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ABSORPTION OF REDUCED IRON OF SMALL PARTICLE SIZE FROM A  
COMMERCIAL SOURCE

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ABSTRACT

The absorption of a commercial brand of small-particle reduced iron was evaluated in 10  
normal subjects. The procedure used was measuring in the same subject the radiolabeled

"true iron absorption" of 60 mg of iron as either ferrous sulfate or ferric ammonium citrate by the hemoglobin incorporation method, as well as by the iron tolerance test (ITT). This consisted of measuring the area under the curve of plasma iron elevations at specified times for 6 hours, or the peak plasma iron, corrected by the plasma iron disappearance rate obtained from measuring plasma iron at specified times for 4 hours after the slow intravenous injection of 0.4 mg of iron as ferric citrate. Only the ITT was used to measure the absorption of 60 mg of reduced iron. Reference dose iron ascorbate absorption was measured in each subject. The absorption of ferric ammonium citrate and reduced iron was expressed as percent of dose and also as absorption percent of that of ferrous sulfate. Mean % geometric "true absorptions" were 39.0 for reference dose, 10.4 for FeSO<sub>4</sub> and 2.4 for ferric ammonium citrate. The later was 23 % that of FeSO<sub>4</sub>. By ITT the mean geometric % absorptions were 7.9, 3.7 and 3.2 for FeSO<sub>4</sub>, ferric ammonium citrate and reduced iron respectively, or 47 and 41% of that of FeSO<sub>4</sub>. Based on these results we propose that the true absorption of the commercial brand of reduced iron tested was 20 %that of FeSO<sub>4</sub>. The use of this method for measuring absorption of unlabeled iron compounds is discussed.

## INTRODUCTION

Food fortification with iron has increased significantly in the last three decades. The utilization of reduced iron for that purpose in the United States has increased from 72 tons in 1970 to 10,124 tons in 1987. Reduced iron constituted 13.2% and 96.7% of the total amount of iron used in fortification, respectively (1). The use of reduced iron as a fortificant of cereals has continued to increase but the total amounts are not available

(personal communication P. Whitiker and L. A. Mejia, 2000). The absorption of fortification iron is of special interest in order to determine what can be expected in terms of iron nutrition in populations consuming different amounts of iron fortified products using various compounds.

A variety of iron products are used for food fortification purposes, including ferrous sulfate, ferrous lactate, ferrous gluconate, ferric ammonium citrate, ferrous fumarate, ferrous succinate, ferric saccharate, ferric orthophosphate, ferric ammonium orthophosphate, ferric pyrophosphate, EDTA iron, hemoglobin, and elemental iron (electrolytic and carbonyl iron) but in amounts much smaller than that of reduced iron (2)

On the other hand, there is the concept of preventive supplementation of vulnerable groups, and inexpensive and well-tolerated sources of iron could be used for this purpose. Viteri, Ali and Tujague (3) have demonstrated that iron reserves can be progressively increased in childbearing-age women by the administration of 30 weekly iron doses of 60 mg as ferrous sulfate consumed in an interval of 7 months (a total of 30 doses). Initially, 16% had low hemoglobin levels but by three months of weekly supplementation no woman had hemoglobin (Hb) levels below 125 g/L. The general idea of preventive supplementation is the safe, long-term intake of a weekly iron-folate (and possibly vitamin A and zinc) supplement by vulnerable groups who can not benefit sufficiently from adequate diets, fortified or not (4)

Studies on the absorption of reduced iron have rendered very dissimilar results in relation to the absorption of ferrous sulfate as a reference. These range from 13 up to 148%. The variability in the absorption of different reduced iron products and of other

iron compounds was shown by Callender over 30 years ago (5, 6). The variability of results are related to the particle size, surface area, and porosity of reduced iron which depends on the manufacturing process (7).

The absorption of commercial reduced iron has been difficult because of the inexistence of commercial reduced iron in an isotopic form. Bjorn-Rasmussen and Hallberg (7) as well as Roe and Fairweather-Tait (8) have produced labeled reduced iron in their laboratories trying to mimic the commercial iron sources. The first authors, however, were clear in pointing out the difficulties in predicting the behavior of commercial reduced iron produced in a micro-scale in the laboratory. Moreover, the majority of the studies on reduced iron absorption have been made adding this iron to different foods making it difficult to establish its true availability in relation to that of ferrous sulfate (please see table 7).

The purpose of the present study was to compare the absorption of an unlabeled commercial source of reduced iron administered alone (without food) at doses compatible with supplementation (60 mg) with those of two other sources of iron whose absorption rates are known: ferrous sulfate and ferric ammonium citrate. Grebe et al. (9) have shown that ferric ammonium citrate is absorbed 22% in relation to ferrous sulfate when both were administered without food. We used the "iron tolerance test" (ITT) to compare the iron absorption from the three compounds and the double radioisotopic absorption of ferrous sulfate and ferric ammonium citrate by hemoglobin incorporation of radiolabeled iron. The absorption of a radiolabeled reference dose of iron ascorbate was also measured in order to adjust the absorptions of the compounds as is customary (10). For

comparison purposes, the absorption of ferrous sulfate by either method was considered 100% and that of the other compounds as a proportion of this absorption.

The source of the commercial reduced iron was a reputable company from which we purchased it without the company being aware of the purpose of such purchase to insure the representativeness of the product.

The ITT measured by integrating the area under the serum iron elevation after an oral dose of the compound, unadjusted or adjusted by the iron disappearance constant after an intravenous iron dose, has been used to measure iron absorption before the development of isotopic methods to determine the "true absorption" or "retention of iron". The true absorption has been measured by the incorporation of radioisotopic iron to circulating hemoglobin; retention has been measured by whole body counting (11). Many of the results from the ITT are considered imprecise given that the individual values obtained correlate poorly with the other two methods (12). However, the mean and ranges of absorption in groups of subjects obtained by the ITT are acceptable when compared to the other methods if adjustments for disappearance rates are used and the absorptions are reported as percent of the ferrous sulfate standard (11, 13-15).

On the other hand, Heinrich (13) and Heinrich and Fischer (14) validated the results of the tolerance test by comparing them to those of whole body counting as a reference method. They, as well as Ekenved (11) were able to obtain correlation coefficients in the order of 0.88 between whole body radioactivity and integrated areas under the serum iron curve or with the peak increments, adjusted or not by disappearance rates, when different iron doses were given to the same subjects.

The utilization of both the ITT and the radioiron incorporation into Hb of a high and a low bioavailability iron compounds (ferrous sulfate and ferric ammonium citrate, respectively) and the ITT for estimating the absorption of non-radiolabeled reduced iron in relation to the previous compounds, constitutes a new approach to estimating the "true absorption" of reduced iron.

## MATERIALS AND METHODS

Healthy adult volunteers between the ages of 18 and 65 years who responded to a public call in the Berkeley, California community were invited to participate in the study. All the procedures from recruiting to the performance of the study were approved by the Committee of the Protection of Human Subjects (CPHS) as well as by the Radiation Safety Committee of the University of California at Berkeley.

Exclusion criteria included pregnancy or the possibility of becoming pregnant within two years, breast feeding, metrorrhagia, gastrointestinal symptoms, suspicions of intestinal malabsorption, chronic inflammatory conditions, current or previous use of therapeutic iron in the last six months, recent blood donation and probability of not being able to finish the full study.

Twenty volunteers entered the study and only ten completed it. The volunteers arrived at the laboratory for screening procedures, signature of informed consent and for scheduling of activities to be undertaken at the penthouse of Morgan Hall at the university campus. Their health status was evaluated and for menstruating women the characteristics of their cycles and birth control methods. They were instructed to fill out a diet history questionnaire and to maintain their usual diet during the whole study period, not to take any supplements except those administered by us and to keep a record of foods consumed the day prior to each test.

The absorption of reduced iron was estimated by the ITT. This test consisted of measuring the area under the plasma iron curve obtained by repeated venous blood sampling for 6 hours after the oral ingestion of 60 mg of reduced iron in a gelatin capsule

accompanied by 125 ml of water. The procedure was performed after an overnight fast. The area under the plasma iron curve was adjusted by the serum iron disappearance rate obtained by the slow intravenous administration of 0.4 mg of iron citrate in a peripheral vein of one arm. Repeated blood samples were obtained from a catheter inserted in a peripheral vein in the other arm. The identical ITT procedure was also performed for ferrous sulfate and ferric ammonium citrate. However, these last two compounds were also labeled with either  $^{55}\text{Fe}$  or  $^{59}\text{Fe}$ . Iron absorption from these two compounds was also measured by their radioactive incorporation into hemoglobin two weeks later. The absorption of a reference dose of 3 mg of iron ascorbate (15 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  + 25 mg Ascorbic acid) administered as a solution in 250 ml of orange juice, was also measured by its incorporation into hemoglobin. In essence, all subjects underwent the 5 following procedures: an intravenous administration of iron citrate; the oral ingestion of the three compounds being tested (ferrous sulfate, ferric ammonium citrate, and commercial reduced iron), and the oral ingestion of the reference iron ascorbate dose. The sequence of these five events was randomly assigned. In half the subjects, three of these events took place at the start of the study and the other two followed two weeks later, after blood was obtained to measure radiolabeled iron incorporation into Hb. In the other half two events took place initially and the other three two weeks later. Each event was separated from the previous one by two days. When two radioactively labeled compounds were administered in sequence, one was labeled with  $^{55}\text{Fe}$  and the other with  $^{59}\text{Fe}$ . Only reduced iron was unlabeled (Table 1). The radioactivity doses administered were 0.3-0.4  $\mu\text{Ci}$  (11.1 - 14.8 mBq) intravenously and 1.0  $\mu\text{Ci}$  (37 mBq) of  $^{59}\text{Fe}$  orally, or 0.7  $\mu\text{Ci}$  (25.9mBq) intravenously and 2.0  $\mu\text{Ci}$  (74 mBq) orally of  $^{55}\text{Fe}$ . Serial 5 ml blood

samples were obtained 10, 15, 30, 45, 60, 90, 120 and 240 minutes after the end of the intravenous infusion, and at 0, 30, 60, 90, 120, 180, 240, 300 and 360 minutes after the oral ingestion of iron.

The blood sampling catheter was kept open between samples by the infusion of 1 ml of saline with 5 u of heparin/100 ml through a 3-way valve. The contents of the valve and the catheter were aspirated before blood sampling for each point in the ITT until about 0.5 ml of blood was recovered. This aspirate was discarded and the 5 ml study sample was subsequently obtained.

A total of 5 doses of 60 mg of ferrous sulfate were also administered to the subjects in order to replace the iron drawn in the five sessions where  $\approx$  55 ml of blood were drawn. Single doses were to be ingested at home by the subjects at the end of the test day, that is at least two days prior to any test.

The following biochemical determinations were performed in each basal sample (time 0): blood hemoglobin, serum iron, total iron binding capacity (TIBC), and plasma ferritin. Percent saturation of TIBC was calculated. Blood hemoglobin was also measured in every sample in order to correct for possible dilution by adjusting all of the values to the basal hemoglobin level.

Blood hemoglobin was measured by the HemoCue System (HemoCue Inc., Angelholm, Sweden). Plasma ferritin was measured by the Spectro Ferritin MT Kits (Ramco Laboratories, Houston, Texas) standardized against WHO standards. Plasma iron and TIBC were measured by the methods published by INACG (16). Iron absorption by incorporation into hemoglobin was measured by liquid scintillation counting (17).

The solubility of ferrous sulfate, ferric ammonium citrate, and reduced iron was measured as indicated by Bjorn-Rasmussen and Hallberg (7), and its particle size was measured in triplicate in a suspension of saline:glycerol mixture (60/40) using a Z1, single channel Coulter particle size counter.

Statistical analyses was done using SPSS (SPSS Inc., Chicago, Illinois). The test used was repeated measures ANOVA using iron compound, method and sequence as grouping factors and absorption as test factor and Tukey's follow-up test with a  $p < 0.05$  as a level of significance.

## RESULTS

The particle size determinations of reduced iron, counted in quintuplicate in a suspension medium consisting of 20 % glycerol in saline solution, showed that 0.56% of particles fell between 10 and 20  $\mu\text{m}$ , 2.62% fell between 5-10  $\mu\text{m}$ , and 96.80% fell below 5  $\mu\text{m}$ . The solubility of the compounds gave the following results: ferrous sulfate 100%, ferric ammonium citrate 91%, and reduced iron 21%. A sample of electrolytic iron from the same manufacturer yielded 56% solubility by the same method (7).

The characteristics of the subjects that completed the study are shown in Table 2 and those of the subjects that did not complete the study are presented in Table 3. Only one woman in the group that did not complete the study could be regarded as anemic (Hb <120 g/L) but 6 had low Hb (<125 >120 g/L) 4 among those who completed the study and 2 among those who did not.

All the subjects ate a varied diet including meat and fish which contributed, on average, 0.7 mg of heme iron/day. Few of the subjects studied consumed coffee or tea with their meals. Most drank bottled sodas and fruit flavored drinks with meals. The basic food components of their diet were rice, pizza, chicken, hamburger, eggs, bread, tortillas, pasta and a small variety of fruits and vegetables. Our semiquantitative estimates of total iron intake indicate an average of 11.1 mg/day among the women studied and of 14.6 mg/day among the men. The diets consumed by the subjects before each test day did not differ from their usual intake (data not shown). Five of the women studied were on oral anovulatory pills and the rest were sexually inactive. None reported having notoriously abundant periods.

Table 4 shows the results of plasma iron, TIBC, percent saturation of TIBC, and ferritin in all basal samples of the subjects who completed the study. All the mean values in the table are within the normal ranges. However, three subjects with low or borderline Hb values presented very low ferritin levels but except for case No. 7 the % saturation of TIBC was above 30. This subject also presented a serum iron level suggesting iron deficiency.

The plasma disappearance rate was  $-1.096\%/minute$  ( $SD= 0.064\%$ ,  $SE= 0.020\%$ ). As expected, the rates were faster in iron deficient than in iron sufficient subjects but given the few subjects studied the correlation between disappearance rates and iron status (Hb, plasma ferritin and % saturation of TIBC) did not reach statistical significance. The disappearance constants were similar to those reported in ferrokinetic studies in the literature (10).

Table 5 presents the results of basal plasma ferritin and the percent absorption of ferrous sulfate, ferric ammonium citrate and reference dose measured by the isotopic method. The geometric means of plasma ferritin and reference dose absorption are indicative of the marginal iron deficiency of the population studied. The absorption of ferric ammonium citrate was 23% of that from ferrous sulfate. The high negative correlation between plasma ferritin and reference dose absorption ( $r=-0.76$ , Figure 1) was what would be expected. However it is surprising that there was no negative correlation between plasma ferritin and ferrous sulfate absorption while it was present with ferric ammonium citrate ( $r=-0.45$ ). There was no significant correlation either between ferrous sulfate and ferric ammonium citrate absorption. Figure 1 also indicates that there is no

correlation between ln plasma ferritin and ln reference dose absorption when the former is higher than 3.33, corresponding to a plasma ferritin level of 28  $\mu\text{g/L}$  in this series.

Table 6 shows the percent absorption of reduced iron, ferrous sulfate, and ferric ammonium citrate estimated either by the area under the plasma iron curve or by peak elevation in the ITT. The results demonstrate the superiority of ferrous sulfate over ferric ammonium citrate in terms of iron absorption, as was the case for radioiron incorporation into Hb (Table 5); the similarity of mean absorption results by the radioiron incorporation and the ITT, and the almost identical results obtained by both methods of estimating absorption by the ITT (correlation coefficients between estimates by area under the plasma iron curve and peak iron values above basal range between 0.97 and 0.98).

## DISCUSSION

Four of the seven women studied presented hemoglobin levels lower than 125 g/L. Three of them had plasma ferritin values lower than 12  $\mu\text{g/L}$ . However, the percent saturation of TIBC was higher than 16% in all cases (mean 30.5%). Therefore, these three subjects were iron depleted based on the presence of low plasma ferritin values (10) and possibly were approximating an iron deficient status. It is evident that the average diet consumed by the students was poor in terms of iron intake

The curve that is drawn by iron plasma levels expresses three events: 1) the amount of iron absorbed at a given time during the initial phase of iron absorption (18); 2) the velocity of transfer of intestinal iron to the plasma pool and 3) its disappearance due to its capture by different tissues. Importantly, the % saturation of TIBC or the unbound TIBC do not influence the plasma iron increments per mg of iron absorbed (11). On the other hand, the absorption measured by radioisotopic iron incorporated into hemoglobin in 2 weeks time only reflects a final point which is the iron utilized in hemoglobin synthesis. In other words, the two methods used in this study measure different events of iron absorption and metabolism. In spite of this, the mean results of percent absorption of ferrous sulfate and ferric ammonium citrate do not differ by the method utilized. Similarly, the area under the curve and the peak values of plasma iron levels corrected by disappearance rates are almost equal. These results are somewhat unexpected given the differences in the events they represent. This fact explains why on an individual basis they do not correlate. The experience of other investigators is similar (11, 12, 15). Thus, the ITT is not a useful method to determine individual amounts of absorbed iron from a given compound. However it can be useful in comparing the

relative absorption of different iron compounds or different doses of the same iron source in the same individual as well as in a group of individuals (11, 15, 19).

The results obtained regarding the absorption of the reference dose, ferrous sulfate and ferric ammonium citrate are similar to those reported by other authors (9, 10, 11, 13, 14).

As has also been described in the literature, we found a negative correlation between plasma ferritin values and the percent of reference dose absorbed (10). However, the correlation between the plasma ferritin level or reference dose absorption and the percent absorption of 60 mg of iron administered as ferrous sulfate by the ITT was positive but failed to reach significance. We expected to find a greater absorption value in subjects exhibiting iron depletion and as a consequence, higher plasma iron levels after dose ingestion. This was not the case possibly due to a greater disappearance rate of plasma iron. This could also alter a possible correlation between reference dose absorption and the values obtained by the ITT.

It is interesting to note that the values obtained for iron absorption by the ITT of ferrous sulfate had a very narrow range. On the other hand, the percent absorption by the ITT of ferric ammonium citrate, with a range of values ten times greater, had a significant correlation with ferritin ( $r=0.45$ ). Still, the correlation between reference dose absorption and that of ferric ammonium citrate also failed to reach significance.

The iron absorption from 60 mg of iron as ferrous sulfate (10%) are in the same range as those reported by Heinrich (14) for subjects whose reference dose absorption was 39%. Also, the relative absorption of iron from ferric ammonium citrate in relation to ferrous sulfate iron measured by Hb radioiron incorporation was 23%, almost identical

to that reported by Grebe et al. (9). The relative absorption of ferric ammonium citrate absorption by the ITT, however, is 47% of that of ferrous sulfate. These results suggest that the "true absorption" of reduced iron is near 20 % of that of ferrous sulfate.

The relation between particle size and percent solubility of reduced iron were also within the same range as those reported by Bjorn-Rasmussen (7), and the absorption of reduced iron in relation to that of ferrous sulfate measured by the same method demonstrates that the commercial product studied was only 46% of that of ferrous sulfate by the ITT.

Table 7 shows the different percent absorption reported in the literature utilizing different compounds and methods. Hoglund and Reizenstein's with low particle size reduced iron administered with low extraction bread gave almost identical results to our own when related to  $\text{FeSO}_4$  absorption even though conditions and amounts given are very different in the two studies (19).

In conclusion, we propose that by the use of combined ITT of unlabeled and radiolabeled compounds and radioiron incorporation into Hb the "true absorption" of unlabeled reduced iron can be estimated in relation to the absorption of  $\text{FeSO}_4$ . By this technique, a common brand of commercial small particle-size reduced iron was absorbed at rates similar to those of ferric ammonium citrate (3.72 and 3.27 % respectively) representing 47 and 41 % of ferrous sulfate iron respectively by the ITT. However the "true absorption" of the reduced iron tested is probably near 20 % of that of ferrous sulfate given the "true absorption" of ferric ammonium citrate. The ingestion of 60 mg of reduced iron on an empty stomach or between meals would be similar to ingesting about 12mg of iron contained in 60 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (that has 20 % iron). The

response to the administration of this brand of reduced iron would be similar to that of similar doses of ferric ammonium citrate. Reduced iron is less reactive than  $\text{FeSO}_4$  and possibly is better tolerated.

The results presented open the door to the use of the method described to measure the absorption of unlabeled compounds (such as different brands of commercial reduced iron) as a fortificant in meals that differ in iron bioavailability .

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TABLE 1: Schematic presentation of the schedule of a subject in one group<sup>⊗</sup>

Procedure	Study days										
	-10	1	4	7	//	21	25	29	//	43	
<b>Blood I (Basal)</b>	√										
<b>*Fe-Citrate I. V.</b>		√A									
<b>*Ferrous sulfate P. O.</b>			√B								
<b>Reduced iron P. O.</b>				√B							
<b>Blood II</b>						√					
<b>*Ferric Ammonium Citrate P. O.</b>								√B			
<b>*Iron Ascorbate P. O. (Reference dose)</b>									√B		
<b>Blood III</b>											√

<sup>⊗</sup> Subjects in the other group had two events between blood I and blood II and three events between blood II and blood III. In either case, events were rotated randomly.

\* Indicates either  $^{55}\text{Fe}$  or  $^{59}\text{Fe}$  radiolabeled compound

A: Five ml of blood obtained before the I. V. infusion and at 10, 15, 30, 45, 60, 90, 120 and 240 minutes after the end of the infusion.

B: Five ml of blood obtained before the oral dose and at 30, 60, 90, 120, 180, 240, 300 and 360 minutes after the ingestion of the capsule.

Bloods I, II and III: 30 ml of blood for measurement of radioactivity incorporated into hemoglobin.

TABLE 2: Characteristics of the subjects who completed the study

Subject No.	Age (years)	Sex	Weight (Kg)	Height (m)	Body Mass Index	Hemoglobin (g/L)
1	20	F	63.6	1.66	23.08	149
2	21	F	65.8	1.68	23.31	120
3	21	F	54.5	1.64	20.26	124
4	18	M	72.6	1.72	24.54	145
5	21	F	61.2	1.64	22.75	137
6	24	M	68.1	1.67	24.41	155
7	18	F	81.7	1.54	34.44	120
8	19	M	83.9	1.87	23.99	155
9	19	F	76.2	1.54	32.13	135
10	20	F	68	1.72	22.98	121
<b>Mean</b>	20.1		69.56	1.67	25.15	136

TABLE 3: Characteristics of the subjects who failed to complete the study

Subject No.	Age (years)	Sex	Weight (Kg)	Height (m)	Body Mass Index	Hemoglobin (g/L)
11	20	F	54	1.59	21.35	141
12	18	F	50	1.57	20.28	126
13	24	M	47.8	1.57	19.41	152
14	22	F	45.6	1.5	20.26	123
15	22	F	47.8	1.57	19.41	132
16	28	M	78	1.72	26.36	150
17	18	F	60	1.57	24.34	121
18	22	F	54	1.62	20.57	132
19	20	F	69	1.72	23.52	119
20	19	F	42.6	1.52	18.43	128
<b>mean</b>	21.3		54.89	1.59	21.39	132

TABLE 4: Basal values for plasma iron, TIBC, % saturation of TIBC and plasma ferritin among the subjects that completed the study.

Subject No.	Plasma Fe µg/L	TIBC µg/L	TIBC saturation %	Plasma ferritin µg/L
1	86	280	30.8	37.2
2	121	243	49.8	1.6
3	120	370	32.3	2.6
4	92	273	33.9	19.1
5	90	286	31.6	27.8
6	82	288	28.4	78.6
7	62	254	22.2	7.1
8	60	251	23.9	39.3
9	95	310	30.7	88.3
10	79	279	28.3	55.4
<b>Mean</b>	86.5	281.47	30.49	
<b>S. D.</b>	20.4	36.39	7.49	
<b>Geometric</b>				
<b>mean</b>				19.57
<b>+ 1 S. D.</b>				80.64
<b>- 1 S. D.</b>				4.76

TABLE 5: Basal ferritin values and % absorption of radiolabeled ferrous sulfate, ferric ammonium citrate and reference dose, measured by Hb radioiron incorporation.

Subject No.	Plasma ferritin $\mu\text{g/L}$	Ferrous sulfate absorption %	Ferric ammonium citrate absorption %	Reference dose absorption %
1	37.2	9.15	4.52	24.39
2	1.6	13.09	13.09	96.69
3	2.6	11.36	9.87	51.97
4	19.1	8.61	0.94	62.69
5	27.8	10.97	2.68	24.37
6	78.6	4.5	0.55	35.09
7	7.1	11.61	8.99	72.31
8	39.3	9.06	4.89	14.86
9	88.3	22.99	1.99	38.36
10	55.4	10.6	0.56	30.95
<b>Geometric mean</b>	19.57	10.39	2.35	38.97
<b>+ 1 S. D.</b>	80.64	15.48	6.75	68.71
<b>- 1 S. D.</b>	4.76	6.96	0.82	21.95

NOTE: Statistically significant correlations were found between plasma ferritin and reference dose absorption ( $r=-0.76$ ) and between plasma ferritin and ferric ammonium citrate absorption ( $r=-0.45$ ).

FIGURE 1

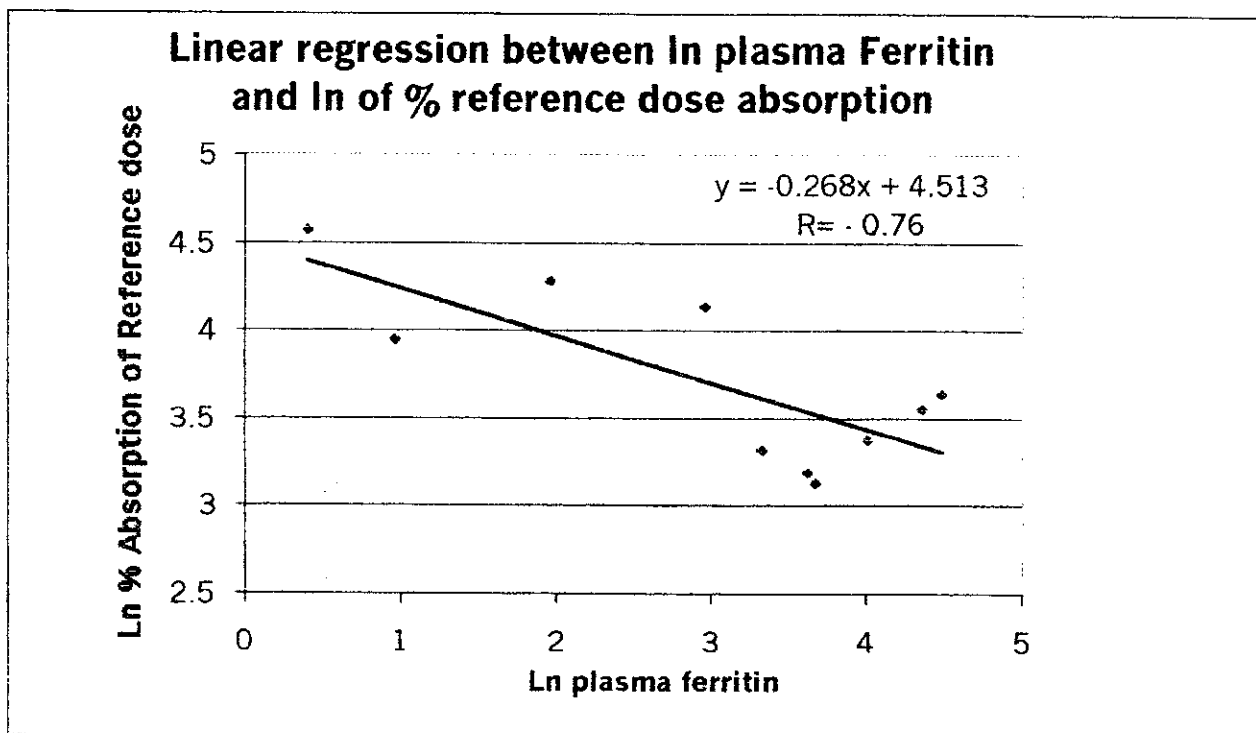


TABLE 6: Percent absorption of ferrous sulfate, ferric ammonium citrate and reduced iron estimated by the "tolerance test" considering both the area under the curve and the peak elevation from baseline plasma values

Subject No	Ferrous sulfate absorption by area	Ferrous sulfate absorption by peak	Ferric ammonium citrate absorption by area	Ferric ammonium citrate absorption by peak	Reduced iron absorption by area	Reduced iron absorption by peak
1	8.1	7.7	3.63	3.55	2.52	2.16
2	8.02	7.7	3.63	3.06	0.67	0.41
3	6.9	6.6	3.99	3.65	1.69	1.52
4	11.93	11.06	8.31	7.93	6.41	6.33
5	7.31	7.36	5.49	5.23	4.58	3.39
6	6.76	5.21	5.08	4.62	4.28	2.23
7	9.04	8.2	2.5	2.36	4.67	3.93
8	14.14	14.13	1.18	0.56	6.13	5.48
9	8.21	7.5	5.91	5.66	4.35	3.6
10	5.01	4.7	4.6	4.33	8.93	7.36
<b>Geometric mean</b>	8.24	7.61	3.97	3.46	3.63	2.86
<b>+ 1 D. S.</b>	11.02	10.48	6.82	7.1	7.76	6.69
<b>- 1 D. S.</b>	6.17	5.52	2.32	1.68	1.7	1.22

NOTE: Mean percent absorption of ferric ammonium citrate and reduced iron expressed as percent of that of ferrous sulfate yield the following results:

#### BY AREA

Ferric ammonium citrate

Reduced iron

48 % (+1 S.D.:96; -1S.D.:24)

44 % (+ 1 S.D.:97; -1 S.D.:20)

BY PEAK

45% (+1 S.D.:115; -1S.D:18).

37% (+ 1 S.D.:88; -1 S.D.:16)

The correlation coefficient between the two ways of estimating absorption were:

For ferrous sulfate: 0.97; for ferric ammonium citrate : 0.98 and for reduced iron: 0.97.

TABLE 7 : Published results on reduced iron absorption by different method sin humans\*.

Dose (mg)	Administration	Absortion as % of FeSO4 or of Ferric Ammonium Citrate	Reference	Notes
1	bread	130 % (FeSO4 dose :Fe 3 mg)	Roe M, et al. 1999 (8)	N=10. Stable isotopes. Fecal monitoring. PSRI:50-20 $\mu$ . (lab. prep.)
10	Water (TT)	120 % (FeSO4 dose : Fe3 mg)	Roe M, et al. 1999 (8)	N=3. PSRI: 10 - 100 $\mu$ . Commercial
1	bread	12 % (arithmetic mean) (Reference dose 5 mgFe as ascorbate)	Callender S, et al. 1968 (5)	N=6. WBC. PSRI ? Lab. prep.
4	Swedish average meal	10% ((Reference dose 5 mg: Fe ascorbate)	Bjorn-Rasmussen, et al. 1977 (7)	N=19. WBC, IIHb PSRI 91% $\leq$ 7 $\mu$ (Lab. prep., and commercial)
11.3	Swedish average meal	20% (Reference dose 3 mg: Fe ascorbate)	Bjorn-Rasmussen, et al. 1977 (7)	N=18. WBC, IIHb PSRI 91% $\leq$ 7 $\mu$ (Lab. prep., and commercial)
40	bread (TT)	31.5% (FeSO4 dose Fe 40 mg with bread)	Vellar O, et al. 1968 (20)	N=18 ITT 2 hrs after intake. TPHR: ? (Lab prep.)
1	bread	45 % (FeSO4: Fe 1 mg with bread)	Hoglund S et al. 1969 (19)	N=13. WBC. PSRI: 97% $\leq$ 5 $\mu$ . (Lab. prep.)
1	bread	15 % (FeSO4: Fe 1 mg with bread)	Hoglund S el al. 1969 (19)	N=9. WBC. PSRI:71% 25 $\mu$ (Lab. prep.)
60	water (TT)	7 - 9 % (Fe ascorbate: Fe3 mg)** 38 - 44 % (FeSO4: Fe 60 mg with water) 83 - 91 % (Ferric Ammonium Citrate: Fe 60 mg with water)	Present study	N=10. PSRI: 97 % $\leq$ 5 $\mu$ Commercially prep.

\* WBC: Whole Body Counter. IIHb: Iron Incorporation into Hemoglobin. PSRI: Particle Size Reduced Iron. ITT: Tolerance test

\*\*Fe absorption from FeSO4 was 27 % of that of the reference dose. This is the expected effect of differences in iron doses administered: 3 mg as iron ascorbate and 60 mg as FeSO4 (11).