

# The Effect of High Ascorbic Acid Supplementation on Body Iron Stores

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The level of assimilation of dietary iron is believed to have an important influence on iron status. To examine the effect of enhancing the availability of dietary iron on iron balance, 17 adult volunteer subjects were given 2 g of ascorbic acid daily with meals for 16 weeks. Serum ferritin levels before and after the study averaged 46 and 43  $\mu\text{g/L}$ , respectively, indicating a negligible effect on iron stores. When vitamin C supplementation was continued for an additional 20 months in five iron-replete and four iron-deficient subjects, serum ferritin determinations again

failed to indicate any significant effect of the vitamin C on iron reserves. These findings were not explained by intestinal adaptation to the enhancing effect of the vitamin, because radioisotopic measurements of nonheme iron absorption showed no reduction in the enhancing effect of 1 g of ascorbic acid after four months of megadoses of vitamin C. It is concluded that altering the availability of nonheme dietary iron has little effect on iron status when the diet contains substantial amounts of meat.

**P**OOR ASSIMILATION of dietary iron is believed to play a major role in the pathogenesis of nutritional iron deficiency. Because heme iron is well absorbed by the intestinal mucosal cells, the most important dietary variable is the intake of nondairy animal foods, including fish and poultry.<sup>1,2</sup> The bulk of dietary iron, however, is in the nonheme form and, in contrast to heme iron, the nature of the meal has a profound effect on iron absorption by the nonheme pathway. In recent years, the application of the extrinsic radioiron tag method<sup>3,4</sup> has provided a much clearer picture of the dietary factors affecting the absorption of nonheme iron.<sup>5-7</sup> The two key enhancers are ascorbic acid and animal tissue, items that are the basis of a model for predicting iron assimilation from different meals.<sup>8</sup> A growing number of dietary iron inhibitors have also been identified, including tea, coffee, bran, egg yolk, calcium phosphate, and EDTA. Because absorption of nonheme iron may vary up to tenfold depending upon the content of enhancing and inhibitory factors,<sup>9</sup> these factors are assumed to play a significant role in iron balance. To date, however, no direct relationship has been demonstrated between the availability of dietary nonheme iron and iron status.

In the present study, the effect of inducing chronic changes in food iron availability on iron stores was assessed in normal subjects whose iron status covered a wide range. Because of the profound effect of vitamin C on the absorption of food iron, heavy supplementation with the vitamin was selected as the means for altering the availability of dietary iron. The effect of ascorbic acid supplementation on iron balance is of interest in developing countries, where its use has been discussed as a means of reducing the prevalence of iron deficiency. The effect of vitamin C on iron balance is also of interest in developed countries such as the United States, where large quantities have been advocated as a means of reducing the frequency or severity of the common cold.<sup>10</sup> If megadoses of vitamin C have

a significant influence on iron balance, progressive accumulation of body iron may occur, particularly in individuals who are genetically susceptible to iron overload. In the present study, iron status was measured serially in normal volunteer subjects placed on 2 g vitamin C daily for periods ranging from four to 24 months.

## MATERIALS AND METHODS

### *Volunteer Subjects*

Seventeen healthy college students between 20 and 30 years of age participated in the study. None of the subjects gave a history of disorders that might influence iron balance or gastrointestinal absorption of iron. The subjects were selected on the basis of preliminary laboratory screening to encompass a broad range of iron status. Of the eight women in the study, three were iron deficient as defined by a transferrin saturation below 15% and a serum ferritin level less than 15  $\mu\text{g/L}$  although only one was anemic (subject W.W., Table 1). Iron deficiency was also present in one male subject who had frequently donated blood before entering the study. The subjects considered their diet to be well balanced and to contain generous quantities of meat and poultry. All subjects gave written informed consent before their participation in the study, and the procedures followed were in accordance with the ethical standards of the Human Subjects Committee of the University of Kansas Medical Center.

### *High-Dose Vitamin C*

The subjects were instructed to take two 500-mg tablets of vitamin C with each of the two largest meals of the day. Seventeen participants took these levels of vitamin C for 16 weeks and nine of them continued in the study for a total of 24 months. No subjective

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Table 1. Baseline Hematologic and Iron Status Measurements

Subject	Sex	Hct (%)	Serum Iron ( $\mu\text{g}/100\text{ mL}$ )	TIBC ( $\mu\text{g}/100\text{ mL}$ )	Transferrin Saturation (%)	Serum Ferritin ( $\mu\text{g}/\text{L}$ )	
						week 0	week 16
1. F.M.*	M	42	187	339	55	256	168
2. J.W.	M	41	126	334	38	189	206
3. W.P.*	M	47	58	306	19	199	46
4. L.W.	F	41	121	316	38	45	18
5. T.B.*	F	37	183	316	58	197	76
6. L.L.	F	40	35	363	10	36	35
7. S.W.	M	42	81	357	23	41	45
8. K.S.	M	40	78	290	27	128	138
9. J.F.*	M	44	108	300	36	109	100
10. R.M.	M	46	99	338	29	146	191
11. B.G.*	M	45	64	261	25	97	110
12. D.B.	F	46	104	296	35	113	100
13. K.C.*	F	37	71	523	14	4	8
14. W.W.*	F	35	27	355	8	3	5
15. C.C.	F	38	31	403	8	4	6
16. C.H.*	F	41	72	333	22	18	16
17. J.B.*	M	40	36	327	11	9	22
Mean		41.3	87	339	32.7	46.3	43.9

\*Long-term study.

side effects of high dose vitamin C were reported. None of the subjects had taken large amounts of ascorbic acid previously, although many participated in the study because they believed that megadoses of vitamin C might be beneficial from a health standpoint and because the ascorbic acid was supplied to them at no cost. The subjects returned to the laboratory every four weeks to have blood samples drawn for determination of serum ferritin levels and to obtain the next month's supply of the vitamin. Compliance was evaluated by interviewing the subjects upon their return to the laboratory each month and again several months after completion of the study. These evaluations indicated that the subjects took at least 90% of the prescribed vitamin supplement.

### Iron Status Measurements

Serum ferritin determinations were used as the basis for monitoring changes in iron status.<sup>11</sup> Two-milliliter venous blood samples were drawn at monthly intervals, and the harvested serum was stored at  $-20^{\circ}\text{C}$  until the conclusion of the study. Measurements were performed with a two-site immunoradiometric assay as originally described by Miles et al.<sup>12</sup> To eliminate interassay variability, all samples from any one subject were measured in the same assay run. Serum iron measurements were performed by the method of the International Committee for Standardization in Hematology (ICSH),<sup>13</sup> and total iron-binding capacity (TIBC) was determined with magnesium carbonate.<sup>14</sup>

### Iron Absorption Measurements

The enhancing effect of 1 g ascorbic acid on nonheme iron absorption was measured before and after 16 weeks of high dose ascorbic acid using double radioiron tags. Four separate tests were performed, and the meal used in each test consisted of dextrimaltose, corn oil, and ovalbumin in amounts sufficient to provide 68 g carbohydrate, 35 g fat, 29 g protein, and 690 kcal.<sup>15</sup> Dibasic calcium phosphate and potassium phosphate were added to adjust the calcium and phosphorus content to 202 and 414 mg, respectively. The total iron content was brought to 4.1 mg by adding labeled ferric chloride immediately before administration. This meal has been used extensively in prior studies of food iron availability including an

investigation of the effect of ascorbic acid on nonheme iron absorption.<sup>10,16-20</sup> The meal was tagged extrinsically<sup>4</sup> with either  $2\ \mu\text{Ci}\ ^{59}\text{Fe}$  or  $5\ \mu\text{Ci}\ ^{55}\text{Fe}$  in 1 mL 0.01N HCl.

On the first day of the paired absorption tests, the subjects, who had fasted overnight, came to the laboratory between 7:00 and 8:00 AM. Blood samples (20 mL) were drawn for measurement of hematocrit, serum iron, TIBC, and background blood radioactivity, and the first test meal was then administered without vitamin C. The next day the second test meal tagged with the alternate form of radioiron was given with 1 g ascorbic acid under identical conditions. Blood (25 mL) was obtained 14 days later to measure incorporated red cell radioactivity. All radioiron assays were performed on duplicate 10-mL samples of whole blood using a modification of the method of Eakins and Brown.<sup>21</sup> Sufficient counts were obtained on each sample to reduce the net counting error for each isotope to  $\pm 2\%$  or less in subjects absorbing more than 1% of the test dose. The percentage of absorption in each subject was calculated from a blood volume estimated from height and weight.<sup>22,23</sup> Red cell incorporation of absorbed radioactivity was assumed to be 80%.<sup>24</sup> Sixteen weeks after the subjects began taking megadoses of ascorbic acid, paired absorption measurements were repeated with the identical protocol; high dose vitamin C was discontinued 24 hours before the first test meal.

Because of the skewed distribution of iron absorption data when expressed as the percentage of absorption, statistical analysis was performed on logarithms and the results retransformed to recover the original units.<sup>25</sup> Significant differences between any pair of iron absorption values were examined by applying paired Student's *t* tests to the differences in log absorption. Differences in serum ferritin levels before and after megadoses of vitamin C were similarly evaluated.

## RESULTS

Serum ferritin levels before and after megadoses of vitamin C are listed together with baseline measurements of iron status in Table 1. The level fell in eight subjects and increased in the remaining nine. The

mean serum ferritin value before supplementation was 46  $\mu\text{g/L}$  and remained virtually unchanged after 16 weeks (mean, 44  $\mu\text{g/L}$ ). It is of interest that of the four subjects whose initial serum ferritin values were below 10  $\mu\text{g/L}$ , only one, the male volunteer, had an increase in the level to within the normal range.

In nine of the original participants, ascorbic acid supplementation was continued for a total of 24 months. Because preliminary analysis indicated that the effect of vitamin C depended on basal iron status, these subjects were considered as two groups according to their original serum ferritin values. The iron-replete group had basal serum ferritin levels ranging from 97 to 256  $\mu\text{g/L}$  (subjects 1, 3, 5, 9, 11; Table 1), while the remaining iron-deficient subjects had initial serum ferritin levels of 3, 4, 9, and 18  $\mu\text{g/L}$  (subjects 13,14, 16, 17).

In the normal subjects there was a slight initial decline in serum ferritin levels but no significant change during 24 months of vitamin C supplementation (Fig 1). In the iron-depleted group, there was a gradual elevation in serum ferritin, from a mean of 6  $\mu\text{g/L}$  at the outset of the study to 35  $\mu\text{g/L}$  after two years. The effect within this group, however, was not consistent (Fig 2). Serum ferritin levels in two of the female volunteers (CH, WW) remained in the iron-deficient range during the entire 24-month period. In the third woman, the serum ferritin level returned to normal at approximately four months but again fell below 20  $\mu\text{g/L}$  during the last few weeks of the study.

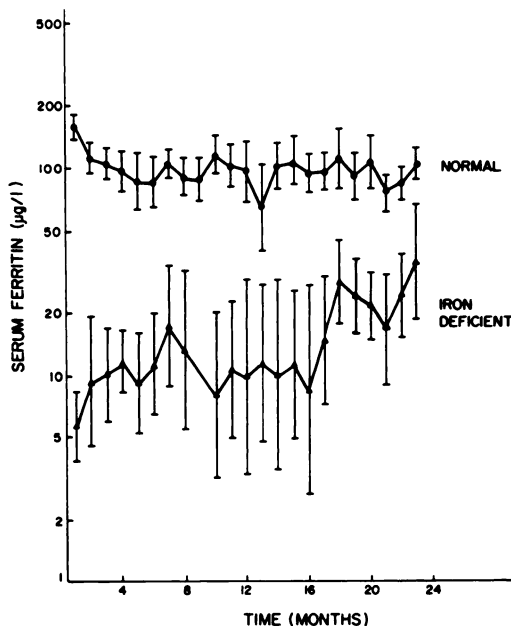


Fig 1. Serum ferritin levels during vitamin C supplementation in normal subjects grouped according to baseline iron status. The vertical bars depict  $\pm 1$  SE.

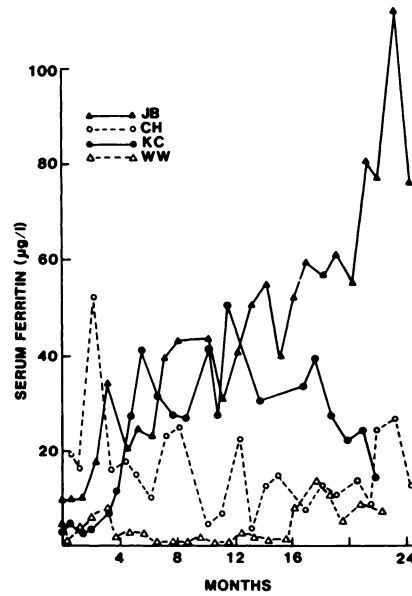


Fig 2. Serum ferritin levels during vitamin C supplementation in four subjects with baseline iron depletion.

The most dramatic change in serum ferritin occurred in the male subject whose ferritin level rose progressively to about 100  $\mu\text{g/L}$  at the end of the two years. This subject, however, had been phlebotomized frequently before the study, and it was not possible to determine whether his progressive increase in iron stores was related to ascorbic acid supplementation or to discontinuing his regular blood donations.

The effect of ascorbic acid on nonheme iron absorption before and after high dose vitamin C is shown in Table 2. As expected, there was a striking increase in mean absorption from 2.57% to 14.24% when the meal was taken with 1 g vitamin C; the absorption ratio with and without ascorbic acid averaged 5.54 ( $t = 10.4$ ,  $P < .001$ ). The effect of vitamin C on nonheme iron absorption was remarkably similar following 4 months of ascorbic acid supplementation. Mean absorption of 1.93% rose to 10.28% with ascorbic acid, giving an absorption ratio of 5.30 ( $t = 7.04$ ,  $P < .001$ ).

It is of interest that absorption from meals both with and without ascorbic acid decreased appreciably after four months of chronic ascorbic acid administration. There was a 25% decrease in absorption from the meal without vitamin C (2.57% to 1.93%), and a similar decrease of 28% from the meal with vitamin C (14.24% to 10.28%). In neither case, however, was the decline statistically significant ( $t = 0.99$ ,  $P = .17$  without ascorbic acid;  $t = 1.49$ ,  $P = .08$  with ascorbic acid).

DISCUSSION

The surprising finding in this study is the negligible effect vitamin C had on iron stores when large quanti-

Table 2. Effect of Chronic High Dose Vitamin C on Iron Absorption

Subject	Iron Absorption				Absorption Ratio			
	Week 0		Week 16		+/- Vit C		Week 16/Week 0	
	- Vit C (A)	+ Vit C (B)	- Vit C (C)	+ Vit C (D)	Week 0 (B/A)	Week 16 (D/C)	- Vit C (C/A)	+ Vit C (D/B)
1. F.M.*	0.25	2.50	0.62	2.50	10.00	4.03	2.48	1.00
2. J.W.	0.50	5.75	0.12	4.25	11.50	35.41	0.24	0.73
3. W.P.*	0.50	2.75	3.00	22.62	5.50	7.54	6.00	8.22
4. L.W.	0.87	14.62	2.00	25.37	16.80	12.68	2.29	1.73
5. T.B.*	1.00	7.12	6.62	5.37	7.12	0.81	6.62	0.75
6. L.L.	1.25	10.25	1.50	6.12	8.20	4.08	1.20	0.59
7. S.W.	1.37	15.37	0.37	5.37	11.21	14.51	0.27	0.34
8. K.S.	2.12	11.50	2.62	6.50	5.42	2.48	1.23	0.56
9. J.F.*	2.50	10.62	1.87	6.75	4.24	3.60	0.74	0.63
10. R.M.	2.75	5.25	0.62	3.62	1.90	5.83	0.22	0.68
11. B.G.*	3.25	14.62	2.50	4.00	4.49	1.60	0.76	0.27
12. D.B.	4.87	17.50	0.75	3.25	3.59	4.33	0.15	0.18
13. K.C.*	6.12	74.12	5.62	59.62	12.11	10.60	0.91	0.80
14. W.W.*	9.00	28.25	1.75	36.50	3.13	20.85	0.19	1.29
15. C.C.	12.87	77.12	14.25	29.87	5.99	2.09	1.10	0.38
16. C.H.*	17.25	29.50	7.50	55.87	1.71	7.44	0.43	1.89
17. J.B.*	35.87	81.25	7.87	19.87	2.26	2.92	0.21	0.24
Mean	2.57	14.24	1.93	10.28	5.54	5.30	0.75	0.72
- 1 SE	1.84	11.02	1.43	7.94	4.70	4.18	0.57	0.58
+ 1 SE	3.58	18.41	2.62	13.31	6.53	6.72	1.00	0.90

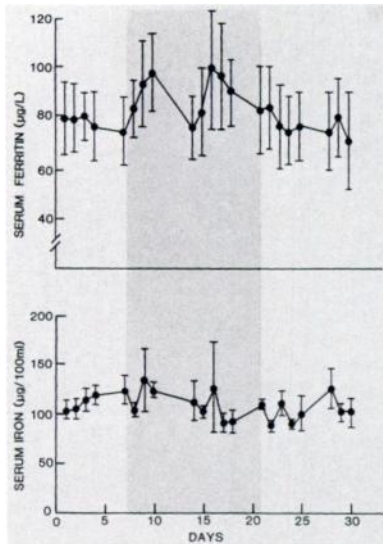
\*Long-term study.

ties of ascorbic acid were included in the diet for up to two years. Nonheme iron absorption from a test meal containing a representative dietary iron content of 4.1 mg increased more than fivefold when given with 1,000 mg ascorbic acid both before and after several months of heavy supplementation. If this fivefold enhancement was maintained for 24 months in a normal adult male with basal absorption of 1 mg iron daily, one would predict an increase in body iron stores of more than 3 g and a parallel increase in serum ferritin of at least 300  $\mu\text{g/L}$ . This estimate must be modified downward, however, for several reasons. It should be reduced by one third because the participants in this study ingested vitamin C with only two meals each day. Moreover, it should be applied only to nonheme dietary iron. While heme iron averages only 5% to 10% of dietary iron intake, it accounts for nearly 30% to 40% of the iron absorbed from the diet because of the high assimilation of heme iron by the mucosal cells.<sup>1</sup> Since ascorbic acid has no effect on the absorption of heme iron (S.R. Lynch, et al, unpublished observations), the predicted increase in stored iron over 24 months should be 1,200 mg or a rise in serum ferritin of at least 100  $\mu\text{g/L}$ , which would have been readily detected.

Another factor that must be considered in these calculations is that the presence of meat greatly diminishes the facilitating effect of vitamin C on nonheme iron absorption. We previously reported nonheme iron absorption ratios with and without ascorbic acid of 4.2

and 3.8 when 100 mg was added to a semipurified meal compared with only 1.7 for a meal containing meat.<sup>10</sup> At the 1,000-mg ascorbic acid level, an absorption ratio of 9.6 without meat was similarly reduced to 3.0 and to 2.3 with a hamburger meal. More recently, we have observed that meat not only diminishes the enhancing effect of ascorbic acid but also flattens the dose-response curve between 100 and 1,000 mg (S.A. Dassenko, unpublished observations). Nevertheless, 1,000 mg ascorbic acid should still enhance the assimilation of nonheme iron from a meal containing meat by 200% to 300%. If this effect is exerted on two thirds of the nonheme iron absorbed by a normal male each day (0.6 mg), a net positive iron balance of at least 0.5 mg daily should occur, or an accumulated total over 2 years of 300 mg iron. Some iron loss occurred during this study as a result of blood sampling for iron absorption and serum ferritin determinations, but this did not exceed 150 mL blood over two years, or 0.1 mg iron daily.

It is unlikely that a true increase in stored iron was obscured in this study by methodologic limitations related to serum ferritin measurements. To enable all assays to be performed at the conclusion of the study, the sera obtained at monthly intervals was stored for periods ranging up to two years. However, loss of immunoreactive ferritin in stored samples would produce an apparent rise in levels and an overestimate of the change in body iron reserves. Moreover, recent



**Fig 3.** Serum ferritin and serum iron levels in two male and two female subjects given 500 mg vitamin C three times a day for two weeks (shaded area). The vertical bars depict  $\pm 1$  SE.

studies by the Iron Panel of the ICSH have shown that the rate of degradation of ferritin in specimens stored at  $-20^{\circ}\text{C}$  is less than 0.3% per year (Worwood et al, unpublished observations). It is doubtful that an increase in stored iron was masked by inherent variability in serum ferritin levels because correlation coefficients between serum ferritin and iron absorption, which are independent measures of iron status,<sup>11</sup> were significant at the 1% level both before and after 16 weeks of megadoses of vitamin C.

A final possibility is that the megadoses of vitamin C induced some redistribution of body iron stores that masked an actual increase in total body iron. In patients with iron overload and secondary ascorbic acid deficiency, parenteral ascorbic acid promotes iron release from the reticuloendothelial cell,<sup>26,27</sup> which is the immediate precursor compartment for circulating ferritin. There was a tendency for serum ferritin levels to fall in iron-replete subjects during the early part of the study (Fig 3), which is consistent with some redistribution from reticuloendothelial stores. However, our subjects were neither iron loaded nor ascorbic acid depleted. Furthermore, we have been unable to demonstrate any acute effects on serum iron or serum ferritin levels when normal subjects are given 500 mg ascorbic acid three times daily for 14 days (Fig 3). A final point is that some initial redistribution of body

iron stores would not obscure a long-term effect on serum ferritin levels.

The present findings are consistent with the established view that body iron is maintained within relatively narrow limits in normal individuals despite wide variations in the type and nature of dietary iron.<sup>5</sup> The mechanism of this regulation in the face of sustained enhancement in iron absorption by ascorbic acid is unknown, but there are several possibilities. The enhancing effect on nonheme iron absorption could have been offset at the level of the mucosal cells if there was an associated reciprocal inhibition in absorption of heme iron. We have recently observed a compensatory rise in heme iron absorption when a potent inhibitor of nonheme iron, such as soy protein, is added to a meal, but we have not been able to demonstrate an inhibiting effect of vitamin C on heme iron absorption (S.R. Lynch, et al, unpublished observations). Another possible explanation is that the enhancing effect of vitamin C is associated with a compensatory decline in mucosal uptake or transport of iron ingested during the day separate from vitamin C ingestion. This type of mucosal regulation is in keeping with the 25% reduction in absorption of nonheme iron that occurred after 16 weeks of vitamin C supplementation. It is also possible that vitamin C enhances iron loss by the gastrointestinal mucosa. Nevertheless, it is still difficult to explain the failure of ascorbic acid supplementation to normalize iron stores in the two subjects who were initially iron depleted.

One reason this study was undertaken was because of concern that massive doses of vitamin C might lead to progressive iron accumulation in iron-replete individuals. Our findings indicate that the regulatory mechanisms that control body iron reserves override any pronounced alterations in food iron availability. They do not, however, exclude the possibility that iron balance is affected in individuals who are genetically susceptible to iron overload. Although hereditary hemochromatosis was once believed to be a rare disorder, recent evidence indicates a frequency of the hemochromatosis gene of nearly 0.07 in certain geographic regions<sup>28</sup> corresponding to a frequency for the heterozygous state of 8% to 10% and for the homozygous state of 3/1,000 of the population.<sup>29</sup> Our present observations do not exclude the possibility that such individuals may be adversely affected by chronic administration of large doses of vitamin C, a point that deserves further study.

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