0.02$), while the corresponding values at 80 mg ascorbic acid/100 g were 7.2 and 19.5%, respectively ($t = 3.4$, $p < 0.02$). The present results confirm the marked inhibitory effect of soy protein on iron absorption and calculations from the absorption figures suggest that such formulas should contain at least 12 mg/100 g iron together with ascorbic acid in a molar ratio of approximately 4:1 if they are to be adequate in terms of iron nutrition. *Am J Clin Nutr* 1984;40:522–527.

KEY WORDS Iron absorption, inhibitory effect of soy, ascorbic acid, infant formulas

Introduction

Iron deficiency is the most commonly recognized nutritional deficiency in both developing countries and affluent societies. It is particularly prevalent among infants and young children because rapid growth imposes large requirements for iron and many infant diets contain only a marginal supply of iron. In this context, the use of infant formulas based on soy as a source of protein is of some concern, since there is evidence that iron absorption in the presence of soy products is poor (1–4). There are three ways in which the low bioavailability of iron in soy products might be improved. First, such products could be fortified with greater amounts of iron; second, the unidentified inhibiting substance could be removed and; third, a promoter of iron absorption, such as ascorbic acid, could be added (5). The present study compares the absorption of iron from a soy-based infant milk formula with that from a similar product based on cows' milk, and examines the effect of varying concentrations of ascorbic acid.

Methods

Subjects

The subjects who participated in the studies were 64 parous Indian housewives living in municipal housing projects in Chatsworth and Merebank, near Durban. While there is a high prevalence of iron deficiency among the women in this community (6, 7), subjects were not selected on the basis of iron deficiency.

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Received February 10, 1984.

Accepted for publication April 17, 1984.

Composition of milk formulas

The soy-based formula used in this study was a soy protein isolate in powder form, designed specifically for infants and children intolerant to lactose or cows' milk. The formula, which contained 6 mg iron/100 g as ferrous sulfate, was prepared for the present studies without ascorbic acid by Nestlé Infant and Dietetic Services, Johannesburg, South Africa. The preparation contained isolated soy protein, vegetable oils, and maltodextrin and was supplemented with minerals and vitamins. The major components were fat (25%), protein (14%), and carbohydrate (55%). It contained 3% minerals, 3% moisture, and had an energy value of 500 kcal/100 g. The normal dilution was 670 kcal/l and it was prepared by dissolving 134 g powder in 900 ml of previously boiled water.

The milk-based formula (Lactogen, Nestlé, Randburg, South Africa) was a commercially available infant formula prepared from cows' milk modified by partial replacement of the butter fat by corn oil and by the addition of carbohydrates, vitamins, and mineral salts. The overall composition was similar to that of the soy formula (24% fat, 16% protein, 52% carbohydrate, 4% minerals, 3% moisture, 490 kcal/100 g). However, it contained no iodine and there were minor differences in the zinc, sodium, potassium, and chloride concentrations. (The zinc concentrations in the soy formula and in Lactogen were 4.5 and 3.8 mg/100 g, respectively). The basic preparation used in these studies contained final concentrations 6 mg iron and 40 mg ascorbic acid per 100 g.

Administration of formulas

Absorption of iron from two different formulas was measured in each group of subjects. The milk drinks were consumed on consecutive mornings after an overnight fast, and no food or drink other than water was permitted for 4 h thereafter. Each portion, prepared by dissolving 50 g of the "milk" powder in 150 ml water, was labeled with 3 μCi of either ^{55}Fe or ^{59}Fe (Radiochemical Centre, Amersham, Berks, England). When ascorbic acid was added this was done together with the isotope. One formula was tested using one isotope, and on the next day the second formula was given labeled with the other isotope. The radioiron, which was prepared in 0.01 N HCl, was stirred into the drink immediately before consumption.

Two weeks later, blood for the determination of ^{55}Fe, ^{59}Fe, Hb, serum iron, unsaturated iron-binding capacity, and serum ferritin was obtained from each subject after an overnight fast. Each person then drank a standard 3-mg dose of iron as $FeSO_4 \cdot 7H_2O$, with 30 mg ascorbic acid added, and labeled with 3 μCi of ^{59}Fe. Only water was permitted during the next 3 h. Blood samples were obtained 14 days later and the absorption of iron from the standard reference dose was estimated from the increment of ^{59}Fe in the blood. This provided a measure of each individual's absorption capacity and made it possible to express iron absorption from the milk formulas in relation to this reference absorption (8). Each subject took part in one experiment only.

Chemical and isotopic techniques

Duplicate 10-ml blood samples and duplicate portions of suitable standard iron solutions were prepared for differential counting of ^{55}Fe and ^{59}Fe using a modification of the method of Eakins and Brown (9). The activities of ^{55}Fe and ^{59}Fe in the processed samples were determined using a liquid-scintillation spectrometer (Packard Tri-Carb Spectrometer model 3375). The ^{59}Fe activity in the 4-ml blood samples collected immediately before the reference iron salt was administered and 2 wk later was assessed in duplicate against suitable standards using a Packard Auto Gamma Scintillation Spectrometer model 5320. The percentage absorption values were calculated on the assumption that 100% of the absorbed radioactivity was present in the Hb of the circulating erythrocytes, and that the blood volume of each subject was 65 ml/kg body weight.

Hb concentrations were determined by the cyanmethemoglobin technique. Serum iron concentration were measured using the International Committee for Standardization in Hematology method (10) as was the unsaturated iron-binding capacity (11). Serum ferritin was quantitated using the enzyme linked immuno-absorbent assay of Conradie and Mbhele (12). Since the percentage iron absorption and serum ferritin concentration showed considerable variation with positive skew, results were expressed as the geometric mean and SD ranges, thus permitting the use of standard parametric statistical methods.

Ethical considerations

Approval for the studies was obtained from the Committee for Research on Human Subjects of the Faculty of Medicine, University of the Witwatersrand, Johannesburg. Written consent was obtained from all subjects after the nature of the investigation had been fully explained to them by an Indian nursing sister. Each subject took part in one experiment only. It was calculated that if each test dose was completely retained, the total whole body radiation dosage would be 143 mrems (13) which is 28% of the annual maximum permissible dose for members of the public (14, 15). In practice, the percentage absorbed was much less, which would make the radiation exposure proportionately less.

Results

In the first three studies (**Table 1**, A, B, and C) the effect of various levels of ascorbic acid on the absorption of iron from soy- and milk-based formulas was compared. In study A the absorption of iron from the standard milk formula containing 20 mg ascorbic acid (40 mg/100 g) was 6.9% (SD range 1.9 to 25.3%), which was more than three times greater than that from the soy formula containing no ascorbic acid (1.5%, SD range 0.6 to 3.7%) ($t = 6.2$, $p < 0.002$). In the next study (B) both formulas had the same ascorbic acid content (20 mg) (40 mg/

TABLE 1
Iron absorption from soy and milk formulas containing 3 mg iron, 50 g formula, and varying amounts of ascorbic acid

Study no	No subjects	Test substance	Ascorbate content	Hb	Transferrin saturation	Serum ferritin	Absorption
			mg	*g/dl**	*%**	*μg/l†*	*%†*
		Soy	0				1.5 (0.6–3.7)
A	13	Lactogen	20	13.4 (±1.1)	27 (±11)	20.4 (5.6–74.8)	6.9 (1.9–25.3)
		Reference	30				23.0 (14.7–36.1)
		Soy	20				2.4 (0.5–11.0)
B	12	Lactogen	20	12.0 (±1.3)	21 (±8)	12.1 (4.0–36.2)	5.3 (1.9–14.6)
		Reference	30				34.0 (18.2–63.5)
		Soy	40				7.2 (3.8–13.6)
C	7	Lactogen	40	12.9 (±1.3)	31 (±11)	20.5 (7.9–53.3)	19.5 (7.9–47.9)
		Reference	30				32.2 (16.4–63.2)
		Soy	0				2.6 (1.1–5.7)
D	11	Soy	20	11.8 (±1.5)	21 (±8)	20.6 (7.8–54.6)	3.4 (1.3–8.8)
		Reference	30				31.0 (22.2–43.5)
		Soy	0				0.6 (0.2–1.7)
E	9	Soy	40	12.7 (±0.7)	31 (±11)	34.8 (18.3–66.3)	4.5 (1.9–10.9)
		Reference	30				35.0 (18.5–67.0)
		Soy	40				4.9 (1.6–15.8)
F	12	Soy	80	12.1 (±1.6)	24 (±9)	16.4 (4.5–59.9)	5.2 (1.5–17.9)
		Reference	30				35.0 (18.0–70.0)

* Mean (±SD).
† Geometric mean (SD range).

100 g) but iron absorption from the milk formula was 5.3% (SD range 1.9 to 14.6%), which was more than twice that from the soy formula (2.4%, SD range 0.5 to 11.0%) ($t = 2.8$; $p < 0.02$). Increasing the ascorbic acid content to 40 mg (80 mg/100 g) in both formulas (study C) only served to emphasize the enhanced iron absorption from the milk-based preparation (19.5%, SD range 7.9 to 47.9% and soy 7.2%, SD range 3.8 to 13.6%) ($t = 3.5$, $p < 0.02$).

The remaining studies (Table 1, D, E, and F) were designed to show the effect of increasing ascorbic acid concentrations on iron absorption from the soy-based formula. Study D demonstrated that the addition of 20 mg ascorbic acid (40 mg/100 g) to the soy formula did not significantly improve the absorption of iron (3.4%, SD range 1.3 to 8.8%) compared to that from the same preparation containing no ascorbic acid (2.6%, SD range 1.1 to 5.7%) ($t = 1.6$, $p > 0.1$). However, increasing the level of fortification to 40 mg ascorbic acid (80 mg/100 g) in study E significantly improved absorption from 0.6% (SD range 0.2 to 1.7%) to 4.5% (SD range 1.9 to 10.9%) ($t = 8.3$, $p < 0.002$). An increase in the ascorbic acid content from 40 to 80 mg (160 mg/100 g) failed to enhance iron absorption further (4.9%, SD range 1.6 to 15.8% and 5.2%, SD range 1.5 to 17.9%, respectively) ($t = 0.4$, $p > 0.6$) (study F).

In a final analysis, an overall comparison was made between the effects of varying doses of ascorbic acid on the absorption of iron from the soy and milk formulas. To make the comparison possible, all the individual results obtained in the six studies were standardized to a reference absorption of 40%, which is the average absorption of subjects with early iron deficiency (8). Since reference absorptions were not obtained in 11 subjects, the analysis was based on the results obtained in 53 patients. The geometric mean iron absorption from the soy-based formula was very low (1.8%, SD range 0.6 to 5.2%) when no ascorbic acid was present and rose only modestly when ascorbic acid was added in concentrations varying between 40 and 160 mg/100 g (**Fig 1**). Statistical comparison of these groups showed that the addition of ascorbic acid in a concentration of 40 mg/100 g did not significantly increase iron absorption (3.3%, SD range 1.2 to 9.6%) ($t = 1.8$, $p > 0.07$) but raising the concentration to 80 mg/100 g did so (6.9%, SD range 2.9 to 16.3%) ($t = 2.4$, $p < 0.02$). Increasing the concentration to 160 mg/100 g did not improve absorption further (7.7%, SD range 2.4 to 24.8%) ($t = 0.4$, $p > 0.7$). In contrast, iron absorption from the milk formula was markedly affected by the concentrations of ascorbic acid present. An increase in the ascorbic acid concentration from 40 to 80 mg/100 g was associated with a more than two-fold increase in iron absorption from 10.5 to 24.2%. An analysis of variance showed that the overall results fell into three groups. In the first group, absorption from the milk-based formula containing 40 mg ascorbic acid/100 g did not differ significantly from absorption from the soy-based formulas containing 80 and 160 mg ascorbic acid/100 g ($F = 1.55$, $p > 0.2$). Absorption from this group was significantly better than that from a second group comprising the soy-based formulas containing either no ascorbic acid or 40 mg/100 g ($F = 25.85$, $p < 0.002$). Finally, iron absorption from the milk-based formula with 80 mg ascorbic acid/100 g was significantly better than that from all other combinations ($F = 15.96$, $p < 0.002$).

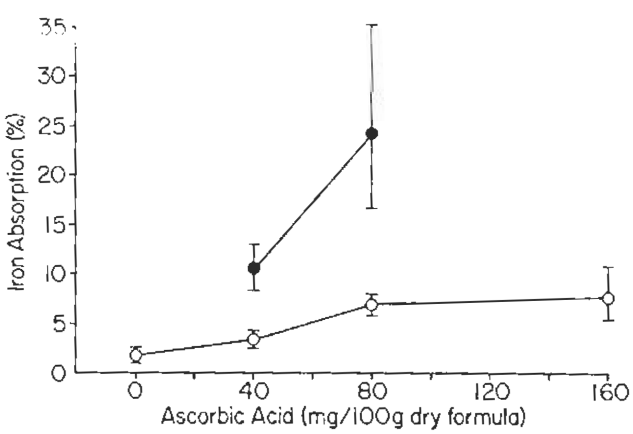

FIG 1. The effect of various concentrations of ascorbic acid on the geometric mean absorption of iron (±SE) from a soy-based infant milk formula containing 3 mg iron as ferrous sulfate and 50 g dry powder (○) and from a similar product based on milk (Lactogen) (●). To make comparisons possible all absorption values were corrected to a reference absorption of 40%. (The geometric mean reference absorptions in the various groups studied varied between 23.0 and 35.0%.)

Discussion

The full-term infant receives a generous and relatively fixed iron supply of 75 mg/kg from the mother (16). During the first 2 months of life the infant relies largely on stored iron for its requirements but the demands of erythropoiesis and growth rapidly deplete these stores and between the 2nd and 6th month there is an increasing dependance on dietary iron which is largely derived from milk, milk products, and soy-based products (17). It is currently estimated that soy-based preparations account for 10 to 20% of infant formula sales in the US; they are used extensively to avoid suspected or proved allergies to milk, while soy is also a major constituent of infant protein supplements (5). However, there is currently some concern as to whether such products may have a deleterious effect on iron nutrition, since there is evidence that soy inhibits iron absorption (1, 4, 18).

Earlier studies on the absorption of iron in the presence of soy products gave conflicting results, with some showing good bioavailability (19, 20) and others relatively poor bioavailability (21, 22). The confusion was resolved in a series of studies by Cook and coworkers (1, 2, 3, 18). They demonstrated that the bioavailability of iron in corn-soy-milk was low (1) and that iron absorption from meals containing isolated soy protein was significantly less than from meals containing egg albumen or casein (2). In further studies they showed that the inhibitor of iron absorption was not removed by purification but could be partially overcome by ascorbic acid (3). This latter observation was of interest because it may explain why Rios et al (23) in an earlier study were not able to show a difference in iron absorption when they compared a milk- and soy-based formula (23). Ascorbic acid was present in both formulas.

The enhancing effect of ascorbic acid on the absorption of iron from vegetable meals has been amply demonstrated in a number of studies (20, 24, 25). The fact that the enhancing effect of ascorbic acid on iron absorption is dose related (26) prompted the present investigation, since it was believed that it might be possible to overcome the inhibitory effect of soy on iron absorption from soy-based formulas by having an appropriate amount of ascorbic acid present in the formula. In these studies a comparison was made between a soy-based formula and a similar formula in which milk was present instead of soy. Several points of interest emerged. Iron absorption from the soy-based formula was very low when no ascorbic acid was present. At the concentration of ascorbic acid which is used for commercial fortification in the products tested (40 mg/100 g), iron absorption was significantly better from the milk formula and the difference was even greater when the ascorbic acid concentration was increased to 80 mg/100 g. The inhibitory effect of soy on iron absorption was still apparent when the concentration of ascorbic acid was increased to 160 mg/100 g formula.

In an attempt to express the present results in figures relevant to iron-deficient subjects, all values were corrected to a reference absorption of 40% (Fig 1). This figure was chosen since it is the amount of iron absorbed by subjects with early iron deficiency (8). If the WHO/UNICEF recommended amount of supplement (100 g) were consumed daily by a 1-yr-old infant then the following amounts of iron would be absorbed from the soy-based formula with increasing amounts of ascorbic acid: no ascorbic acid, 0.11 mg, 40 mg/100 g, 0.20 mg; 80 mg/100 g, 0.41 mg; and 160 mg/100 g, 0.46 mg. In contrast, figures of 0.63 and 1.45 mg were obtained with the milk formula when the levels of ascorbic acid fortification were 40 and 80 mg/100 g, respectively. When these figures are considered against the background of the 1-yr-old infant's daily iron requirements of 1.5 mg, it can be seen how inadequate the absorption figures for the soy formula actually were. For such products, which represent an important potential source of dietary protein, to be adequate from the point of view of iron nutrition, certain adjustments seem appropriate. For example, the soy-based formula tested in the present study was found to be inadequate with regard to the amounts of both iron and ascorbic acid that it contained. More satisfactory absorption figures might be anticipated if the level of iron fortification were to be doubled from 6 to 12 mg/100 g and the weight ratio of ascorbic acid to iron of approximately 6 to 1 (molar ratio 2:1) were to be increased to 12 to 1 (molar ratio 4:1). While all these figures are based on limited experiments with single meals and on the assumption that absorption data obtained in adults can be applied to infants, they at least provide some guidelines for future studies.

The authors are grateful for the generous assistance of Nestlé South Africa.

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