

Ascorbic acid: effect on ongoing iron absorption and status in iron-depleted young women¹⁻³

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ABSTRACT The effect of ascorbic acid on iron retention from a diet with predicted low iron bioavailability (containing minimal meat and ascorbic acid) was investigated in iron-depleted premenopausal women. Eleven women were depleted of storage iron (indicated by serum ferritin) through a combination of diet (5.0 mg Fe/2000 kcal for 67–88 d) and phlebotomy. They then consumed a diet containing 13.7 mg Fe/2000 kcal, supplemented with placebo or ascorbic acid three times daily (1500 mg total) with meals for 5.5 wk. Ascorbic acid improved apparent iron absorption (balance method) [$38 \pm 2\%$ ($\bar{x} \pm \text{SEM}$) vs $27 \pm 2\%$]. Ascorbic acid also improved hemoglobin, erythrocyte protoporphyrins, and serum iron but not hematocrit, serum ferritin, iron-binding capacity, or transferrin saturation. In iron-depleted women consuming a diet with predicted poor iron availability, ascorbic acid supplementation enhanced body iron retention for 5.5 wk. *Am J Clin Nutr* 1990;51:649–55.

KEY WORDS Iron absorption, iron depletion, ascorbic acid, iron balance, iron bioavailability

Introduction

The enhancing effect of ascorbic acid on the absorption of nonheme iron has been observed repeatedly (1, 2) by use of methods that measure iron absorption from a single meal. Because body iron content is regulated chiefly by intestinal absorption rather than by excretion (3), the enhancement of absorption by ascorbic acid would be expected, over time, to result in greater body iron stores. Cook et al (4) investigated whether ongoing ascorbic acid supplementation with meals would affect serum ferritin, an indicator of body iron stores. Their healthy, free-living subjects had no change in serum ferritin after ingesting 2 g ascorbic acid/d with meals for 16 wk, which suggested that body iron stores were not affected. This finding was somewhat paradoxical because the enhancing effect of ascorbic acid on iron absorption from a meal was documented in these subjects at both the beginning and end of the experiment. In a similar report (5), 100 mg ascorbic acid three times daily with meals for 8 wk did not affect the serum ferritin concentrations of 25 volunteers, as compared with a control group given placebos. The subjects in both studies (4, 5) may have been consuming diets with substantial amounts of meat, and thus their diets may have already promoted nonheme iron absorption without ascorbic acid supplementation.

The purpose of this study was to determine whether iron bal-

ance and biochemical indices could be improved by adding ascorbic acid to a diet providing poorly available iron. This was investigated in young adult women depleted of iron, who represent a group likely to benefit from enhanced iron absorption.

Methods

Subjects

Eleven healthy, premenopausal, eumenorrheic women with low to moderate serum ferritin values (8.5–55 $\mu\text{g/L}$), who gave informed consent, were studied in a controlled metabolic unit environment. They were aged 22–36 y with a height of 167 ± 8 cm ($\bar{x} \pm \text{SD}$) and weight of 67 ± 17 kg. Participants were chaperoned on trips outside the metabolic unit to help ensure compliance. This research was approved for human subjects by the USDA Human Studies Review Committee and by the University of North Dakota's Radioactive Drug Research Committee and Institutional Review Board.

Experimental protocol

Participants were depleted of body iron stores by ingesting a low-iron diet and undergoing phlebotomy until serum ferritin values decreased to $< 8.5 \mu\text{g/L}$ (Fig 1). Depending on individual responses this required 67–88 d. As persons became depleted they began an iron-repletion diet, with their low iron status maintained by continued phlebotomy. Iron loss caused by phlebotomy was reduced after 88 d. From days 109–175, participants' diets were supplemented with placebo or ascorbic acid, 500 mg three times daily (1500 mg total), with major meals. From days 149–175 all participants received a supplement of 50 mg Fe/d as ferrous sulfate in time-release capsules (Fig 1). At the completion of the study, participants were provided with a 2-mo supply of iron supplements (50 mg/d). They were requested to take all the supplemental iron, then obtain

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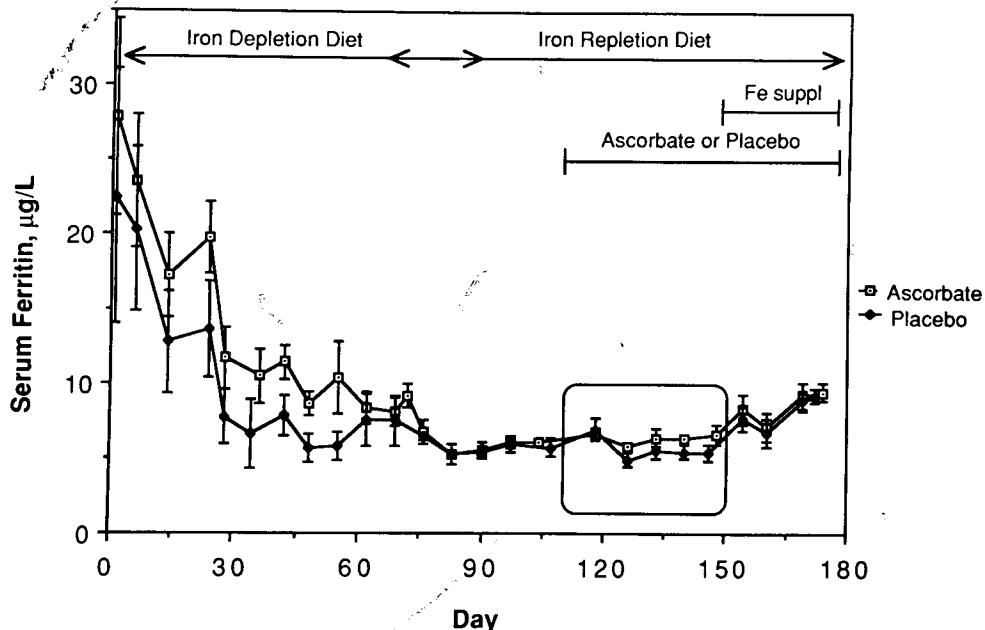


FIG 1. Effect of ascorbic acid on serum ferritin. The mean \pm SEM is shown for the seven participants supplemented with ascorbic acid and the four participants fed a placebo during the periods indicated. Data in the box were examined for the effects of ascorbic acid by repeated measures of analysis of covariance (correcting for individual differences existing immediately before the ascorbic acid treatment). Ascorbic acid did not affect serum ferritin significantly.

hemoglobin and hematocrit measurements through their personal physicians at the expense of the research center.

During iron depletion half the participants received 800 mg supplemental calcium/d. Calcium supplements did not affect iron balance or indicators of iron status, allowing this prior treatment to be ignored when evaluating the effects of ascorbic acid. Treatments were assigned randomly at the beginning of the study, with the constraint that ascorbic acid was assigned with the same frequency to persons with or without the calcium pretreatment. Of 13 original participants in the study, 2 left for personal reasons (unrelated to the research), which left 4 in the placebo group and 7 in the ascorbic acid group.

Diets

Both diets consisted of a 3-d rotating menu cycle. The iron-depletion diet provided 5.0 mg iron (analyzed)/2000 kcal. In accordance with the model of Monsen et al (6) the diet consisted of one medium and three low-iron-availability meals, achieved by limiting the amounts of meat, poultry, fish, and ascorbic acid. Iron absorption from this diet would be expected to be 5–10% in women who have no iron stores (6).

The iron-repletion diet provided 13.7 mg iron (analyzed)/2000 kcal, with predicted poor iron availability similar to that of the iron-depletion diet (Table 1). The additional iron was principally from vegetable sources.

Energy intakes were adjusted individually to maintain body weights within 2% of initial values. Energy adjustments were made by proportionally adjusting the amounts of all foods.

To blind the ascorbic acid treatment to the volunteers, the placebo group received sugar-free lemonade concentrate added to fruit juices, and the ascorbic-acid-treatment group received an identical amount of sugar-free lemonade with ascorbic acid and additional aspartame sweetener.

⁵⁹Fe absorption

To determine absorption of ⁵⁹Fe, 3.7 KBq of the isotope was added to the juice of a test breakfast on days 8, 31, 85, and 148. The test breakfast (from one day of the rotating menu) contained 32 g cranberry/apple juice, 200 g milk (2% fat), and french toast consisting of 32 g unenriched white bread, 8 g egg, 32 g milk, 1.6 g vanilla, 4 g sugar, 0.016 g cinnamon, 12 g syrup, and 12 g butter. This meal provided 0.8 ± 0.2 mg Fe ($\bar{x} \pm$ SD, analyzed). Ascorbic acid supplements were not included in the test meal.

Retention of ⁵⁹Fe was measured by whole-body counting. Retained activity was measured within a few hours of the test meal and weekly thereafter. Counting measurements were corrected for differences in body size and radioisotope distribution by the method of Cohn et al (7). Absorption was estimated by extrapolating back to the time of the meal along the linear portion of a semilogarithmic retention plot (the natural logarithm of whole-body radioactivity vs time) and correcting for physical decay of the isotope (8). Corrections were made for residual activity remaining from previous meals by extrapolating forward along the linear portion of the retention plot.

Analyses

Diets, blood, menses, and excreta were collected with care to avoid trace mineral contamination. Duplicate diets were prepared for analysis throughout the study. Iron balance was determined by analysis of 6-d composites of diets and excreta. Samples of the diet and fecal composites were digested with concentrated nitric and 70% perchloric acids by method (II)A of the Analytical Methods Committee (9). Menstrual samples were extracted from sanitary products with 0.12 mol HCl/L, followed by digestion with nitric and perchloric acids. The iron

TABLE 1
Sample iron-repletion menu*

Breakfast	Lunch	Dinner	Evening snack
50 g Grape juice	40 g Orange juice	150 g Apple juice	40 g Orange juice
70 g Eggs	25 g Pork round	25 g Chicken	111 g Pumpkin cake
0.5 g Bouillon	25 g Egg noodles, dry	20 g Rice, dry	200 g Milk, 2%
40 g Whole-wheat bread	30 g Green beans	40 g Carrots	
5 g Margarine	14 g Cheddar cheese	1 g Onion flakes	
10 g Strawberry jelly	1 g Bouillon	4 g Bouillon	
	50 g Lettuce	5 g Butter	
	15 g French dressing	5 g Cornstarch	
	40 g Whole-wheat roll	60 g Whole-wheat roll	
	5 g Butter	30 g Cheddar cheese	
	35 g Cashews	20 g Vanilla wafers	

* A representative day of a 3-d-cycle menu with food amounts corresponding to 2000 total kcal. The diet contains 13.7 mg Fe (analyzed)/2000 kcal and 63 mg ascorbic acid (calculated)/2000 kcal.

content of the digestates was determined by inductively coupled argon plasma emission spectrophotometry. Analytical accuracy was monitored through periodic analyses of bovine liver standards (National Institute of Standards and Technology, Gaithersburg, MD). The analyzed values were $98 \pm 4\%$ of certified values for iron ($n = 49$).

Biochemical measurements on fasting blood samples included the following: plasma iron measured colorimetrically with prior precipitation by trichloroacetic acid (10), serum ferritin measured by radioimmunoassay (11), percent transferrin saturation calculated from the iron and total-iron-binding capacity (12), erythrocyte free protoporphyrin measured by ethyl acetate-acetic acid extraction (13), and zinc protoporphyrin measured by hematofluorometry (14).

Statistics

The effects of ascorbic acid were evaluated for days 108–148, when ascorbic acid supplements were given without additional iron supplementation (Fig 1). A repeated measures analysis of variance (ANOVA) was used to test for differences attributable to time, ascorbic acid supplementation, or an interaction between time and ascorbic acid (15). Blood chemistry values were corrected for individual differences existing immediately before the ascorbic acid supplementation period by using a repeated measures analysis of covariance (ANCOVA). Pearson correlations were used to determine the relationship between serum ferritin and ^{59}Fe absorption (15).

Results

General response to protocol

As planned, the participants' body iron stores, as indicated by serum ferritin, were depleted by day 88 of the study (Fig 1). Very little recovery in serum ferritin was evident on the repletion diet, even during the final 26 d of iron supplementation (Fig 1). A general accounting of changes in body iron content during the study is provided in Fig 2. The participants were able to retain more iron from either diet than was excreted but had a net reduction in body iron during the depletion phase because of blood loss by phlebotomy (Fig 2). Menstrual iron

loss was a minor portion of the total iron loss (Fig 2). Although it appeared that there was a net positive accumulation of iron by the end of the study, this cannot be concluded with confidence because of the high variability associated with cumulative balance data. As discussed further below, the clinical laboratory data did not support the possibility (suggested in Fig 2) that the participants' body iron status at the end of the study was better than that at entry.

Follow-up measurements indicated that iron status was probably restored to at least those concentrations seen at admission by the iron supplements provided at the end of the study. Only two participants provided follow-up measurements of hemoglobin and hematocrit; these values were normal and essentially identical to their concentrations at admission. A third participant reported only a serum ferritin measurement. Her iron stores were appreciably improved, as indicated by serum ferritin values of 8.5 $\mu\text{g/L}$ at admission, 9.3 $\mu\text{g/L}$ at discharge, and 48 $\mu\text{g/L}$ 6 mo after discharge.

Effect of ascorbic acid on iron absorption, balance, and clinical values

Because of differences in energy intakes, persons treated with ascorbic acid consumed slightly more iron than those in the placebo group (16.3 vs 13.7 mg, respectively) (Table 2). Despite this difference the ascorbic acid group excreted no more total iron in the feces than did the placebo group, and they had significantly less fecal iron as a percentage of dietary iron (Table 2). Thus, apparent iron absorption was significantly improved by the ascorbic acid supplementation (38% vs 27%) (Table 2). The effect of ascorbic acid on apparent absorption (defined as dietary minus fecal iron) was evident by the second 6-d balance period after the start of ascorbic acid supplementation (day 121) and persisted for the 5.5 wk of ascorbic acid supplementation until the equilibration was interrupted by iron supplementation (Fig 3).

Although balance measurements indicated that more iron was retained by persons fed supplemental ascorbic acid, this was not as clear from their blood chemistries. The ascorbic acid group had slightly better iron status than did the placebo group, as indicated by concentrations of hemoglobin (Fig 4), erythrocyte free protoporphyrin (not shown), zinc protoporphyrin

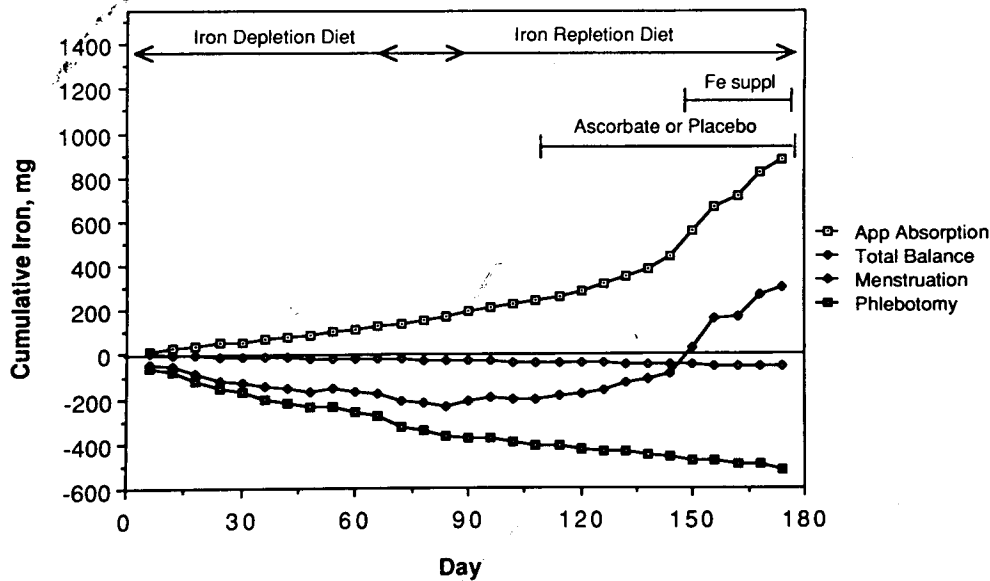


FIG 2. Components of cumulative iron balance. Average data for all participants ($n = 11$) are shown. Apparent absorption (App Absorption) is dietary iron minus fecal iron. Total Balance is dietary iron minus iron lost through fecal and urinary excretion, menstruation, and phlebotomy. Body surface losses are not included. Note that the cumulative balance data are characterized by a high degree of variability.

(Fig 5), and serum iron (Fig 6). However, hematocrit, serum ferritin, total-iron-binding capacity, and percent transferrin saturation were all unaffected by ascorbic acid.

Absorption of ^{59}Fe

Absorption of ^{59}Fe was not affected by ascorbic acid when the supplement was not given with the test meal. The declining iron status of the participants was evidenced by greater absorption of administered ^{59}Fe as the study continued. Absorption of ^{59}Fe administered on days 8, 31, 85, and 148 was $14 \pm 4\%$, ($\bar{x} \pm \text{SEM}$) $17 \pm 2\%$, $22 \pm 3\%$, and $24 \pm 3\%$, respectively. The

natural logarithm of serum ferritin was negatively correlated with ^{59}Fe absorption ($r = -0.33$, $p < 0.05$) when data for the entire study was used, indicating more efficient iron absorption when body iron stores were reduced.

Discussion

The enhancing effect of ascorbic acid on apparent iron absorption is generally consistent with the model of Monsen et al (6) for predicting iron absorption from diets. The percent of iron absorbed from the ^{59}Fe -labeled meals was greater than that predicted from the model ($\sim 23\%$ observed vs 5–10% predicted for women with depleted iron stores). Reasons for this difference may include the acute depletion by means of diet and phlebotomy, the very low iron content (0.8 mg) of the test meal, and the addition of ^{59}Fe to juice rather than to solid foods in the meal (16). It is more difficult to compare the apparent absorption (balance method) results in this context because fecal iron includes both unabsorbed dietary iron and endogenous iron excreted through the gastrointestinal tract. However, iron excretion is thought to be relatively constant and unaffected by iron status (3). Therefore, the apparent absorption measurements of 38% and 27% for persons receiving ascorbic acid and placebo, respectively, probably represent an actual difference in absorption of 11% of the dietary iron. This is congruent with a predicted iron absorption of 20% for the ascorbic acid group and 5–10% for the placebo group (6).

The present study clearly demonstrates that ascorbic acid enhances iron absorption over a period of several weeks in women who have been iron depleted. This response was evident from the balance data before changes occurred in serum iron status indicators. Participants treated with ascorbic acid retained an average of 2.3 mg additional iron daily from a diet containing ~ 15 mg Fe (Table 2). Over the 40-d period of ascor-

TABLE 2
Effect of ascorbic acid on iron balance*

	Ascorbic acid group	Placebo group	<i>p</i>
Dietary iron (mg/d)†	16.3 ± 0.12	13.7 ± 0.1	NS
Fecal iron (mg/d)	10.0 ± 0.3	9.7 ± 0.4	NS
Urinary iron (mg/d)	0.048 ± 0.004	0.017 ± 0.005	NS
Iron balance (mg/d)‡	6.3 ± 0.3	3.9 ± 0.4	0.06
Apparent absorption (mg/d)§	6.3 ± 0.3	4.0 ± 0.4	0.06
Dietary iron in feces (%)	62 ± 2	73 ± 3	0.05
Apparent absorption (%)	38 ± 2	27 ± 2	0.05

* $\bar{x} \pm \text{SEM}$.

† The analyzed dietary iron content was 13.7 mg/2000 kcal. Variation from this value reflects individual differences in energy required for weight maintenance. The ascorbic acid group, with body weights of 69 ± 17 kg, required 2400 ± 400 kcal whereas the placebo group, with body weights of 65 ± 18 kg, required 2000 ± 400 kcal.

‡ Iron balance = dietary iron - fecal iron - urinary iron.

§ Apparent absorption = dietary iron - fecal iron.

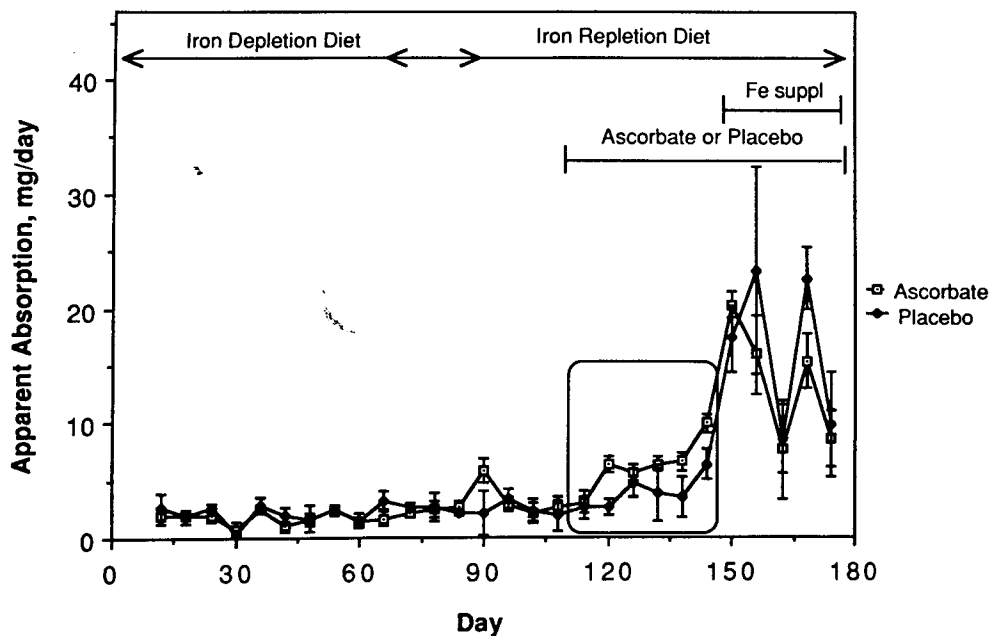


FIG 3. Effect of ascorbic acid on apparent iron absorption. The mean \pm SEM is shown for the seven participants supplemented with ascorbic acid and the four participants fed a placebo during the time periods indicated. The box indicates the data examined by repeated measures of analysis of variance, to test for the effect of ascorbic acid. An artifactual difference occurred at day 90 because of the change to the iron-repletion diet.

bic acid supplementation (without additional iron supplementation), this difference apparently resulted in an average of 92 mg of additional body iron for each person receiving ascorbic acid. If this additional iron was added to body iron storage pools, serum ferritin values for the ascorbic acid-supplemented group would be at least 10 μ g/L greater than those in the placebo group, when using the general guideline that 120 μ g stor-

age Fe/kg corresponds to 1 μ g serum ferritin/L (3). This did not occur (Fig 1), probably because the additional iron retained by the ascorbic acid group was used to replenish hemoglobin and serum iron pools, rather than to replenish storage iron pools associated with serum ferritin. This explains why hemoglobin, serum iron, and erythrocyte free and zinc protoporphyrin responded to the enhanced iron retention associated with ascor-

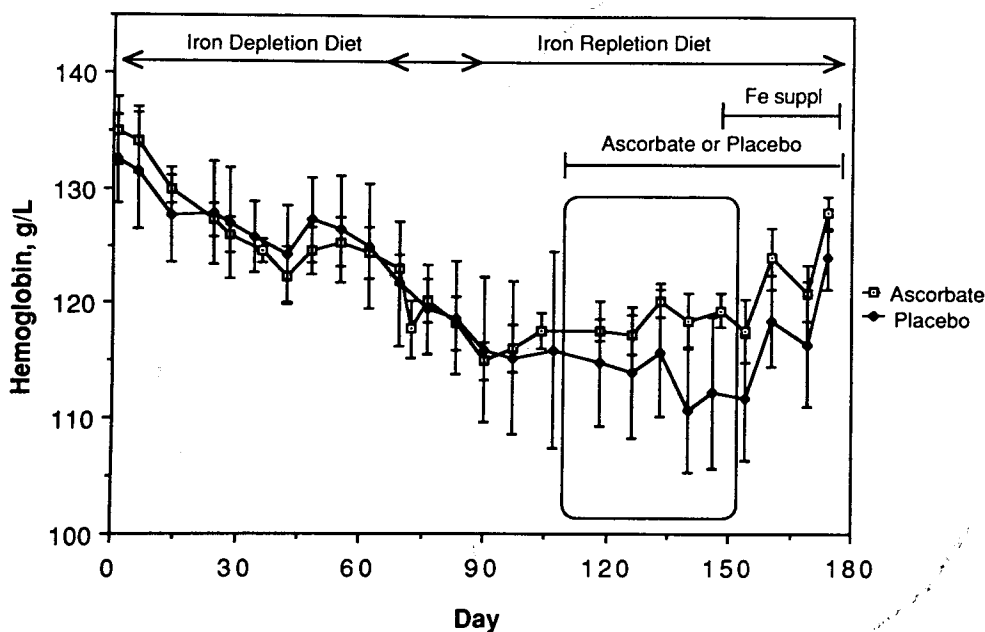


FIG 4. Effect of ascorbic acid on hemoglobin. See explanation for Figure 1. Ascorbic acid affected hemoglobin significantly ($p < 0.05$).

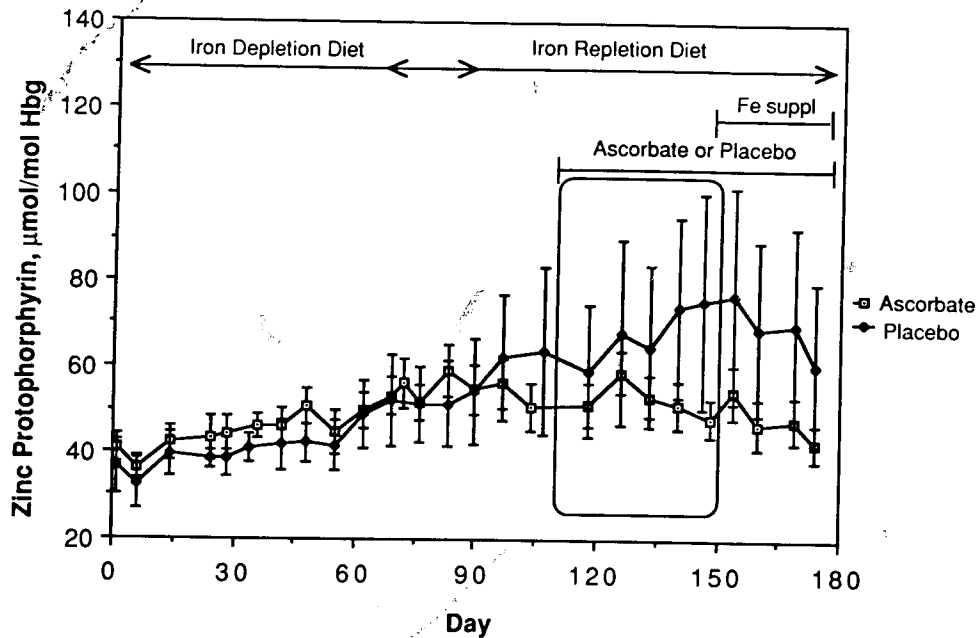


FIG 5. Effect of ascorbic acid on erythrocyte zinc protoporphyrin. See explanation for Figure 1. Ascorbic acid affected erythrocyte zinc protoporphyrin significantly ($p < 0.05$).

bic acid supplementation while other biochemical indicators did not respond within this limited time period.

In the studies by Cook et al (4) and Malone et al (5), persons supplemented with ascorbic acid for several weeks did not respond with improved serum ferritin values despite the demonstration that ascorbic acid enhanced iron absorption from single meals both at the beginning and end of the former experiment (4). These studies raised questions about the applicability

of results with single meals to situations that attempt to enhance iron availability for a longer period. The purpose of the present study was to determine if ascorbic acid would enhance iron absorption over a period of several weeks in women who were iron depleted. The best indicator of the enhancement of iron absorption by ascorbic acid in the present study was iron balance. This method was not used in the previous studies (4, 5), so it is not known whether iron balance would be improved

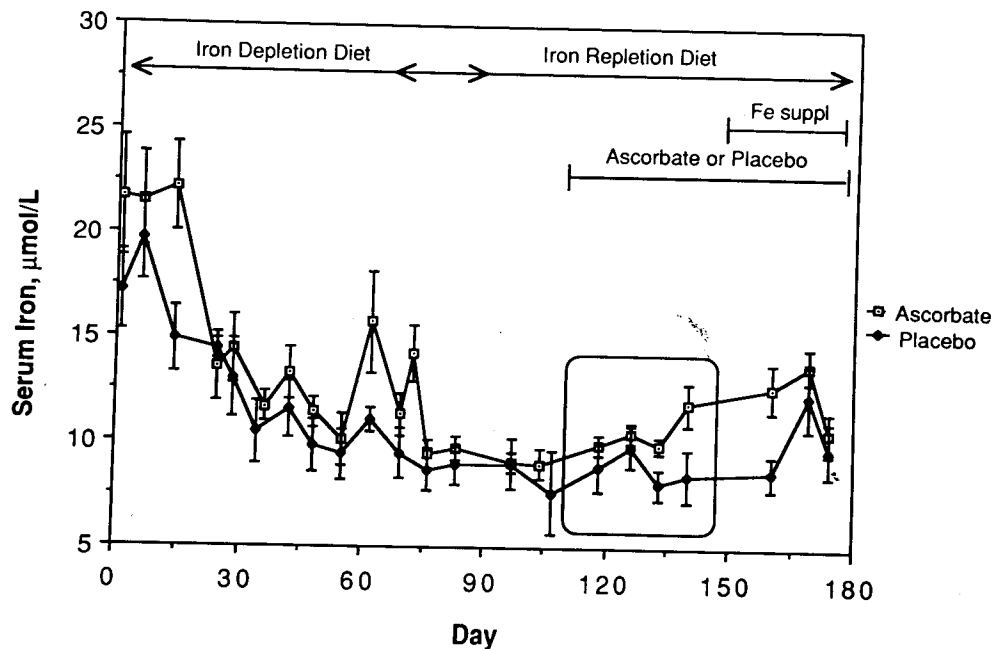


FIG 6. Effect of ascorbic acid on serum iron. See explanation for Figure 1. Ascorbic acid treatment affected serum iron marginally ($p < 0.06$).

by supplementing the usual diets of nondepleted adults with ascorbic acid. We are not aware of any published reports of the effect of ascorbic acid supplementation on iron balance in persons with normal iron status.

One would conclude from observing serum ferritin concentrations that ascorbic acid supplements did not improve iron status in the present study even though the iron balance results indicate otherwise. Serum ferritin may not be a good indicator of changing iron status under these conditions. Jacobs et al (17) reported gradual recoveries of hemoglobin and transferrin saturation but little recovery of serum ferritin over several months in three men who had blood removed by repeated phlebotomy. This finding is in contrast to the 6-mo follow-up serum ferritin reported (through a private physician) by one of the participants in the present study. The lack of a response in ferritin values to ascorbic acid during the present study can be explained by a need for iron in erythrocyte hemoglobin formation (Figs 4 and 5). However, this does not explain the results of Cook et al (4) or Malone et al (5) because they studied persons with normal iron status (about one-fourth of Cook's participants and one-third of Malone's participants had serum ferritin values $< 12 \mu\text{g/L}$). It is impossible to say whether the lack of effect of ascorbic acid on serum ferritin in previous reports (4, 5) was because of a relative insensitivity of serum ferritin to changing iron status or because there was no actual change in iron status. Because the participants in previous studies may have already been consuming diets that promoted nonheme iron absorption, minimizing any further effect of supplemental ascorbic acid, there may have been no real change in iron status. Further research employing the balance method would clarify whether ascorbic acid supplementation will improve ongoing iron retention of persons with low to normal iron status who consume diets with either low or average amounts of meat and ascorbic acid.

The results of this study suggest that iron-depleted women are capable of absorbing as much as 25% of the iron from a diet with iron principally in a nonheme form and with low amounts of ascorbic acid and meat (substances known to enhance iron absorption). In addition, the supplementation of such a diet with ascorbic acid further enhances the body's retention of iron on an ongoing basis, over a period of ≥ 5.5 wk. ■

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