CONTROL OF IRON DEFICIENCY

DSAN-C-0045

University of Kansas Medical Center

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Current Year Costs: $79,205

Narrative Summary

The objective of this AID contract is to develop intervention strategies to combat nutritional iron deficiency. The following five areas of activity were proposed in the original contract: (1) factors affecting iron bioavailabil ity of food, (2) iron availability from diets in developing countries (LDC diets), (3) development of iron fortification systems, (4) food technology, and (5) pilot fortification programs. With few exceptions, these studies involve measurements of radioiron absorption in man. A total of three-hundred and twenty iron absorption measurements have been performed during the past year, using 80 volunteer subjects.

During the period of this report research was completed in a number of areas that have been the subject of study in the earlier part of the contract period. Studies in the area of factors affecting iron bioavailability of food were designed to complete our evaluation of the effect of soy products. In the earlier investigations it was demonstrated that soy products inhibited the absorption of nonheme iron from meals composed of semipurified ingredients and infand food supplements. The results of the recent investigations demonstrate that soy products also

produce a significant decrease in the bioavailability of nonheme iron from a meat meal. The effect depends on the amount of soy used and is considerably smaller when the meal includes other foods that exert a similar inhibitory effect. The diminuition in nonheme iron availability is partially offset by the high iron content of soy flour which increases the overall iron content of the meal and by a reciprocal rise in the availability of heme iron. Nevertheless, when 30 g soy flour (dry weight) was eaten with 70 g beef, the mean absorption was 0.27 mg compared with a mean absorption of 0.57 mg from 100 g beef. On the other hand, when soy flour was substituted for meat at the level currently used in the school lunch program and eaten in a mixed meal, there was only a 23% decrease in the actual amount of iron absorbed.

Studies of <u>iron availability from diets in developing countries</u> were extended by measuring the availability of iron from two types of sorghum and a number of legumes. Cereal and legume foods form the bulk of the staple diets in many developing countries. While the availability of iron from cereals such as wheat, corn, and rice has been studied extensively, there are fewer observations on various legumes and very few for sorghum. This series of studies demonstrated that iron was poorly available from both red and white sorghum as well as from black beans, lentils, mung beans, and split peas. Differences in availability were relatively small and percentage absorption values were very similar to those for soybeans and cornmeal.

With reference to the <u>development of fortification systems</u> studies on the availability of fortification iron added to rice prior to cooking were completed. Earlier investigations suggested that iron absorbed into rice might be poorly absorbed and relatively little affected by enhancers of iron absorption in the meal. This would make rice

a poor vehicle for fortification iron since, if correct, the observations would suggest that fortification iron might not enter the common nonheme iron pool. However, these earlier studies involved the use of iron coated onto rice using a shellac to prevent loss during the washing of the rice prior to cooking. The current studies demonstrate that this is not the case and indicate that fortification iron added to rice before cooking enters the nonheme common pool in the meal. We now believe that the earlier results were the result of incomplete release of the fortification iron because of inadequate dissolution of the coating. The present studies demonstrate that rice remains a suitable vehicle for fortification iron.

Finally, a pilot study was undertaken using iron incorporated into a hydrodynamically balanced compound (Hoffmann-LaRoche Company) that allows delayed release to occur in the gastrointestinal tract. Iron in this form was significantly better absorbed than the common pool of iron when this preparation was taken together with a relatively inhibitory meal. This approach has a considerable potential advantage for the administration of both fortification and supplementation iron to people in developing countries since it might allow the iron to be released in the upper gastrointestinal tract after the meal containing inhibitors of iron absorption has passed further down the gastrointestinal tract.

A. General Background

Iron deficiency anemia is widely recognized as the most prevalent nutritional deficiency in the world. While the impact of the anemia which accompanies iron lack has not been defined fully, severe iron deficiency is known to impair work capacity, increase the risk of maternal and infant morbidity and mortality, reduce resistance to infection, and impair the physical and mental growth of school-age children.

Two major approaches have been discussed as intervention strategies to combat iron deficiency anemia. The most direct approach is iron supplementation or the supply of medicinal iron to children or pregnant women. However, iron supplementation programs are costly to administer and usually fail due to lack of compliance. An alternative to iron supplementation is some form of dietary manipulation: either adding more iron to the diet (iron fortification) or adding a substance that increases food iron availability (enhancement fortification). A critical question at present concerns the efficacy of either or both of these procedures because their effectiveness has never been clearly established. Furthermore, because no single method of iron delivery will be suitable in all LDCs, several fortification systems should be made available which could be applied in specific situations with minimal modification.

The purpose of the present project is to develop methods of iron delivery that would be suitable for field testing and eventual implementation on a national level. The optimal approach will depend on the level of dietary iron and the degree of its availability. At one extreme, diets with lowiron content but high availability would be best suited to iron fortification. The best strategy may be to employ both these approaches in tandem.

B. Statement of Project Objectives

The development of an effective food intervention strategy to combat iron deficiency requires consideration of the absorption of a particular form of iron, its stability when added to a particular food vehicle, and the effect that it may have on the vehicle itself. The selected food vehicle must be consumed in sufficient quantities by the deficient population, be compatible with the form of added iron, and be centrally processed to permit adequate regulation of the fortification process.

Before selecting a particular fortification strategy, a number of studies must be conducted in human subjects with radioisotopes to determine basal iron availability of the diet, to measure absorption of fortification iron, and to estimate the gain in iron balance that would be achieved with implementation of the strategy. Field trials will eventually be required to establish the effectiveness of a given strategy but such trials are costly and should be limited to those approaches that appear most promising.

The present project as originally submitted was to conduct research in 5 closely related areas:

(1) Evaluation of factors affecting food iron availability.

(2) Measurement of iron availability from LDC diets.

(3) Development of effective systems of iron or enhancement fortification.

(4) Food technology studies to establish iron/vehicle compatibility.

(5) Pilot fortification trials to measure the efficacy of the intervention strategy.

There has been no major change in the basic objectives as defined in the original contract. Efforts in developing iron fortification systems depend largely on isotopic studies of food iron absorption. These studies are still the core of the present contract. During this year studies of factors affecting food iron absorption which commenced earlier in the contract period were complete. It has been possible to answer most of the questions raised by earlier observations.

C. Accomplishments to Date

The body of this progress report is contained in the following appendices:

Appendix A: Iron absorption studies

Appendix B: Publications

A detailed description of nine iron absorption studies conducted during the past year are contained in Appendix A. The studies are presented under the headings of Abstract, Rationale, Design, Results, and Comment. The following is a list of these studies:

Study	Pg.	# Subjects	Title
39	14	3	Pilot study of iron absorption, hydrodynamically balanced system (HBS).
40	21	9	Effect of soy (high level) on absorption of heme and nonheme iron from beef.
41	29	7	Effect of soy (low level) on nonheme iron absorption from beef alone and hamburger meal.
42	37	11	Rice fortification iron exchange I.
43	45	12	Effect of soy (low level) on absorption of heme and nonheme iron from hamburger meal.
44	52	9	Bioavailability of iron from red sorghum, white sorghum, and corn meal.
45	59	10	Iron bioavailability from soybeans, black beans, and lentils.
46	66	10	Iron bioavailability from mung beans, split peas, and cornmeal.
47	74	9	Rice fortification iron exchange II.

Three of the studies performed during the past year were done to complete the series of observations on the effect of soy products on iron absorption. In these studies, soy flour was used to replace a portion of the meat in a simple meat meal and in a more complex meal that included a bun, french fries, and a milkshake. The marked reduction in nonheme iron absorption seen previously with semipurified meals and infant food supplements was again observed when a large quantity of soy flour (30 g dry weight to 70 g ground beef) was added to a pure meat meal, nonheme iron absorption falling from 24.82% to 0.87% (Study 40). A smaller amount of soy (30 g hydrated weight), equal to that currently employed in the school lunch program, had a somewhat less marked effect. Nevertheless, there was still a significant fall in percent nonheme iron absorption; the mean values being 16.74 and 3.19% respectively (Study 41). When the beef burger was eaten together with french fries and a milkshake, nonheme iron availability was also markedly reduced even in the absence of soy (2.36%). Nevertheless, the addition of soy produced a further small fall in nonheme iron availability to 1.63%. However, since the iron present in the soy increased the overall nonheme iron content of the meal, there was no difference in the actual amount of nonheme iron absorbed (0.08 mg vs. 0.07 mg). A further interesting observation was made, namely that in the presence of soy, percentage heme iron absorption showed a reciprocal increase. For example, in the mixed meal in which the level of soy substitution used was comparable to that used in the school lunch program (Study 43), percentage heme iron absorption rose from a mean of 33.1% to 42.1%. Thus the negative effect of soy on nonheme iron availability was ameliorated both by an increase in the nonheme iron content of the meal and by an increase in percentage absorption of heme iron. The sum of

these opposing effects would mean that if soy flour were added to a hamburger in a mixed meal at the level currently employed in the school lunch program, mean absorption from this meal would have fallen from 0.56 mg to 0.43 mg. This is a small but significant reduction in iron absorption due largely to withdrawal of 30% of the heme from the meal, an effect which is not entirely compensated for by the increase in percentage absorption of heme iron.

Legumes and cereal products are the dietary bulk staples of many developing countries. The demonstration that iron in soybeans and soy products is in general relatively poorly available made a comparison with other legumes as well as with cereal important. Wheat and rice have been studied extensively and were not included in this series of observations. Studies 44, 45, and 46 demonstrate that the availability of iron from soybeans and four other commonly eaten legumes, black beans, lentils, mung beans, and split peas as well as from corn and two types of sorghum is very similar. This information is summarized on the third graph in Study 46. Valid comparisons between these three studies were made possible by the inclusion of a reference meal with each. It can be seen by this graph that the meal:reference ratio was relatively similar in all cases ranging between 0.3 and 1.1. In fact, it is noteworthy that the ratios obtained for two cornmeal studies lie at the extremes of the range for the series of observations. This set of experiments has led us to the conclusion that cornmeal, sorghum, and a number of legumes including soybeans do not differ significantly in their effect on

nonheme iron absorption. All are relatively poor sources for available iron. The legumes including soybeans may have some advantage in terms of iron nutrition because of the higher intrinsic iron content.

Studies on the suitability of rice as a vehicle for fortification iron were completed (Studies 42 and 47). While technical difficulties made the interpretation of Study 42 unsatisfactory, Study 47 clearly demonstrated that fortification iron introduced into rice prior to cooking and absorbed into the rice during the cooking process shares the properties of the common pool with respect to bioavailability. This study demonstrates that the poor absorption previously observed with iron coated onto rice was due to incomplete release of iron from the coated grains and not to the poor availability of iron cooked into rice. It therefore reflected a technical problem in the coating procedure and not a property of rice. This is an important result and indicates that rice may still be a suitable vehicle for fortification iron if the technical problems of adding the fortificant can be solved.

The final study carried out during this year (Study 39) was performed in collaboration with Hoffmann-LaRoche Company and represents a pilot investigation of a new and potentially attractive means of overcoming the inhibitory effect of food on fortification or supplementary iron. Hoffmann-LaRoche Company has developed a hydrodynamically balanced system that allows delayed release of iron to occur in the gastrointestinal tract thus separating iron added to a meal in the form from the rest of the components because of different rates of passage through the gastrointestinal tract. The limited number of observations performed in this study suggest that the iron in the

hydrodynamically balanced system may be absorbed two to three times as well as ferrous sulfate introduced into the same meal. The possibility of reduced side effects and better absorption in the presence of food would be major advantages of such a product in any supplementation program.

Dissemination and Ultilization of Research Results

The findings that have been developed under this contract have been communicated through formal presentations at national and international meetings, through exchange on an informal basis between investigators visiting the iron nutrition unit in Kansas City, and by reciprocal visits by Kansas investigators. The following is a summary of these various avenues of communication.

1. Presentations

a. Cook: "Biochemical Determinants of Food Iron Availability"; XII International Congress of Nutrition; San Diego CA; 20 August 1981.

b. Morck: "Inhibition of Food Iron Absorption by Soy Products"; XII International Congress of Nutrition; San Diego CA; 22 August 1981.

c. Cook: "Rapid Assessment of Iron Status"; Fifth International Conference on Proteins of Iron Storage and Transport; La Jolla CA; 26 August 1981.

d. Lynch: "An In Vitro Method for Predicting Iron Availability"; Fifth International Conference on Proteins of Iron Storage and Transport; La Jolla CA; 26 August 1981.

e. Cook: "The Effect of Plant Protein on Iron Bioavailability"; International Nutritional Anemia Consultative Group (INACG) Annual Meeting; Santiago, Chile; 11 December 1981.

f. Cook: "Bioavailability of Iron from Cereal and Legume Diets"; Nutrition Foundation Food and Nutrition Liaison Committee Meeting; Naples FL; 22 January 1982.

g. Lynch: "Relationship between Iron Stores and Food Iron Absorption"; FDA Nutrition Division Seminar; Washington DC; 6 May 1982.

h. Lynch: "Iron Status in the Elderly"; American Society for Clinical Nutrition; Washington DC; 8 May 1982.

2. Scientific Publications

The following articles have been published or accepted for publication during the fourth year of this contract. Copies are included in Appendix B.

a. Cook JD, Morck TA, Skikne BS, Lynch SR. Dietary inhibitors of iron absorption in man. In: Wagner PA, Kirk JR, eds. Proceedings of the Florida Symposium on Micronutrients in Human Nutrition. Gainesville: University of Florida, 1981;99-105.

b. Cook JD, Morck TA, Lynch SR. The inhibitory effect of soy products on nonheme iron absorption in man. Am J Clin Nutr 1981;34:2622-2629.

c. Morck TA, Lynch SR, Skikne BS, Cook JD. Iron availability from infant food supplements. Am J Clin Nutr 1981;34:2630-2634.

d. Cook JD. Current definitions of deficiency anemia. In: Oski FA, Pearson HA, eds. Iron Nutrition Revisited - Infancy, Childhood, Adolescence. Report of the Eighty-Second Ross Conference on Pediatric Research. Columbus OH: Ross Laboratories, 1981;25-29.

e. Cook JD, Morck TA, Skikne BA, Lynch SR. Biochemical determinants of iron absorption. In: Harper AE, David GK, eds. Nutrition in Health and Disease and International Development: Symposia from the XII International Congress of Nutrition. Progress in Clinical and Biological Research, Volume 77. New York: Alan R. Liss, 1981;323-331.

f. Morck TA, Cook JD. Factors affecting the bioavailability of dietary iron. Cereal Foods World 1981; 26:667-672.

g. Cook JD, Skikne BS. Serum ferritin: a possible model for the assessment of nutrient stores. Am J Clin Nutr 1982;35:1180-1185.

h. Cook JD. Clinical evaluation of iron deficiency. Seminars in Hematology 1982;19:6-18.

i. Pintar J, Skikne BS, Cook JD. A screening test for assessing iron status. Blood 1982;59:110-113.

j. Morck TA, Lynch SR, Cook JD. Reduction of the soy-induced inhibition of nonheme iron absorption. Am J Clin Nutr 1982;36:219-223.

k. Lynch SR, Cook JD, Morck TA. In vitro measurement of food iron absorption. Proceedings of the Fifth International Conference on Proteins in Iron Metabolism (in press).

l. Cook JD, Morck TA, Skikne BS, Lynch SR. The importance of animal products in human iron nutrition. In: Beitz DC, Hanson RG, eds. Animal Products in Human Nutrition. New York: Academic Press (in press).

m. Lynch SR, Finch CA, Monsen ER, Cook JD. Iron status of elderly Americans. Am J Clin Nutr (in press).

n. Morck TA, Lynch SR, Cook JD. Inhibition of food iron absorption by coffee. Am J Clin Nutr (accepted).

o. Pootrakul P, Skikne BS, Cook JD. The use of capillary blood for measurements of circulating ferritin. Am J Clin Nutr (accepted).

p. Lynch SR, Morck TA. Iron deficiency anemia. Contemporary Issues in Clinical Nutrition (accepted).

Statement of Expenditures and Obligations

Category	Total Awarded	Expended Thru 7/82	Balance
Personnel	271,983	229,180	42,803
Travel	29,480	21,638	7,842
Shipping	24,000	-	24,000
Equipment	38,644	22,100	16,544
Supplies	38,644	39,427	783
Volunteers	67,500	41,950	25,550
Contracts	12,915	10,640	2,275
Publication	2,500	3,000	500
Subtotal	485,666	367,935	117,731
Indirect	163,190	135,405	27,785
Total	648,856	503,339	145,517

A no-cost extension for the completion of the objectives of this contract has been requested which will extend the contract end date to 31 October 1983.

APPENDIX A

-IRON ABSORPTION STUDIES-

STUDY 39

PILOT STUDY OF IRON ABSORPTION HYDRODYNAMICALLY BALANCED SYSTEM (HBS)

ABSTRACT

Gastrointestinal side effects are a major obstacle to the implementation of iron supplementation programs. Hoffmann-LaRoche Company has developed a hydrodynamically balanced system (HBS) that allows delayed release to occur in the gastrointestinal tract. A decrease in side effects is anticipated. Absorption from hydrodynamically balanced capsules was 1.44, 1.21, and 2.14% for th ee different formulations. In comparison, Feosol taken with the same meal as only absorbed at the 0.6% level. This pilot study suggested HBS iron should be carefully evaluated since a lower frequency of side effects and better absorption in the presence of food would be major advantages in any supplementation program.

RATIONALE

The major obstacle in iron supplementation programs at the present time is gastrointestinal side effects associated with the administration of oral iron. One approach to improving therapeutic efficacy (increasing absorption and/or reducing side effects) is to administer the iron in a manner which delays its release within the gastrointestinal tract. In the present study, we evaluated a hydronamically balanced system (HBS) in which iron is incorporated into a substance that absorbs water and has a lesser density than the gastric contents. It therefore remains in the proximal portion of the stomach which in turn retards gastric emptying. At the same time, iron is released from the preparation providing a constant flow to the small intestine. In the present study three formulations of HBS were compared with conventional iron (liquid ferrous sulfate).

DESIGN

The HBS was radiolabeled in the Kansas laboratory by personnel from Hoffmann-LaRoche; details of this preparation are unavailable. The products were labeled as follows: HBS-1 (JLT258-160), HBS-3 (JLT9258-74), HBS-4 (JLT-9258-75). The alternate form of radioiron was liquid ferrous sulfate (Feosol elixir, SmithKline), 5 ml containing 50 mg iron, labeled with radioiron by the addition of 1 uCi $^{59}FeSO_4$. In addition to the iron preparations, all four meals used in this study consisted of a McDonald's Egg McMuffin, 1 oz 40% bran flakes, 8 oz 2% milk, 4 g sugar, and 6 oz coffee.

RESULTS

Although studies were performed in only three individuals, the differences were great enough to suggest a distinct advantage to the HBS preparation. The preparations are listed as slow, medium, and fast in the attached table, reflecting the predicted rate of dissolution. Mean absorption values averaged 1.44, 1.21, and 2.14% respectively and

all were significantly higher than Feosol which averaged only 0.60%. These results are only rough indicators of the difference but suggest that HBS may offer a distinct therapeutic advantage.

DISCUSSION

This pilot study requires more extensive evaluation of HBS and further studies are underway.

STUDY 39. Iron Absorption from Hydrodynamically-Balanced System (HBS) and Feosol.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) HBS Slow (A)	HBS Med (B)	HBS Fast (C)	Feosol (D)	ABSORPTION RATIO A/D	B/D	C/D
1	24	42	47	0.52	0.40	1.03	0.23	2.26	1.73	4.47
2	24	42	40	1.41	1.35	2.67	1.06	1.33	1.27	2.51
3	26	44	78	4.12	3.33	3.58	0.93	4.43	3.58	3.84
MEAN*	25	43	53	1.44	1.21	2.14	0.60	2.37	1.99	3.51
			-1 SE	0.79	0.65	1.47	0.37	1.67	1.47	2.96
			+1 SE	2.62	2.24	3.12	0.99	3.36	2.71	4.18

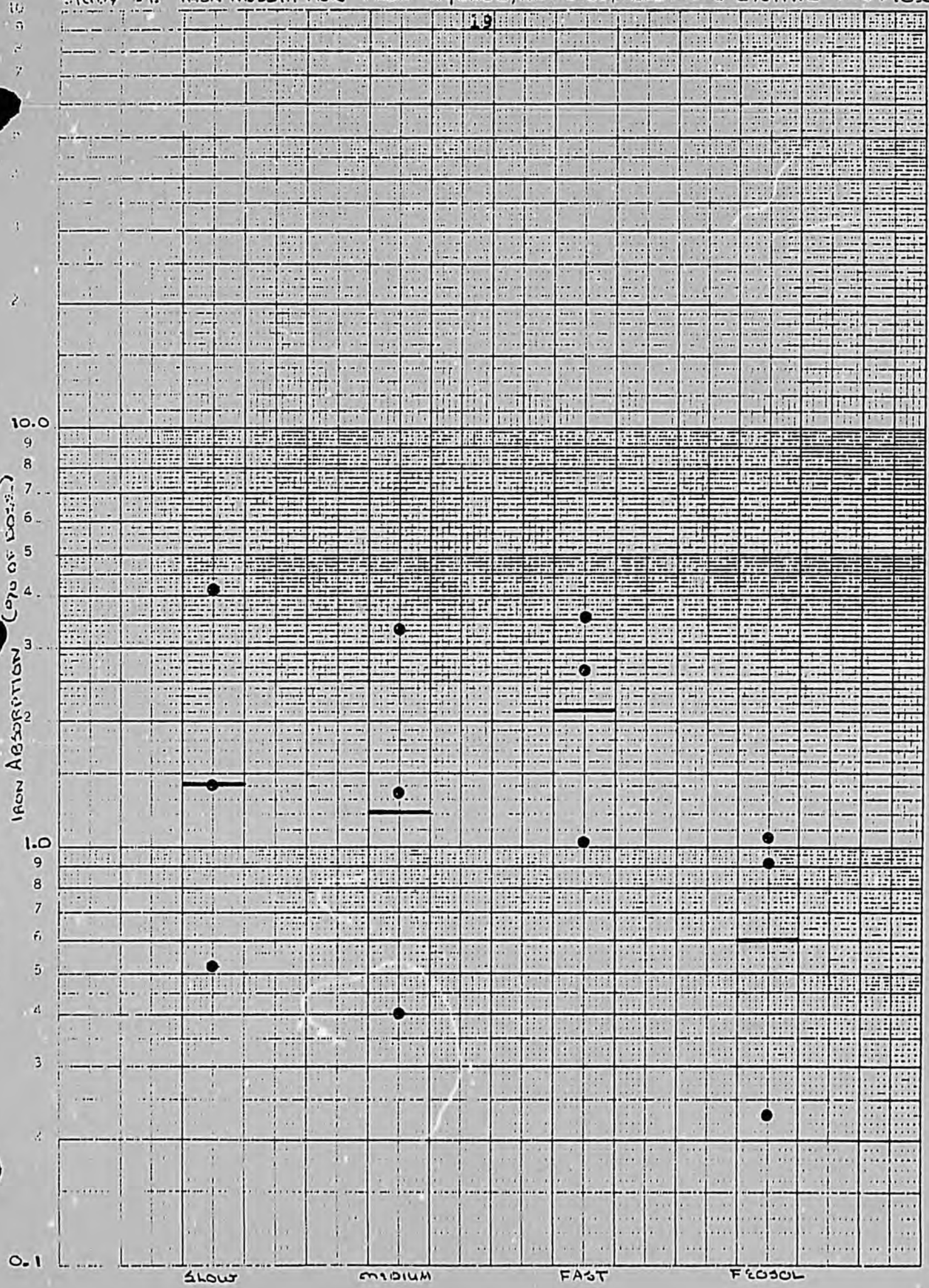
* Geometric means except Age and Packed Cell Volume

STUDY 39. Iron Absorption from Hydrodynamically-Balanced System (HBS) and Feosol.

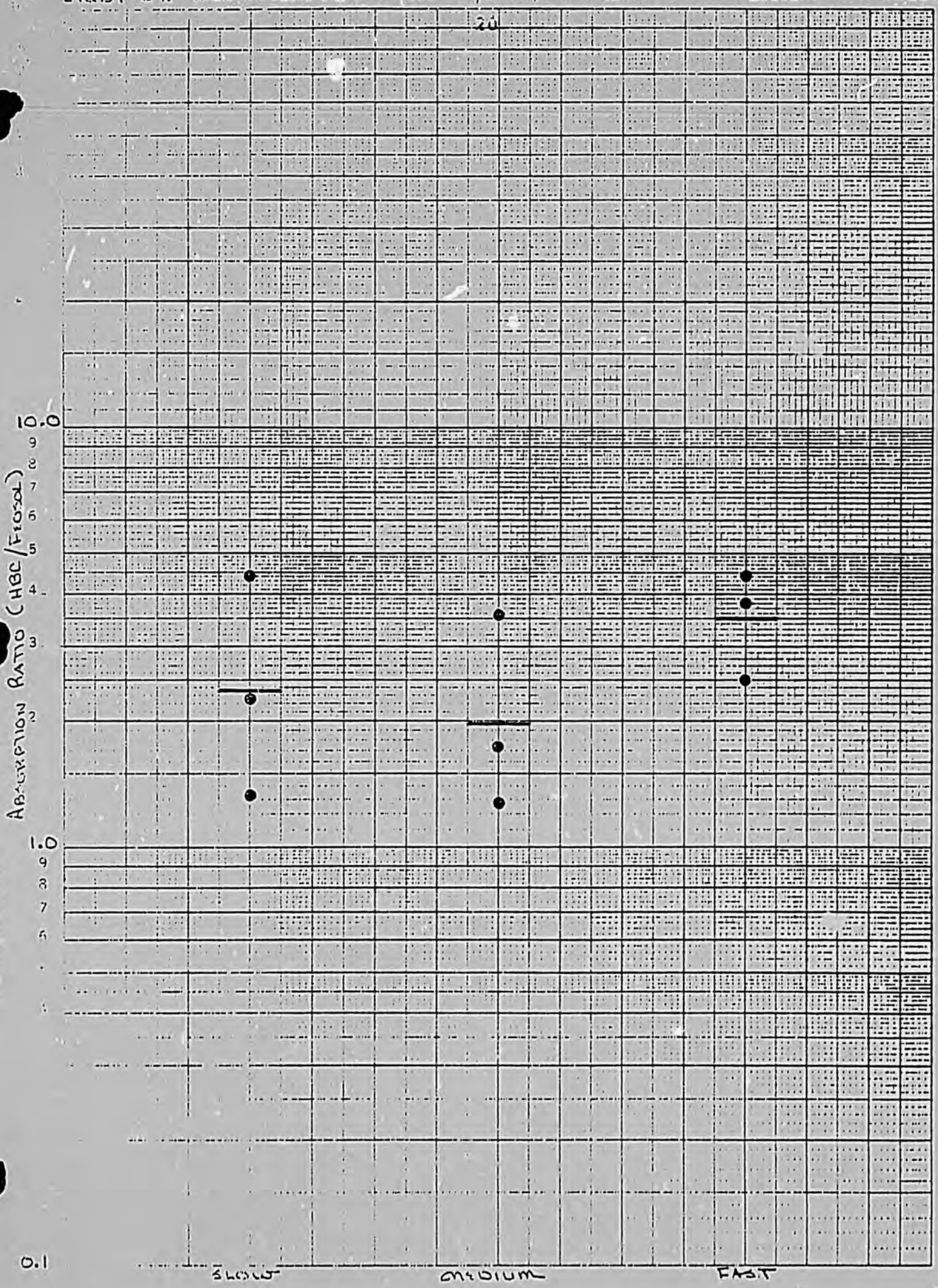
	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.19	0.67	2.37	0.57	1.99	3.51
-1 SE	1.11	0.52	1.67	0.44	1.47	2.96
+1 SE	1.27	0.88	3.36	0.73	2.71	4.18
1/Ratio	0.84	1.48	0.42	1.76	0.50	0.28
Student's *t*	2.61	1.47	2.48	2.19	2.25	7.30
p value	0.011	0.053	0.012	0.018	0.017	0.0002
Correlation* (r)	0.99	0.95	0.81	0.98	0.87	0.95
p value	0.0003	0.007	0.047	0.002	0.028	0.006

* log scale

STUDY 34. IRON ABSORPTION FROM HYDROLYZED/ALKALIZED SYSTEMS WITH FEOSOL.



STUDY 31. IRON ABSORPTION FROM HYDROGEN-MANNITOL-GLUCONATE-SALT SYSTEM WITH FROSOL



STUDY 40

EFFECT OF SOY (HIGH LEVEL) ON ABSORPTION OF HEME AND NONHEME IRON FROM BEEF

ABSTRACT

Previous studies have demonstrated inhibition of nonheme iron absorption by soy products. In this study we compared the absorption of both heme and nonheme iron from meals consisting of 1) 100 g ground lean beef and 2) 70 g ground lean beef mixed with 30 g (dry weight) textured soy flour. Percentage absorption for nonheme and heme iron was 24.82 and 0.87, and 17.03 and 27.13 from the beef and beef + soy meals respectively, demonstrating a reciprocal increase in percentage heme iron absorption. A mean of 0.57 mg iron was absorbed from the beef-only meal and 0.27 mg from the beef + soy meal.

RATIONALE

Several prior studies from this laboratory have demonstrated that a number of soy products markedly inhibit the absorption of nonheme iron. The majority of these studies were performed with meals containing no meat and therefore did not examine the effect on iron absorption of substituting meat products with soy. One obvious effect would be a reduction in heme content of the meal. Because the absorption of heme iron is several times higher than nonheme iron, even a modest decrease in heme content could produce a substantial reduction in composite absorption of heme and nonheme iron. The present study was undertaken to determine the effect on heme and nonheme iron absorption of substituting part of the meat in a complete meal.

DESIGN

Nine volunteer subjects participated in this study. It involved the administration of two meals simultaneously labeled with extrinsic tags of heme and nonheme radioiron. The first meal consisted of 100 g ground lean beef which had been broiled for 5-7 minutes immediately prior to administration. The 100 g meat contained 2.6 mg iron, 1.2 mg in the form of heme. Ferric chloride, 0.1 mg iron tagged with ^{59}Fe, was added in a volume of 0.5 ml 0.01N HCl to the meat just prior to serving. Similarly, ^{55}Fe-labeled heme iron isolated from a pathogen-free rabbit was added in a volume of 0.5 ml directly to the hamburger. The second meal consisted of 70 g ground lean beef that had been mixed prior to cooking with 30 g (dry weight) textured soy flour (Supra -50A, Ralston Purina). Iron content was 5.24 mg, 0.84 mg as heme. The second meal was tagged with radioiron and administered in a manner identical to the first.

RESULTS

When the meal consisted only of beef, the absorption of both heme and nonheme iron was exceptionally high in these iron replete subjects. Heme iron absorption averaged 17.0% as compared to 24.8% absorption of nonheme iron. Indeed, the absorption of nonheme iron was significantly higher than that of heme iron (mean ratio A/B=1.46, $t=3.48$, $p < 0.01$).

When a portion of the beef was substituted with soy, a profound decrease in nonheme iron absorption, to a mean of 0.87%, was observed. Paradoxically, heme absorption actually increased in the soy-containing meal from 17.0 to 27.1%, a difference that was highly significant ($t=5.96$, $p < 0.001$).

The significance of these changes in percentage absorption is illustrated by calculating the absolute absorption of heme and nonheme iron from these meals:

	Beef Meal			Beef + Soy Meal		
	Heme	Nonheme	Total	Heme	Nonheme	Total
Iron Content (mg)	1.20	1.50	2.70	0.84	4.50	5.34
Iron Absorbed (mg)	0.20	0.37	0.57	0.23	0.04	0.27

COMMENT

There was a dramatic reduction in the absorption of nonheme iron when soy was added to beef in this study. However, this fall should be interpreted in the light of the very high availability of nonheme iron from a meal containing only meat. The addition of any food other than animal tissue to meat will produce a sharp fall in absorption although perhaps not to the extent observed with soy.

Moreover, while percentage absorption decreased dramatically, the change in absolute absorption of nonheme iron in the soy-containing meal was somewhat less pronounced.

Of considerable theoretical interest from the point of view of mucosal iron transport is the finding that the addition of soy to this meal actually improves the percent absorption of heme iron. Therefore, the absolute quantity of heme iron absorbed from this meal increased slightly. It seems possible that the apparent facilitating effect of soy on heme absorption is exerted by control mechanisms within the mucosal cell. If control of the absorption process is exerted in the exit pathway from the mucosal cell to the circulation, it is conceivable that a reciprocal relationship could exist beween the absorption of heme and nonheme iron. Thus, a decrease in mucosal uptake of nonheme iron would be accompanied by a rise in the absorption of heme. If this concept is valid, a sharp increase in the absorption of nonheme iron might inhibit the absorption of heme iron and preliminary evidence to support this has been obtained in more recent studies with ascorbic acid. The overall effect of adding this relatively large amount of a soy product to the meat-only was to reduce iron absorption by a factor of approximately two.

It should be noted that the level of soy substitution in this study was substantially higher than the level at which soy is used as a meat extender in the present school lunch program. In our study, 30% of textured soy flour was added on the basis of dry weight rather than hydrated weight which provides a level of substitution roughly two and one-half times that currently used in the US.

STUDY 40. Effect of Textured Soy Flour on Nonheme and Heme Iron Absorption from Beef.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Nonheme -Soy (A)	Nonheme +Soy (B)	Heme -Soy (C)	Heme +Soy (D)	ABSORPTION RATIO +/- Soy Nonheme B/A	Heme D/C
1	25	45	98	9.08	0.41	6.57	14.16	0.04	2.15
2	25	44	80	18.10	0.40	19.15	29.00	0.02	1.51
3	24	45	108	19.55	0.93	14.10	23.85	0.05	1.69
4	24	41	40	23.02	0.78	18.60	18.07	0.03	0.97
5	25	49	42	27.30	1.70	29.15	56.08	0.06	1.92
6	23	48	74	32.33	0.62	14.18	25.88	0.01	1.82
7	25	47	58	32.52	0.91	20.43	36.28	0.02	1.77
8	24	46	31	33.61	1.01	19.50	27.23	0.03	1.39
9	27	50	20	50.21	2.66	22.26	31.46	0.05	1.41
MEAN*	25	46	54	24.82	0.87	17.03	27.13	0.04	1.59
			-1 SE	21.09	0.71	14.81	23.79	0.03	1.47
			+1 SE	29.22	1.01	19.59	30.95	0.04	1.72

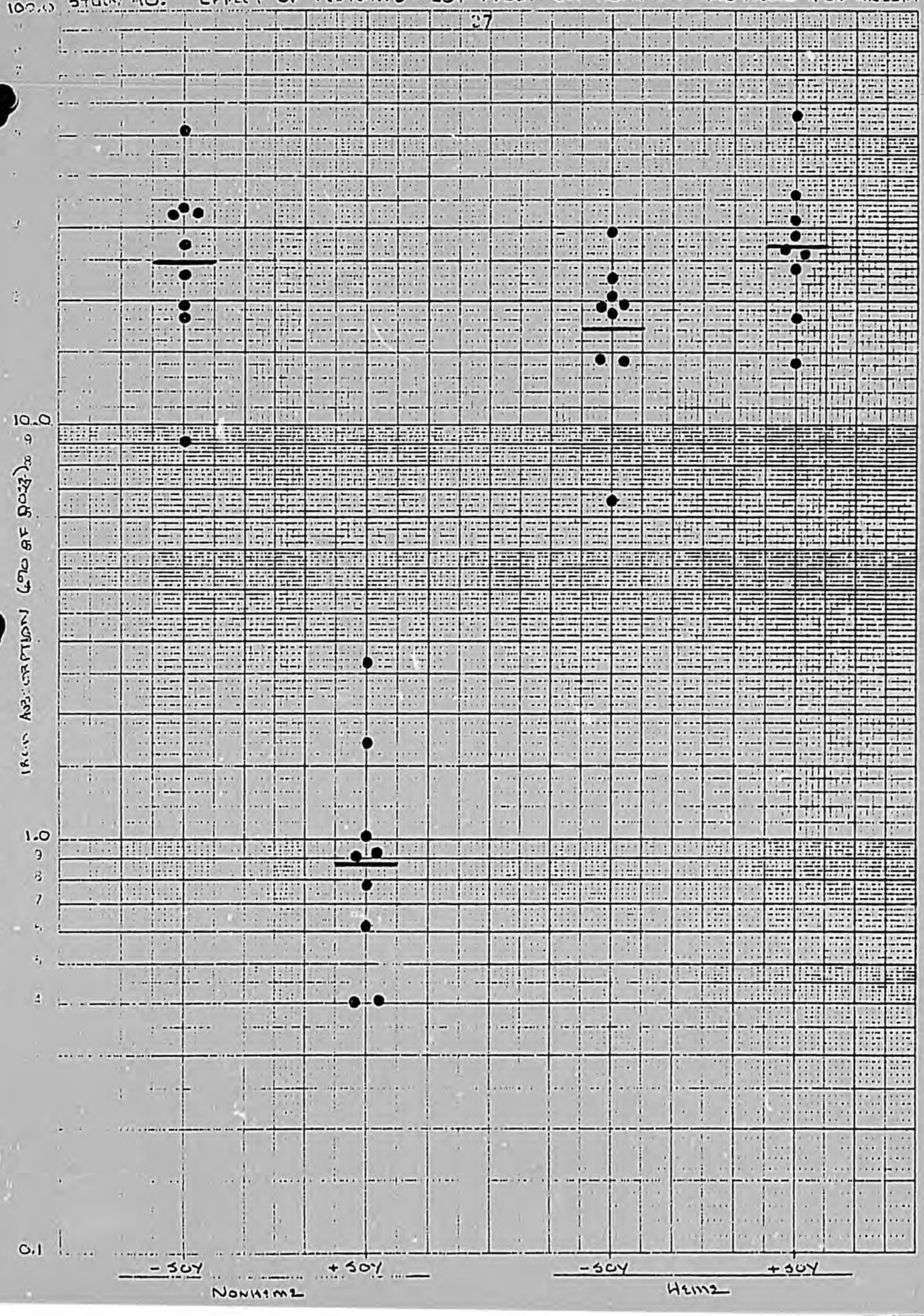
* Geometric means except Age and Packed Cell Volume

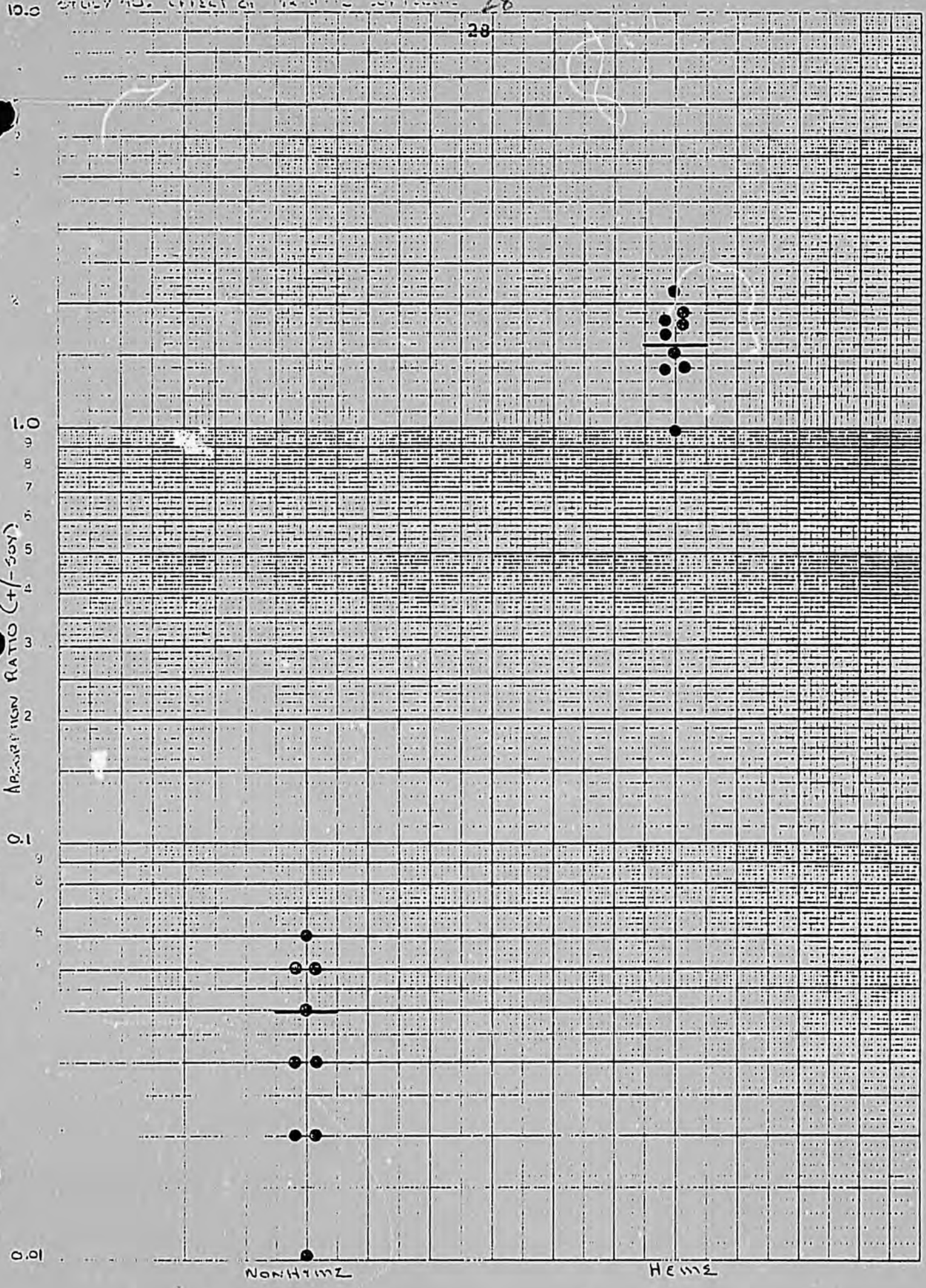
STUDY 40. Effect of Textured Soy Flour on Nonheme and Heme Iron Absorption from Beef.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	28.31	1.46	0.91	0.05	0.03	0.63
-1 SE	24.74	1.31	0.80	0.04	0.03	0.58
+1 SE	32.40	1.62	1.05	0.06	0.04	0.68
1/ Ratio	0.04	0.69	1.09	19.43	30.95	1.59
Student's *t*	24.79	3.48	0.67	19.16	20.81	5.96
p value	$<10^{-5}$	0.004	0.262	$<10^{-5}$	$<10^{-5}$	0.0002
Correlation* (r)	0.753	0.755	0.605	0.655	0.595	0.836
p value	0.009	0.009	0.042	0.028	0.045	0.002

* log scale

STUDY NO. EFFECT OF TEXTURED SOY FOOD ON NONHEME AND HEME IRON ABSORPTION




10.0
1.0
0.1
0.01
ABSORPTION RATIO (+/- SOY)
NONHEME
HEME

STUDY 41

EFFECT OF SOY (LOW LEVEL) ON NONHEME IRON ABSORPTION FROM BEEF ALONE AND A HAMBURGER MEAL

ABSTRACT

Earlier studies demonstrating an effect of soy on nonheme iron absorption from mixed meals involved the use of 30 g dry weight soy flour. In this study, we examined the effect of adding 12 g textured soy flour (the level of soy substitution currently employed in the school lunch program) on nonheme iron absorption from 1) a beef burger and 2) a beef burger together with a milkshake and french fries. Mean absorption levels were 16.74% and 3.19% for the beef burger without and with soy respectively and 2.36 and 1.63% for the complete meal without and with soy respectively. As a result, absolute nonheme iron absorption fell from 0.25 to 0.08 mg in the beef meal and from 0.08 to 0.07 mg in the composite meal.

RATIONALE

In the previous study a profound fall in the absorption of nonheme iron was observed when soy was added to a meal containing only beef. Since this reduction may represent the effect of adding any food substance other than animal tissue to beef, the present study was undertaken to determine whether the fall in absorption of nonheme iron is less pronounced when an additional source of food is ingested with beef.

DESIGN

Seven volunteers participated in this study, one of whom was markedly iron deficient. Four separate meals were served. The first meal (A) consisted of 100 g cooked ground lean beef tagged with 0.1 mg iron as ferric chloride and 2 uCi ^{59}Fe. The second meal (B) contained 70 g ground beef that had been mixed with 30 g hydrated textured soy flour (only 12 g dry product as compared to 30 g used in Study 40).

In the second pair of meals, the beef (C) and beef + soy (D) meals were consumed with a vanilla milkshake (145 ml) and french fries (68g).

RESULTS

When soy was added to the meal containing only ground beef, percentage absorption fell significantly from 16.74 to 3.19% (mean ratio D/A=0.19, $t=5.48$, $p < 0.001$). Surprisingly, an even greater decrease in percentage absorption was observed when the milkshake and french fries were added to the beef meal (mean absorption 2.36%, mean ratio C/A=0.14, $t=4.39$, $p < 0.01$). The partial substitution of meat with soy produced a further modest decrease to a mean of 1.63%, a reduction of 31% which was marginally significant ($t=2.09$, $p=0.04$). The addition of soy increased the iron content of

both types of meal. As a result there was a somewhat smaller fall in the absolute amount of nonheme iron absorbed from the meal containing ground beef only while nonheme iron absorption from the two complex meals was very similar.

	Beef		Standard Meal	
	-Soy	+Soy	-Soy	+Soy
Nonheme iron content (mg)	1.50	2.45	3.30	4.25
Nonheme iron absorbed (mg)	0.25	0.08	0.08	0.07

COMMENT

It is apparent from this study that the addition of other food items to a beef meal produces a sharp reduction in percentage absorption of nonheme iron. The inhibiting effect of soy in this study was no more pronounced than that of french fries and a milkshake. Moreover, the substitution of soy for meat in the complex meal reduces percent nonheme iron absorption moderately but does not significantly alter the actual quantity absorbed because of an increase in meal iron content.

It is also noteworthy that when only 12 g of textured soy flour rather than 30 g is added to beef, the reduction in percentage nonheme iron absorption is less pronounced. In study 40, a decrease of 96% was observed at the higher level of soy as compared to 81% in the present study with the level of soy substitution currently employed in the school lunch program.

STUDY 41. Effect of Soy-Extended Beef on Iron Absorption From Beef-Only and Standard Meals.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (µg/l)	IRON ABSORPTION (% of dose) Beef -Soy (A)	Beef +Soy (B)	Standard Meal -Soy (C)	Standard Meal +Soy (D)	ABSORPTION RATIO +/- Soy B/A	+/- Soy D/C	STD/Beef C/A	STD/Beef D/B
1	26	47	122	7.51	2.37	0.85	0.61	0.31	0.71	0.11	0.25
2	27	44	142	13.38	1.15	0.65	0.88	0.08	1.35	0.04	0.76
3	25	45	158	14.91	1.07	1.16	0.65	0.07	0.56	0.07	0.60
4	26	39	2	16.00	11.27	25.78	18.12	0.70	0.70	1.61	1.60
5	23	47	126	19.23	2.37	1.10	0.88	0.12	0.80	0.05	0.37
6	30	43	93	22.06	4.80	3.78	1.11	0.21	0.29	0.17	0.23
7	25	44	65	36.30	9.17	6.02	5.15	0.25	0.85	0.16	0.56
MEAN*	26	44	64	16.74	3.19	2.36	1.63	0.19	0.69	0.14	0.51
			-1 SE	13.94	2.24	1.43	1.01	0.14	0.58	0.09	0.40
			+1 SE	20.10	4.56	3.90	2.66	0.26	0.83	0.22	0.66

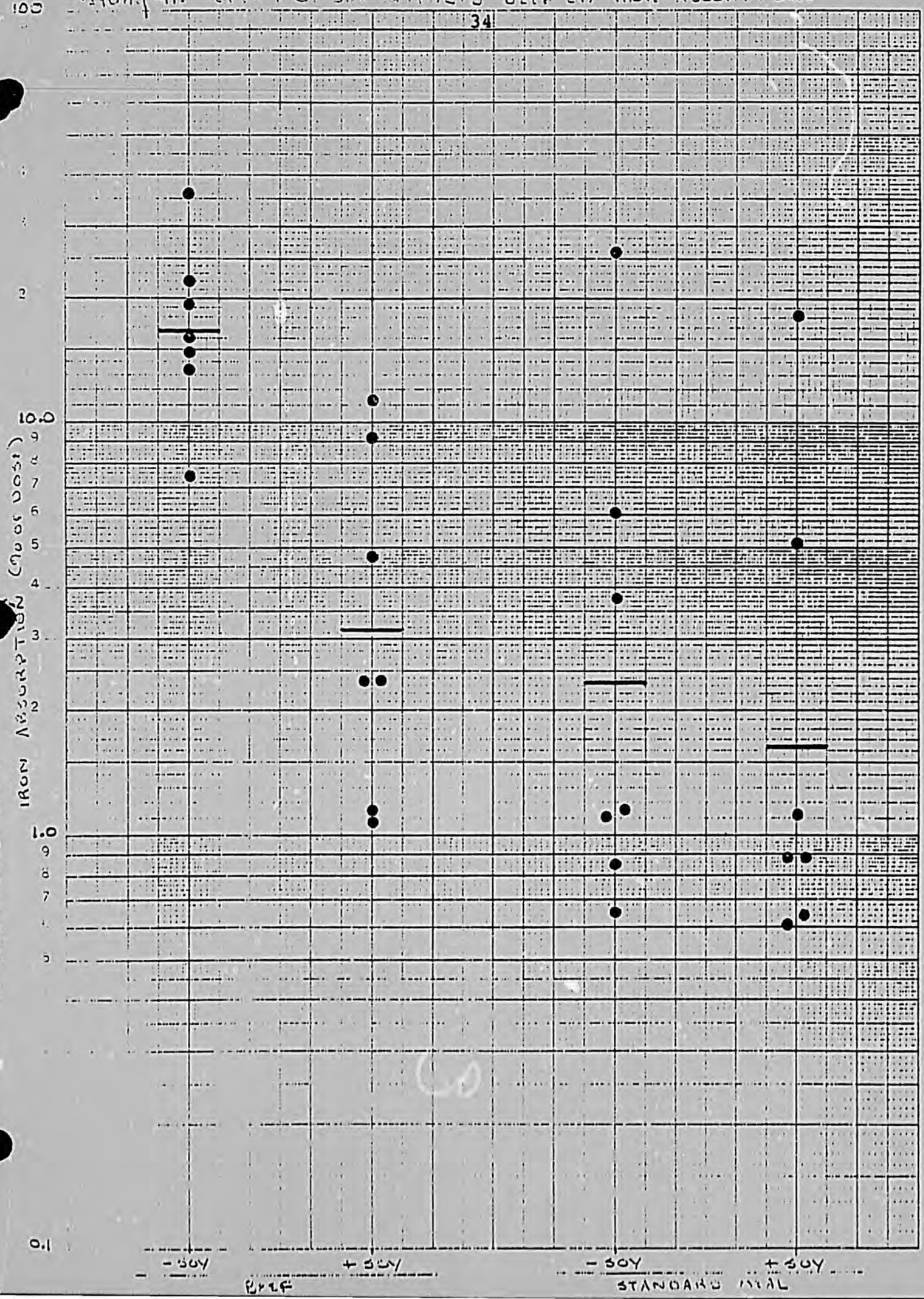
* Geometric means except Age and Packed Cell Volume

STUDY 41. Effect of Soy-Extended Beef on Iron Absorption From Beef-Only and Standard Meals.

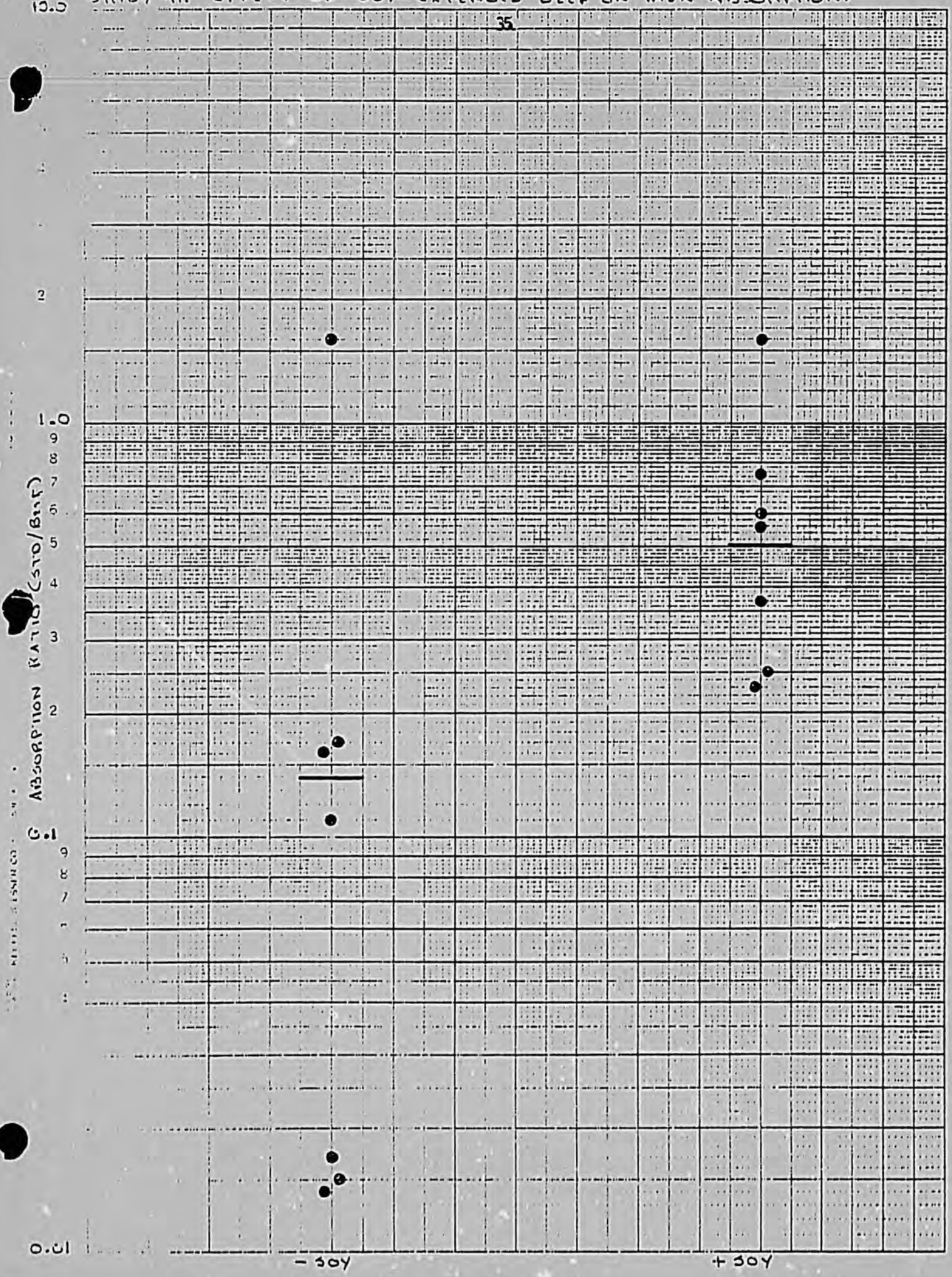
	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	5.23	7.08	10.22	1.35	1.95	1.44
-1 SE	3.87	4.53	6.60	1.07	1.51	1.21
+1 SE	7.08	11.06	15.80	1.71	2.52	1.72
1/ Ratio	0.19	0.14	0.10	0.74	0.51	0.69
Student's *t*	5.48	4.39	5.32	1.30	2.63	2.09
p value	0.0008	0.002	0.0009	0.120	0.020	0.041
Correlation* (r)	0.523	0.474	0.436	0.910	0.860	0.937
p value	0.114	0.141	0.164	0.002	0.006	0.0009

* log scale

Study 41. Effect of 50V extended beef on iron absorption.

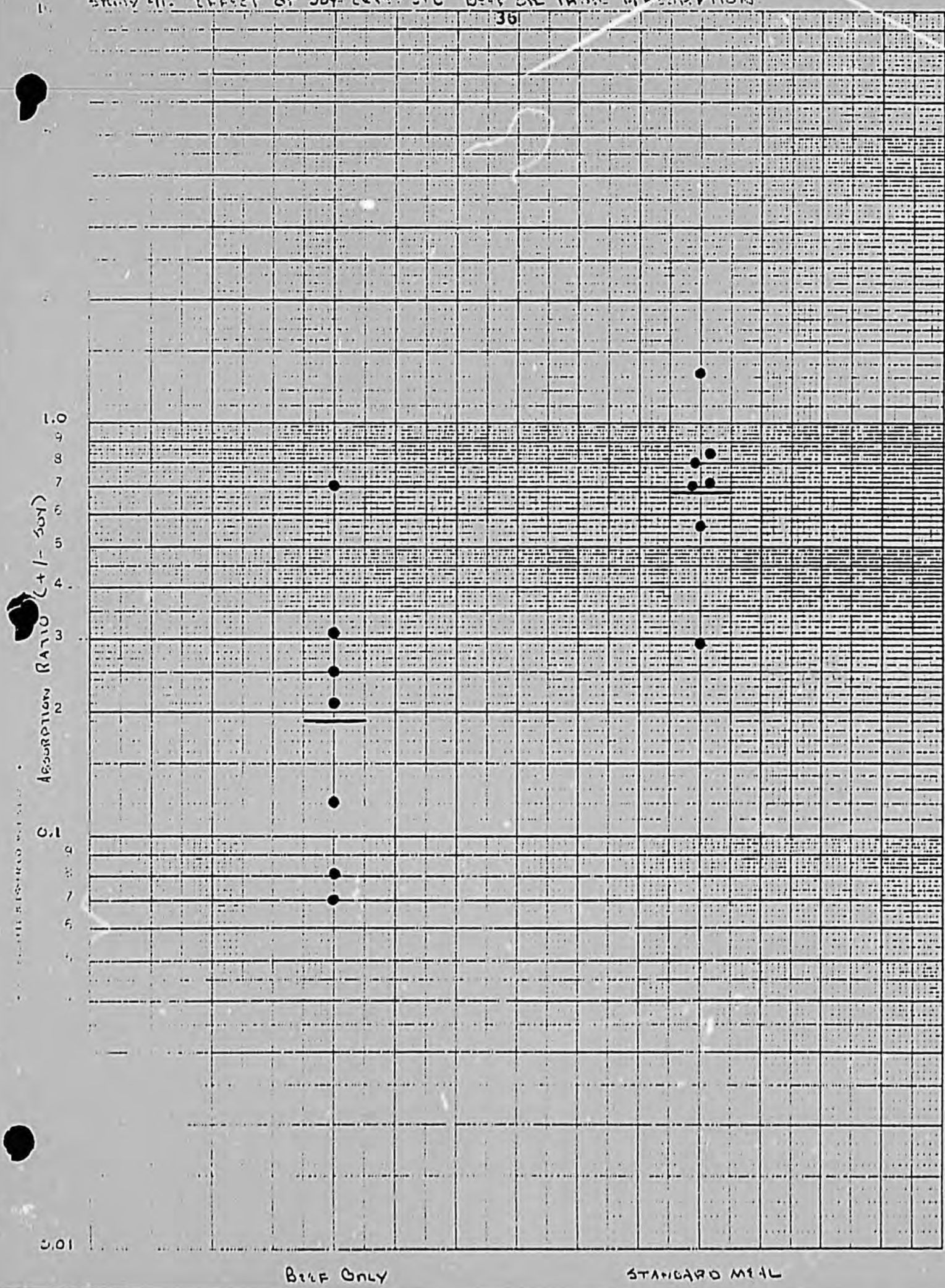


STUDY 41. EFFECT OF SOY-EXTENDED BEEF ON IRON ABSORPTION.



STUDY 41. EFFECT OF SOY-EXTENDED BEEF ON IRON ABSORPTION

36



STUDY 42

RICE FORTIFICATION IRON EXCHANGE I

ABSTRACT

A previous study (34) demonstrated that fortification iron coated onto the surface of rice grains by the use of a shellac did not exchange completely with the common pool of nonheme iron in the meal. This could have resulted either from incomplete release from the coating or incomplete exchange between iron cooked into rice grains and the rest of the iron in the meal. This study was designed to compare the absorption of iron cooked into rice and rice noodles with the absorption of the common iron pool in the rest of the meal. Iron was better absorbed from the common pool than from the label cooked into the rice. However, discrepancies between the standards prepared for the rice experiment make the result difficult to interpret.

RATIONALE

In last year's report, two separate studies were described in which rice was coated with $FeSO_4$ by a process developed by Hoffmann-LaRoche. This shellaking procedure was designed to prevent the loss of the mineral coat during washing of the food and to release fortification iron during the cooking process. In the first attempt, the shellac coat prevented complete dissolution of the added iron. In a second study (34), a thinner coat was applied which appeared to more completely release the iron during cooking. However, when absorption of the iron coated onto the rice was compared to absorption of iron added to another meal item, a marked difference in absorption was observed. This suggested that iron released during the cooking process might have been absorbed into the rice grain and thus failed to exchange completely with the common pool of nonheme dietary iron. If so, this would represent a major obstacle to the use of rice for iron fortification.

The present study was designed to determine whether radioiron added to the water used to cook rice undergoes complete isotopic exchange with a second form of nonheme iron added to the meal. The format of the study was similar to that of Study 34 except that radioiron was added to the cooking water as an extrinsic tag rather than used to coat the rice grain.

DESIGN

This study was performed in 11 normal subjects who were fed two separate meals. The meals differed only in respect to the form of the rice. Each contained a quarter pound of beef (113 g uncooked weight), 80 g applesauce, and 100 g rice (uncooked weight). The first meal was prepared by adding

1600 g washed rice to a large cooker containing 1900 ml water, 20 g salt, and 34 g margarine. Sixteen ml radioiron containing 64 mg iron as $FeSO_4$ was added to the water; the food was stirred thoroughly and then cooked for 35 min. The cooking water was almost totally absorbed by the rice. The second radioiron label (4.0 mg iron as $FeCl_3$ per person) was mixed with the applesauce prior to serving.

In the second meal rice noodles, rather than whole grain rice, were served. As in the first meal, sufficient radioactive $FeSO_4$ was added to the cooking water to provide the equivalent of 4 mg iron per serving. The noodles were added to the boiling water which was immediately removed from the heat and allowed to cool. All the water was absorbed by the noodles within approximately 5 min. The alternate form of radioiron was added as $FeCl_3$ (4.0 mg iron) to the applesauce.

RESULTS

Values for percentage absorption are listed in the accompanying tale. In the meals containing whole grain rice (A, B), mean absorption of the $FeSO_4$ added to the cooking water averaged 1.39% compared to 1.75% for the iron added to the applesauce. This 26% difference in absorption was statistically significant ($t=3.14$, $p=0.005$). From meals containing rice noodles (C, D), mean absorption of the $FeSO_4$ used for cooking averaged 1.43% as compared to a mean of 2.23% for $FeCl_3$ added to the applesauce. This 56% difference in absorption was also statistically significant ($t-7.62$, $p < 0.001$).

COMMENT

The findings in this study were unexpected. While it was anticipated that a difference in the absorption of nonheme iron might be observed with whole grain rice for reasons described above, it was not expected that this would occur with rice noodles. One would expect rice noodles to be digested rapidly and provide no barrier to complete isotopic exchange. Two sets of radioiron counting standards were prepared in this study and there were some discrepancies between them. It is possible that the aberrant results may have been the result of an error in the preparation of counting standards. The study was therefore repeated (study 47).

STUDY 42. Absorption from Rice Meal and Noodle Meal with Double Extrinsic Tags.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Rice Water (A)	Rice Food (B)	Noodle Water (C)	Noodle Food (D)	ABSORPTION RATIO B/A	D/C
1	25	44	80	0.45	0.56	1.01	1.67	1.24	1.65
2	24	50	115	0.52	0.86	0.92	1.71	1.65	1.85
3	24	46	70	0.70	1.06	0.86	1.53	1.51	1.77
4	23	45	138	0.78	0.85	0.76	1.12	1.08	1.47
5	26	44	106	0.88	1.05	0.85	1.08	1.19	1.27
6	23	42	121	0.98	1.42	1.13	1.95	1.44	1.72
7	23	42	124	1.28	1.46	1.00	1.66	1.14	1.66
8	23	44	58	2.26	2.26	1.88	2.21	1.00	1.17
9	23	46	103	2.53	2.18	1.31	1.46	0.86	1.11
10	23	46	152	4.92	5.40	4.80	8.37	1.09	1.74
11	24	46	14	9.55	19.20	7.65	15.20	2.01	1.98
MEAN*	24	45	86	1.39	1.75	1.43	2.23	1.26	1.56
			-1 SE	1.04	1.29	1.14	1.73	1.17	1.47
			+1 SE	1.86	2.37	1.80	2.87	1.36	1.65

* Geometric means except Age and Packed Cell Volume

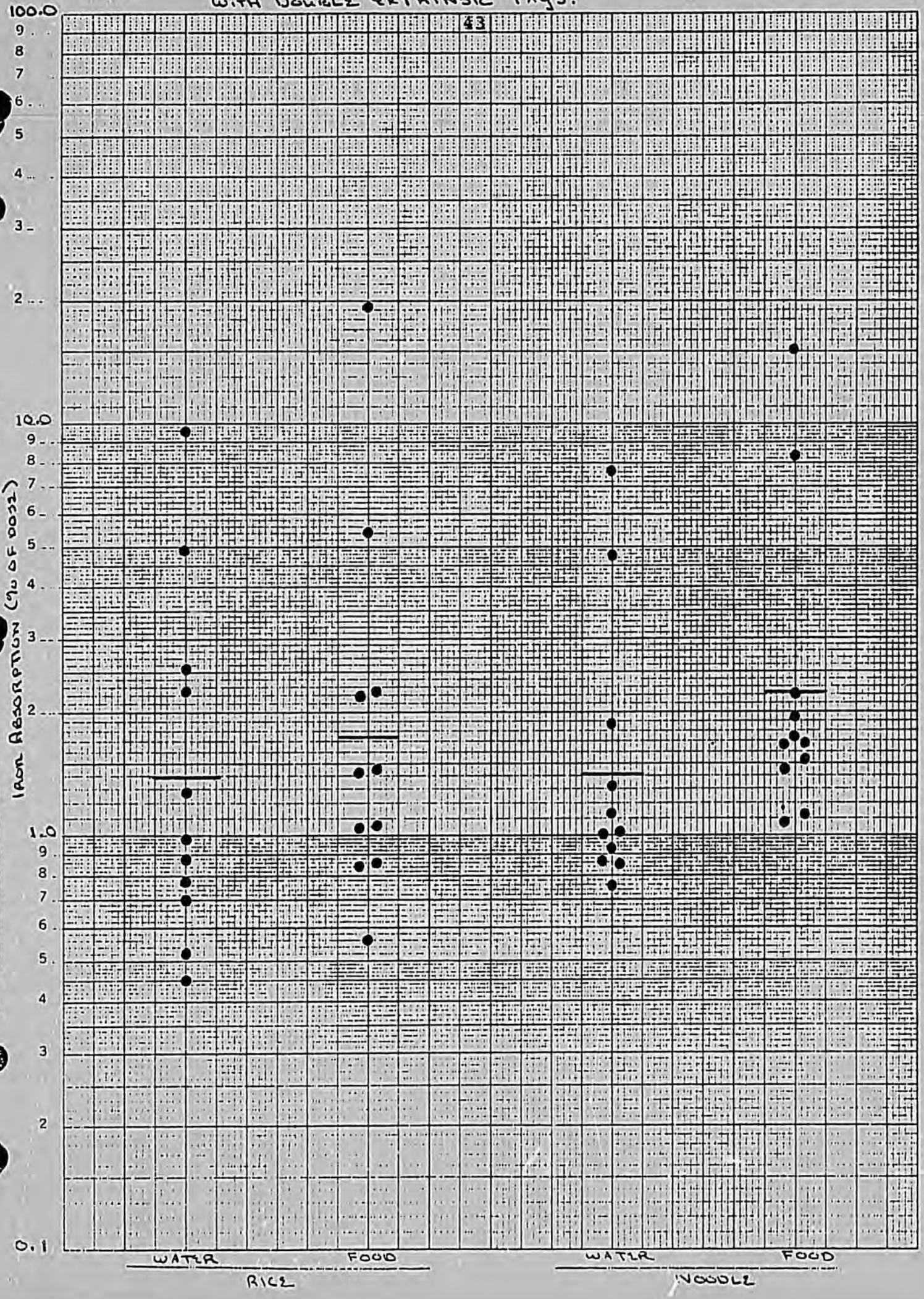
STUDY 42. Absorption from Rice Meal and Noodle Meal with Double Extrinsic Tags.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.79	0.97	0.62	1.22	0.78	0.64
-1 SE	0.74	0.86	0.53	1.09	0.69	0.60
+1 SE	0.85	1.10	0.73	1.37	0.89	0.63
1/ Ratio	1.26	1.03	1.61	0.82	1.28	1.56
Student's *t*	3.14	0.25	2.98	1.81	1.93	7.62
p value	0.005	0.40	0.007	0.05	0.04	$<10^{-5}$
Correlation* (r)	0.97	0.92	0.84	0.95	0.91	0.98
p value	$<10^{-5}$	$<10^{-5}$	0.0006	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$

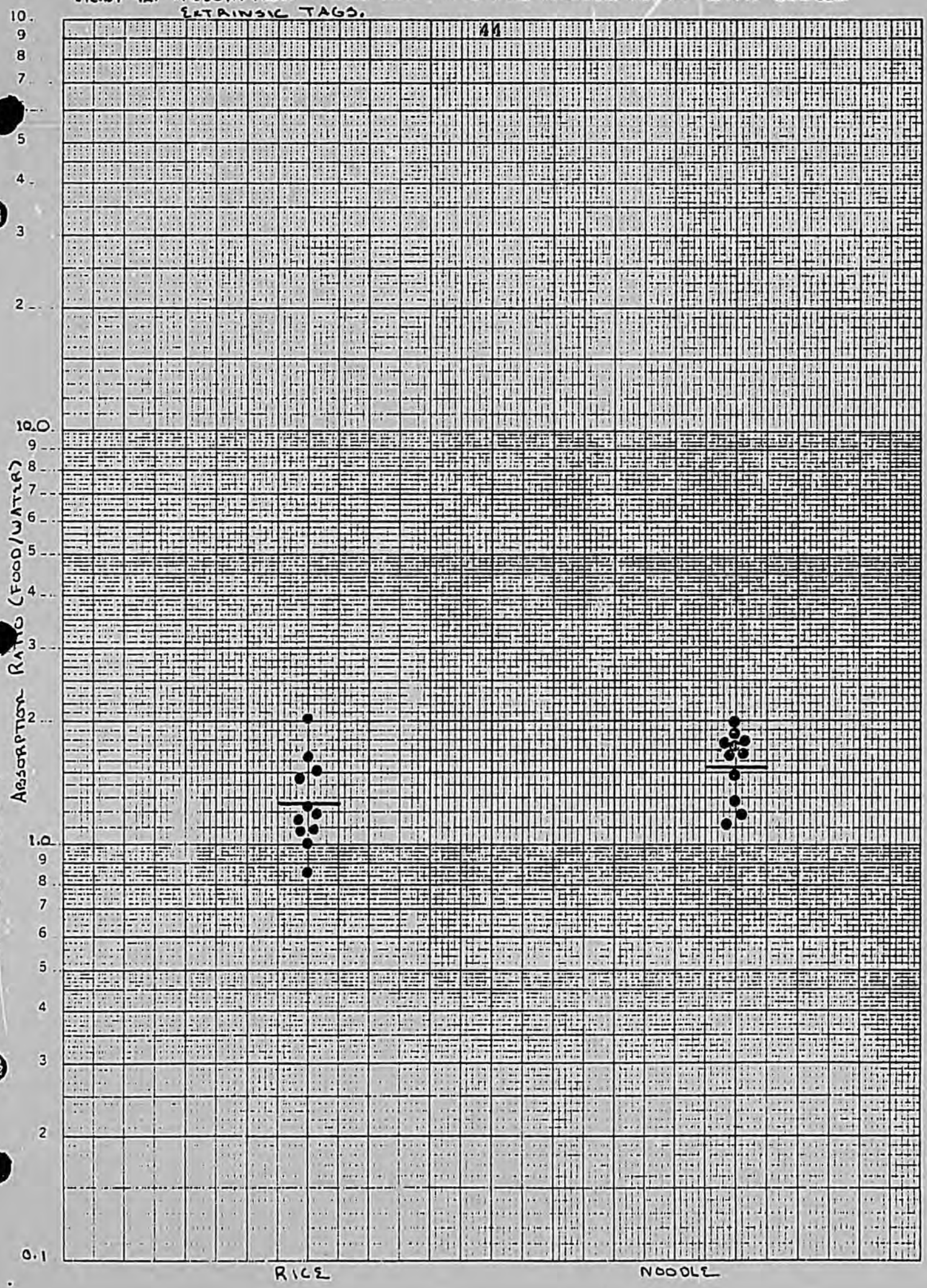
* log scale

43

WITH DOUBLE EXTRINSIC TAGS.



STUDY 12. ABSORPTION FROM RICE MEAL AND NOODLE MEAL WITH DOUBLE EXTRINSIC TAGS.



STUDY 43

EFFECT OF SOY (LOW LEVEL) ON ABSORPTION OF HEME AND NONHEME IRON FROM A HAMBURGER MEAL

ABSTRACT

The effect of 30 g soy flour (hydrated weight) added to 70 g beef eaten in a meal which included a bun, french fries, and a milkshake on absorption of heme and nonheme iron was examined. Mean percentage nonheme iron absorption fell from 5.05 to 1.90 while mean percentage heme iron absorption rose from 33.1 to 42.1. As a result, total iron absorption of the meal without soy was 0.56 mg, and 0.43 mg with soy. The marked effect of soy on percentage nonheme iron absorption was ameliorated by an increase in nonheme iron content as a result of the iron present in the soy as well as an increase in percentage absorption of heme iron.

RATIONALE

The previous two studies (40 and 41) demonstrated that the addition of a large quantity of soy flour to a meal of beef alone or to a complex meal reduced nonheme iron availability substantially. In this study a lower level of soy was employed, similar to that currently used in the school lunch program. A complex meal was used since the addition of other foods also reduced nonheme iron absorption (study 41). The data obtained in this study more closely predict the effect of soy extension as it is currently practiced in this country.

DESIGN

A total of 12 volunteer subjects were employed in this study. Three had marginal iron reserves. The study involved the administration of two separate meals tagged simultaneously with heme and nonheme extrinsic labels. The first meal contained 100 g lean beef (broiled for 10 min), french fries, and a vanilla milkshake. In the meal designated as "plus" soy, 70 g of beef was mixed with 12 g textured soy flour and 18 ml water to provide a 30% substitution of beef with hydrated soy. Both meals were tagged by adding simultaneously 0.1 mg iron as $^{59}FeCl_3$ and 5 uCi ^{55}Fe-labeled heme prepared in rabbits.

RESULTS

In the beef-only meal, percentage absorption of nonheme iron averaged 5.05% and fell significantly to 1.90% with the soy-extended hamburger. This 62% fall was highly significant ($t=5.63$, $p<0.001$). There was a reciprocal rise in heme iron absorption from 33.10% to 42.10% ($t=2.83$, $p=0.008$).

The effect on net iron absorption is outlined in the following table:

	Standard Meal			Standard Meal + Soy		
	Heme	Non-	Total	Heme	Non-	Total
Iron Content	1.20	3.20	4.24	0.84	4.15	4.99
Iron Absorbed	0.40	0.16	0.56	0.35	0.08	0.43

COMMENT

The effect of soy addition in this study is very similar to that observed in two previous studies (40,41). A sharp decrease in percent nonheme iron absorption is accompanied by a reciprocal rise in the percent absorption of heme iron. This presumably reflects some reciprocal control within the mucosal cell as discussed in Study 40. As a result, the effect of a decrease in the heme iron content of the meal is partially but not completely offset by improved absorption. In addition, the effect of soy on nonheme iron absorption is partially compensated for by an increase in nonheme iron content. Nevertheless, overall iron absorption from the soy-extended meal is only 77% that from the pure beef meal.

STUDY 43. Effect of Soy (School Lunch Program Levels) on Nonheme and Heme Iron Absorption.

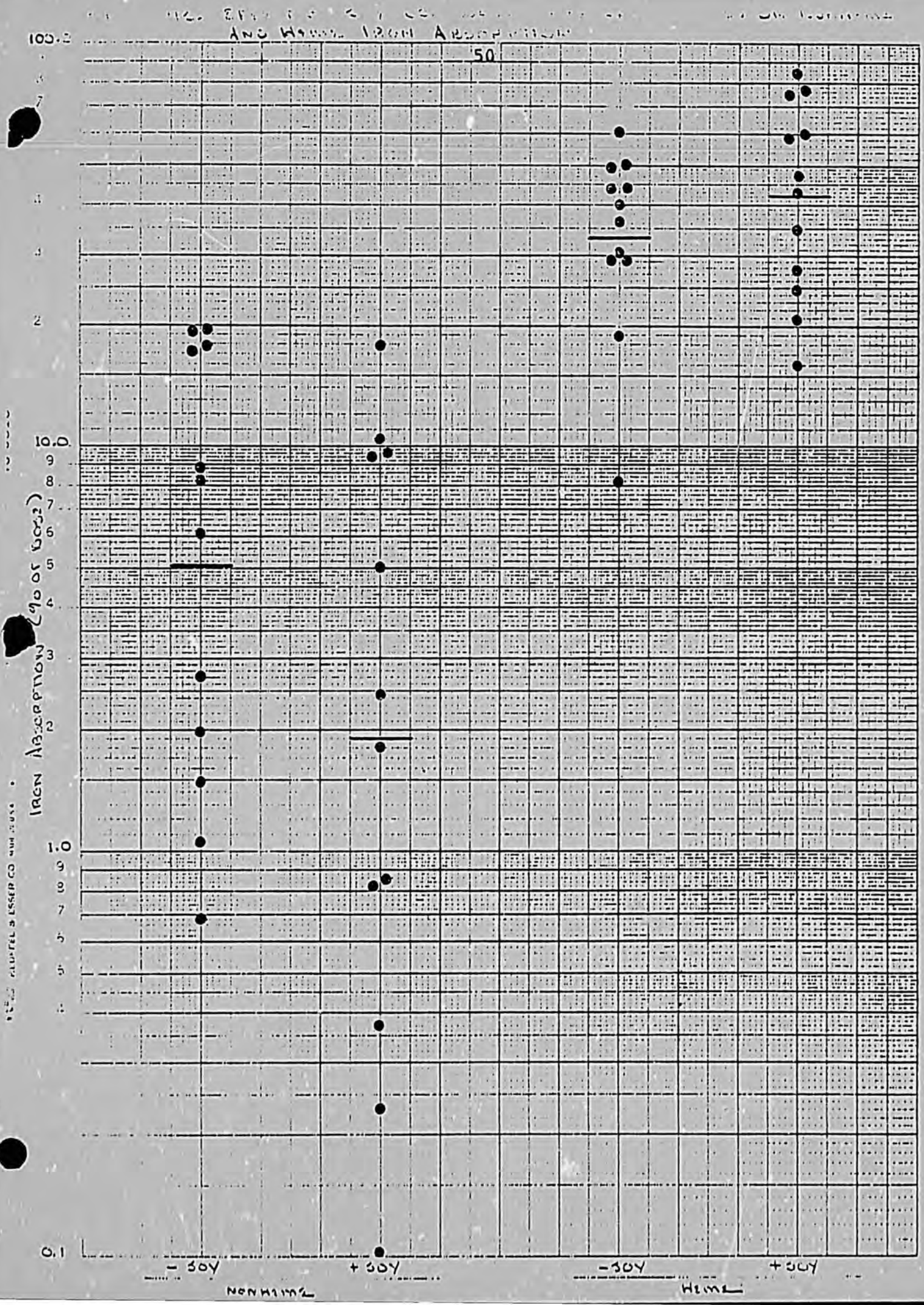
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION Nonheme -Soy (A)	IRON ABSORPTION Nonheme +Soy (B)	IRON ABSORPTION Heme -Soy (C)	IRON ABSORPTION Heme +Soy (D)	ABSORPTION RATIO +/- Soy Nonheme B/A	ABSORPTION RATIO +/- Soy Heme D/C
1	24	47	118	0.68	0.23	8.23	16.17	0.33	1.94
2	27	42	104	1.05	0.10	29.25	27.81	0.09	0.95
3	18	42	87	1.47	0.37	18.87	21.73	0.25	1.15
4	21	46	78	1.98	0.85	44.18	47.68	0.42	1.07
5	24	48	85	2.71	0.82	29.30	35.43	0.30	1.20
6	18	46	78	6.08	2.46	36.76	43.15	0.40	1.17
7	19	47	31	8.20	5.00	49.52	59.82	0.60	1.20
8	18	45	89	8.83	1.80	30.63	25.27	0.20	0.82
9	18	44	48	17.05	9.70	60.63	58.77	0.56	0.96
10	18	41	10	17.80	9.42	48.97	75.06	0.52	1.53
11	18	45	15	19.08	17.90	44.05	85.31	0.93	1.93
12	18	44	5	19.42	10.41	39.92	76.55	0.53	1.91
MEAN*	29	45	44	5.05	1.90	33.10	42.10	0.38	1.27
			-1 SE	3.54	1.16	28.36	35.98	0.32	1.17
			+1 SE	7.20	3.09	38.63	49.26	0.45	1.38

* Geometric means except Age and Packed Cell Volume

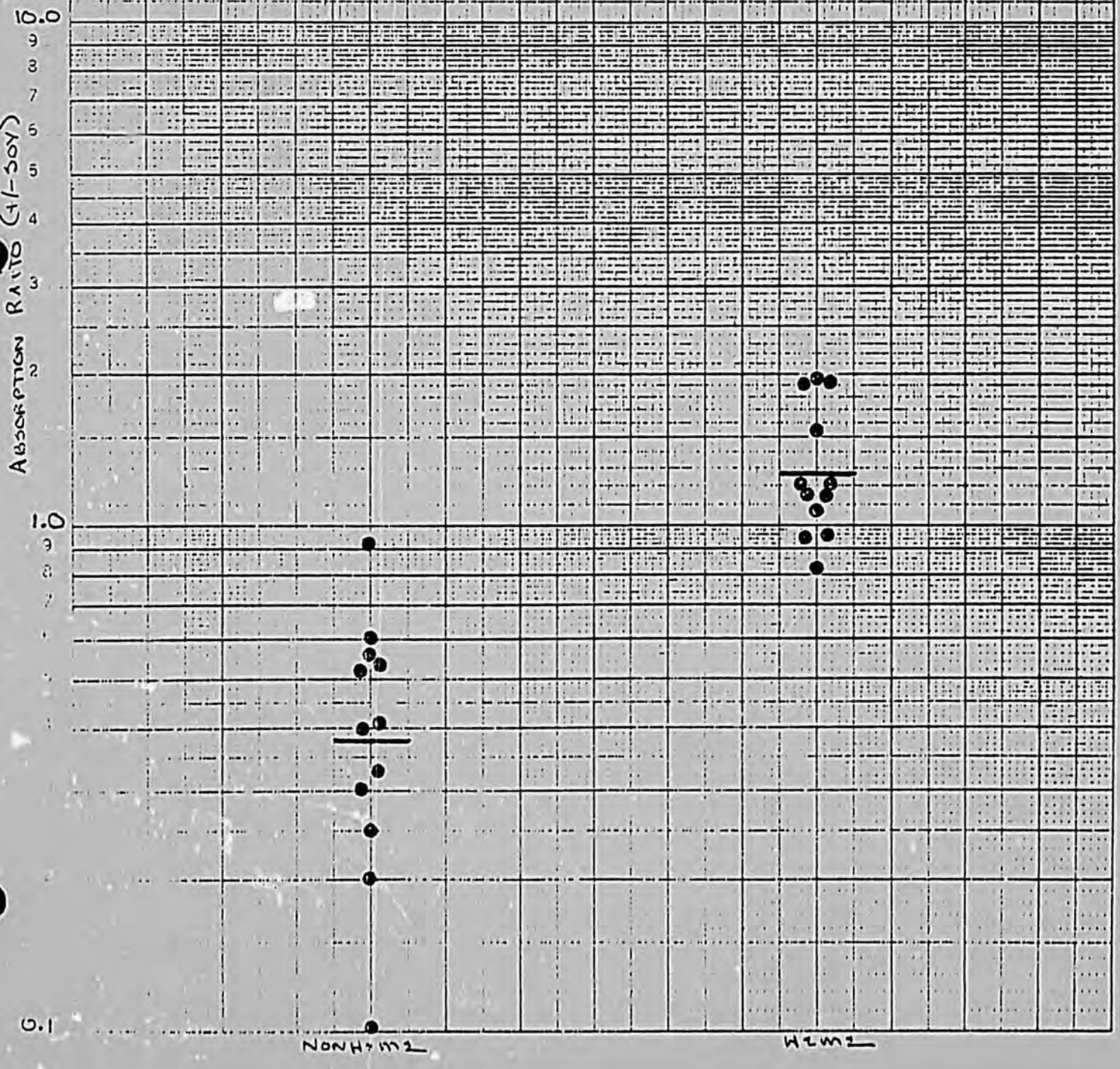
STUDY 43. Effect of Soy (School Lunch Program Levels) on Nonheme and Heme Iron Absorption.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	2.66	0.15	0.12	0.06	0.06	0.79
-1 SE	2.23	0.12	0.09	0.04	0.03	0.72
+1 SE	3.16	0.20	0.15	0.04	0.06	0.86
1/ Ratio	0.38	6.55	8.33	17.41	22.14	1.27
Student's *t*	5.63	7.33	8.91	7.28	8.65	2.83
p value	0.0001	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	$<10^{-5}$	0.008
Correlation* (r)	0.963	0.761	0.838	0.711	0.872	0.851
p value	$<10^{-5}$	0.002	0.0003	0.005	0.0001	0.0002

* log scale


50
100.0
10.0
1.0
0.1
IRON ABSORPTION (% OF BOD2)
- 30Y
+ 30Y
NON HIME
- 30Y
+ 30Y
HIME

STUDY 113. EFFECT OF SOY (SCHOOL LUNCH PROGRAM) BASED ON NONHEME AND HEME IRON ABSORPTION.



STUDY 44

BIOAVAILABILITY OF IRON FROM RED SORGHUM, WHITE SORGHUM, AND CORNMEAL

ABSTRACT

The extrinsic tag method was used to measure nonheme iron absorption from red sorghum porridge, white sorghum porridge, cornmeal porridge, and a reference dose of ferrous ascorbate. Iron was poorly absorbed from each of the three grain types, mean values being 0.81%, 0.61%, and 0.95% respectively. Mean reference absorption was 8.91%. This study demonstrated low iron availability from sorghum and cornmeal. There was no significant difference between the two types of sorghum.

RATIONALE

Sorghum is an important staple in some developing countries. While iron availability from other staples (corn, rice, wheat) has been studied extensively, only a few observations on sorghum are available. It is generally believed that red sorghum contains more tannin than white. The former is usually considered less palatable but is grown because less damage is done to the crops by birds. The present study was undertaken to determine whether there is any difference in iron availability from red and white sorghum and whether this is associated with a variation in tannin level.

DESIGN

Sorghum meal was obtained from LeSotho, Africa by Dr. Fred Barret (USDA). Four separate test meals were administered to each of nine male volunteers. In meals A, B, and C radioiron was added to a 90 g serving of porridge. The porridge was made with 15 g red sorghum flour (meal A), 15 g white sorghum flour (meal B), or 15 g cornmeal (meal C), which was mixed with 25 ml cold water and 0.5 g salt and then added to 50 ml boiling water and cooked 10 min. Sugar (4.4 g) was added to each meal. Meal D was the standard reference meal (3 mg $FeSO_4$ with 18.9 mg as ascorbic acid in 50 ml water labeled with 1 uCi ^{59}Fe).

RESULTS

Nonheme iron was poorly absorbed from all the porridge meals, geometric mean values being 0.81% for red sorghum, 0.61 for white sorghum, and 0.95% for cornmeal. There was no statistical difference in absorption between the different meals.

COMMENT

This study demonstrated low iron availability from both types of sorghum. There was no difference in absorption between red sorghum and white sorghum. Moroever, assay for tannic acid carried out at Kansas State University demonstrated some polyphenyl compounds in both samples but no tannin in either of them.

STUDY 44. Bioavailability of Iron from Red Sorghum, White Sorghum, and Cornmeal.

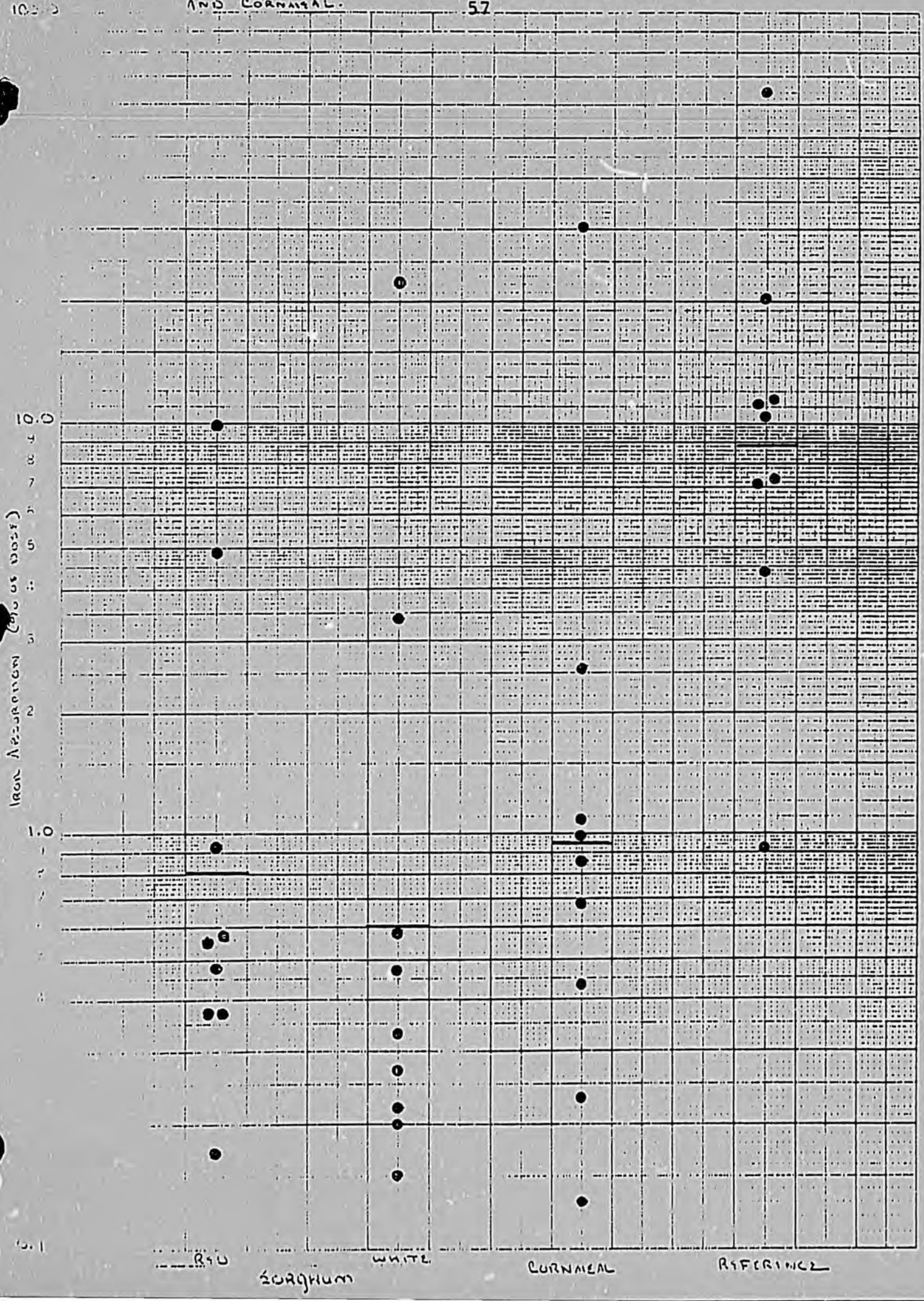
Subject	Age	Packed Cell Volume (%)	Ferritin (µg/l)	IRON ABSORPTION (% of dose) Sorghum Red (A)	Sorghum White (B)	Cornmeal (C)	REF (D)	ABSORPTION RATIO A/B	A/C	B/C
1	27	42	157	0.17	0.20	0.23	10.41	0.85	0.73	0.86
2	25	49	143	0.37	0.47	0.98	11.35	0.78	0.37	0.47
3	20	43	61	0.37	0.22	1.17	7.13	1.68	0.31	0.18
4	23	44	77	0.48	0.33	0.43	20.12	1.45	1.11	0.76
5	18	44	21	0.55	0.15	2.55	11.08	3.66	0.21	0.05
6	32	44	90	0.57	0.27	0.85	7.37	2.11	0.67	0.31
7	20	45	77	0.92	0.58	0.13	0.92	1.58	7.07	4.46
8	18	46	31	4.86	3.42	0.68	4.37	1.42	7.14	5.02
9	19	47	17	9.92	22.31	30.96	64.16	0.44	0.32	0.72
MEAN*	22	45	59	0.81	0.61	0.95	8.91	1.32	0.85	0.64
			-1 SE	0.52	0.35	0.56	6.09	1.08	0.55	0.40
			+1 SE	1.25	1.05	1.61	13.05	1.63	1.31	1.03

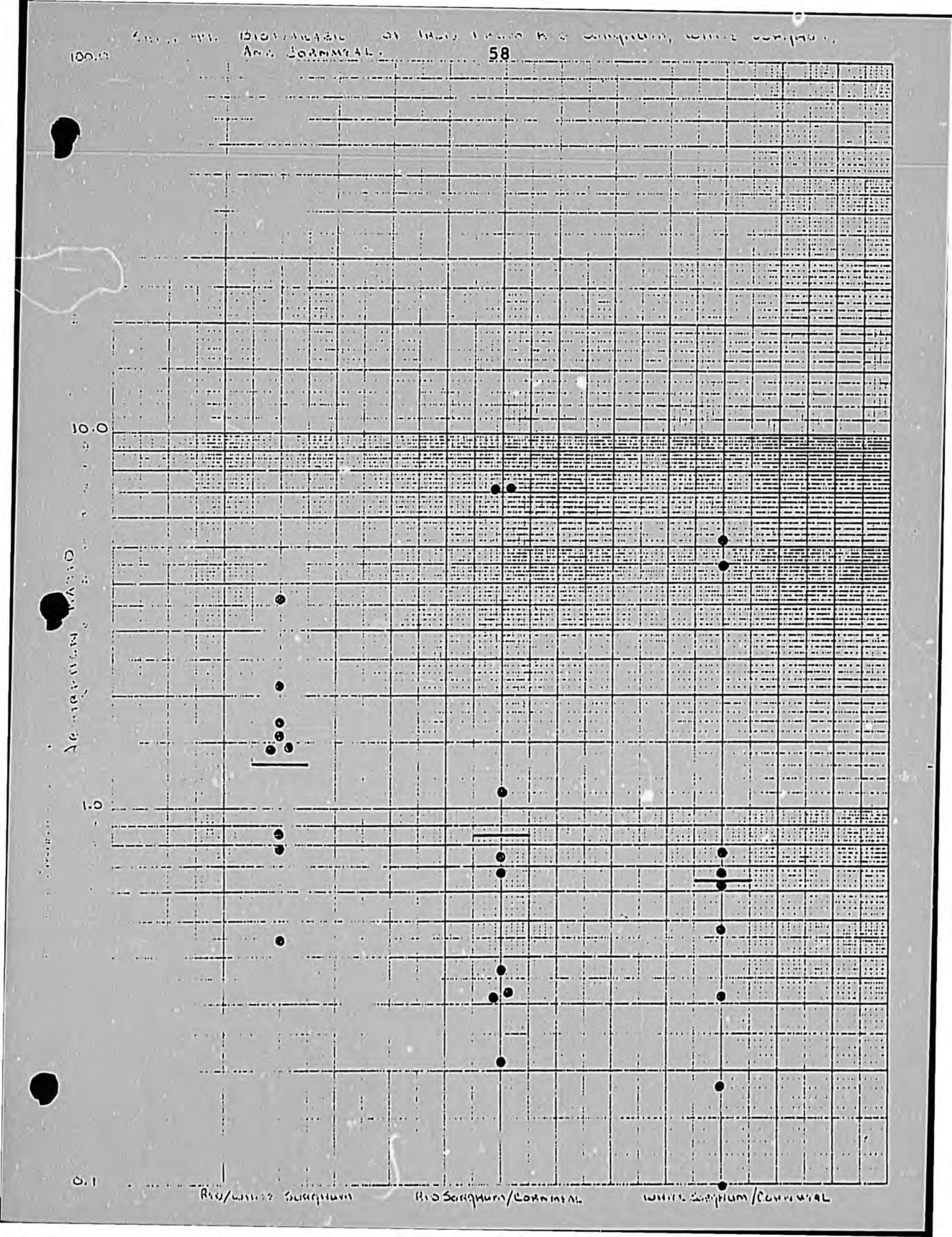
* Geometric means except Age and Packed Cell Volume

STUDY 44. Bioavailability of Iron from Red Sorghum, White Sorghum, and Cornmeal.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.32	0.85	0.09	0.64	0.07	0.11
-1 SE	1.08	0.55	0.05	0.40	0.04	0.08
+1 SE	1.63	1.31	1.15	1.03	0.12	0.15
1/ Ratio	0.75	1.18	10.97	1.56	14.55	9.32
Student's *t*	1.38	0.37	4.69	0.95	4.95	6.54
p value	0.103	0.36	0.0008	0.186	0.0006	0.0001
Correlation* (r)	0.94	0.60	0.22	0.61	0.36	0.76
p value	0.0001	0.042	0.281	0.040	0.172	0.009

* log scale


10.0
1.0
0.1
Iron Absorption (% of Dose)
Red Sorghum
White
Cornmeal
Reference


100.0
10.0
1.0
0.1
Red/White Sorghum
Red Sorghum/Cornmeal
White Sorghum/Cornmeal

STUDY 45
IRON BIOAVAILABILITY FROM SOYBEANS, BLACK BEANS, AND LENTILS

ABSTRACT

This study and the next (46) were designed to compare nonheme iron absorption from identical meals prepared from different legumes. A reference dose of ferrous acorbate was included in both studies to allow a direct comparison. Mean values for iron absorption from soup prepared from soybeans, black beans, and lentils were 1.66%, 0.84%, and 1.2% respectively. Absorption was highest from the soybean soup and lowest from the black bean soup. Although this difference is statistically significant at the 5% level, the actual variation is small, suggesting that the bioavailability of iron in different legumes is comparable.

RATIONALE

Early studies showed a wide variation in reported levels of iron absorption from soybeans. More recently, studies carried out under this contract have demonstrated relatively uniform, poor availability of nonheme iron from soybeans and purified soy products. Previous studies have in most instances also demonstrated relatively low iron availability from other legumes. The purpose of this study and the following one (46) was to compare iron availability from soybeans with that from similar legumes. They were selected to provide approximately the same quantity of protein and intrinsic iron.

DESIGN

Each subject ate four separate meals. The first three, A, B, and C, were prepared as a soup consisting of the legume seasoned with bay leaves, red pepper, garlic powder, onions, margarine, salt, and pepper. Radioiron was added as $^{55}FeCl_3$ or $^{59}FeCl_3$ to the soup as an extrinsic tag in each meal and each subject received 100 g of the legume plus 50 ml of the supernatant broth in which it was cooked (total serving 150 g). The iron content of all meals was made up to 4 mg by the addition of $FeCl_3$. The legume sources were soybeans (meal A), black beans (meal B), and lentils (meal C). The fourth meal (meal D) was a reference dose of ferrous ascorbate.

RESULTS

Iron was poorly absorbed from each of the meals, the highest absorption being obtained with soybeans and the lowest with black beans. Although the difference between these two meals was statistically significant at the 5% level, only 0.03 mg more iron was absorbed from the soybean soup.

COMMENT

This study demonstrates that there is little difference in the amount of iron absorbed from soybeans and that absorbed from other legumes. Although these legumes contain appreciable quantities of iron, the bioavailability is low. This does not appear to be a unique property of the soybean but rather a general finding among legumes.

STUDY 45. Bioavailability of Iron from Soybeans, Blackbeans, and Lentils.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Soybeans (A)	Blackbeans (B)	Lentils (C)	REF (D)	ABSORPTION RATIO A/B	A/C	B/C
1	20	50	73	0.30	0.46	0.20	3.35	0.65	1.50	2.30
2	21	46	79	0.40	0.11	1.76	18.66	3.63	0.22	0.06
3	20	49	62	0.87	1.88	0.71	35.00	0.46	1.22	2.64
4	23	46	132	1.20	0.40	0.41	3.60	3.00	2.92	0.97
5	20	49	91	1.41	0.13	2.35	15.20	7.83	0.60	0.07
6	19	46	20	1.55	1.26	1.61	11.73	1.23	0.96	0.78
7	18	46	23	2.53	1.73	1.38	52.80	1.46	1.83	1.25
8	18	42	80	4.42	4.63	1.81	31.60	0.95	2.44	2.55
9	20	46	52	5.31	1.06	2.38	37.58	5.00	2.23	0.44
10	20	47	86	9.81	2.48	2.86	15.28	3.95	3.43	0.86
MEAN*	20	47	61	1.66	0.84	1.20	16.32	1.97	1.38	0.70
			-1 SE	1.16	0.57	0.91	12.11	1.46	1.06	0.46
			+1 SE	2.36	1.23	1.58	22.00	2.65	1.79	1.07

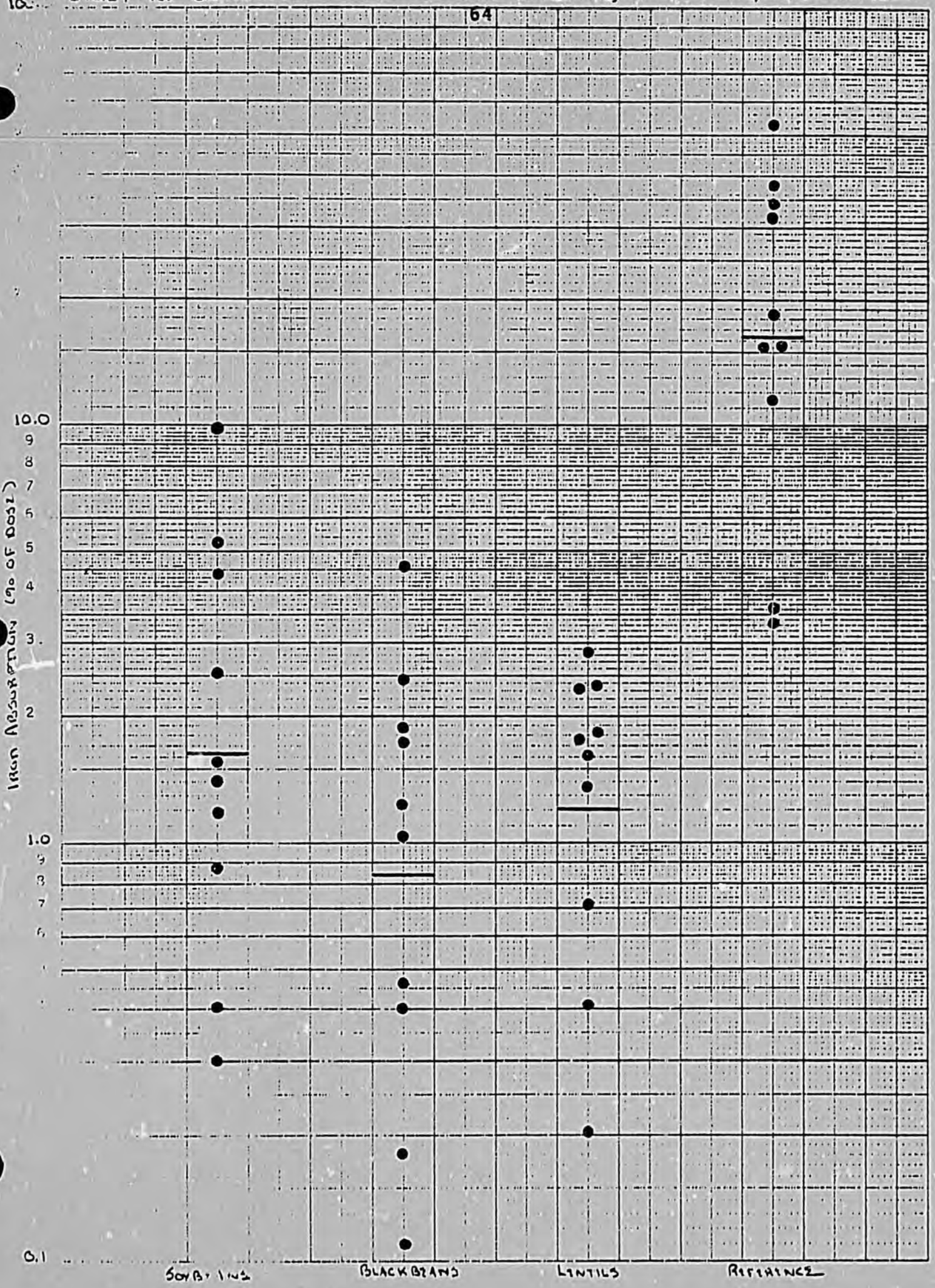
* Geometric means except Age and Packed Cell Volume

STUDY 45. Bioavailability of Iron from Soybeans, Blackbeans, and Lentils.

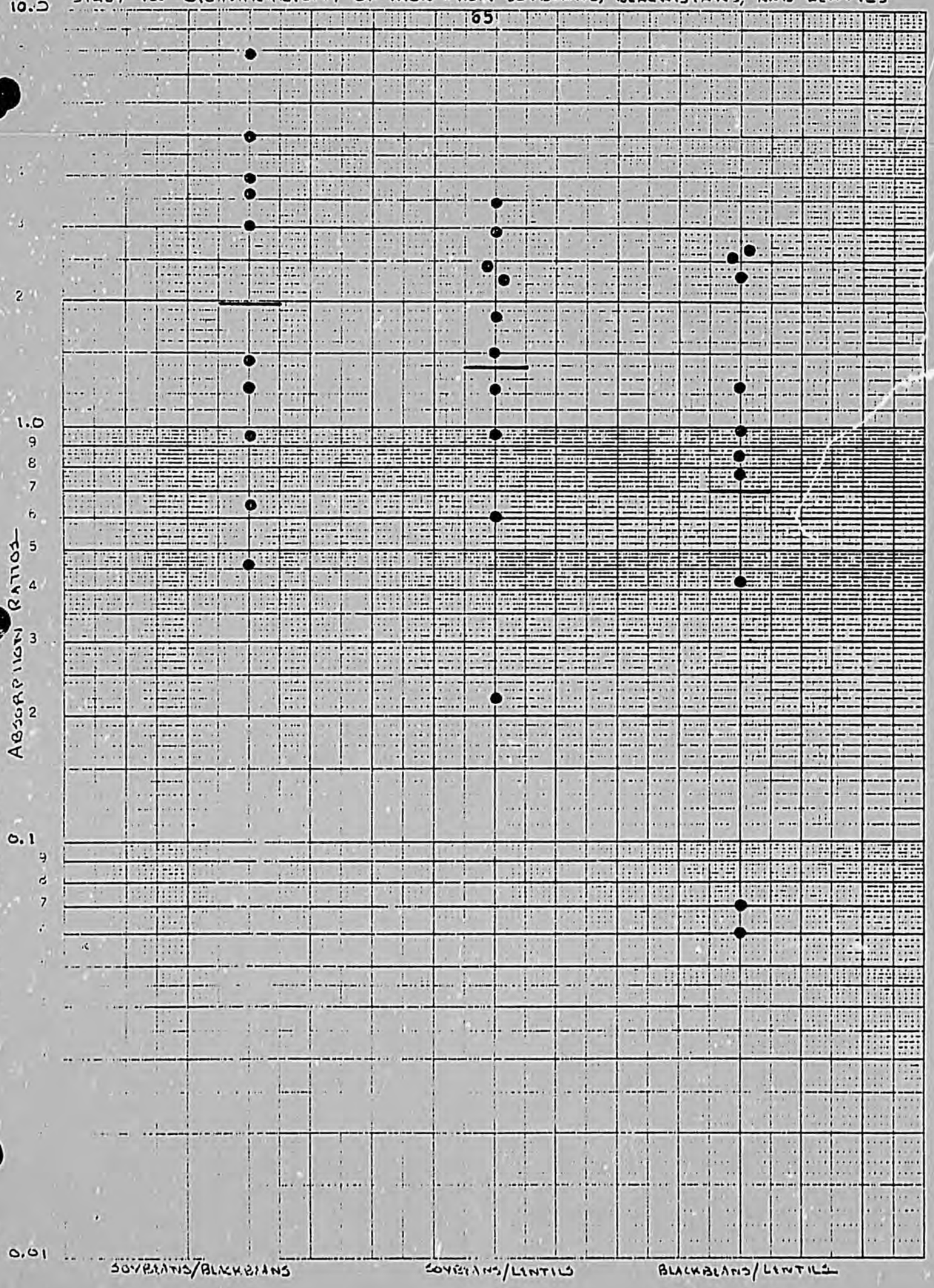
	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.97	1.38	0.10	0.70	0.05	0.07
-1 SE	1.46	1.06	0.07	0.46	0.04	0.06
+1 SE	2.65	1.79	0.14	1.07	0.07	0.09
1/ Ratio	0.51	0.73	9.83	1.43	19.36	13.52
Student's *t*	2.28	1.22	6.83	0.84	8.14	11.13
p value	0.024	0.127	$<10^{-5}$	0.21	$<10^{-5}$	$<10^{-5}$
Correlation* (r)	0.67	0.68	0.48	0.18	0.44	0.67
p value	0.017	0.016	0.080	0.311	0.101	0.017

* log scale

STUDY 46. BIOAVAILABILITY OF IRON FROM SOYBEANS, BLACK BEANS, AND LENTILS.



STUDY 115. BIOAVAILABILITY OF IRON FROM SOYBEANS, BLACKBEANS, AND LENTILS



STUDY 46

IRON BIOAVAILABILITY FROM MUNG BEANS, SPLIT PEAS, AND CORNMEAL

ABSTRACT

The present study is an extension of study 45 and provided additional information about iron availability from legumes. Cornmeal was included in this study to provide a comparison with a cereal. Mean absorption values were 1.91% for mung beans, 1.09% for split peas, 0.54% for cornmeal. The reference absorption was 18.82%. Thus, studies 45 and 46 taken together demonstrate that the bioavailability of iron from different legumes is very similar and somewhat better than that from unpurified cornmeal.

RATIONALE

Study 46 represents an extension of study 45 and was conducted for the same reasons. An additional comparison was made in this study with cornmeal mush prepared from unpurified cornmeal. This meal was added to provide a comparison with a commonly eaten cereal.

DESIGN

Iron absorption studies were carried out in ten male volunteers using the extrinsic tag method. Each subject ate four meals, one of which (meal D) was the reference dose of $FeSO_4$ (3 mg iron as $FeSO_4$ with 18.9 mg ascorbic acid in 50 ml water). The first two meals were prepared as a soup having a composition identical to that used in study 45 with the exception that the legumes in this study were mung beans (meal A) and split peas (meal B). The third meal (meal C) was a cornmeal mush prepared from 480 g cornmeal, 16 g salt, 140 g sugar, 73.1 g margarine, and 2,400 ml water. Each subject received a 150 g serving. The iron content of these meals was somewhat higher than that in study 45, each being made up to 7.8 mg by the addition of $FeCl_3$.

RESULTS

Geometric mean iron absorption values obtained were 1.91% for mung beans, 1.09% for split peas, and 0.54% for cornmeal. The reference absorption of 18.82% was very similar to that in study 45 (16.32%).

COMMENT

Iron absorption from legume-based soups was relatively low but very similar to the values obtained in study 45. A

direct comparison is possible since the reference absorption values of 18.82 and 16.32% are very similar. In addition, the mean serum ferritin concentrations of 52 ug/l and 61 ug/l imply similar iron stores. A significantly lower mean percentage absorption was obtained from cornmeal. However, it should be noted that the same cornmeal used in study 45 yielded considerably better availability. The reason for the very poor availability for this preparation of cornmeal is not clear.

It is evident that the bioavailability of iron in soybeans is very similar to that in other legumes and in unpurified cornmeal. This is demonstrated on the following graph which depicts the meal:reference ratios for all observations from studies 44, 45, and 46. The meal:reference ratio excludes the effect of iron stores, making observations in different subjects directly comparable. The results of these three studies led us to the conclusion that the availability of iron in legumes is comparable to that in cereals. The higher iron content of cereals would provide some advantage for iron nutrition. Furthermore, the inclusion of soybeans in cereal-based diets would carry no particular disadvantage for iron absorption.

STUDY 46. Bioavailability of Iron From Mungbeans, Split Peas, and Cornmeal.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Mungbeans (A)	Split Peas (B)	Cornmeal (C)	REF (D)	ABSORPTION RATIO A/B	A/C	B/C
1	20	44	36	0.76	0.27	0.58	25.31	2.81	1.31	0.46
2	19	45	81	1.07	0.77	0.32	14.28	1.38	3.34	2.40
3	19	44	77	1.13	0.80	0.48	31.35	1.41	2.35	1.66
4	20	45	157	1.75	2.30	0.26	43.67	0.76	6.73	8.84
5	18	44	65	1.87	0.96	1.28	20.47	1.94	1.46	0.75
6	18	46	38	2.17	1.53	0.25	7.15	1.41	8.68	6.12
7	18	45	37	2.20	0.98	1.31	21.38	2.24	1.67	0.74
8	18	44	19	3.37	1.23	0.70	12.93	2.73	4.81	1.75
9	18	45	47	3.43	1.62	0.48	34.06	2.11	7.14	3.37
10	18	46	51	3.91	2.32	0.67	9.18	1.68	5.83	3.46
MEAN*	19	45	52	1.91	1.09	0.54	18.82	1.74	3.53	2.03
			-1 SE	1.60	0.89	0.45	15.55	1.54	2.82	1.50
			+1 SE	2.27	1.34	0.65	22.78	1.97	4.41	2.74

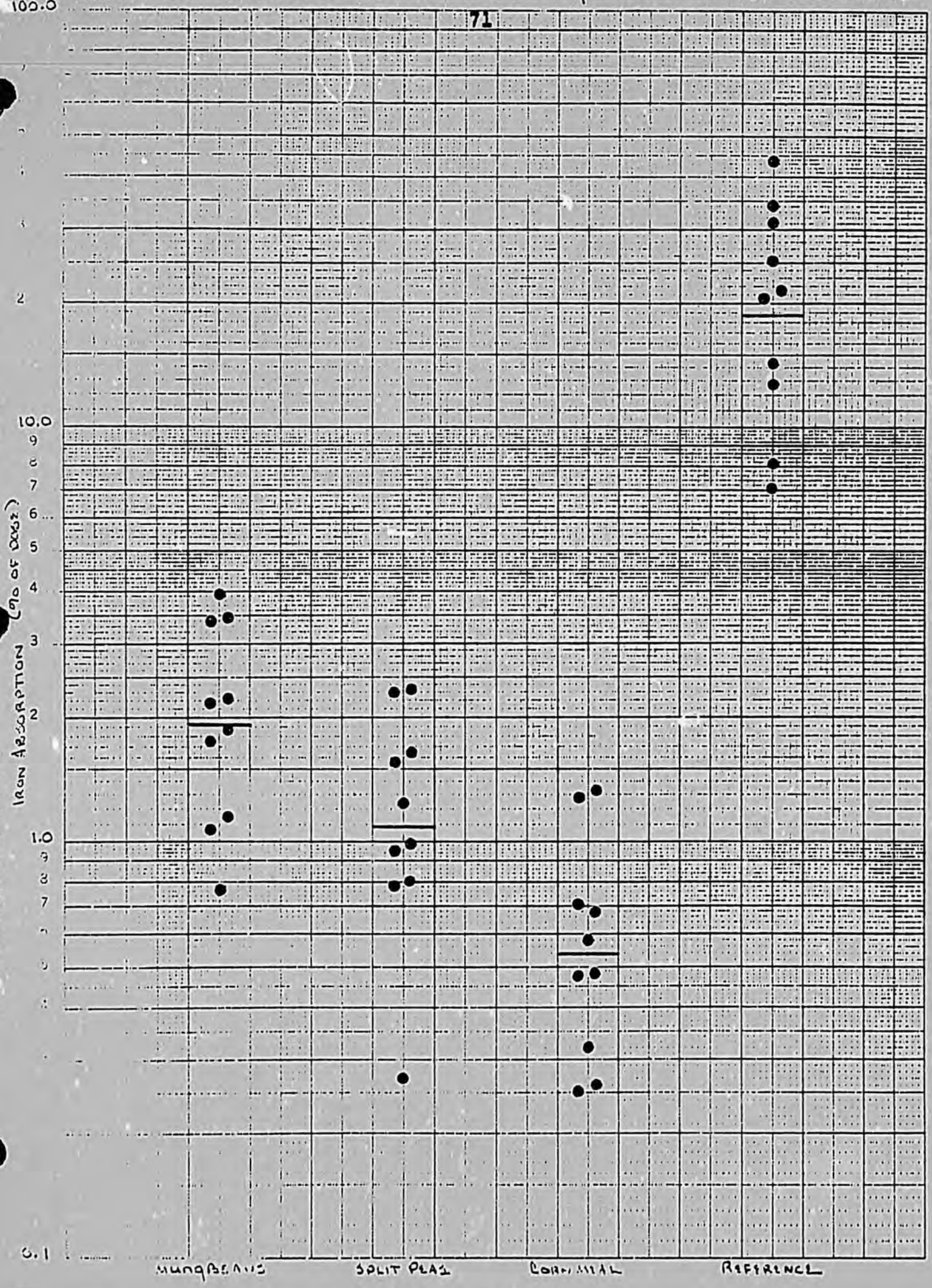
* Geometric means except Age and Packed Cell Volume

STUDY 46. Bioavailability of Iron from Mungbeans, Split Peas, and Cornmeal.

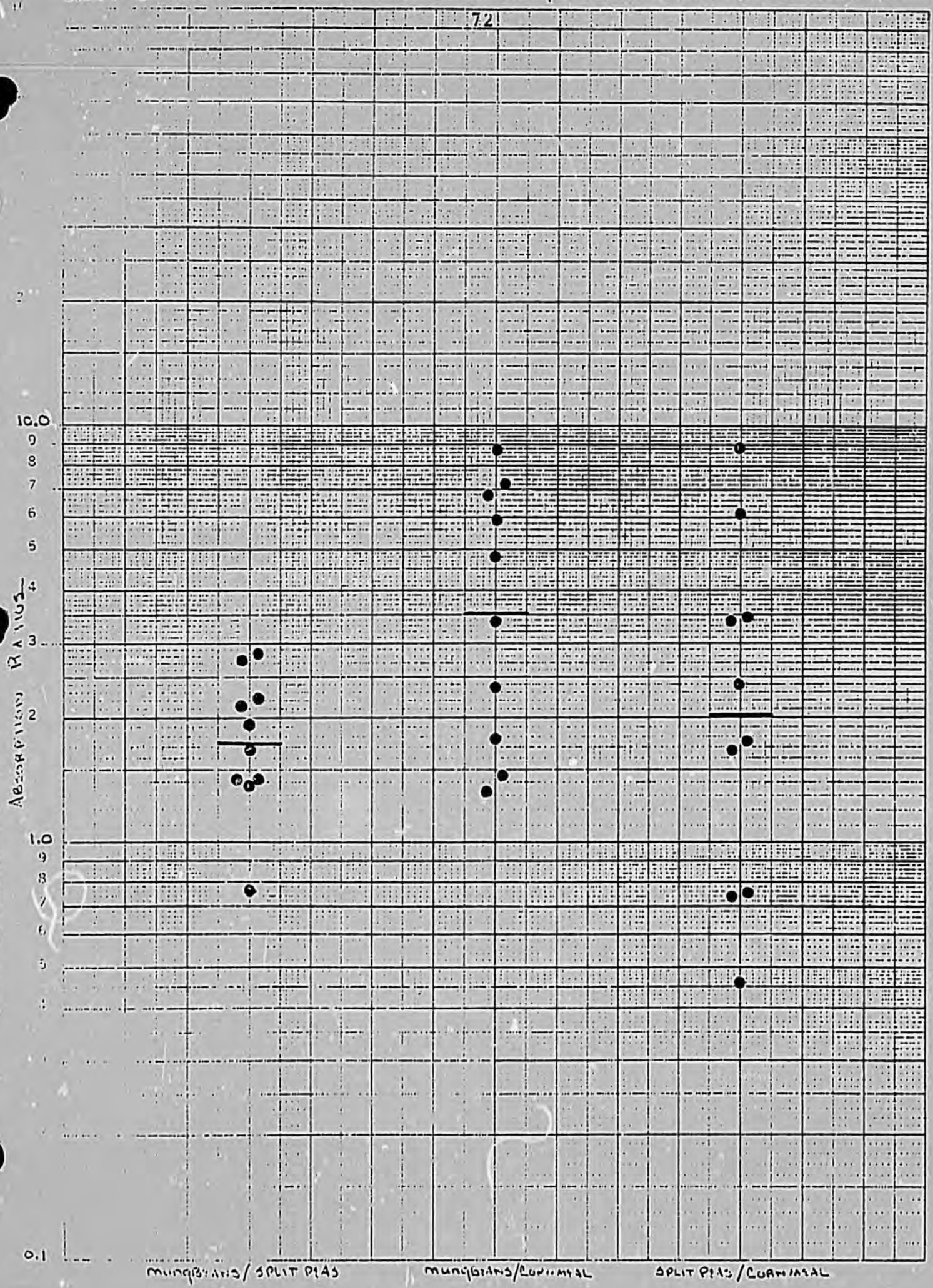
	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.74	3.53	0.10	2.03	0.06	0.03
-1 SE	1.54	2.82	0.08	1.50	0.04	0.02
+1 SE	1.97	4.41	0.14	2.74	0.08	0.04
1/ Ratio	0.57	0.28	9.85	0.49	17.14	34.73
Student's *t*	4.48	5.60	7.67	2.34	9.44	13.51
p value	0.0008	0.0002	$<10^{-5}$	0.0219	$<10^{-5}$	$<10^{-5}$
Correlation* (r)	0.789	0.210	0.345	0.223	0.185	0.023
p value	0.0033	0.280	0.164	0.267	0.303	0.474

* log scale

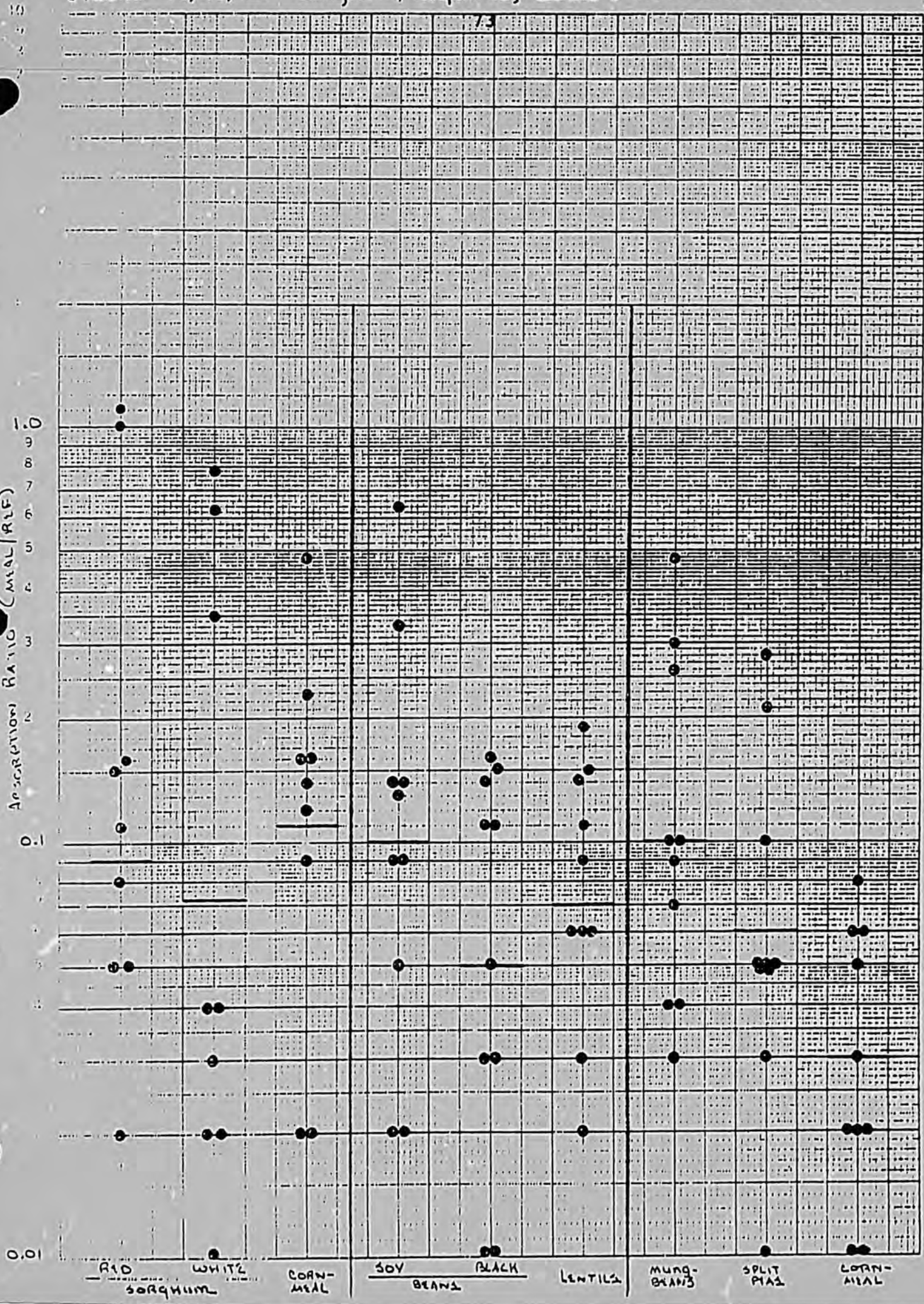
STUDY 46. BIOAVAILABILITY OF IRON FROM MUNG BEANS, SPLIT PEAS, AND CORNMEAL.



STUDY 46. BIOAVAILABILITY OF IRON FROM MUNGBEANS, SPLIT PEAS, AND CORNMEAL.



STUDIES 44, 45, 46. SORGHUM, LEGUMES, CORN : REFERENCE DOSE



STUDY 47

RICE FORTIFICATION IRON EXCHANGE II

ABSTRACT

This is the final study in a series to determine whether fortification iron added to rice prior to cooking enters the nonheme common pool. Absorption of radioiron cooked into rice or rice noodles was compared with the absorption from another tag put into another component of the meal. Mean percentage absorptions for the rice meal were 2.17% for iron cooked into the rice and 1.96% for the other common pool tag; with the noodle meal, the values were 2.6 and 2.9%. These results indicate that fortification iron added to rice before cooking enters the nonheme common pool in the meal.

RATIONALE

The rationale for this study was outlined in Study 42. The design of the present study was the same except that in addition to the meal containing rice, applesauce, and meat, 50 mg ascorbic acid was added in the form of 4 oz fresh orange juice. It was thought that by examining isotopic ratios at a higher level of assimilation, the sensitivity of the comparison would be enhanced.

DESIGN

The meals employed in this study and their preparation are exactly as that described in Study 42 with the exception that 4 oz orange juice was ingested with both meals.

RESULTS

Mean absorption of $FeSO_4$ added to the water used to cook rice (meal A) averaged 2.17%, nearly identical to the mean of 1.96% from $FeCl_3$ added to the applesauce. The mean ratio B/A=0.90 was not statisticaly significant (student's t=1.34, p=0.11).

Results with the meal containing rice noodles were very similar. Absorption of $FeSO_4$ added to the cooking water averaged 2.60% as compared to a mean of 2.90% with the iron added to the applesauce. The mean ratio of 1.12 did not reflect a significant difference in absorption of these two iron forms (t=1.46, p=0.09).

COMMENT

The present study is the last in a series to determine whether iron used to coat rice or added to cooking water is absorbed to the same extent as nonheme iron in the complete meal. There are perhaps several reasons for differences observed in earlier studies. When $FeSO_4$ was used to coat the rice grain, the difference in absorption may have resulted from incomplete dissolution of the iron coat.

Why a difference was also observed when iron is simply added to the cooking water (Study 42) is not clear, but technical problems encountered in the preparation of the standards is a possible explanation. Nevertheless the present study suggests that rice is a feasible vehicle for fortification and that fortification iron present during the cooking process is just as available as iron in the meal with which it is consumed. The procedure used for rice coating by Hoffmann-LaRoche however is not suitable in its present form, and it is clear that an alternate approach to this food technology problem will be required if rice is pursued as a vehicle for fortification.

STUDY 47. Absorption from Rice Meal and Noodle Meal with Double Extrinsic Tags.

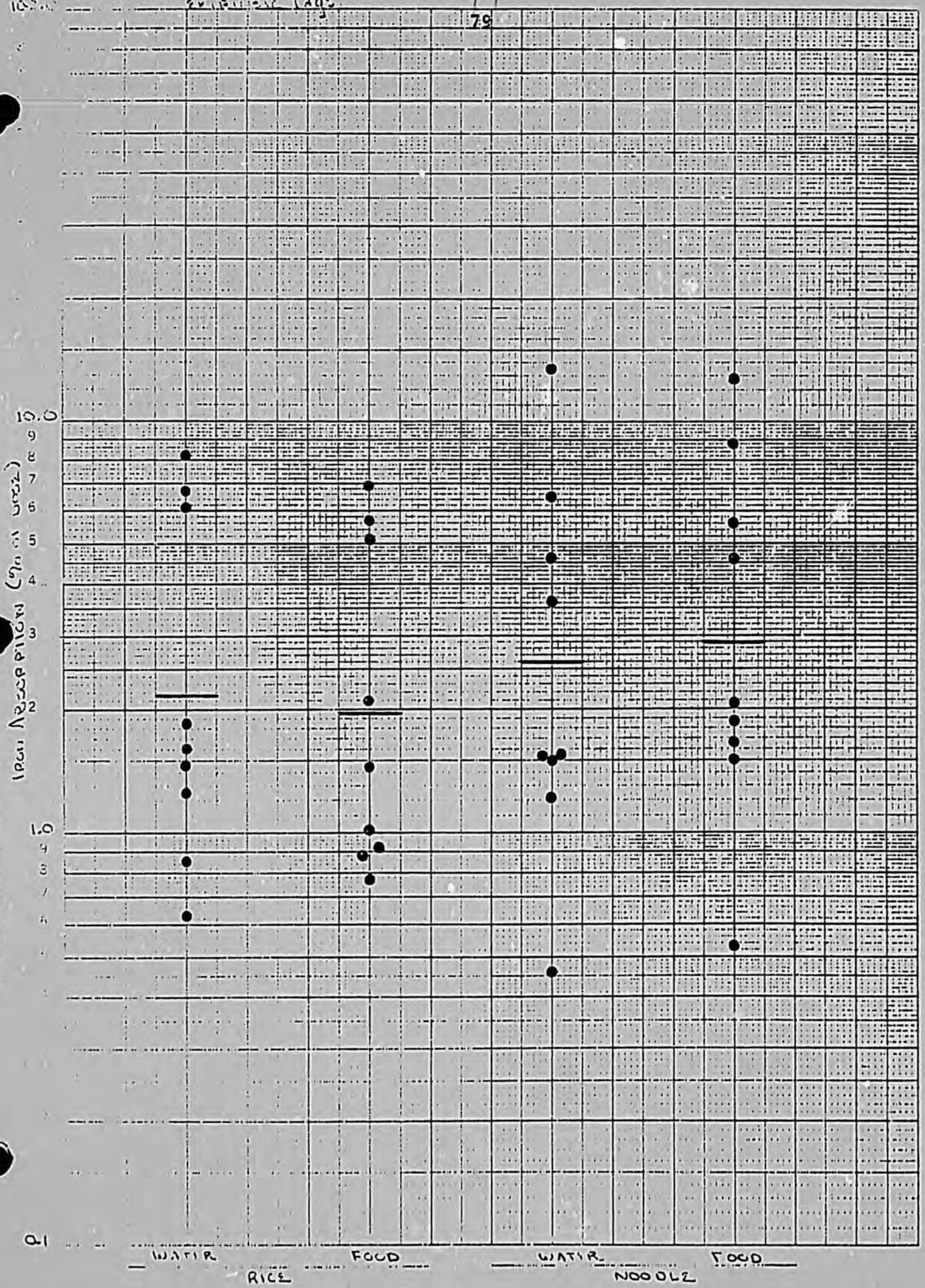
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (µg/l)	IRON ABSORPTION (% of dose) Rice Water (A)	Rice Food (B)	Noodle Water (C)	Noodle Food (D)	ABSORPTION RATIO B/A	D/C
1	32	40	55	0.63	0.91	0.46	0.53	1.44	1.15
2	42	44	136	0.85	0.77	1.51	2.06	0.90	1.36
3	19	45	31	1.25	0.87	1.52	1.66	0.69	1.09
4	24	44	139	1.43	1.02	1.50	1.88	0.71	1.25
5	23	43	54	1.60	1.46	1.21	1.50	0.91	1.23
6	28	46	75	1.86	2.10	3.66	5.61	1.12	1.53
7	23	45	48	6.08	5.13	6.48	8.81	0.84	1.35
8	26	44	20	6.76	5.72	4.63	4.63	0.84	1.00
9	23	46	13	8.16	6.90	13.65	12.86	0.84	0.94
MEAN*	27	44	63	2.17	1.96	2.60	2.90	0.90	1.12
			-1 SE	1.59	1.46	1.85	2.08	0.84	1.04
			+1 SE	2.95	2.62	3.64	4.06	0.97	1.31

* Geometric means except Age and Packed Cell Volume

STUDY 47. Absorption from Rice Meal and Noodle Meal with Double Extrinsic Tags.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.11	0.83	0.75	0.75	0.67	0.89
-1 SE	1.03	0.71	0.64	0.62	0.57	0.83
+1 SE	1.19	0.99	0.87	0.91	0.80	0.97
1/ Ratio	0.90	1.20	1.34	1.33	1.48	1.12
Student's *t*	1.34	1.08	1.90	1.49	2.29	1.46
p value	0.11	0.15	0.05	0.09	0.03	0.09
Correlation* (r)	0.97	0.87	0.89	0.33	0.86	0.97
p value	$<10^{-5}$	0.001	0.0007	0.003	0.002	$<10^{-5}$

* log scale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9
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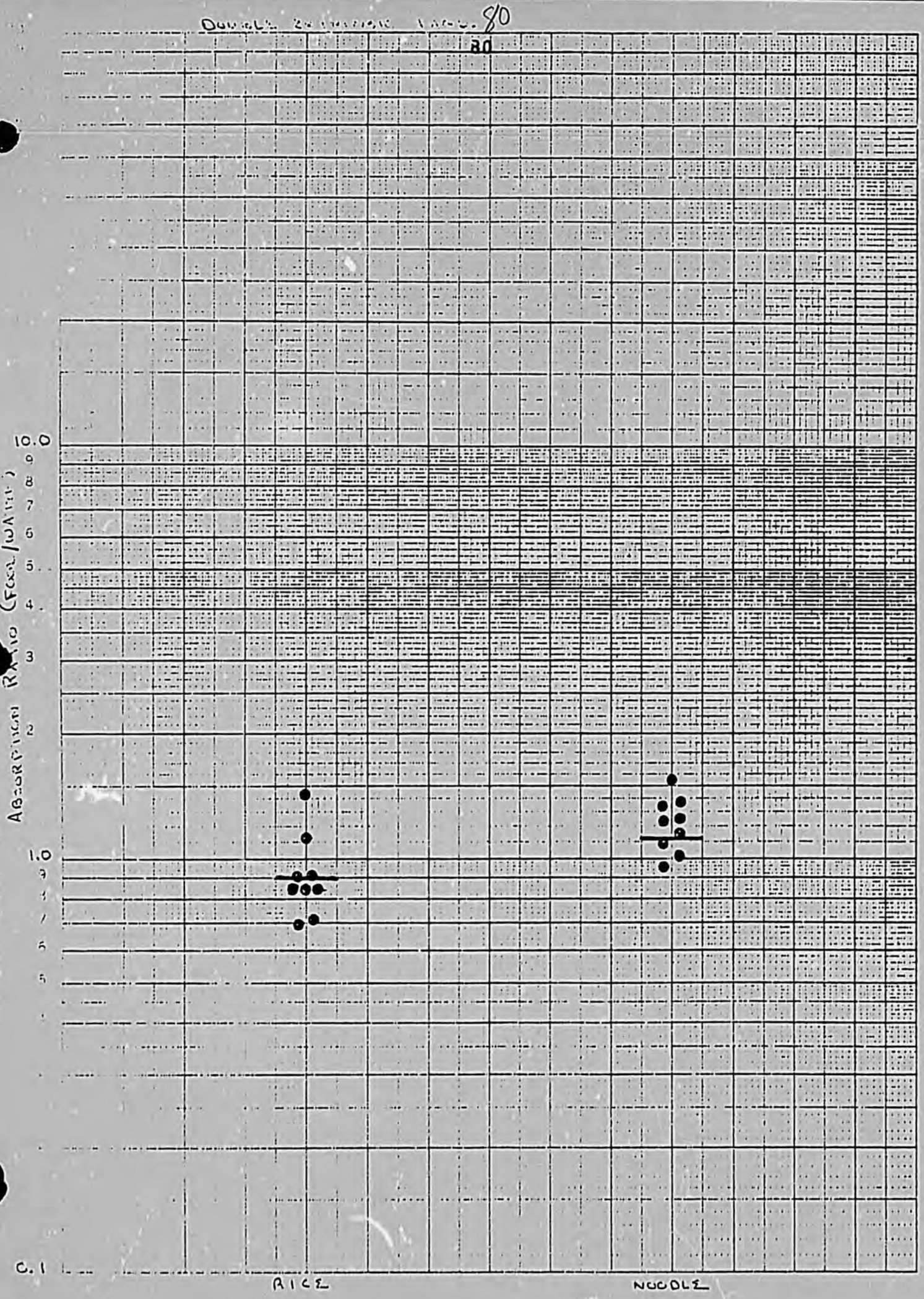
WATER
FOOD
RICE
WATER
FOOD
NOODLE


80
10.0
1.0
0.1
RICE
NOODLE

APPENDIX B

-PUBLICATIONS-

From: Cook JD, Morck TA, Skikne BS, Lynch SR. Dietary inhibitors of iron absorption in man. In: Wagner PA, Kirk JR, eds. Proceedings of the Florida Symposium on Micronutrients in Human Nutrition. Gainesville: University of Florida, 1981;99-105.

#85

DIETARY INHIBITORS OF IRON ABSORPTION IN MAN

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It is now well known that when several food items are ingested in the same meal, a common pool of nonheme iron is formed, the absorption of which is measured by extrinsic radioiron tagging (Cook et al, 1972; Bjorn-Rasmussen et al, 1972). Studies of food iron absorption with this technic have focused primarily on dietary constituents that enhance the absorption of nonheme iron. Of these, ascorbic acid and animal tissue including fish and poultry are of overriding importance. In fact, a method has recently been proposed for predicting iron absorption from a meal based on its content of ascorbic acid and animal tissue (Monsen et al, 1978). This model is an important advance because it focuses on iron availability rather than total iron content. However, the model is oversimplified because many dietary factors, especially substances that inhibit iron absorption, are not taken into account. These inhibitory factors are the subject of the present review.

In evaluating the effect of an inhibitor or an enhancer of iron absorption, it is necessary to choose a basal meal that is presumably 'neutral' with respect to iron absorption. The degree of inhibition/enhancement will undoubtedly depend on the choice of this reference meal. For example, the effect of an inhibitor may be much less dramatic in a meal that also contains an enhancer such as ascorbic acid or animal protein. The use of baseline

meals with different levels of availability undoubtedly explains some of the conflicting reports on the potency of dietary inhibitors. The major known inhibitors of food iron absorption are listed in the following table.

Dietary Inhibitors of Iron Absorption

	potency
Egg	++
Bran	+++
Phytate	±
EDTA	±
Ca/PO_4	±
Tea	+++
Coffee	++
Protein	
Ovalbumin	+
Soy	+++

Eggs. Early studies employing biosynthetic tagging indicated that iron in egg, especially in yolk, is poorly assimilated. For example, the addition of an egg to a meal consisting of bread reduced percentage absorption from 5.1 to 0.9% (Elwood et al, 1968). Peters et al (1971) reported that the major inhibitory effect was in egg yolk. The addition of egg white to ferrous ascorbate reduced percentage absorption from 33 to 14% whereas the addition of yolk caused a decrease to 3.9%. The inhibiting effect is attributed to vitellin, the major phosphoprotein complex in egg yolk. A less dramatic inhibiting effect has been observed in more recent studies. When a boiled egg was added to a continental breakfast, Rossander et al (1979) observed a de-

crease in absorption that was not statistically significant. In a study by Cook and Monsen (1976a), a powdered egg was found to be no more inhibitory than whole milk or egg albumin. These inconsistencies may relate to the nature of the meal used to examine the effect of egg.

Bran. In the classic chemical balance studies performed by Widdowson and McCance (1942), it was noted that less iron is absorbed from a diet containing brown bread than one containing white bread. In later studies using extrinsic radioiron tagging, the degree of inhibition in nonheme iron absorption was proportional to the amount of bran added. Bjorn-Rasmussen (1974) reported that the addition of 1.7, 3.3, 6.7, and 10% bran produced a decrease in absorption of 18, 31, 57, and 54% respectively. In studies done in our laboratory, we observed a 74% decrease in percentage absorption of nonheme iron, from 2.4 to 0.6%, when 12 g bran was added to a meal containing a milkshake and muffins. When the meal contained an absorption enhancer the inhibiting effect was less pronounced. For example, when meat was added to the meal containing 12 g bran, absorption decreased from 3.5 to 1.7% (51%). When 100 mg ascorbic acid was added to the meals, absorption with bran fell from 9.3 to 3.9%, a 58% decrease (unpublished results).

Most workers have attributed the inhibiting effect of bran to its phytate content. We attempted to assess the role of phytate by incubating bran at 37°C for 16 hours to allow destruction of phytate by endogenous enzymatic hydrolysis. Although the phytate content was reduced from 139 to 9 mg, the inhibiting effect was very similar to that of whole bran. In another study, dephytinized bran was separated into soluble, phosphate-rich and insoluble, high fiber fractions. Studies with these two components demonstrated that the major inhibitory effect resided with the soluble fraction.

EDTA. Interest in the effect of EDTA on iron absorption has been stimulated by its suggested use for iron fortification. A significant inhibition in absorption was observed when Na_2EDTA was added to a

meal containing either common food items or semi-purified ingredients. Nonheme iron absorption decreased by 20 and 50% with molar ratios for EDTA:iron of 1:1 and 2:1 respectively (Cook and Monsen, 1976b).

More recent studies, however, have suggested that EDTA may sometimes enhance iron absorption. Layrisse and Martinez-Torres (1977) observed that the absorption of 5-50 mg iron as NaFeEDTA was twofold higher than when iron was added as ferrous sulphate. Viteri et al (1978) reported 0.6% absorption when 5 mg NaFeEDTA was added to a milk-rice-sugar formula, compared to 0.3% when ferrous sulfate was added. The effect of EDTA on nonheme iron absorption is apparently related to both the total content of EDTA and its molar ratio to iron. When the molar ratio of EDTA:iron is less than 1, absorption from the meal may be enhanced, particularly when basal availability is low.

Calcium and Phosphate. There are conflicting reports on the effect of inorganic calcium and/or phosphate on iron absorption. Peters et al (1971) suggested that phosphate compounds strongly inhibit iron absorption. More recently, Cook and Monsen (1976) observed a 50% decrease in iron absorption when 178 mg calcium and 138 mg phosphorous were added to a meal containing semipurified ingredients. However, separate addition of soluble calcium or phosphates had little effect on iron absorption. The formation of an insoluble complex of calcium and phosphate may have bound and insolubilized the iron. Additional studies in this area are necessary because of the extensive use of phosphate compounds as food additives.

Tea and Coffee. Tea is one of the most potent inhibitors of food iron absorption. So striking is its inhibitory effect that regular consumption of the beverage has been proposed as a means of decreasing the absorption of dietary iron in patients with iron loading disorders (Alarcon et al, 1979). In the original description of its inhibiting effect, the addition of a cup of tea to a meal containing radiolabeled bread decreased percentage absorption from 10 to 2% (Disler et al, 1975a). In a

subsequent study in rats, no inhibiting effect was seen with tannate-free tea or with caffeine whereas tannins extracted from tea and tannic acid produced a marked inhibition of iron absorption (Disler et al, 1975b). This effect is due to the formation of a black insoluble precipitate of iron tannate. Tannins are widely distributed throughout the vegetable kingdom and may be responsible, at least in part for the low availability of iron in these foods.

We have observed in our laboratory a similar inhibiting effect of coffee. When a cup of coffee was taken with a meal containing meat, a 40% decrease in absorption was observed, compared to a 65% decrease when taken with tea. These observations are relevant to the possible use of sugar as an iron fortification vehicle because a large quantity of sugar is consumed in these beverages.

Protein. Because animal tissue enhances iron absorption, protein is generally regarded as having an enhancing effect on iron absorption. However, studies with a semipurified meal containing dextrimaltose, corn oil, and egg albumin, have demonstrated that of these, egg albumin is the only significant inhibitor (Monsen and Cook, 1979). When the protein source was omitted from the meal, absorption increased from 3.8 to 9.6%; when the protein level was doubled, absorption decreased significantly from 2.3 to 1.4%. These results indicate that if protein *per se* has an effect on iron absorption, it is an inhibitory one.

We have recently studied the effect of other semipurified proteins and have found an even more striking inhibition by soy protein. When various soy products (including fat-free soy flour, textured soy protein, and isolated soy protein) were substituted for ovalbumin, a 70-80% inhibition in absorption was observed. Similarly, a 50-60% decrease was seen when soy protein was substituted for 30% of the meat protein. These findings have implications for iron fortification programs because infant protein supplements contain high concentrations of soy protein.

Summary

Whereas the effect of certain dietary enhancers of iron absorption such as ascorbic acid and animal tissue are well defined, the effects of inhibiting substances in our diet have not yet been fully characterized. The most potent inhibitors are egg, bran, tea, coffee, and soy products. The effect of these substances should be included in models to estimate food iron availability.

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#175

A Screening Test for Assessing Iron Status

By Jean Pintar, Barry S. Skikne, and James D. Cook

Intervention strategies to combat iron deficiency anemia in developing countries may hasten the development of iron overload in patients with an inherited defect in hemoglobin synthesis. This risk could be diminished if there was a rapid and simple method available for detecting iron overload in population screening programs. We have developed such a method, which is in effect a semiquantitative ferritin measurement based on a modification of a two-site enzyme-linked immunoassay. The assay requires only 2 drops of whole blood and a total incubation time of 90 min. The procedure, which can readily distinguish iron deficiency from even a modest increase in storage iron, has a potentially wide application in settings where a prompt assessment of iron status is required.

IRON DEFICIENCY is highly prevalent in many developing countries, affecting from 10% to 30% of the population in some areas. In order to alleviate this nutritional deficiency, additional iron must be supplied to the population either with the diet (iron fortification) or as iron medication (iron supplementation). However, a significant concern with these intervention strategies is that certain individuals may be adversely affected by a higher iron intake. For example, in Thailand where iron deficiency is very common, about 1% of the population have inherited abnormalities in hemoglobin synthesis (e.g., thalassemia major, hemoglobin E-thalassemia) that may lead to early death from iron overload.[1] Many of these disorders are associated with a hypochromic microcytic anemia, making it difficult to distinguish them morphologically from iron-deficiency anemia.

Serum ferritin levels provide a clear separation between iron-deficiency anemia (below 20 μg/liter) and thalassemia major (greater than 500 μg/liter.)[2] Most conventional radioimmunometric assays for serum ferritin require several hours to perform and require laboratory facilities that are seldom available in developing countries. We describe a rapid screening test of iron status that takes advantage of the striking difference in serum ferritin levels in anemic patients with iron deficiency and iron overload.

MATERIALS AND METHODS

Human Ferritin

This was purified from human liver tissue using a modification of the method of Mazur and Shorr.[3] Following six recrystallizations with cadmium sulfate, the material was passed through a Sephadex G200 column and concentrated by ultracentrifugation at 100,000 *g* for 2 hr. The ferritin button was dissolved in normal saline containing 0.2% sodium azide and stored at 4°C. The concentration of ferritin protein was determined by the Lowry method[4] using bovine serum albumin (BSA) as the standard.

Rabbit Antiferritin

Rabbits were immunized by injecting 200 μg ferritin with complete Freund's adjuvant at weekly intervals for 3 wk followed by one final injection 3 wk later. The titer of whole antisera used in these studies was 1:32. Crude antisera were stored at −20°C.

Antiferritin Antibody

This was isolated by affinity chromatography using cyanogen bromide-activated Sepharose 4B as described by Gonyea.[5] The protein yield was assessed by determining absorbance at 280 nm. Ten milliliters whole antisera yielded approximately 15 mg protein, which was then diluted to a final concentration of 10 mg/ml.

Enzyme-Conjugated Antibody

Purified antiferritin was conjugated with alkaline phosphatase using a modification of the method of Theriault and Page.[6] Ten milligrams purified antiferritin was added to 0.6 ml alkaline phosphatase in 3.2 *M* ammonium sulfate (alkaline phosphatase from calf mucosa, 5050 U/ml; Sigma Chemical Co., St. Louis, Mo.). This mixture was dialyzed against 1 liter phosphate-buffered saline (PBS) (15 mmole/liter pH 7.2, containing 0.2% sodium azide). The antibody–enzyme mixture was then mixed with 100 μl glutaraldehyde solution (10 g/liter water) and allowed to stand 2 hr at room temperature. After further dialysis against PBS, the material was applied to a Sephadex G200 column that had been previously equilibrated with PBS. Eluted fractions with the highest protein content as measured by absorbance at 280 nm were pooled; the conjugate was diluted 1:10 with 5% BSA in 0.05 *M* Tris-HCl, pH 8.0, and stored at 4°C.

Preparation of Solid Phase

The test was performed using polyvinyl chloride microtiter plates (Dynatech Laboratories, Alexandria, Va.). The solid phase was prepared by adding to each well whole rabbit antiferritin serum diluted 1:1250 with 0.2 *M* sodium bicarbonate, pH 9.2. After standing overnight at 4°C, the serum was aspirated and the wells washed 3 times with PBS. The washed plates were covered with sealing film (Dynatech Laboratories, Alexandria, Va.) and were stored in this manner for up to 12 wk at 4°C.

Assay Procedure

In the first step, two drops containing approximately 35 μl serum are added with a Pasteur pipette to each well followed by the same volume of BSA-buffer (30 g BSA/liter PBS). Standards are similarly prepared by adding 2 drops ferritin solution to each well followed by 2 drops normal rabbit serum. After allowing the plates

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to stand at room temperature for 15 min, the wells are aspirated and rinsed 3 times with PBS.

In the second step of the assay, 4 drops conjugated antiferritin (diluted 1:100 in BSA buffer) are placed in each well and the plates allowed to stand for 15 min. The wells are again aspirated and rinsed 3 times with PBS.

In the final step, each well is filled with substrate solution containing p-nitrophenylphosphate (PNP) prepared by dissolving 2.0 g PNP in 100 ml 1 *M* diethanolamine containing 0.1 g/liter magnesium carbonate, pH 10.0. After allowing the plates to stand at room temperature for 1 hr, the reaction is stopped by adding one drop of 1 *M* sodium hydroxide to each well. The intensity of the yellow color in each well is estimated visually against a white background. When the test is performed with whole blood rather than serum, capillary blood is drawn into heparinized microhematocrit tubes (Fisher Scientific, St. Louis, Mo.). The contents of one capillary tube are placed in each well followed by 2 drops of BSA-buffer. For standards, 1 drop ferritin solution is added to each well together with 1 drop buffer and 2 drops heparinized whole rabbit blood. The plates are incubated, washed, and aspirated as described above.

RESULTS

In the initial evaluation of the assay, serum from venous blood was used for both the screening test and quantitative measurements of serum ferritin using a conventional immunoradiometric assay (IRMA).[7] The color intensity in the screening test with each sample was compared to ferritin standards containing 0, 20, 50, 100, 200, and 500 μg/liter. Color intensity of each well was assessed independently by 5 individuals. The results of a study on 90 sera are shown in Fig. 1. While some overlap was observed in the screening test, the feasibility of the approach was established.

In a second evaluation, attention was focused on the screening assay as a means of distinguishing iron deficiency from iron overload in patients with anemia. To further simplify the procedure, the need for centrifugation was eliminated by using heparinized whole blood obtained by fingerstick. The results with the screening test were compared to serum ferritin levels measured with IRMA on venous blood drawn simultaneously. On the basis of ferritin standards containing 20 and 200 μg/liter, the color was judged to be in one of three categories: low (<20), normal (21–200), or elevated (>200); color was estimated by 5 observers on samples from 38 patients with a wide range in serum ferritin.

Using the broader ranges of low, normal, and high.

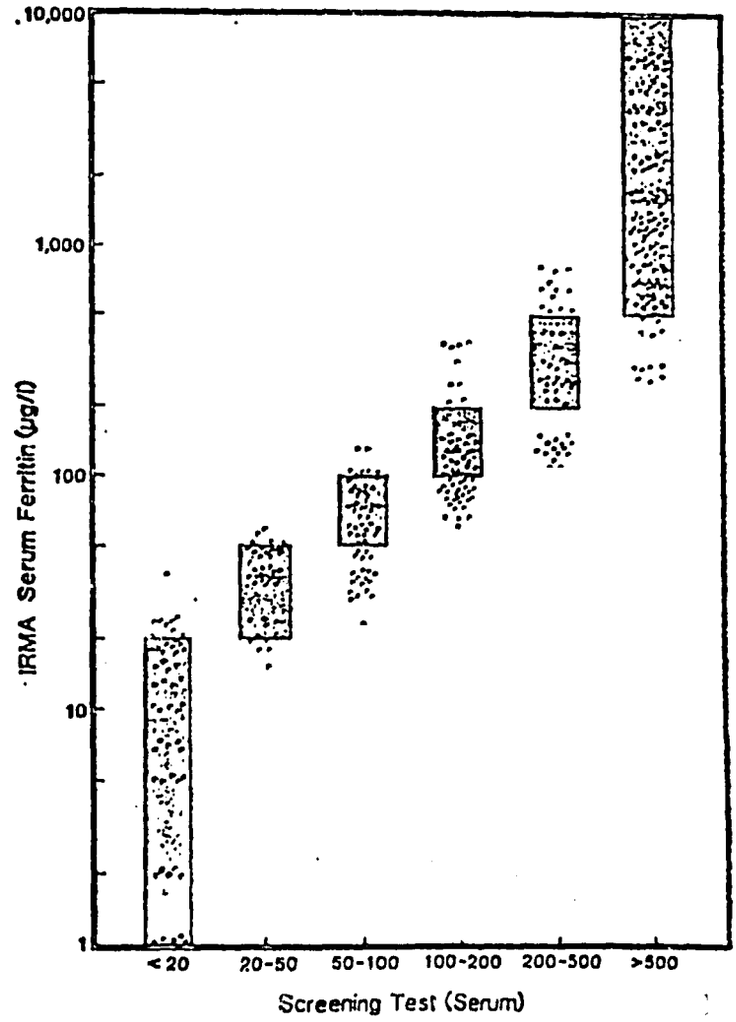

Fig. 1. Results of screening test performed on sera compared with serum ferritin measured by IRMA.

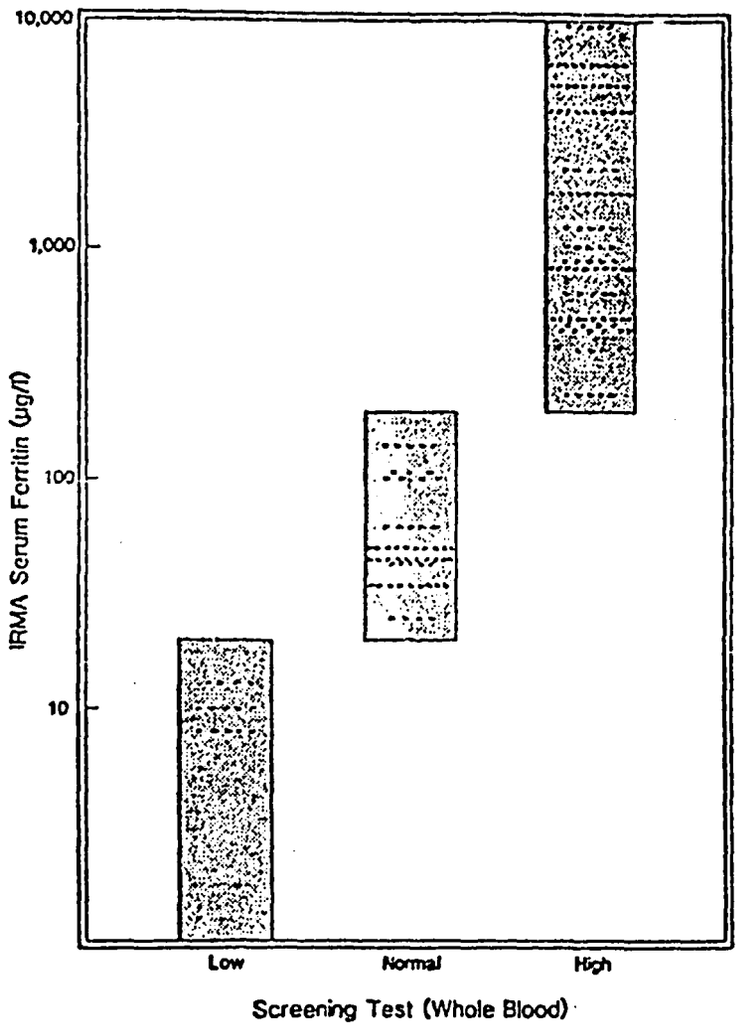

Fig. 2. Results of screening test performed on whole blood compared with serum ferritin measured by IRMA.

excellent agreement was obtained between the screening test and the IRMA method (Fig. 2). Of 4 samples with low serum ferritin (8, 10, 13, and 20 µg/liter), 90% were correctly placed by the screening test, and in no instance was the ferritin estimated as high (Table 1). Of 12 sera with ferritins between 21 and 200 µg/liter, 92% were correctly assigned. With 22 sera containing over 200 µg/liter serum ferritin, 98% were identified correctly and the ferritin was not judged low in any of these.

DISCUSSION

The prime objective of this study was to develop a simplified test that could readily distinguish iron-deficiency anemia from anemia associated with iron overload. This objective was achieved; in no case was a low ferritin considered high or vice versa (Table 1). It should be noted that not all patients with iron deficiency anemia have ferritin values below 20 µg/liter. When iron lack is associated with inflammation, infection, or liver disease, serum ferritin levels as high as 60 µg/liter may occur in anemic patients with absent iron stores.[8] However, because ferritin levels in patients with significant iron overload are invariably above 500 µg/liter, the screening test can still distinguish iron deficiency from iron excess.

Table 1. Comparison of Screening Test With IRMA Serum Ferritin Levels

Serum Ferritin (µg/liter)	No. of Samples	Screening Test Low	Normal (% of observations)	High
≤ 20	4	90	10	—
21–200	12	3	92	5
> 200	22	—	2	98
Total	38			

An important advantage of the screening test is that it can be performed without laboratory facilities and is therefore suitable for use in field work and in rural health clinics. Although sophisticated laboratory facilities are required to prepare the materials for the assays, the coated plates and reagents can be stored for at least 12 wk without appreciable deterioration. From a practical aspect, the screening test involves a total incubation time of 90 min, although one person can easily perform 15–20 tests/hr. This incubation time could be shortened appreciably with certain modifications in the procedure. For example, we have found

that incubation at 37°C rather than room temperature allows a decrease in the incubation time with substrate from 60 to 30 min. We did not employ the higher temperature since this would be inconvenient in field work. An alternate approach would be to employ double monoclonal antibodies against ferritin, one to prepare the solid phase and one for enzyme conjugation. This would allow simultaneous addition of the unknown and the enzyme-conjugated antibody, and thereby eliminate one phase of the test. The paradoxical fall in the dose–response curve seen with IRMA when large doses of standard are used is not seen with the screening test, and color development is not appreciably altered when whole blood is used because the red cells are removed after the first incubation step. However, it is necessary to ensure that the volume of standard solution used is approximately equivalent to the serum content of whole blood.

Although the screening test was designed to distinguish between the extremes in iron status in a setting with limited laboratory facilities, it has several potential applications in the United States. Since all causes of anemia except for iron deficiency are associated with an increase in serum ferritin, the absence of color in the screening test would provide strong presumptive evidence of iron deficiency. By performing the test in all patients admitted to hospital with low hemoglobin levels, a more costly hematologic evaluation could be avoided. Another application would be to detect latent iron deficiency in nonanemic patients in segments of the population where iron deficiency is uncommon. If performed in conjunction with stool tests for occult blood as part of the annual medical examination, the early recognition of a gastrointestinal malignancy would be facilitated.

A screening test for iron status might also be useful in a community blood bank where potential donors are presently screened by hemoglobin or hematocrit determinations, which are relatively insensitive measures of iron deficiency. The test would facilitate the identification of nonanemic donors with latent iron deficiency who may develop frank anemia with continued blood donations. Another useful application for this test is in those segments of the population susceptible to iron deficiency, such as women of childbearing age. Since many women are reluctant to take iron supplements during pregnancy because of associated gastrointestinal side effects, the screen could be used in prenatal clinics to identify those women with a specific need for iron therapy.

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#28

Original Research Communications—general

The inhibitory effect of soy products on nonheme iron absorption in man[1-3]

James D. Cook, M.D., Timothy A. Morck, Ph.D., and Sean R. Lynch, M.D

ABSTRACT Radioiron absorption studies were performed in male volunteer subjects to determine the effect on nonheme iron absorption of various semipurified proteins. When egg albumen and casein were substituted in protein-equivalent quantities in a semisynthetic meal, similar mean absorptions of 2.5 and 2.7% were observed. In contrast, isolated soy protein reduced absorption sharply, to an average of 0.5%. When egg albumen in the semisynthetic meal was replaced with full fat soy flour, textured soy flour, and isolated soy protein, absorption fell from 5.5 to 1.0, 1.9, and 0.4%, respectively, indicating an inhibitory effect by a wide range of soy products. The effect of substituting textured soy flour for meat in a meal containing a hamburger, french fries, and a milkshake was also evaluated. With 3:1 and 2:1 ratios of meat to unhydrated textured soy flour, absorption decreased by 61 and 53%, respectively. The soy products tested in this study have a pronounced inhibitory effect on the absorption of nonheme iron. *Am. J. Clin. Nutr.* 34: 2622–2629, 1981.

KEY WORDS Iron absorption, isolated soy protein, textured soy flour, full fat soy flour

Introduction

The majority of clinical studies has shown that protein facilitates the absorption of dietary nonheme iron. Animal tissue including fish and poultry are especially important not only because they contain assimilable heme iron (1–3) but also because they enhance absorption of nonheme iron from foods eaten in the same meal (4–7). However, not all sources of protein enhance iron absorption. For example, egg yolk has been shown to impair iron absorption (8, 9) and dairy products in general have a relatively inhibitory effect on nonheme iron absorption (5).

We recently reported the results of iron absorption studies in which the effect of doubling or deleting the sources of carbohydrate, fat, and protein in a semisynthetic meal were examined (10). Egg albumen, the protein source, had an inhibiting effect on iron absorption whereas the content of fat or carbohydrate had little influence. To determine whether this inhibition was unique to egg albumen, we examined in this study the effect of other semipurified proteins including casein and isolated soy protein. Several soy products were found to inhibit markedly the absorption of nonheme iron in human subjects.

Methods

Subjects

Three separate studies were performed in 36 men ranging in age from 18 to 50 yr. Study I (15 subjects) compared the effect of different semipurified proteins; study II (10 subjects) examined the influence of various soy products on iron absorption; study III (11 subjects) evaluated the effect on iron absorption of adding soy protein to meat. All of the volunteers were in good health and denied a history of gastrointestinal disorders that might affect iron absorption. Iron status was normal,

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with the exception of one subject who had a serum ferritin of 12 ng/ml indicating depleted iron stores (11). Written consent was obtained from each volunteer and all procedures were carried out in accordance with the standards of the Human Subjects Committee of the University of Kansas Medical Center.

Absorption measurements

Three or four separate iron absorption tests were performed in each subject using double sequential radioiron labels. Test meals were administered between 7 and 9 AM after an overnight fast, and only water was allowed for the subsequent 3 h. All meals were labeled extrinsically by adding varying quantities of $FeCl_3$ in 1.0 ml 0.01 N HCl containing either 2 μCi ^{59}Fe or 5 μCi ^{55}Fe (12, 13). On the 1st day of each study, blood was drawn for measurements of serum ferritin (14) and background blood radioactivity. The first pair of test meals were tagged separately with either ^{59}Fe or ^{55}Fe and administered on 2 consecutive days. Blood was obtained 14 days later to measure incorporated red cell radioactivity and a second pair of separately tagged meals were again given on successive days. Subjects in study I were given only one additional test meal after the initial pair of measurements. A final blood sample was drawn 2 wk after the last test meal to measure the increase in circulating red cell radioactivity. All radioiron measurements were performed on duplicate 10-ml samples of whole blood using a modification of the method of Eakins and Brown (15). Percentage absorption was calculated on the basis of blood volume estimated from height and weight (16) and an assumed red cell incorporation for absorbed radioactivity of 80% (17).

Test meals

All meals in studies I and II were comprised of semipurified ingredients (Table 1). They represent modifications of the semisynthetic meal used in previous studies (5, 18) except that no inorganic calcium or phosphate was added. In study I, each meal contained 68 g carbohydrate derived from dextrimaltose, 35 g fat from corn oil, and 29.4 g protein from various sources. The protein in meal A was supplied as egg albumen (Monark Egg Corp., Kansas City, MO), in meal B as sodium caseinate (National Casein Sales, Chicago, IL), and in meal C as isolated soy protein (ISP) (Supro 710, Ralston Purina Co., St. Louis, MO). ISP is the spray-dried washed precipitate that is recovered after the water extract of defatted soybean flour has been adjusted to pH 4.5, the isoelectric point of the soy protein.

In study II, the carbohydrate and fat components of the meals were the same as for study I, but each meal contained only 14.7 g protein. This was supplied in meals A through D as albumen, full fat soy flour (El Molino Mills, City of Industry, CA) (FSF), textured soy flour (Supro 50A, Ralston Purina Co.) (TSF), and ISP respectively. TSF is obtained by the extrusion of defatted soy flour. Sufficient $FeCl_3$ was added to each meal in studies I and II to provide a final iron content of 4.1 and 4.0 mg, respectively.

The components of each of the meals in studies I and II were thoroughly mixed and served as a drink with the exception of the TSF meal (meal C, study II). In the latter, the dry TSF granules were hydrated in a styrofoam bowl with twice their weight of water and warmed for 45 s in a microwave oven. The fat and carbohydrate components were mixed and a portion poured over the TSF granules which were then eaten with a spoon. The remaining carbohydrate and oil mixture which contained the radioiron was taken as a drink.

The effect of partial substitution of meat with TSF was examined in study III. Meals A, B, and C each contained 55 g french fried potatoes, 180 ml vanilla milkshake, a meat patty containing varying amounts of TSF, and a bun. Meal A contained 100 g lean ground beef (precooked weight). In meal B, 30 g dry TSF granules were added to 100 g beef while meal C contained the same quantity of TSF but only 70 g beef. This resulted in weight ratios of meat to unhydrated TSF of approximately 3:1 in meal B and 2:1 in meal C and protein ratios of 1.2 and 0.8, respectively. In meals B and C, TSF was moistened with an equal quantity of water and then mixed with the raw ground beef. All patties were broiled for 5 to 7 min per side, refrigerated overnight, and heated in a microwave oven immediately before serving. The hamburger meals were tagged extrinsically by adding 0.1 mg iron as labeled $FeCl_3$ to the milkshake. The final test (meal D) in study III consisted of a reference dose of 3 mg iron as $FeSO_4$ and sufficient

TABLE 1
Protein and iron content of semipurified meals

	Meal	Protein substitute			Iron		
		Source	Weight	Protein content	Protein substitute	$FeCl_3$	Total
			g	g	mg	mg	mg
Study I							
	A	Albumen	36.7	29.4	0.4	3.7	4.1
	B	Casein	32.6	29.4	0.3	3.8	4.1
	C	Soy (ISP)	33.9	29.4	4.0	0.1	4.1
Study II							
	A	Albumen	18.4	14.7	0.1	3.9	4.0
	B	FSF	40.4	14.7	3.4	0.6	4.0
	C	TSF	28.6	14.7	3.3	0.7	4.0
	D	ISP	17.0	14.7	2.0	2.0	4.0

freshly prepared ascorbic acid solution to give a 2:1 molar ratio of ascorbate to iron (6, 19). The purpose of this measurement, which has been used extensively in prior iron absorption studies in human subjects, is to standardize measurements of nonheme iron absorption in subjects with varying iron status.

Statistical analysis

Because of the highly skewed distribution of iron absorption measurements when expressed as a percentage of the administered dose, absorption data were converted to logarithms for statistical analysis and the results were reconverted to antilogarithms to recover the original units (20). When comparing any two test meals in the same study, a paired *t* test was used to determine whether mean log absorption values differ significantly from 0 which is equivalent to testing whether the mean ratio in percentage absorption differ from 1.

Results

The effect of three semipurified protein sources on iron absorption (study I) is shown in Table 2. Geometric mean absorption in meal A containing egg albumen averaged 2.49%. This is somewhat higher than previously observed values for this meal, which presumably reflects the omission of inorganic calcium and phosphate (21). Mean absorption from meal B containing sodium caseinate was nearly identical to that with egg albumen, averaging 2.74% (mean ratio B/A = 1.10). In contrast, absorption from meal C containing ISP was much lower, averaging only 0.46% (mean ratio C/A = 0.19, $p < 0.001$).

A comparison of the effect of different soy products on iron absorption (study II) is summarized in Table 3. Mean iron absorption from meal A containing egg albumen averaged 5.5%. This value is higher than that from the similar meal in study I, reflecting the lower albumen content in this study (10). In all three meals containing soy products in study II, iron availability was markedly reduced and in all instances the differences with meal A were highly significant. With FSF (meal B) absorption averaged 0.97% (mean ratio B/A = 0.18). Inhibition of iron absorption from TSF (meal C) was somewhat less pronounced, averaging 1.91% (mean ratio C/A = 0.35). The lowest iron absorption occurred in meal D containing ISP with a mean absorption of 0.41% (mean ratio D/A = 0.08). Thus, when soy products were substituted for albumen in equivalent protein quantities, percentage nonheme iron absorption was reduced by 82, 65, and 92% with FSF, TSF, and ISP, respectively. In study II, the difference in absorption between the three meals containing soy products were also statistically significant. Absorption with TSF was significantly higher than with either FSF (mean ratio C/B = 1.95, $p < 0.01$) or ISP

TABLE 2
Effect of protein concentrates on iron absorption (study I)

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption			Absorption ratio	
				ALB* (A)	Casein (B)	Soy (C)	B/A	C/A
		%	ng/ml		% of dose			
1	29	47	61	0.97	1.00	0.16	1.03	0.17
2	26	50	34	1.26	1.11	0.07	0.88	0.06
3	27	46	59	1.40	1.40	1.70	1.00	1.22
4	25	45	164	1.41	1.21	0.56	0.86	0.40
5	26	43	31	1.43	4.12	0.36	2.94	0.25
6	25	46	46	1.55	1.83	0.18	1.19	0.12
7	25	44	94	1.63	6.43	0.30	4.00	0.18
8	25	43	51	1.80	1.06	0.27	0.59	0.15
9	25	49	88	2.26	2.41	0.95	1.08	0.42
10	28	50	48	3.78	1.26	0.52	0.33	0.14
11	30	43	55	4.51	6.03	0.56	1.35	0.12
12	24	44	37	4.82	8.37	1.16	1.75	0.24
13	25	46	45	6.25	4.43	0.12	0.71	0.02
14	26	48	114	6.50	2.78	1.03	0.43	0.16
15	26	44	12	7.51	20.45	4.97	2.78	0.66
Mean†	26	46	53	2.49	2.74	0.46	1.10	0.19‡
			−1 SE	2.08	2.16	0.35	0.92	0.15
			+1 SE	2.98	3.47	0.62	1.31	0.24

* ALB, egg albumen.
† All values are geometric means except age and packed cell volume.
‡ $p < 0.001$.

(mean ratio C/D = 4.62, $p < 0.001$). Absorption with FSF was also significantly higher than with ISP (mean ratio B/D = 2.37, $p < 0.01$).

The effect of partial substitution of TSF for meat was examined in study III (Table 4). With meat alone (meal A), absorption averaged 3.2% with a range of 0.48 to 14.61%. When 30 g TSF was added to the same quantity of meat (meal B), absorption fell to 1.24%, a highly significant decrease (mean ratio B/A = 0.39, $p < 0.001$). When the meat content was reduced in proportion to the quantity of added soy (meal C), absorption was slightly higher, averaging 1.51%. The difference in absorption from this meal and meal A containing no soy was also highly significant (mean ratio C/A = 0.47, $p < 0.001$). Thus, in meals containing a meat to soy ratio of 3:1 and 2:1, the decrease in absorption due to TSF averaged 61 and 53%, respectively. In these iron-replete men, reference absorption (meal D) averaged 19.88%. Absorption of nonheme iron from meals A,

TABLE 3
Effect of various soy products on iron absorption (study II)

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption				Absorption ratio		
				ALB* (A)	FSF (B)	TSF (C)	ISP (D)	B/A	C/A	D/A
		%	ng/ml	% of dose						
1	24	44	83	2.01	0.81	1.00	0.16	0.40	0.49	0.07
2	21	46	105	3.28	0.91	0.90	0.52	0.27	0.27	0.15
3	35	40	149	3.46	0.40	1.71	0.28	0.11	0.49	0.08
4	24	46	59	3.75	1.52	3.30	0.61	0.40	0.88	0.16
5	42	42	94	4.11	0.46	1.47	0.13	0.11	0.35	0.03
6	25	47	30	5.53	0.57	2.40	0.17	0.10	0.43	0.03
7	24	44	112	6.08	1.21	2.82	0.33	0.19	0.46	0.05
8	24	46	46	8.37	4.67	2.73	1.27	0.55	0.32	0.15
9	34	46	86	14.03	0.66	1.06	1.55	0.04	0.07	0.11
10	25	42	26	18.36	1.85	4.43	0.72	0.10	0.24	0.03
Mean†	28	44	69	5.50	0.97	1.91	0.41	0.18‡	0.35‡	0.08‡
			−1 SE	4.42	0.77	1.60	0.31	0.14	0.28	0.06
			+1 SE	6.83	1.23	2.27	0.54	0.23	0.43	0.09

* ALB, egg albumen.
† All values are geometric means except age and packed cell volume.
‡ $p < 0.001$.

TABLE 4
Effect of soy protein on iron absorption from a meal containing beef (study III)

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption				Absorption ratio		
				100 g beef (A)	Beef:soy 3:1 (B)	Beef:soy 2:1 (C)	REF* (D)	B/A	C/A	B/C
		%	ng/ml	(% of dose)						
1	36	44	60	0.48	0.13	0.25	17.80	0.27	0.52	0.52
2	22	44	70	1.43	1.16	0.18	14.05	0.81	0.75	1.07
3	18	39	33	2.30	1.16	1.22	17.91	0.50	0.53	0.95
4	19	44	59	2.41	0.78	0.60	7.73	0.32	0.24	1.30
5	18	48	41	3.10	1.06	0.85	31.95	0.34	0.27	1.24
6	18	46	61	3.47	1.55	3.25	23.43	0.44	0.93	0.47
7	19	49	60	3.76	1.31	1.75	13.71	0.34	0.46	0.74
8	37	41	51	3.81	0.88	0.91	7.88	0.23	0.23	0.96
9	18	48	61	3.91	0.82	0.91	19.46	0.20	0.23	0.90
10	20	46	31	10.76	6.47	8.08	36.85	0.60	0.75	0.80
11	29	49	27	14.61	7.87	15.62	95.83	0.53	1.06	0.50
Mean†	23	45	48	3.20	1.24	1.51	19.88	0.39‡	0.47‡	0.82‡
			−1 SE	2.43	0.89	1.06	16.02	0.34	0.40	0.73
			+1 SE	4.21	1.72	2.17	24.68	0.44	0.56	0.91

* REF, reference dose.
† All values are geometric means except age and packed cell volume.
‡ $p < 0.001$.

B, and C therefore averaged 16, 6, and 8% of the reference dose absorption, respectively.

Discussion

The inclusion of soy products caused a significant inhibition of iron absorption from all meals in this study (Fig. 1). The immediate question posed by these results is the contrast with earlier investigations that indicated a relatively high bioavailability for soybean iron. For example, animal studies using the hemoglobin-repletion method in rats have shown a relative biological value of 60 to 80% for soy products (22). However, it is known that iron absorption measured in small laboratory animals does not always reflect bioavailability for human beings. For example, Fitch et al. (23) observed that rhesus monkeys developed iron deficiency anemia when placed on diets containing ISP but not when fed casein-based diets with comparable levels of protein and iron. These workers also demonstrated that radioiron absorption from ISP was less than half that from casein.

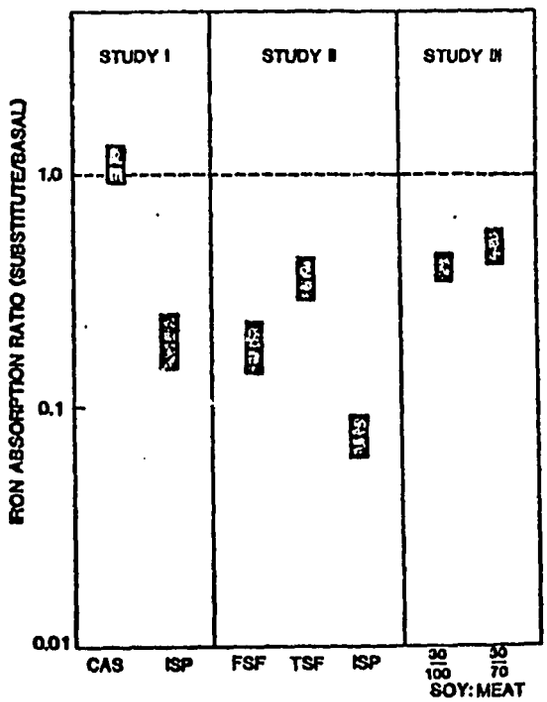

FIG. 1. The effect of semipurified proteins on nonheme iron absorption. Absorption ratios of soy product: albumen are plotted for studies I and II. For study III, absorption ratios are plotted for meals containing varying weight ratios of soy to meat. The height of each *bar* represents ±1 SE.

A number of human studies have also shown relatively high iron availability from meals containing soybean that had been hydroponically labeled with radioiron. For example, Moore (24) reported a mean absorption of 18% in seven normal subjects and values of 20 to 25% in patients with iron deficiency anemia. In a study comparing iron absorption from a variety of food items, a mean absorption of 11% was observed in 17 subjects given intrinsically labeled soy (19). Sayers et al. (25) later reported a mean absorption of 20% from intrinsically labeled soybean meals given to five iron-deficient subjects.

However, not all human studies have shown this relatively high absorption of intrinsically labeled soybean. Ashworth et al. (26), in a study of 10 infants, observed a geometric mean absorption of 2.6% for boiled soybean; when the soybean was baked before administration to 16 infants, mean absorption was 6.7%. Bjorn-Rasmussen et al. (27) recorded an absorption of 2.62% when soybeans were given to 15 normal subjects, three of whom were women. Geometric mean absorption in this study was 1.53%, a value well within the range of absorption observed in the present study.

The cause of this wide difference in iron absorption from hydroponically labeled soybean is unknown. In four separate studies with different batches of the same variety of tagged soybean, absorption ranged from 2.7 to 11.0% (28) (Table 5). This broad range is not explained by differences in iron status because the absorption ratio of soybean to reference varied from 0.11 to 0.46.

Other workers have not demonstrated diminished absorption from soy-based diets. In a study by Rios et al. (29), in 4- to 7-month-old infants, mean absorption of fortification

TABLE 5
Absorption of hydroponically-tagged soybean

No. of subjects	Iron absorption*		Absorption ratio
	Soybean	Reference	
17	11.0	25.8	0.46
15	2.7	25.5	0.11
7	9.8	44.3	0.22
11	5.7	38.0	0.15
Mean	6.1	29.4	0.21

* All values are geometric means.

iron added to a soy-based formula was 5.4% as compared to 3.4% from a milk-based formula. The relatively high proportion of fortification iron (ferrous sulfate) in this study may have masked an inhibiting effect. Young and Janghorbani (30) recently measured iron absorption from a soy-based diet using a fecal balance technique and ^{58}Fe as an extrinsic tag. An exceptionally high absorption of 21 ± 6% was observed which the investigators attributed to a generous but unspecified ascorbic acid intake with each meal. Van Stratum and Rudrum (31) found no effect on serum iron levels, iron binding capacity, or transferrin saturation in 92 healthy volunteers fed a diet containing 25% protein derived from soy protein foods. Since these iron parameters do not become abnormal until iron stores are exhausted (32), and since iron deficiency develops slowly in the absence of blood loss, one would not expect these measurements to show a difference.

This variability in iron absorption from soybean meals is perplexing. One difference between previous studies and ours is that our meals were extrinsically tagged whereas most earlier studies used intrinsically tagged soybean. However, this observation does not explain the low absorption in this study because in each instance where a discrepancy has been noted between extrinsic and intrinsic tagging, the extrinsic label was better absorbed. In 11 subjects given intrinsically tagged soybean, absorption averaged 6.3% from the intrinsic label as compared to 7.8% from an extrinsic label in the same meal (12). The 27% higher absorption in that study may be explained by the fact that the extrinsic tag was taken as a drink and not mixed with the food; other workers have observed extrinsic to intrinsic tag ratios of 1.04 with soybean meals (27). It therefore seems unlikely that the lower absorption values for soy products observed in the present study relate to the extrinsic tag method.

Other differences between earlier studies and this report lie in the nature of the meals and the methods used for preparing them. The semisynthetic meal contained both fat and carbohydrate whereas soybean was administered alone in many prior studies. Moreover, in some of the latter the soybeans were baked; it is noteworthy that in the studies by Ashworth et al. (26), baking resulted in a more than 2-fold increase in percentage iron absorption from soybean. We have also found a significant increase in iron absorption from baked soy products (T. A. Morck, S. R. Lynch, and J. D. Cook, unpublished observations). Processing soybean to obtain higher protein-containing products cannot be excluded as a factor in the inhibition of iron absorption. Although TSF rather than FSF gave the highest iron absorption in study II, (1.91%), the product with the highest protein content (ISP) gave the lowest iron absorption (0.41%). Since all three meals in study II were prepared on an equal protein basis, the results suggest that the processing of whole beans to obtain the three products altered their influence on food iron absorption.

While our results demonstrate that soy products may, under some circumstances, depress iron absorption significantly, the mechanism of the inhibition is unknown. Phytate is believed to have a strong inhibiting effect on iron absorption (33). We did not measure phytate levels of the soy fractions but they are known to contain relatively high amounts of phytate; ISP has been reported to contain 1.86% phytic acid (22). However, in a recent study with whole wheat bran, we found that the inhibiting effect of bran was not lessened when it had been dephytinized (34). Moreover, while fully iron saturated phytate is insoluble at neutral pH, it has been shown that more than half of the iron in wheat bran is present in the form of monoferric phytate, an iron complex soluble at neutral pH (35). Monoferric phytate is absorbed by dogs equally as well as food iron (36). Further studies will be necessary before phytate can be implicated in the inhibition of nonheme iron absorption caused by soy products.

The implications of our findings in relation to iron status in the population are uncertain because of the complexity of dietary factors that influence nonheme iron absorption. In this country, soy is increasingly used as a meat extender and in meals containing meat, nonheme iron is readily available (3). In addition, the heme portion of meat is absorbed independently, and is not impaired by factors that reduce nonheme iron absorption (2). Therefore, one would expect the inhibiting effect of soy in people consuming a relatively large proportion of animal tissue to be quite limited.

Inhibition of iron absorption by soybean derivatives is more likely to be an important problem in areas where soy constitutes the major source of protein, especially if the diet contains little meat or ascorbic acid. We have recently reported that infant protein supplements such as corn-soya-milk, which contain a high proportion of soy protein, may not supply sufficient iron for normal nutritional needs of children (37). There is little doubt that soy is a valuable and relatively inexpensive source of dietary protein. The inhibitory effect on iron absorption reported here does not detract from this important property but does emphasize the need to evaluate the adequacy of iron in diets containing significant amounts of soy products. It seems likely that the deleterious effects with respect to iron nutrition could be circumvented by the addition of fortification iron to these diets or perhaps the addition of an enhancer of iron absorption such as ascorbic acid.

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Iron availability from infant food supplements[1-3]

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ABSTRACT Radioiron absorption tests are performed in human volunteer subjects to measure iron absorption from five infant food supplements. These products included corn-soya-milk, corn-soy blend, wheat-soy blend, wheat protein concentrate blend, and whey-soy drink mix. In iron replete adult males, mean percentage absorption ranged from 0.6 to 1.4%. By relating these results to absorption from a reference dose of inorganic iron, it was estimated that iron-deficient infants would absorb between 1.7 and 4.1% of the iron contained in the infant foods. These results indicate that this fortification iron is poorly absorbed, and probably supplies only about half of the daily iron requirement for iron deficient children between 6 months and 3 yr of age. *Am. J. Clin. Nutr.* 34: 2630–2634, 1981.

KEY WORDS Iron, corn-soya-milk, infant foods

Introduction

Food supplements are distributed to undernourished infants and children in developing countries as part of the Food for Peace Program of the U.S. Department of Agriculture. Corn meal, wheat flour or protein concentrate, soy flour, nonfat dried milk, and sweet cheese whey are combined in varying proportions. The foods are distributed in dry form and are either diluted in water and consumed as a drink or porridge, or mixed with other foods to enhance protein and caloric intake.

Iron, as ferrous fumarate, is added at a level of 7.8 mg iron/50 g. Ferrous fumarate is well absorbed in the fasting state (1), but its assimilation when added to food has not been adequately studied. In the present study, extrinsic radioiron tagging was used to measure iron absorption from the following five infant food supplements: corn-soya-milk (CSM) (2), corn-soy blend (CSB) (3), wheat-soy blend (WSB) (4), wheat protein concentrate blend (WPC) (5), and whey-soy drink mix (WSD) (6). The protein content of these foods is listed in Table 1.

Methods

Studies were performed in 27 adult male volunteers ranging in age from 18 to 45 yr. All subjects gave written informed consent before their participation in the study and procedures followed were in accord with the ethical standards of the Human Subjects Committee of the University of Kansas Medical Center. None had a history of hematological abnormalities or disorders known to influence gastrointestinal absorption. Iron status was judged to be normal in 25 of the subjects on the basis of hematocrit, transferrin saturation, and serum ferritin. Two had reduced iron stores as determined by serum ferritin levels of 12 and 13 μg/l.

The subjects were divided into two groups of 13 and 14 individuals each. Four separate iron absorption tests were performed in each subject using dual radioiron labels. In both groups, one of the test meals consisted of CSM, the most widely distributed infant food supplement. The second and third test meals differed in the two studies, consisting of WSB and WPC in study 1 and CSB and WSD in study 2. In both, the fourth test meal was a reference dose (REF) of inorganic radioiron containing 3 mg iron as ferrous sulfate and 19 mg freshly dissolved ascorbic acid. The effect of differences in iron status between the two study groups was minimized by expressing iron absorption from the infant foods as a ratio with respect to REF absorption. CSM was administered in both studies to gauge the accuracy of this adjustment.

All test meals were administered between 7 and 9 AM after an overnight fast and water alone was allowed for 3 hr. The infant foods were prepared the day before serving, weighed into individual portions, and refrigerated overnight. Each meal contained 50 to 59 g dry product, 20 g sucrose, 1 g salt, and 170 to 225 ml water. Mineral and vitamin mixes were included in the product formulation and brought the total content to 9 mg iron and 20 mg ascorbic acid per serving. The meals were

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TABLE 1
Percentage of total protein contributed by major ingredients

	CSM	CSB	WSB	WPC	WSD
Soy	45	63	55	61	74
Cornmeal	27	37			
Milk	28				
Wheat			45	39	
Whey					26
Total %	100	100	100	100	100

tagged extrinsically by adding 0.1 mg iron as ferric chloride in 1 ml 0.01 N HCl and either 2 μCi ^{59}Fe or 5 μCi ^{55}Fe (7). WSD was served as a cold beverage and the remaining foods were heated in a microwave oven and served as a hot porridge.

Blood was drawn on the 1st day of the study for measurement of serum iron, iron-binding capacity, serum ferritin, and background radioactivity. Meals A and B, tagged separately with either ^{59}Fe or ^{55}Fe, were then administered on 2 successive days. Blood was obtained 14 days later to measure incorporated red cell radioactivity and meals C and D were then given on 2 successive days. A final blood sample was drawn 2 wk later to measure the rise in circulating 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 (8). Percentage absorption in each subject was calculated on the basis of a blood volume estimated from the height and weight (9). Red cell incorporation of absorbed radioactivity was assumed to be 80% (10). Serum iron was measured by the ICSH method (11), iron-binding capacity by a magnesium carbonate technic (12), and serum ferritin by a 2-site immunoradiometric assay (13).

Because of the highly skewed distribution of iron absorption data when expressed as a percentage of the administered dose, statistical analysis was performed on logarithms and the results transformed to antilogarithms to recover the original units (14). One-way analysis of variance was used to compare absorption from the five infant foods after adjustment for REF absorption.

Results

Iron status measurements and iron absorption values for both studies are listed in Table 2. Iron absorption from all supplements was remarkably low; nearly two-thirds of the absorption values were less than 1%. Geometric mean values ranged from 0.6% for CSM in study 2 to 1.4% for the same food in study 1. Absorption from the REF dose averaged 26.8 and 13.4% in studies 1 and 2, respectively. This difference is presumably related to differences in iron status even though mean serum ferritin levels in the two groups do not reflect such a difference.

Absorption data were corrected for differences in iron status by expressing each value as a ratio with respect to REF absorption (Fig. 1). The accuracy of this correction can be assessed by comparing the absorption ratios obtained for CSM in study 1 and 2. Uncorrected percentage absorption from CSM differed by 60% in the two studies whereas the absorption ratios of 0.052 and 0.043 differed by less than 20% (Table 3). One-way analysis of variance was performed on logarithms of absorption ratios and showed marginal significance ($F = 2.70$, $P < 0.05$). However, Duncan's multiple range tests (15) failed to show a significant difference between any of the infant supplements.

Discussion

An important advantage of expressing absorption in relation to a reference dose is that percentage absorption can be predicted in subjects with differing iron status. The relatively low means for REF absorption of 27 and 13% in the present study are typical values for iron replete individuals. A much higher percentage REF absorption of about 60% would be observed in the iron-deficient children who would receive these food supplements. Iron absorption from each of the foods has been calculated for REF absorption of 60% in Table 3. Values range from 1.7% with both WSB and WPC to 4.1% for CSB; intermediate values of 3.1 and 2.6% were obtained with CSM and 2.9% for WSD. The corrected values are remarkably similar despite variations in the composition of each supplement. Thus, children who take 100-g supplement daily might derive only 0.30 to 0.74 mg iron daily from these infant foods.

The extremely low assimilation of iron contained in infant foods was unexpected. Nevertheless, these results are comparable to those obtained in a previous study in Jamaican infants reported by Ashworth and March (16). Absorption from CSM averaged 6% in that study compared to mean REF absorption of 64%. When adjusted to 60% REF absorption, CSM absorption was 5.7% in comparison with lower values of 2.6 and 3.1 observed in the present study. Much of the disparity between the two studies can be explained by the fact that arithmetic means rather than geometric means were calculated in the report by Ashworth and March (16). Geometric values are invariably lower than arithmetic means, especially when percentage

TABLE 2
Iron bioavailability from infant food supplements

Subject	Age	Packed cell volume	Serum iron	TS*	Serum ferritin	Iron absorption			
		%	μg/dl	%	ng/ml	% of dose			
						CSM	WSB	WPC	REF
Study 1									
1	18	48	164	41	56	0.58	0.72	0.71	10.86
2	18	47	104	23	58	0.62	0.70	1.58	32.33
3	24	51	113	31	113	0.65	0.28	0.42	18.96
4	20	48	172	46	105	0.70	0.40	0.45	27.18
5	18	46	84	21	37	0.71	0.47	1.51	42.88
6	31	45	142	39	107	1.00	0.61	0.33	11.46
7	19	50	106	29	39	1.03	0.35	0.67	23.40
8	19	46	119	34	44	1.28	1.22	0.47	19.66
9	20	46	100	25	54	2.16	0.91	1.20	51.46
10	20	49	123	34	40	2.17	0.58	0.40	23.00
11	21	49	89	24	67	2.22	0.45	0.31	33.25
12	18	50	134	40	41	4.10	1.93	0.97	46.83
13	20	45	150	39	13	12.51	9.68	7.08	49.35
Mean†	21	48	123	33	52	1.40	0.76	0.77	26.82
						CSM	CSB	WSD	REF
Study 2									
1	26	44	144	35	126	0.03	0.58	0.37	11.35
2	45	41	136	49	86	0.12	0.28	0.13	3.11
3	27	41	156	46	62	0.27	0.45	0.38	11.37
4	24	48	118	28	24	0.33	0.50	0.62	12.87
5	27	44	99	25	81	0.41	0.25	0.23	1.22
6	22	42	118	36	79	0.47	0.38	0.17	5.70
7	27	42	69	21	26	0.66	0.46	0.28	17.00
8	25	46	173	44	28	0.71	1.28	0.80	14.71
9	25	44	140	38	30	0.76	0.75	0.68	23.78
10	24	47	86	23	72	1.13	1.86	1.25	18.42
11	29	40	108	30	43	1.32	1.77	1.50	32.82
12	24	42	112	3[illegible]	65	1.56	1.80	5.67	41.53
13	42	46	168	57	56	2.17	3.73	0.82	17.27
14	31	45	94	25	12	4.22	10.40	4.63	62.47
Mean†	28	44	123	35	48	0.57	0.92	0.65	13.36

* Transferrin saturation.
† Values for serum ferritin and iron absorption tests are geometric means.

absorption is low. Their data can be corrected to geometric means by assigning values of 0.1% to two subjects reported as absorbing no iron. When adjusted to REF absorption of 60%, absorption from CSM in iron-deficient children averages 3.1% as compared to values of 2.6 and 3.1% obtained in iron-replete males in the present study. The remarkable similarity in results obtained in subjects with widely different iron status underscores the importance of REF corrections. It also demonstrates the validity of measuring food iron availability in subjects who may differ greatly from the population that will consume the food. Further validation will be difficult because of the concern in administering radioisotopes to children.

Another aspect of methodology relating to the present study is that absorption of the fortification iron, ferrous fumarate, was measured by adding an extrinsic radioiron tag (ferric chloride) rather than using radiolabeled ferrous fumarate. Previous studies have shown that complete exchange occurs between dietary iron and fortification iron when the latter is added as ferric chloride or ferrous sulfate (17, 18). However, an extrinsic tag may not exchange completely with fortification iron when the latter is highly insoluble such as ferric orthophosphate or sodium iron pyrophosphate (17). There is no evidence that an extrinsic tag is *less* well absorbed than conventional forms of added fortification iron. If the extrinsic tag did not exchange

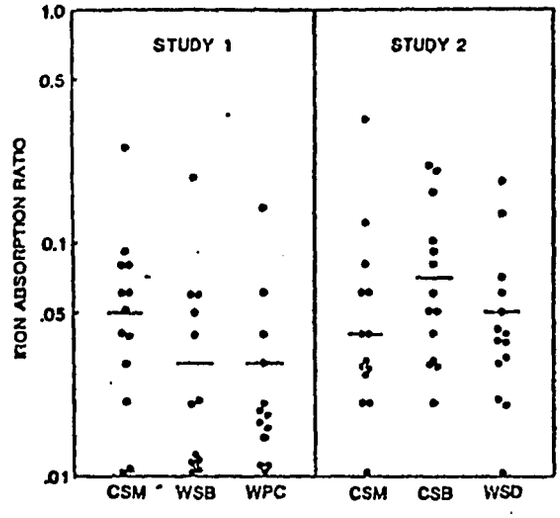

FIG. 1. Absorption of iron from infant food supplements expressed as a ratio with respect to absorption from a standard reference dose of inorganic iron. Ratios are plotted on a logarithmic scale. The *horizontal bars* depict geometric means.

TABLE 3
Iron absorption from infant food supplements adjusted for iron status

Study	Test meal	Iron absorption	Meal:REF	Adjusted absorption*	
				%	mg iron
		% of dose			
1	CSM	1.40	0.052	3.12	0.56
	WSB	0.76	0.028	1.68	0.30
	WPC	0.77	0.028	1.68	0.30
2	CSM	0.57	0.043	2.58	0.46
	CSB	0.92	0.069	4.14	0.74
	WSD	1.96	0.049	2.94	0.52

* Corrected to 60% REF absorption and 18 mg Fe/100 g dry product.

completely with ferrous fumarate in this study, falsely high rather than low absorption values would have been observed.

The recommended intake of iron is 15 mg/day for children 6 months to 3 yr of age (19, 20). These values are based on the assumption that 10% of dietary iron is absorbed by the average child although two to three times this amount may be absorbed by iron-deficient children (21, 22). The results of the present study indicate that the iron added to infant food supplements may not fulfill the daily requirement of 0.7 to 1.0 mg absorbed iron (23). Since we estimated that iron-deficient children would absorb only 0.3 to 0.7 mg iron from 100 g of product, the WHO/UNICEF recommended quantity of 80 to 100 g CSM/day might satisfy only about one-half of their iron requirements. It is unlikely that the typical diet in a developing country would furnish the balance of daily iron needs.

There are at least two factors that could account for the low availability of fortification iron added to infant foods. Calcium and phosphorous salts impair the absorption of food iron when combined in the same meal but not when added separately (24). Specifications for the mineral mixes used in each of the five products tested in this study require the inclusion of either tricalcium phosphate or a combination of calcium carbonate and dicalcium phosphate. We have recently observed a substantial increase in iron absorption from CSM when these minerals are omitted (T. A. Morck, S. R. Lynch, and J. D Cook, unpublished observation). A second possibility for low absorption in this study is the presence of soy protein. We have noted a striking inhibition of food iron absorption with a variety of soy products ranging from whole soybean to isolated soy protein (25). The importance of this inhibitory effect of soy on iron absorption from infant foods cannot be assessed from the data contained in this report because soy protein contributed between 45 to 75% of the total protein in each of the five infant foods examined.

Poor availability of fortification iron is not in itself adequate justification for eliminating calcium and phosphate or soy protein from infant foods. Availability could be improved by increasing the level of ascorbic acid in the vitamin mix (26). Alternatively, it may be possible to add a form of iron, the absorption of which is less inhibited by food. Recent studies suggest that sodium iron EDTA may be less inhibited than other iron salts in certain diets and may therefore enhance iron absorption from infant foods (27, 28).

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Current Definitions of Deficiency Anemia

James D. Cook, MD

Deficiency (nutritional) anemia is defined as a decrease in an individual's hemoglobin concentration below normal because of an inadequate supply of essential hematopoietic nutrients. One limitation of this definition is the difficulty in determining what constitutes a "normal" hemoglobin. The customary practice in prevalence studies is to select a certain cut-off level of hemoglobin below which anemia is considered to be present. However, there are many problems with this approach. It is difficult to reach agreement about the best criteria in a given segment of the population. Another problem is that hemoglobin levels vary widely in normal subjects, and the cut-off level for hemoglobin is therefore far below the mean hemoglobin for a particular age and sex. Because nutritional anemia is usually mild, a marked overlap in hemoglobin occurs between normal and anemic individuals. Even small changes in criteria can lead to vastly different estimates of the prevalence of nutritional anemia.

Some comment should be made about the value of the hemoglobin per se. Although several nutrients are required for normal hematopoiesis, the prevalence of iron deficiency so exceeds deficiencies of other nutrients that iron deficiency has become synonymous with nutritional anemia. Focusing only on the hemoglobin level implies that a low hemoglobin is the only important consequence of iron lack, a dubious assumption in the pediatric age group.

Moreover, a decrease in hemoglobin in a population does not necessarily indicate a nutrient deficiency. For example, infection may contribute significantly to anemia in children. Finally, the hemoglobin is one of the least sensitive and last affected parameters in iron deficiency.

Difficulties in determining the prevalence of nutritional anemia have recently prompted alternative methods of analysis. I will review three of these in some detail: therapeutic iron trial, distribution analysis, and multiple iron parameters.

Therapeutic Trial

The most convincing evidence of nutritional anemia is the response in hemoglobin levels following administration of the deficient nutrient. Therapeutic iron trials have provided valuable information about the distribution of hemoglobin levels in normal and anemic individuals. A classic study was reported several years ago by Garby,[12] who obtained baseline hematocrits in Swedish women and then administered oral iron for 3 months. Anemic individuals were defined as those showing a significant increase in hematocrit following iron therapy. When the frequency distributions of the original hematocrits in anemic and normal individuals were plotted, it became obvious that there was a marked overlap in hematocrit levels between the two populations. Using the best single hematocrit level to distinguish the two groups, it was found that 17% of anemic women (those showing a significant increase in hematocrit following oral iron) were incorrectly classified as normal, while more than 20% of normal women (no response to iron therapy) had been incorrectly classified as anemic.

The therapeutic trial is the most sensitive approach to determining the prevalence of nutritional anemia, but it has limitations. Iron administration must be supervised to assure compliance. When performed in a population, the best approach is to obtain serial measurements in the same individual (as opposed to successive random sampling), and this increases the cost of the study enormously. A serious limitation in infancy is that developmental changes in hemoglobin may obscure a response to oral iron. However, despite these limitations, the therapeutic trial is the "gold standard" against which other measurements of nutritional anemia should be measured.

Distribution Analysis

In contrast to many laboratory parameters, hemoglobin levels in a normal population have a gaussian distribution. Distribution analysis is based on the assumption that in a population with a significant prevalence of nutritional anemia, the observed frequency distribution of hemoglobin is actually comprised of two separate populations of normal and anemic individuals. This double population can easily be detected by plotting the cumulative distribution of hemoglobin levels on probability paper. In the absence of significant anemia, as in adult males, this plot is linear, whereas a pronounced deviation from linearity occurs in the presence of nutritional anemia. This approach was first used in a collaborative study of nutritional anemia in Latin America performed several years ago.[3] A computer-searching technique was used to simulate the observed frequency distribution, assuming two populations of normal and anemic individuals. The results were similar to those of Garby. In pregnant women, approximately 30% of truly anemic women were misclassified as normal, while the number of normal women misclassified as anemic actually exceeded the number of truly anemic women. Distribution analysis has been further developed by Habicht and co-workers[4] to analyze the difference in hemoglobin concentrations between black and white women in HANES I (Health and Nutritional Examination Survey).

Distribution analysis is not the total solution for determining prevalence of nutritional anemia, but it offers advantages over the use of single criteria. One advantage is that the prevalence survey itself is used to determine the normal hemoglobin level within that population, rather than using an empirical prede-

termined value. The main limitation is that several hundred hemoglobin values are needed to obtain reliable estimates. This may be a problem with prevalence studies in infancy, because developmental changes in hemoglobin require pooling of data in relatively narrow age groups.

Multiple Iron Parameters

Among the most important advances in iron nutrition during the past decade has been the development of more specific and sensitive laboratory measures of iron status. There is now abundant evidence to indicate that the optimal approach for measuring iron status is to perform a battery of laboratory parameters, including serum iron, iron-binding capacity, transferrin saturation (TS), free erythrocyte protoporphyrin (FEP), mean corpuscular volume (MCV), and serum ferritin (SF). I will focus my remarks on only TS, FEP, and SF.

TS and FEP are measures of iron-deficient erythropoiesis, that is, a deficiency in iron supply to developing red cells. TS is better known, but is more difficult to perform and subject to error from iron contamination. Additional drawbacks are the wide diurnal variations in normal individuals and the abrupt changes in levels with infections. Some of these problems are eliminated with FEP measurements; these have been greatly facilitated in recent years by the development of simplified techniques of analysis. FEP increases only after several weeks of iron deprivation, and returns slowly to normal following iron therapy. I have been impressed by the similarity in the information provided by TS and FEP measurements in adults.

An exciting new advance in the field of iron nutrition is the development of immunoradiometric measurements of serum ferritin as a measure of iron status. Several reviews of serum ferritin have been published recently.[5-7] In normal individuals, SF is directly proportional to body iron stores.[4] SF measurements are valuable because they permit one to distinguish between true iron deficiency and the anemia of chronic infection, a distinction that cannot be made by TS and FEP. SF decreases in true iron deficiency but increases with chronic inflammation, and therefore usually provides a clear-cut distinction between these two most common causes of hypochromic, microcytic anemia.

A low SF value is absolutely specific for iron deficiency (12 ng/ml is considered by most as the optimal cut-off point), although a normal value does not always exclude iron deficiency. SF is also the most sensitive test for detecting early iron deficiency, but does not fall further as iron depletion progresses. This may be a reason that SF is not always considered the best test for diagnosing iron deficiency in infants and young children, a majority of whom have no iron stores and uniformly low SF. An additional problem with SF in children is the frequent occurrence of inflammation or infection; SF often fails to detect iron deficiency when it coexists with inflammation.

Three stages of iron deficiency can be defined on the basis of these laboratory measurements (Figure). Storage iron depletion is defined by a fall in SF below 12 ng/ml without changes in other iron parameters. The second stage is iron-deficient erythropoiesis, when iron supply to the marrow is curtailed; this stage is characterized by an abnormal TS and/or FEP. The third and final stage, iron deficiency anemia, is characterized by a significant fall in circulating hemoglobin, all other iron parameters having become abnormal at this stage.

The exact manner in which these various iron measurements should be used to determine the prevalence of iron deficiency is still uncertain. There is, however,

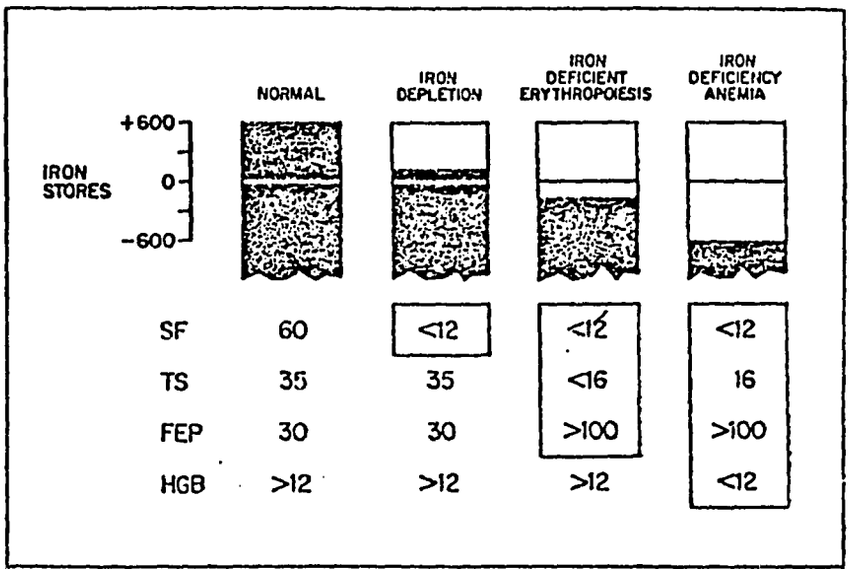

Figure. Characterization of three stages of iron deficiency based on hemoglobin and three iron parameters.[10] (©*Am J Clin Nutr*, American Society for Clinical Nutrition. Reprinted with permission.)

an advantage in performing a battery of measurements. Evidence for this is derived from a study performed in more than 1,500 subjects living in the northwest US.[9] Frequency distribution of TS, FEP, and SF, like the hemoglobin, did not show a discrete population of iron-deficient subjects. Moreover, the use of only one parameter as a measure of iron deficiency did not facilitate the identification of patients with iron deficiency anemia. For example, the prevalence of anemia in the total survey based on hemoglobin alone was 8.3%. When only one of the three iron parameters was used to identify iron deficiency, the prevalence of anemia increased to only 10.9%. However, when two out of three and three out of three iron parameters were abnormal, 27% and 63% of the subjects, respectively, were anemic. This study underscores the importance of performing a battery of iron status measurements to detect iron deficiency anemia.

In a subsequent report, we attempted to estimate iron status by using a combination of these iron parameters on the assumption that they identify different stages of iron deficiency.[10] In iron-replete subjects with SF above 12 ng/ml, iron stores (in milligrams) were estimated by multiplying the SF by 10. At the other extreme of iron deficiency anemia, body iron was estimated from the hemoglobin level on the assumption that a 1-g decrease in the adult corresponds to a deficiency of about 150 mg iron. When the hemoglobin falls below normal (12 g/100 ml hemoglobin in women), a deficiency in body iron of at least 300 mg has already occurred. Lower hemoglobin levels can be used for estimating the deficit in body stores, providing the other iron parameters have established that the cause of the low hemoglobin is, in fact, iron deficiency. TS and FEP were also incorporated into the analysis to obtain information at intermediate degrees of iron deficiency. When results of a survey of 426 normal women were analyzed in this manner, the distribution of calculated iron stores approached a gaussian distribution when plotted on probability paper, providing some tentative support for these estimates.

When one applies this type of analysis, iron deficiency should be the only major cause of anemia in the sampled population. By providing an estimate of body iron in all individuals, it may prove to be the optimal way of determining the prevalence of iron deficiency.

Summary

A major limitation in prevalence studies of nutritional anemia has been the use of single cut-off points of hemoglobin to define anemia. The therapeutic iron trial and distribution analysis of hemoglobin levels circumvent some of these problems when the hemoglobin is used as the only parameter of iron nutrition. However, evidence has been obtained in recent studies to indicate that the optimal approach for estimating the prevalence of iron deficiency is to employ a battery of iron parameters in tandem with the hemoglobin determinations.

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Discussion

DR GARRY: Can the estimate of iron stores you used for adults, namely, storage iron (mg) = SF (ng/ml) x 10, be used in children also?

DR COOK: I don't know of any phlebotomy studies that have been done in children, so I don't have a direct answer to that.

DR SIIMES: The cord bloods of newborns have the same levels of ferritin as adult males, but the newborn obviously has a remarkably lower total iron store. By this calculation, one comes to a straight line. We have published a nomogram that plots the relation between ferritin and milligrams of storage iron per kilogram of body weight (Figure). This plot is applicable to infants and children, as well as adults. The correlation between the log of serum ferritin and iron stores determined as the milligrams per kilogram of body weight seems to be quite clear.

DR COOK: Studies in adults with iron overload suggest a value of 8 rather than 10. The number may vary, depending on the clinical setting.

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BIOCHEMICAL DETERMINANTS OF IRON ABSORPTION

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Poor assimilation of dietary iron is both the major cause of nutritional iron deficiency and the major obstacle in designing food fortification programs to alleviate this deficiency. Studies in the past decade have shown that when several food items are ingested in the same meal, a common pool of nonheme iron is formed. In contrast to earlier studies with single food items, this approach has provided some insight into the specific biochemical determinants of iron availability. Nevertheless, many foods influence iron absorption by mechanisms that remain undefined. The factors that have been identified are shown in the following table:

Biochemical Determinants of Food Iron Absorption

	Potency
Enhancers	
Heme*	+++
Ascorbic Acid	+++
Amino Acids	+
Inhibitors	
Tannins	+++
Ca/PO_4	++
Protein	+
Phytate	+
Fiber	+
Ferritin*	+

*Intrinsic availability

ENHANCERS

Heme Iron. The most important biochemical distinction in regard to dietary iron is the proportion that is in the form of hemoglobin and myoglobin. Although this heme iron constitutes only 5-10% of dietary iron, it accounts for nearly one-third of the iron absorbed each day because of its high availability. Since heme iron enters the mucosal cell as an intact porphyrin complex, its absorption is little affected by the composition of the meal. In a normal individual with iron stores of 500 mg, absorption of heme iron is about 25% as compared to 5% of nonheme iron (Monsen et al, 1978). About 40% of the iron in animal foods (including poultry and fish) is in the form of heme, giving a total intake of about 1 mg per day (Bjorn-Rasmussen et al, 1974). Heme is considered an enhancer in this discussion not because of its effect on the absorption of iron ingested in the same meal, but because of its high intrinsic availability.

Ascorbic Acid. In contrast to heme iron, absorption of nonheme iron is profoundly influenced by the composition of the meal. Ascorbic acid is the most potent enhancer of nonheme iron absorption known and has no ceiling to its enh ncing effect, even at megadose levels (Cook and Monsen, 1977). When the relative increase in iron absorption from a meal is plotted against the molar ratio of ascorbic acid/dietary iron, the dose response curve has two components (Lynch and Cook, 1980). The break occurs at a molar ratio of ascorbate/iron of about 7.5 which corresponds to about 100 mg ascorbic acid in an average meal. Above this level, the enhancing effect of ascorbic acid is less pronounced. Ascorbic acid facilitates iron absorption by forming a soluble iron chelate at low pH which remains soluble at the higher pH of the small intestine (Conrad and Schade, 1968)

So marked is the enhancing effect of ascorbic acid on iron absorption that it has been proposed as a dietary fortificant to render food iron more available. While this approach is attractive in theory, there are a number of practical problems. About one-third of added ascorbic acid is inac-

tivated by boiling and almost all is lost by baking. Moreover, when both ascorbic acid and fortification iron are added to a vehicle such as salt, discoloration occurs. The relatively high cost of ascorbic acid as compared to iron is an additional constraint in developing countries. Even if ascorbic acid is not suitable for fortification, its value should be stressed in educational programs designed to improve iron status. The absorption of iron in a meal can be increased three times by including 100 ml orange juice (Callender et al, 1970) and up to seven times by including fresh fruit such as papaya (Layrisse et al, 1974).

Amino Acids. Animal tissue is an important constituent of the diet, not only because of its heme iron content but also because of its ability to enhance absorption of iron in foods ingested in the same meal. Substitution of animal protein in a meal containing only nonheme iron produces a two- to four-fold increase in iron absorption (Cook and Monsen, 1976). This is true for a variety of animal foods but does not include dairy products such as milk, cheese, and eggs. One gram meat is roughly equivalent in its enhancing effect to 1 mg ascorbic acid (Monsen et al, 1978).

Despite many years of investigation, the biochemical factor responsible for the enhancing effect of animal tissue has not been identified. In early studies, cysteine was believed to be responsible for the enhancing effect of fish (Martinez-Torres and Layrisse, 1970), but this has not been confirmed in later studies. Bjorn-Rasmussen and Hallberg (1979) have recently shown that the enhancing effect of meat is not mediated by an increase in gastric secretion and that the enhancing effect resides with the insoluble residue rather than with a water extract of meat. Since amino acids are known to enhance iron absorption (Forth and Rummel, 1973), the meat effect may relate to the ease with which animal foods are digested in the intestinal tract to polypeptides or amino acids.

INHIBITORS

Until recently, little attention was given to

factors that inhibit the absorption of dietary iron. Moreover, as with animal foods, the biochemical mechanism by which certain foods reduce iron assimilation has not been identified. The following are the most important inhibitors.

Tannins. The pronounced inhibitory effect of tea on iron absorption was first reported by Disler et al (1975a; 1975b). These workers showed that a single cup of tea reduces the absorption of ferric chloride from 22 to 6% and in a meal with several ingredients from 11 to 2.5%. In animal studies, this inhibiting effect did not exist with tannate-free tea or with caffeine but it could be reproduced exactly with either tannic acid or with tannins extracted from the tea. The South African workers concluded that the inhibiting effect results from the formation of a black, insoluble precipitate of iron tannate. We have observed a similar but less pronounced inhibiting effect of coffee which is apparently due to tannins as well (Morck et al, unpublished observations). Tannins are widely distributed in vegetable foods and may be responsible for the low iron assimilation from these foods. Tea has such a marked inhibitory effect that its use has been proposed as a means of reducing the absorption of dietary iron in patients with thalassemia major or intermedia (de Alarcon et al, 1979).

Calcium/Phosphate. Animal studies have led to conflicting views on the effect of these minerals on iron absorption. In human studies, egg yolk has been shown to markedly inhibit iron absorption, an effect that is attributed to vitellin, the major phosphoprotein complex in egg yolk. Peters et al (1971) concluded that phosphate compounds, either as inorganic phosphate or in phosphate-containing meals, are among the most important inhibitors of iron absorption. The effect is presumed to be due to the formation of highly insoluble ferric phosphate.

In studies with a semipurified meal (Monsen and Cook, 1976), the separate addition of a soluble calcium salt and a soluble phosphate salt had little effect on iron absorption. However, pronounced in-

hibition was observed when both were added simultaneously, an effect that probably resulted from co-precipitation of iron by an insoluble complex of calcium phosphate.

Protein. The effects on iron absorption of protein, carbohydrate, and fat remain controversial. The bulk of information is derived from animal studies in which the proportion of these macronutrients is altered while maintaining a constant caloric intake. It has been difficult to determine in these studies whether the observed effect is due to an increase in one constituent or the reduction of another.

Data on the effect of protein have been inconsistent. The enhancing effect of meat has often been ascribed to protein per se but without direct evidence. In fact, recent studies have shown that certain semipurified proteins may actually inhibit absorption. For example, when carbohydrate, fat, or protein were either doubled or eliminated from a semipurified meal, a significant effect was observed only with the protein source, egg albumen. When the latter was omitted, absorption increased from 3.8 to 9.6%, but fell from 2.3 to 1.4% when the level was doubled (Monsen and Cook, 1979). More recently, a striking inhibitory effect of soy protein has been observed (Cook et al, 1981). When full-fat soy flour, textured soy protein, or isolated soy protein were substituted for egg albumen, absorption was inhibited 70-80%. A significant but less dramatic inhibition was also observed when textured soy protein was used as a meat extender. The mechanism for this inhibition has not been defined.

Phytate. In the early 1940's, in a study using a chemical balance technic, it was observed that less iron is absorbed from a diet containing brown bread than one containing white bread (Widdowson and McCance, 1942). Modern studies with extrinsic tagging have confirmed that bran inhibits iron absorption in proportion to the amount added to a meal (Bjorn-Rasmussen, 1974). It has been widely assumed that the effect is due to its phytate

content. However, Sharpe et al (1950) observed that while sodium phytate added to a meal reduced absorption, naturally-occurring phytate did not depress iron absorption significantly. Morris and Ellis (1976) found that more than half the iron in wheat bran is present as monoferric phytate. In contrast to fully saturated iron molecules, monoferric phytate is soluble at neutral pH but insoluble in an acid environment. Studies in dogs (Lipschitz et al, 1979) and in humans (Simpson et al, 1981) have demonstrated that radiolabeled monoferric phytate is relatively well absorbed.

In the study by Simpson et al (1981), examining the inhibitory effect of bran, the phytate content seemed to have little effect on absorption. When 12 g whole bran was added to a meal containing a milkshake and muffins, absorption was reduced from 2.4 to 0.6%. When whole bran was incubated at 37°C to destroy phytate by enzymatic hydrolysis and included in a similar meal, the inhibitory effect of bran was not diminished. Thus, while naturally-occurring phytate may reduce iron absorption, its effect is less pronounced than previously assumed.

Fiber. The effect of fiber on iron absorption has not been extensively studied in man, but there is reason to believe that it is inhibitory. In a recent study in humans (Cook et al, unpublished observations), two separate meals were given that were nearly identical with respect to macronutrient composition but maximally different with respect to fiber content - 5.1 g vs. 0.3 g. Absorption from the low fiber meal averaged 6.1% as compared to 3.0% from the high fiber meal. Thus, at these two extremes, the inhibiting effect of fiber was relatively modest. Furthermore, studies with pectin and hemicellulose have failed to demonstrate an inhibiting effect of these fiber sources (Cook et al, unpublished observations). It would appear that fiber has relatively little effect on iron absorption.

Ferritin. The nonheme iron fraction of animal tissue is well absorbed. However, when purified radiolabeled ferritin was administered to human

subjects, absorption averaged less than 1% as compared to 2% from a maize meal in the same subjects (Layrisse et al, 1975). On the other hand, when 2 mg ferritin iron was administered with veal muscle, percentage absorption increased markedly from 1.3 to 12%. The low availability of purified ferritin therefore appears to be an artifact of protein purification.

SUMMARY

The technic of extrinsic radioiron tagging has greatly assisted in the identification of specific biochemical determinants of food iron absorption. Nevertheless, there remains a large gap between our knowledge of the intrinsic availability of iron in certain foods and the specific biochemical factors that account for these effects. The enhancing effect of ascorbic acid and animal tissue on iron absorption is well established if not well understood. As yet, there is only scant information on the biochemical factors that cause inhibition of iron absorption by tannins, calcium/phosphate, protein, phytate, and fiber.

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#91

Factors Affecting the Bioavailability of Dietary Iron

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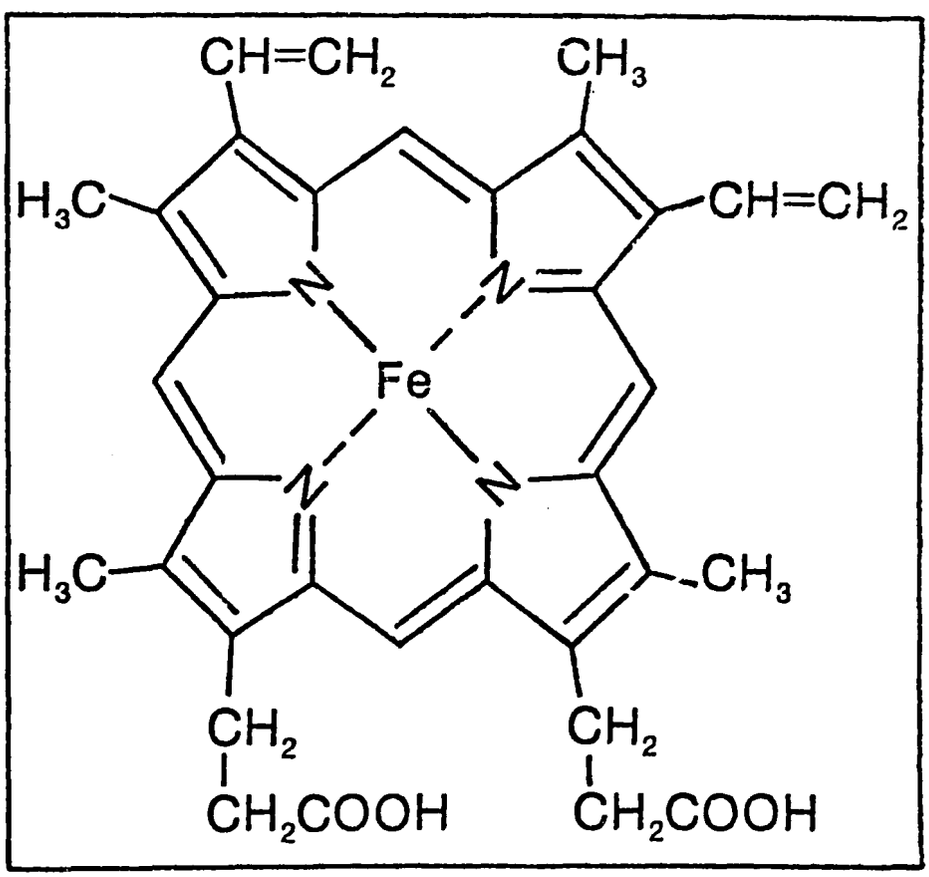

Iron is efficiently recycled in the body and little is excreted because its function in proteins of oxygen and electron transport is essential to life. Primary responsibility for maintaining adequate levels of this nutrient rests in an intestinal absorptive system that is attuned to the changing needs of the individual. The amount of iron absorbed from food is indirectly related to iron status. As iron stores fall, absorption increases, and once stores are replete, absorption falls to a low level. The few milligrams of iron necessary to replace daily losses must be absorbed from a diet that, in Western countries, usually contains a severalfold excess of this element. The fact that iron deficiency anemia is still a common condition indicates that not all of the iron present in this diet is capable of being absorbed.

Sources of Dietary Iron

Both the source and chemical form of dietary iron can markedly affect its availability for absorption. A mixed Western diet contains four sources of iron: food of animal origin, food of vegetable origin, fortification iron, and contamination iron (Table I). For most of the world's population, animal-derived foods are not available, and fortification iron is not yet widely distributed. In the vegetable category, the staples rice, maize, wheat, and beans have either moderate or poor iron availability (1). Therefore, it is not surprising that iron deficiency is prevalent among populations subsisting solely on these foods.

The factors that affect bioavailability of dietary iron will be presented here primarily in the context of a Western diet in which the amount of iron absorbed is not limited by the total iron content of the diet.

There are two major chemical forms of iron in a mixed diet, and each is absorbed by a different mechanism. Heme, containing iron in a porphyrin ring structure (see line art), is found in hemoglobin and myoglobin and accounts for nearly 40% of the iron present in animal tissue, including fish and poultry (2). Because of the porphyrin complex, heme is more soluble in the neutral conditions of the small intestine than in the acid of the stomach. It is taken up by inside (3). Absorption of heme iron is high, averaging from 15% for iron-replete men to 35% in those lacking iron stores (2).

The 1-mg iron per day provided by heme is a small proportion (5–10%) of total daily iron intake. However, due to its high bioavailability, it provides nearly one third of the iron absorbed each day from a mixed diet (4). Because of its unique absorptive mechanism and solubility at elevated pH, heme iron availability is unaffected by dietary components that either enhance or inhibit the availability of the nonheme iron present in the rest of the diet.

Nonheme iron is present in foods of vegetable origin and also accounts for the

Source	Bioavailability	Examples
Food of Animal Origin		
Heme	High	Hemoglobin, myoglobin
Nonheme	High	Muscle tissue, liver, fish
	Low	Eggs, purified ferritin
Food of Vegetable Origin		
Nonheme	Moderate	Wheat, maize, beans
	Low	Rice, spinach
Fortification Iron		
Nonheme	High	Ferrous salts (sulfate, fumarate)
	Moderate	Ferric orthophosphate
	Low	Sodium iron pyrophosphate
Contamination Iron		
Nonheme	High	Beer fermented in iron drums
	Low	Ferric hydroxide, ferric oxide

the upper small intestinal mucosa in a soluble form.

Other dietary constituents aid or interfere with nonheme iron solubility and, thus, absorption. For example, although nonheme iron in meat is generally well absorbed, purified ferritin has low bioavailability when added to a vegetable meal (5). This may be an artifact due to chemical alteration during purification; however, it more likely demonstrates that meat digestive products are needed to facilitate ferritin absorption.

Other sources of nonheme iron are compounds added to fortify the diet with additional iron above its endogenous level. The most common sources are soluble iron salts or small-particle elemental iron. When taken without food, ferrous salts are better absorbed than ferric forms (6). This is probably related to the fact that ferric iron is insoluble in aqueous solution above pH 3, whereas the majority of ferrous iron remains soluble at pH 8.

Particle size is an important determinant of elemental iron solubility and availability for absorption. However, electrolytic iron has a higher bioavailability than hydrogen-reduced iron of the same particle size, probably due to the greater surface area of its dendritic- crystal structure (7).

Solubility alone cannot accurately predict bioavailability of different iron compounds used for fortification of wheat flour. Four soluble forms of iron were added to dinner rolls, and iron absorption was measured in human subjects (8). Reduced iron was absorbed the same as ferrous sulfate, but ferric orthophosphate and sodium iron pyrophosphate were only about 30 and 50% as available, respectively (8).

The iron content of the diet also can be increased by contamination during harvesting, processing, cooking, or storing food. Under the acidic conditions occurring in fermentation of maize and sorghum beer, iron was extracted from cast-iron drums and was shown to be well absorbed from the beer (9). At the other extreme, soil contamination of teff when it is threshed under the hoofs of cattle was reported to account for most of the 200–300 mg daily iron intake of Ethiopians (10). Oxides and hydroxides of iron, common insoluble environmental forms, do not exchange with soluble radioiron salts used to measure food iron absorption in humans. Therefore, the actual nutritional value of contaminant iron cannot be directly determined (11).

Predicting the Bioavailability of Dietary Iron

Methods for predicting availability of dietary iron in vitro have concentrated on duplicating the chemical environment of the stomach and upper small intestine. Although use of human gastric juice has been reported, more often food is incubated with hydrochloric acid containing pepsin at 37°C (12). After adjusting the solution to pH 7.5, the fraction of iron that was ionizable (reacting with α, α-dipyridyl) was found to correlate well with percentage iron absorbed in human studies; soluble iron, containing complexed as well as ionizable iron, did not (13). Other techniques determine the proportion of ionic iron in the ferric or ferrous state, thus providing a useful monitor of chemical changes occurring during food processing (14).

Although the amount of dietary iron that is chemically suited for absorption can be determined using in vitro techniques, animal studies are necessary to measure the actual absorption and utilization of food iron. Growing animals, depleted of iron stores through phlebotomy or by consuming an iron-deficient diet, are fed a diet containing graded levels of iron provided by a food item or an iron salt. The hemoglobin, hematocrit, or body weight response to this added iron is then compared to the response observed in a comparable group of animals fed identical levels of iron from highly available ferrous sulfate. In this manner the biological value of the iron contained in the test ingredient can be calculated relative to ferrous sulfate (15). Radioisotope retention by animals fed meals labeled with radioiron is added iron exchanges completely with iron in the diet (16,17).

Iron bioavailability studies conducted in rats have provided much useful information concerning foods and iron compounds that are good or poor sources of iron. However, using animals to assess food interactions with dietary iron and to estimate its bioavailability in a complex meal is more difficult. Recent data suggest that a primate model may prove useful for these purposes (18).

The very small amount of iron absorbed relative to the iron content of the diet makes chemical iron balance studies in humans very difficult. Any analytical or sampling error may mask the effect on iron balance attributed to the diet.

Direct measurement of iron absorption using minute quantities of radioisotopes of iron has generated the most meaningful data on iron absorption in humans. Absorption is determined two weeks after a test meal by either measuring erythrocyte incorporation or whole-body retention of radioiron (4). Although at first radioiron was biologically incorporated into the food under study, it was subsequently found that a soluble form of inorganic iron added extrinsically to a meal exchanges completely with other dietary nonheme iron released into a common pool of available iron in the acidic environment of the stomach (19,20). The addition of biosynthetically tagged heme iron likewise labels that component of dietary iron, enabling the proportional absorption of each iron form in a complex meal to be determined (21).

In some studies, a standardized dose of ferrous sulfate and ascorbic acid is included as one test meal, the absorption of which is based solely on the level of iron stores in each subject (22). By expressing iron absorption from a test meal as a ratio to the amount of ferrous ascorbate absorbed in the same individual, intersubject variability due to differences in iron status can be minimized. Additionally, this ratio is useful in predicting the amount of iron that would be expected to be absorbed by people differing in iron status from those in the test population. Adjusting the data to a reference dose absorption of 40% provides an index of how much iron in the test meal would be absorbed by subjects without any iron stores (11).

Dietary Components Affecting Bioavailability

Although an iron salt may exhibit high availability when fed alone, adding it to a meal such as maize, wheat, or black beans reduces the level of iron absorption considerably (19). Conversely, when a glass of orange juice is included in a breakfast meal, iron absorption can be

increased 2½ times (23).

These examples demonstrate that iron absorption from a complex meal represents the summation of all enhancing and inhibiting components present in that meal. The iron absorption enhancers and inhibitors that have been studied most extensively in humans and their relative potencies are shown in Table II. Certain sugars, amino acids, and amines released during enzymatic digestion may improve iron availability by decreasing both the precipitation and polymerization of iron in aqueous solutions, probably by interfering with the formation of water bridges with iron molecules. Substances capable of forming chelates with iron promote absorption by maintaining the metal in a soluble, absorbable state (24). Other agents such as tannates, phytates, phosphates, oxalates, and carbonates form insoluble precipitates and macromolecules with iron that have poor availability (25). Two factors of major nutritional significance that have been shown to consistently increase iron availability are ascorbic acid and meat or animal tissue.

Enhancers

Ascorbic Acid. When ascorbic acid is added to a meal, the increase in iron absorption is proportional to the dose, even at levels as high as 1 gm (26,27). Facilitation of iron absorption appears to operate in two ways. As a reducing agent, ascorbic acid maintains the normally ferric form of food iron in a more soluble ferrous state. Secondly, under acidic conditions in the stomach, ascorbic acid forms a complex with iron that remains soluble as the pH increases in the upper small intestine (28).

The addition of orange juice containing 40–50 mg ascorbic acid to a breakfast meal of bread, egg, and tea or coffee was found to increase iron absorption from 3.7 to 10.4% (29). Ascorbic acid also was shown to account for the sevenfold increase in iron absorption from maize porridge when papaya was included in the meal (30). Because oxidation and heating destroys much of the ascorbic acid in food during storage and cooking (31), the ascorbic acid content of raw foods may not accurately predict the degree of iron absorption enhancement expected from processed foods.

Animal Tissue. Besides contributing highly available heme iron, animal tissue in the diet produces a twofold to fourfold increase in the absorption of nonheme iron. This phenomenon applies to a variety of meat products, but eggs, milk, and cheese either have no effect or are somewhat inhibitory to iron absorption (32). The exact mechanism by which animal tissue promotes iron absorption is still unknown, but it has been suggested that amino acids and/or polypeptides arising from proteolytic digestion might chelate dietary nonheme iron, thereby facilitating its absorption.

Martinez-Torres and Layrisse observed a twofold increase in iron absorption when 100-g fish was added to a meal of black beans (33). They found this same magnitude of effect when the total amino acid content of the fish was added in purified form or when 210-mg cysteine (the amount present in 100-g fish) was added alone. Like ascorbic acid, cysteine also is a reducing agent.

Recently, studies by the same researchers have shown enhancement of iron absorption from maize, black beans, and soybeans when this level of cysteine was added after cooking the test meals (34).

Other workers have shown increased iron absorption when beef, fish, chicken, or calf thymus was added to a maize meal, but they noted no response for egg white, cysteine, or a water extract of beef (35). They proposed that meat aids iron absorption by counteracting luminal factors that otherwise would interfere with the transport of iron to the mucosal cell surface.

Inhibitors

Much of the early work in iron nutrition focused on factors that would increase the amount of iron absorbed from the diet. Basal meals in these studies consisted of staple foods such as corn, beans, or rice, which, by themselves, exhibit low intrinsic iron bioavailability.

To better assess inhibitory factors in the diet, chemically defined meals were formulated using purified sources of protein, fat, and carbohydrate; ferric chloride was added to bring the iron content to 4 mg. Adding to this meal almost any nonmeat food that did not contain ascorbate usually resulted in a drop in iron absorption. The biochemical mechanism for this inhibition remains unclear in most instances. The following are the food constituents that have been most extensively studied in their effect on iron absorption in humans.

Table II. Food Components Affecting Dietary Iron Bioavailability

Food Components	Potency
Enhancers	
Ascorbic Acid	+++
Animal Tissue	+++
Inhibitors	
Tea	+++
Soy Products[a]	+++
Coffee	++
EDTA	++
Calcium/Phosphate Salts	++
Eggs	
Yolk	++
Albumen	++
Wheat Bran	++
Phytate	±
Fiber	±

[a] When the major proportion of dietary protein is provided by soy.

Tea. Disler and co-workers were the first to report the dramatic reduction in iron absorption when tea was drunk with a meal (36). A single cup of tea reduced absorption of ferric chloride from 22 to 6%, and even with a complex meal, tea caused iron absorption to fall from 11 to 2.5%. Tannins extracted from tea leaves by hot water were shown to account for this inhibition. Neither caffeine nor tannate-free tea had any effect on iron absorption in rats, whereas tannic acid or purified tannins extracted from tea produced the same degree of inhibition as brewed tea itself (37). This effect is probably due to formation of an insoluble iron-tannate complex in the gut that renders a significant proportion of dietary iron unavailable for absorption. So great is the inhibitory effect of tea, it has been proposed as one way to reduce dietary iron absorption in patients with iron overload diseases (38).

Coffee. Even in the presence of supplemental ascorbic acid, coffee as well as tea inhibits iron absorption (39). In our own studies, coffee has proved to be about two thirds as inhibitory as tea.[1] When subjects drank coffee an hour after a meal, iron absorption was reduced the same amount as when coffee was taken with a meal. Greater inhibition was seen with stronger coffee.

Although the mechanism of the coffee effect has not been identified, polyphenolic compounds are present in coffee and could possibly act in the same manner as the chemically related tannins in tea. Evidence that tea and coffee may act differently on iron absorption has been presented by recent in vitro data. When added to a suspension of cooked pinto beans, tea reduced the amount of soluble iron by 88%. Coffee, on the other hand, did not decrease the amount of iron in solution but was responsible for oxidizing all to the ferric state (40).

Soy Products. Based on studies using human subjects, we have recently reported a substantial inhibition of iron absorption associated with soy products (41). When substituted for egg albumen as the protein source in a semi-purified diet, full-fat soy flour, textured soy flour, and isolated soy protein reduced iron absorption by 82, 65, and 92%, respectively. Similarly, a 50 to 60% decrease was observed when textured soy flour was added to ground beef in a hamburger meal. Follow-up studies showed that the inhibitory effect attributed to isolated soy protein could not be completely eliminated by extensive heating or by adding the enhancers ascorbic acid or meat to the meal.[1] The precise mechanism behind this soy effect remains to be determined, but it appears to influence iron absorption to a greater degree when soy provides all of the

[1] T. A. Morck, S. R. Lynch, and J. D. Cook. Unpublished data.

protein in a meal.

Ethylenediaminetetraacetic acid (EDTA). The ability of the chelator EDTA to bind iron and keep it soluble at neutral pH is well-known, but the ratio of chelate to iron seems to play a major role in determining whether this iron can be effectively utilized by the body.

A significant inhibition in absorption was observed when Na_2EDTA was added to meals comprised of semi-purified ingredients or common food items. When molar ratios for EDTA:iron were 1:1 and 2:1, nonheme iron absorption was decreased by 20 and 50%, respectively (42).

An ability of EDTA to enhance iron absorption, however, has been shown in more recent studies in which absorption of 5–50 mg iron as preformed NaFeEDTA was twofold higher than when iron was added as ferrous sulfate (43). A similar response relative to ferrous sulfate was seen when this iron chelate was added to a milk-rice-sugar formula (44).

The effect of EDTA on iron absorption appears to depend on both the absolute amount of EDTA in the diet as well as the molar ratio of EDTA to iron. When this molar ratio is less than 1, absorption from the meal may be enhanced, especially when basal availability of food iron is low (45).

Calcium/Phosphate. In animal studies, calcium and phosphate compounds have been reported to decrease nonheme iron availability (46,47). Monsen and Cook demonstrated that, when a semi-purified meal was fed to humans, the addition of either calcium chloride or potassium phosphate alone did not affect iron absorption (48). However, when both were added simultaneously to the meal, iron absorption was reduced 53–73%. These results may be explained by the adsorption of iron onto the insoluble calcium phosphate complex formed in the alkaline environment of the small intestine, rendering it unavailable for absorption.

Peters et al studied the effect of various phosphate compounds on iron absorption in humans and also under in vitro conditions (49). They reported that both inorganic and protein-bound phosphate formed an insoluble ferric phosphate complex that was unavailable for absorption.

Eggs. Although eggs contain approximately 1 mg of iron, the iron is poorly available to humans and the egg yolk further inhibits absorption of other dietary iron (29). The addition of egg white to ferrous ascorbate reduced iron absorption from 33 to 14%, and added yolk dropped absorption even further to 3.9% (49). The vitellin fraction of yolk, high in phosphoprotein, was believed to account for this inhibition.

Cook and Monsen used a semi-purified diet containing egg albumen in human studies to assess the effect of various animal proteins on iron absorption. Powdered egg produced the same level of iron absorption as whole milk, cheese, or the unsubstituted basal diet, indicating that not all animal-derived protein enhances iron absorption (32). The same workers subsequently showed that doubling the quantity of egg albumen in the meal caused a drop in iron absorption from 2.3 to 1.4%. On the other hand, omitting it completely from the formulation increased iron absorption by nearly threefold (50). Taken together, these studies show that both the white and the yolk of hen's eggs can inhibit the absorption of nonheme iron contributed by other foods eaten in the same meal.

Wheat Bran. In an early human study using chemical balance methods, Widdowson and McCance showed that more iron was absorbed from white bread than from brown bread, even though brown bread contained 50% more iron (51). More modern studies employing an extrinsic radioiron tag have demonstrated that iron absorption is reduced in direct proportion to the amount of bran added to a meal (52).

Because wheat bran is high in phytate, a compound known to bind iron and other trace minerals, this was proposed as the inhibitory compound in bran. More than half of the iron in wheat bran is present as monoferric phytate (53). In contrast to saturated ferric phytate, monoferric phytate remains soluble at neutral pH and its iron has been found to have relatively high bioavailability in rats, dogs, and man (54,55). However, only when purified sodium phytate is added to meals is iron absorption reduced; endogenous food phytate has no effect (51,56). In other human studies, iron absorption was diminished to the same extent when either dephytinized bran or whole bran was added to wheat flour muffins (55). Therefore, it was proposed that the inhibitory effect of bran was not necessarily due to phytate, but was perhaps associated with its high fiber content.

Fiber. In a recent review of the effects of fiber on trace mineral balance in man, Kelsay concluded that iron balance was unaffected by dietary fiber (57). However, a study in humans was conducted using dual radioiron tags added to two meals having identical nutrient composition but containing either 5.1 or 0.3 g crude fiber calculated from food tables. Iron absorption averaged 3% from the high-fiber meal, and twice that level was absorbed from the low-fiber meal.[2] Further unpublished studies by the same group of investigators confirmed the bran inhibition of iron absorption but failed to show any effect due to pectin or purified cellulose.

Under in vitro conditions, iron reportedly was bound by acid detergent fiber and neutral detergent fiber from wheat and maize (58). Pectin, but not cellulose, also exhibited high iron binding activity in vitro and reduced by 40% the amount of iron absorbed by a group of iron-overloaded patients with hemochromatosis (59).

Obviously, the effect of fiber on iron absorption has not been completely clarified. Studies using purified fiber sources may not be representative of the interaction of iron with foods that are naturally high in fiber.

Model for Estimating Iron Availability

A practical model has been proposed for estimating the amount of iron likely to be absorbed from any given diet (2). By

[2]Cook et al. Unpublished data.

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knowing how much meat and ascorbic acid is in the food consumed, meals are classified as having low, medium, or high iron availability. Estimates can then be made of the amount of heme and nonheme iron that would be absorbed by people having different levels of iron stores. This value is much more physiologically useful than the total iron content of the diet.

Dietary factors that inhibit iron absorption may vary in their degree of potency, but they clearly outnumber the two identified enhancers. It is hoped that, as new information accumulates, it can be incorporated into an updated model that addresses the interaction of both enhancers and inhibitors in determining the net bioavailability of iron in the diet.

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Serum ferritin: a possible model for the assessment of nutrient stores[1-3]

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Introduction

The importance of iron as a nutrient was first recognized by the anemia that always accompanies a severe deficiency of the metal. The subsequent search for methods to detect milder degrees of iron deficiency has been facilitated by the existence of two highly specialized iron proteins—transferrin for the transport of iron between cells, and ferritin for the storage of iron within cells. The tissue concentration of ferritin or total nonheme iron in accessible sites of iron storage such as the liver or bone marrow is a reliable measure of body iron stores. However, the difficulty in obtaining samples from these tissues has limited their usefulness for assessing iron status.

In 1972, Addison et al. (1) first reported that minute amounts of ferritin could be detected in normal human serum using a sensitive immunoassay and that the concentration of this circulating ferritin is closely correlated with body iron stores. Serum ferritin measurements have proven to be an invaluable tool for evaluating iron status, both in clinical practice and in prevalence surveys. However, despite its practical value, the physiological significance of this tiny extracellular iron compartment remains obscure. Several comprehensive reviews have been published recently on ferritin biochemistry (2–5), and on both the methodological (6, 7) and clinical implications (6, 8, 9) of serum ferritin measurements. The following overview of these findings is presented in the hope that extrapolations to the assessment of other body nutrients may become apparent.

Biochemistry of tissue ferritin

Roughly two-thirds of body iron stores are contained in ferritin, the soluble fraction of tissue nonheme iron. The remainder exists as insoluble hemosiderin, an heterogeneous material composed largely of denatured ferritin and small amounts of sugar, sialic acid, lipid, and other proteins. Hemosiderin, which is visible by light microscopy, is the end point of the intracellular storage iron pathway. The concentration of tissue ferritin closely parallels that of hemosiderin although the ratio of ferritin to hemosiderin is higher in hepatic parenchymal cells and lower in reticuloendothelial (RE) cells in bone marrow and spleen.

Ferritin is an ubiquitous protein that is present throughout the plant and animal kingdom. While it is probably present in all cells, the concentration is too low in many tissues to measure it without the use of sensitive immunological assays. Although studies of ferritin biochemistry began in the early 1940's (10), the exact biochemical structure is still an area of active investigation. The characterization of ferritin has been facilitated by the fact that the protein is highly soluble in water, resistant to heating to 70 to 75°C, and readily crystallized with cadmium sulfate. It is a spherical protein consisting of an outer shell of 24 identical or nearly identical subunits, each with a molecular weight of 18,500 daltons. The molecular weight of the assembled iron-free protein apoferritin is about 445,000 daltons.

Iron is incorporated into ferritin by passing through tiny channels in the protein coat and is then deposited on a central core of highly insoluble ferric oxide phosphate micelles. This dense iron core provides a convenient means of assessing the cellular distribution of ferritin by electron microscopy. The iron con-

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tent of ferritin may vary considerably and iron may account for up to 20% of the protein's dry weight or about 4500 iron atoms/molecular apoferritin. The maximum molecular weight of the iron saturated protein is about 900,000 daltons. The ultrastructure of ferritin crystals has been extensively studied and amino acid sequencing of horse spleen ferritin has recently been completed (Crichton RR, unpublished observations). The biosynthesis of ferritin, including the manner in which iron is incorporated, has been investigated in detail. It has been demonstrated in animal studies that iron administration can induce a 6-fold increase in hepatic ferritin synthesis which is mediated at the posttranslation level. A similar increase in synthesis occurs in response to acute inflammation, an observation that has led to the suggestion that ferritin is an acute phase reactant.

A productive avenue of recent investigations stems from the observation that ferritin is not an homogeneous protein on isoelectric focusing but may contain as many as 20 isoferritin bands. The most popular view at present is that this microheterogeneity is genetic in origin and results from the existence of at least two ferritin subunits of slightly different size and electrical charge (Fig. 1) (11). The subunit that predominates in the more acidic bands has been designated the "H" subunit since it is seen predominantly in ferritin isolated from heart. The "L" subunit

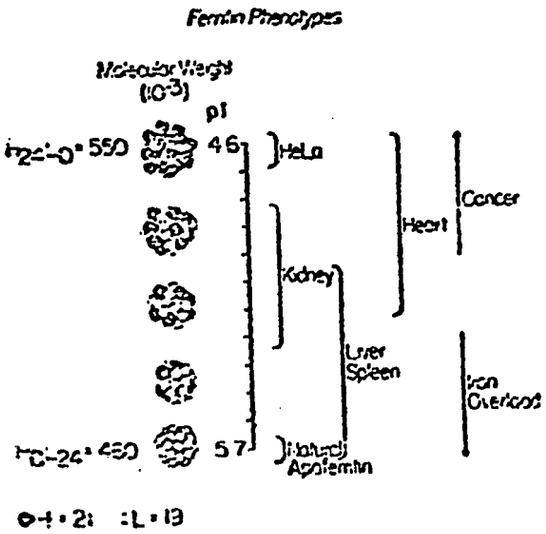

FIG. 1. A proposed model to explain the different isoferritins found in the body (11).

predominates in the more basic isoferritins that are seen in liver ferritin. It is suggested that the assembled ferritin molecule contains varying proportions of H and L subunits ranging from the holopolymer of L in the most basic isoferritin to approximately 30% L and 70% H in the most acidic ferritin. This genetic hypothesis predicts the existence of 23 heteropolymers and two holopolymers, which is entirely consistent with the number of bands observed on isoelectric focusing. The existence of different ferritin subunits has been of some help in identifying the tissue source of circulating ferritin and in explaining certain immunological differences in immunoassays of serum ferritin.

Serum ferritin concentrations in health and disease

In considering methods for measuring iron status, it is convenient to divide body iron into compartments. The essential or functional compartment is that iron contained in Hb, myoglobin, and certain iron-containing enzymes. A much smaller compartment of storage iron exists only to replace any iron loss from circulating hemoglobin. The majority of clinical measurements to detect iron deficiency (e.g., Hb concentration, red cell indices, transferrin saturation, free erythrocyte protoporphyrin) becomes abnormal only after iron stores are completely exhausted; there are only a few approaches that can detect changes in the storage compartment. The most precise quantitative procedure is to phlebotomize the subject at weekly intervals until significant anemia develops. The original level of iron stores can then be calculated by subtracting the induced deficit in circulating Hb from the amount of iron removed by phlebotomy. Another procedure is to measure iron absorption which varies inversely with iron stores. Iron stores can also be evaluated qualitatively by examining a sample of bone marrow or liver with light microscopy after staining for iron or quantitatively by chemical analysis of nonheme iron. However, these invasive approaches are justified only in a clinical setting to identify the cause of frank anemia or to diagnose iron storage disease.

In comparison to these rather cumbersome measurements the serum ferritin level pro-

vides a simple quantitative measurement of body iron stores. There are many studies that indicate that serum ferritin is a measure of iron reserves. For example, the mean serum ferritin level in various surveys in developed countries is 90 to 95 μg/l in men and 25 to 30 μg/l in women (8). These values are in exact proportion to average values of 900 and 300 mg storage iron determined in several earlier studies by quantitative phlebotomy. The serum ferritin level falls progressively during pregnancy when iron stores are diminishing, and this decline can be prevented by iron supplementation (12). A rapid fall in serum ferritin concentration occurs during the first 6 months of life, consistent with developmental changes in storage iron during this period (13). In normal blood donors, serum ferritin has been shown to correlate closely with the frequency of blood donation (14). A close inverse relationship has been observed in normal subjects between percentage absorption or iron and serum ferritin (15, 16). Finally, iron stores in normal men measured directly by quantitative phlebotomy have shown a high correlation with serum ferritin; pooled values from regression analysis in different studies indicate that 1 μg/l serum ferritin is equivalent to about 10 mg storage iron (17–19).

A close relationship between serum ferritin and body iron stores has also been demonstrated in various clinical studies. In patients with aplastic anemia, the serum ferritin has been shown to correlate with the number of previous blood transfusions (17, 20). The serum ferritin level is markedly increased in patients with idiopathic hemochromatosis and the level correlates closely with hepatic iron concentration (21). Furthermore, regular therapeutic venisections in these patients induce a predictable and orderly fall in the serum ferritin level. In patients with thalassemia major, plasma ferritin is known to correlate with both hepatic iron concentration and the number of previous transfusions (22). It should be noted, however, that the decline of iron stores and serum ferritin may be much different in normal individuals than in patients with iron overload.

The single most important facet of serum ferritin levels from both a clinical and a nutritional standpoint is that a low serum ferritin in a patient with a low Hb is diagnostic of iron deficiency anemia. In a total of 154 cases of uncomplicated iron deficiency reported in the literature, values above the lower cutoff level of 12 μg/l were observed in only 2.6% of patients who had no stainable iron on bone marrow examination (23). In hospitalized patients, however, the diagnostic value of the serum ferritin is diminished by the fact that a variety of clinical disorders such as infection, chronic inflammatory disease, malignancy, and liver disease produces an independent 2- to 4-fold elevation in serum ferritin at any given level of storage iron. Thus, while a serum ferritin below 12 μg/l is diagnostic of iron deficiency, levels as high as 60 μg/l may be seen when iron deficiency and inflammation or liver disease occur in the same patient (23).

One of the most important applications of serum ferritin measurements is in evaluating the iron status of a population. The serum ferritin measurement has several advantages over previous laboratory determinations. It is a more sensitive index of iron deficiency because in contrast to transferrin saturation or free erythrocyte protoporphyrin, the serum ferritin reflects changes in iron stores before they are completely exhausted. The serum ferritin also has greater specificity than the transferrin saturation or free erythrocyte protoporphyrin which do not distinguish between iron deficiency anemia and the anemia of chronic disease which is associated with a block in RE iron transport. Another advantage is that serum ferritin reflects changes in iron status within the iron replete segment of the population, a facet that is particularly important when monitoring the impact of iron fortification or supplementation programs. Moreover, the serum ferritin will not only indicate a change in median iron stores, but will also identify individuals who may be at risk of developing iron overload. One limitation of serum ferritin levels in population studies is that once iron stores have become depleted, the level no longer reflects the severity of the iron lack. It should therefore not be used to replace but rather to complement other iron storage parameters. Indeed, measurements of Hb concentration, transferrin saturation, free erythrocyte protoporphyrin, and serum ferritin were combined in a recent study to obtain a frequency distribution of iron stores in the entire population (24). This

type of analysis furnishes more information than the traditional approach based on the proportion of individuals falling below certain specified cutoff points. However, it may not be possible to determine the frequency distribution of body iron in populations where infection, inflammation, or protein-calorie malnutrition are present because of their separate effect on iron status parameters.

Physiological significance of serum ferritin

Because of the undisputed practical value of serum ferritin measurements in clinical and nutritional studies, a concerted effort has been made to determine the biological significance of the circulating protein that is designed to sequester iron intracellularly. Studies to determine the kinetics and biochemical nature of serum ferritin in human subjects have been difficult because of the low concentration of the protein. Despite this technical problem, a considerable amount of information has been obtained.

The source of serum ferritin appears to be the RE cell. When an RE-cell-label such as heat-damaged red blood cells tagged with ^{59}Fe are injected into rats, labeled ferritin appears in the circulation within minutes whereas no such labeling follows the injection of a parenchymal cell tag (25). Additional support for the RE cell origin of serum ferritin is the intimate relationship in clinical studies between marrow RE cell stores and serum ferritin, even when the latter is disproportionately elevated by inflammation or liver disease (20). However, in certain diseases such as viral hepatitis and malignancy, parenchymal cell distruction may be associated with spillage of intracellular ferritin into the circulation (8).

When purified ferritin is injected intravenously into rats, the protein is cleared exclusively by the parenchymal cell of the liver (26, 27). Despite the large molecular size of the protein, very rapid clearance half-times on the order of 5 to 15 min have been observed in the rat (25), dog (28), and rabbit (Worwood M, unpublished observations). A study in newborn infants has also demonstrated a half-time of 5 to 10 min which lengthens slightly when ferritin concentration is increased (27). This rapid turnover would be consistent with a sizable pathway of internal iron transport if the circulating protein was saturated with iron. However, the pattern of serum ferritin on sucrose density centrifugation resembles that of the iron-free protein apoferritin (29), and Worwood et al. (30) reported that the iron content of ferritin isolated from the serum of patients with transfusional iron overload was less than one-tenth that of tissue ferritin. It should also be noted that most of the ferritin kinetic studies were performed in animals with purified ferritin rather than with native serum ferritin. In a recent study in human volunteer subjects who were injected with ^{125}I-labeled ferritin which had been isolated from the serum of patients with iron overload, a clearance half-time of several hours was observed (Worwood M, unpublished observations). This lower turnover is more in keeping with relatively low day-to-day variability in serum ferritin levels.

In attempting to define the nature and origin of serum ferritin, emphasis has been placed on the isoferritin profile of the circulating protein and its immunological properties as evaluated with antisera against heart, liver, spleen, or tumor. Serum ferritin is generally composed of the more basic ferritins and thereby resembles the natural apoferritin of the liver (8, 29). However, major shifts in the isoferritin profile may be seen in various disease states. For example, patients with massive iron overload have only the more basic isoferritins but during the course of therapeutic iron depletion, a gradual increase in acidic ferritin occurs (31). Diagnostic possibilities have been raised because of the immunological differences observed in serum ferritin from malignant tissue and normal individuals, but the subject is complex. Several comprehensive reviews of the area have been published recently (3, 6, 8, 9).

Recent information has been obtained that the isoferritin profile of ferritin in serum is related to carbohydrate content (8, 32, 33). It was observed that about two-thirds of ferritin in serum of normal subjects binds to concanavalin A whereas no such binding occurs with tissue ferritin from heart, spleen, or liver or with serum ferritin in patients with hepatic necrosis. Moreover, when ferritin that had been isolated from the serum of patients with hemochromatosis was treated with neuraminadase which releases sialic acid residues, microheterogeneity was completely abol-

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ished. The isoferritin profile of serum ferritin therefore appears to be a function of glycosylation rather than differences in subunit composition.

These observations may have important implications for the physiological significance of serum ferritin. In normal subjects, it would appear that much of the circulating protein enters the plasma by secretion from the RE system and that carbohydrate residues are added during this process. This could account for the low iron content of the ferritin in serum since there would be scant opportunity for iron incorporation during this secretory process. The nonglycosylated fraction of circulating ferritin may represent direct release of cytosolic ferritin, a suggestion that is in keeping with the observation that most of the ferritin circulating in patients with extensive hepatic necrosis does not bind to concanavalin A. Apparently, most of the ferritin in plasma is synthesized on polyribosomes bound to the endoplasmic reticulum, and is therefore excreted from the cell whereas most apoferritin synthesis takes place on free polyribosomes and is consequently retained within the cell. The sialic acid content of circulating ferritin may help not only in identifying its origin but, since the carbohydrate content of plasma proteins often affect their survival in plasma, glycosylation may determine the rate and destination site of ferritin cleared from the circulation.

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Reduction of the soy-induced inhibition of nonheme iron absorption[1-3]

Timothy A. Morck, Ph.D., Sean R. Lynch, M.D., and James D. Cook, M.D.

ABSTRACT Recent studies in normal men have demonstrated that soy products have a pronounced inhibitory effect on the absorption of nonheme dietary iron. The purpose of the present investigation was to explore approaches to overcoming this inhibition. A modest but significant increase in iron absorption was observed when test meals containing either isolated soy protein or whole soybeans were baked at 200°C. The addition of 100 mg ascorbic acid to a meal containing isolated soy protein had a more dramatic effect, increasing mean absorption from 0.6 to 3.2%. A similar but less pronounced increase was observed when meat was added to the isolated soy protein meal. These results indicate that the inhibitory effect of soy can be reversed to some extent by substances known to enhance nonheme iron absorption. *Am J Clin Nutr* 1982;36:219-228.

KEY WORDS Iron absorption, soy protein, soybeans, ascorbic acid, meat

Introduction

In a recent study comparing the effects of semipurified proteins, we observed that several soy products inhibited nonheme iron absorption (1). When protein equivalent amounts were substituted for egg albumen (EA) in a meal containing no meat products, decreases in iron absorption ranged from 65 to 92%. Similarly, when 30 g dry textured soy flour was added to a meal containing either 100 or 70 g ground beef, absorption was reduced by 61 and 53%, respectively. Since soy products are a good source of high quality protein, the feasibility of counteracting their inhibitory effect on iron absorption by dietary manipulation merits careful consideration. Two approaches were examined in this report: *1)* extensive heating of soy products to destroy possible inhibitory compounds, and *2)* adding enhancers of iron absorption to meals containing soy products to improve iron availability.

Methods

Subjects

Twenty-six male subjects ranging in age from 18 to 50 yr participated in three separate iron absorption studies. All were in good health and denied a history of gastrointestinal disorders that might affect iron absorption. Iron status was uniformly normal with the exception of one volunteer who had a serum ferritin level of 2 μg/l, indicative of markedly reduced iron stores. Written informed consent was obtained from each volunteer before the study. Experimental procedures were in accordance with the standards of the Human Subjects Committee at the University of Kansas Medical Center.

In study I (nine subjects), iron availability from whole soybeans and from a meal containing isolated soy protein (SP) was determined both before and after extensive baking of the soy component. The other two studies, each with seven subjects, examined the effect of adding the known iron absorption enhancers ascorbic acid (study II) and beef (study III) to semipurified meals containing either EA or SP as the protein source.

Absorption measurements

Double sequential radioiron labels were used to measure iron absorption from four separate test meals in each subject. All meals were administered between 7 and 9 AM after an overnight fast, and only water was allowed for the subsequent 3 h. The absorption of nonheme iron was measured by adding variable quantities of $FeCl_3$ in 1 ml 0.01 N HCl labeled with either 2 μCi ^{59}Fe or 5 μCi ^{55}Fe to the test meals. Percentage absorption was determined from incorporated red cell radioactivity as described previously (1, 2).

Test meals

With the exception of meals C and D in study I which contained whole soybeans, all test meals consisted of a

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mixture of semipurified ingredients which were thoroughly mixed and served as a drink (SS meal). This meal contained corn syrup solids (Dri-Sweet 36, Hubinger Co., Keokuk, IA) and corn oil which provided 66.8 g carbohydrate and 34.8 g fat, respectively. The protein component, a variable under study, consisted of either 18.4 g spray-dried EA (Monark Egg Corporation, Kansas City, MO) or 16.9 g SP (Supro 710, Ralston Purina Co., St. Louis, MO). Both of these products provided 14.7 g protein per meal. Their iron content differed significantly (EA:10.9 ppm, SP:117.2 ppm).

In the first part of study I (meals A and B) the effect of baking SP on iron absorption from the SS meal was examined. A slurry of SP in water was prepared (1:3 by weight). It was spread onto a lightly greased cookie sheet and baked for 1 h at 200°C. The dried residue was finely ground with a mortar and pestle and added to the other meal ingredients. Baking destroyed the water dispersibility of SP resulting in a gritty precipitate that settled out of the liquid meal while standing. Taste was not appreciably altered. Ferric chloride providing 2.0 mg iron and the radioiron label were added to both meals bringing the total iron content to 4.0 mg per serving.

The effect of heating whole soybeans was studied in the second part of study I (meals C and D). Dried soybeans (Arrowhead Mills, Inc., Herford, TX) were first soaked in water overnight. This water was discarded and fresh water added together with diced onions, salt, monosodium glutamate, and bay leaves. After boiling for 2 h, the liquid was decanted and 100-g portions of boiled soybeans were weighed out. Additional 100-g portions were placed on greased cookie sheets and baked at 200°C for 45 min with periodic turning. Average weight per serving of these light brown, crunchy, toasted soybeans was 38 g, indicating a 62% loss of moisture. Individual portions of boiled or baked beans (iron content after preparation: 13.0 and 14.6 ppm, respectively) were served with 100 ml deionized water containing 3.0 mg iron.

Erythrocyte radioiron concentration was measured by the method of Eakins and Brown (3) and serum ferritin by the 2-site radioimmunometric technique (4). The frequency distribution of iron absorption measurements when expressed as a percentage of the administered dose is logarithmic (5). Absorption data were therefore converted to logarithms for statistical analysis and the results reconverted to recover the original units. When comparing absorption between any pair of test meals in the same subjects, a paired *t* test was used to determine whether mean log of the meal ratios differed significantly from zero. This is equivalent to testing whether the mean ratio in percentage absorption differed from 1.0.

Results

Effect of baking (study I)

Mean absorption from the SS meal containing uncooked SP (meal A) averaged 0.64% (Table 1). When SP was baked (meal B), mean absorption increased to 1.28%. The absorption ratio B/A (1.99) was highly significant ($p < 0.001$). A similar but less pronounced increase in absorption was observed when boiled soybeans (meal C) were further

TABLE 1
Effect of baking isolated SP and whole soybeans on iron absorption (study I)

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption: Isolated SP, Uncooked (A)	Iron absorption: Isolated SP, Baked (B)	Iron absorption: Whole soybean, Boiled (C)	Iron absorption: Whole soybean, Baked (D)	Absorption ratio: B/A	Absorption ratio: D/C	Absorption ratio: C/A	Absorption ratio: D/B
	yr	%	*μg/l*	*% of dose*							
1	22	41	14	0.26	0.51	0.63	0.96	1.96	1.52	2.42	1.88
2	27	46	172	0.37	1.16	1.37	0.72	3.13	0.52	3.70	0.62
3	20	45	55	0.52	1.08	1.38	2.63	2.07	1.90	2.65	2.43
4	29	46	132	0.53	1.48	0.85	0.97	2.79	1.14	1.60	0.65
5	19	45	61	0.68	2.45	0.18	0.92	3.60	5.11	0.26	0.37
6	35	44	35	0.70	1.15	4.06	7.07	1.64	1.74	5.80	6.14
7	18	46	32	0.85	1.67	1.65	1.65	1.96	1.00	1.94	0.98
8	25	46	139	0.87	0.83	1.02	1.82	0.95	1.78	1.17	2.19
9	20	46	56	2.10	2.61	1.43	2.02	1.24	1.41	0.68	0.77
Mean*	24	45	46	0.64	1.28	1.06	1.60	1.99†	1.50‡	1.65§	1.25§
			−1 SE	0.53	1.08	0.80	1.26	1.73	1.23	1.21	0.93
			+1 SE	0.78	1.52	1.41	2.02	2.30	1.84	2.25	1.67

* Geometric values except age and packed cell volume.
† $p < 0.001$.
‡ $p < 0.05$.
§ p = NS.

TABLE 2
Effect on iron absorption of 100 mg ascorbic acid (AA) added to semisynthetic meals made with isolated SP and with EA

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption				Absorption ratio			
				Isolated SP		EA					
				Alone (A)	+AA (B)	Alone (C)	+AA (D)	B/A	D/C	A/C	B/D
	yr	%	μg/l	% of dose							
1	24	41	2	0.23	4.91	5.77	11.53	21.34	1.99	0.03	0.42
2	26	42	38	0.33	1.93	6.37	8.67	5.84	1.36	0.05	0.22
3	33	42	49	0.36	1.25	8.93	11.75	3.47	1.31	0.04	0.10
4	50	43	72	0.43	1.56	3.36	7.65	3.62	2.27	0.12	0.20
5	24	45	15	0.46	11.01	3.53	10.33	23.93	2.92	0.13	1.06
6	19	47	29	0.48	3.68	2.06	5.17	7.66	2.50	0.23	0.71
7	24	47	130	0.80	3.40	4.90	12.43	4.25	2.53	0.16	0.27
8	25	44	72	0.82	2.47	3.20	8.15	3.01	2.54	0.25	0.30
9	26	42	24	0.82	2.97	13.38	19.82	3.62	1.48	0.06	0.14
10	20	44	32	0.90	4.32	4.90	14.86	4.80	3.03	0.18	0.29
11	23	45	36	1.45	4.53	6.63	8.68	3.12	1.30	0.21	0.52
Mean*	27	44	31	0.56	3.20	5.05	10.19	5.69†	2.02†	0.11†	0.31†
			−1 SE	0.47	2.66	4.31	9.14	4.55	1.83	0.09	0.26
			+1 SE	0.66	3.84	5.91	11.37	7.10	2.23	0.14	0.39

* Geometric values except age and packed cell volume.
† $p < 0.0001$.

baked (meal D). Mean absorption from meal C averaged 1.06% and increased to 1.60% after baking (meal D), (ratio D/C = 1.50, $p < 0.05$).

Effect of ascorbic acid (study II)

The effect of adding 100 mg ascorbic acid to the SS meal containing either SP (meals A and B) or EA (meals C and D) was examined. The total iron content of each serving was adjusted to 4.0 mg by the addition of 2.0 and 3.9 mg iron as ferric chloride to SP and EA meals, respectively. Absorption from the SP meal without ascorbic acid was very low, averaging 0.56% (Table 2). When ascorbic acid was added, absorption increased significantly to 3.20% (meal ratio B/A = 5.69, $p < 0.001$). Absorption of 5.05% iron from meal C containing EA was much higher than from meal A containing SP. With the addition of ascorbic acid (meal D), absorption increased over 2-fold to 10.19% (D/C = 2.2, $p < 0.001$).

The value of 0.11 for mean absorption ratio A/C indicates an 89% inhibition of iron absorption when SP was substituted for EA in the SS meal. The degree to which 100 mg ascorbic acid reduced this inhibitory effect is indicated by the absorption ratio B/C = 0.63. Although iron absorption from meal B with SP and ascorbic acid remained 37% lower than from meal C with EA alone, this difference was not statistically significant.

Effect of beef (study III)

A 100-g ground beef patty was broiled and served with the SS meal containing SP (meal B) or EA (meal D). Meals A and B contained 2.1 mg nonheme iron derived from SP while EA provided 0.2 mg iron in meals C and D. In meals B and D, the beef contributed 1.5 mg total iron and 0.5 mg nonheme iron. Sufficient $FeCl_3$ was added to all the meals to raise the iron content to 5.6 mg.

When beef was added to the EA meal, absorption increased by only 26% (5.94 to 7.47%; $p < 0.05$) (Table 3). However, absorption increased nearly 4-fold, from 0.36 to 1.44% ($p < 0.001$) when beef was added to the meal containing SP. Absorption from meal B (SP + beef) expressed as a ratio to that of meal C (EA alone) equals 0.25 showing a highly significant difference ($p < 0.001$). Therefore, the enhancing effect of beef failed to completely reverse the inhibiting effect of SP in this meal.

semisynthetic meals made with isolated SP and with EA

Subject	Age	Packed cell volume	Serum ferritin	Iron absorption				Absorption ratio			
				Isolated SP		EA					
				Alone (A)	+Beef (B)	Alone (C)	+Beef (D)	B/A	D/C	A/C	B/D
	yr	%	μg/l	% of dose							
1	23	45	89	0.11	0.28	2.05	4.12	2.54	2.00	0.05	0.06
2	28	44	76	0.12	1.01	3.90	6.68	8.41	1.71	0.03	0.15
3	23	50	39	0.18	0.75	4.02	8.67	4.16	2.15	0.04	0.08
4	21	44	80	0.35	0.88	1.65	1.27	2.51	0.76	0.21	0.69
5	24	46	94	0.75	3.45	6.22	4.07	4.60	0.65	0.12	0.84
6	19	44	45	0.77	2.36	17.48	20.71	3.06	1.18	0.04	0.11
7	19	45	18	1.95	8.48	45.61	51.03	4.34	1.11	0.04	0.16
Mean*	22	45	55	0.36	1.44	5.94	7.47	3.90†	1.26‡	0.06†	0.19§
			−1 SE	0.24	0.94	3.79	4.75	3.33	1.05	0.05	0.13
			+1 SE	0.55	2.20	9.30	11.76	4.58	1.50	0.08	0.28

* Geometric values except age and packed cell volume.
† $p < 0.0001$.
‡ p = NS.
§ $p < 0.01$.

Discussion

We recently reported an inhibitory effect of soy products on nonheme iron absorption (1). The observations made in that report, although similar to findings described here, are at variance with some earlier studies in which much better absorption from soy-based meals was recorded (6, 7). It seemed possible that the discrepancy might have resulted from differences in the methods used to prepare test meals containing soybeans. In the earlier investigations, ground soybeans were boiled in water to a thick paste and then baked at 150 to 200°C for 45 to 60 min. Iron absorption from these baked soybean meals was approximately 2.6 times greater than absorption from boiled beans in a later study performed in Jamaican children (8). In the present study, baking soybeans produced only a modest 50 to 100% increase in iron absorption and the iron was still considerably less available than it was in the SS meals containing EA. It should also be noted that extensive heating is not a practical solution for preparations like SP since unacceptable changes in their functional properties may occur such as the reduced water dispersibility noted in this study.

Another approach to counteracting the inhibitory effect of soy is to add substances that enhance the absorption of nonheme iron. The two that are most effective are ascorbic acid and meat (2). When 100 mg ascorbic acid were added (study II), iron absorption from the SP meal was only slightly lower than from the meal with EA and no ascorbic acid (3.20 versus 5.05% or 0.13 versus 0.21 mg iron), a difference that is not statistically significant. However, when beef was added to the meal with SP (study III), only 1.44% (0.07 mg) nonheme iron was absorbed compared to 5.94% (0.33 mg) from the meal containing EA alone. It is apparent from this study that the small enhancing effect of beef would be of little practical value for counteracting the inhibition caused by SP.

Our observations indicate that ascorbic acid partially reverses the soy-induced inhibition of nonheme iron absorption although the increase in amount of iron absorbed may be considerably less than when the same amount of ascorbic acid is added to a less inhibitory meal. For example, the addition of ascorbic acid to the soy-based meal in study II resulted in a more than 5-fold increase in percentage absorption compared to only a 2-fold increase when it was added to the meal with EA. However, the absolute increase in absorbed iron was only 0.11 mg in the case of the soy meal as compared to 0.2 mg with EA.

There are several practical limitations to the use of ascorbic acid for enhancing iron

absorption. It is expensive and unstable in oxidizing environments. Sayers et al. (6) reported no improvement in iron absorption when 100 mg ascorbic acid was added to soy flour before it was boiled and baked; 85% of the reduced form of the vitamin was lost during baking. The enhancing effect of ascorbic acid is better preserved when the food is only boiled (6). Furthermore, much of the vitamin would probably be lost during storage.

Another approach to overcoming the low availability of iron in soy-containing meals that should be considered is the addition of fortification iron to the meal. This would supply additional iron if percentage absorption remained constant. Because of the low availability of iron in these meals, relatively large amounts of fortification iron would be needed. The use of iron in a form such as sodium iron EDTA merits consideration since absorption of iron from this compound is less affected by inhibitory ligands in cereals than more conventional forms of fortification iron (9).

It is clear from our studies that the adverse effect of soy products on nonheme iron absorption can be modified. However, neither extensive heating nor the addition of the major enhancers of iron absorption completely reverses it. Our studies emphasize the need for a better understanding of the factor or factors responsible for this effect in order to develop effective strategies for combatting it.

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THE IMPORTANCE OF ANIMAL PRODUCTS IN HUMAN IRON NUTRITION

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THE IMPORTANCE OF ANIMAL PRODUCTS IN HUMAN IRON NUTRITION

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I INTRODUCTION

Long before iron deficiency was recognized as the major cause of hypochromic anemia, many clinicians believed that a high intake of meat would produce a hematologic reponse. Increasing the intake of meat in the diet is not a satisfactory method of treating patients with iron deficiency anemia. Nevertheless, there is mounting evidence that the intake of animal foods is a major determinant of iron status. Recognition of the importance of animal protein in iron nutrition is based largely on the results of food iron absorption studies in human subjects. Because these measurements form the cornerstone of our present knowledge of iron balance, they will be reviewed before discussing the importance of animal foods in iron nutrition.

II MEASUREMENTS OF IRON ABSORPTION IN MAN

Early studies of food iron absorption were based largely on chemical balance measurements; absorption was calculated as the difference between iron intake and fecal iron loss. This approach proved to be unreliable because the small differences between iron intake and iron loss are often obscured by iron contamination. Most of our present knowledge of iron nutrition is based on isotopic measurements of iron absorption which have been used extensively since their introduction in the late 1940's.

A. Radioiron Absorption

There are several ways of determining percentage absorption of orally administered radioiron: fecal radioiron balance, plasma radioiron

tolerance curves, whole-body counting, and measurements based on incorporated red cell radioactivity. When assessing iron balance in patients with various clinical disorders, whole-body counting is the optimal method because the results are not affected by the distribution of absorbed radioactivity. However, when studying food iron absorption in normal individuals, measurements of red cell radioactivity are preferable because as many as four separate iron absorption tests can be performed in the same subject using dual isotopes of iron (^{55}Fe and ^{59}Fe). By calculating the ratio of absorption from different meals in the same subject, the effect of differences in iron status between subjects can be eliminated. Moreover, any errors in estimating blood volume from height and weight or assuming that all subjects incorporate the same proportion of absorbed radioactivity into circulating red cells are eliminated by calculating the ratio of iron absorption in a series of tests in the same subject.

One drawback in food iron absorption studies is that percentage absorption of iron may vary markedly from study to study depending on the iron status of the subjects (Cook et al., 1969). This problem can be circumvented by measuring absorption in all subjects from a standard reference dose consisting of 3 mg inorganic iron as ferrous sulfate. By relating food iron absorption to reference dose absorption in each subject, the effect of differences in iron status of the subjects can be eliminated.

B. Biosynthetic Tagging

The most important aspect of food iron absorption is not the method

for determining isotopic retention but rather the technic used for tagging dietary iron. Early studies were performed with single food items that had been biosynthetically labeled with radioiron (Moore, 1964). Vegetable foods were labeled by growing them in hydroponic media containing radioiron and animal foods were tagged by injecting radioiron intravenously several months before sacrifice. Studies with these foods provided the first evidence that iron bioavailability of animal foods is much higher than vegetable foods (Layrisse et al., 1969; Martinez-Torres and Layrisse, 1974). Thus, iron absorption from staple foods (maize, black beans, and wheat) averaged 3-5% while absorption from animal foods, (meat, liver, and fish), ranged from 15-30%.

A major limitation of studies with biosynthetically labeled foods became apparent when two foods tagged separately with ^{59}Fe and ^{55}Fe were administered in the same meal. It was found that iron absorption of one food item could be markedly affected by the presence of a second food. For example, when labeled meat was administered with biosynthetically tagged maize, absorption of meat iron was diminished while absorption of maize iron was greatly enhanced (Martinez-Torres and Layrisse, 1971). It was obvious from these mixing studies that data obtained with biosynthetically tagged foods are of limited importance because of the complexity of the average diet. It was necessary to develop a technic to measure absorption from a complete meal.

C. Dietary Nonheme Iron

A major advance in studies of food iron absorption was the development in the early 1970's of extrinsic tagging to measure absorption of dietary

heme and nonheme iron. This approach was based on the observation that if a small quantity of inorganic radioiron is simply added to a vegetal food at the time of its administration, absorption is nearly identical to that of a biosynthetic, or intrinsic, tag. Moreover, if a meal containing several vegetal foods is labeled extrinsically after thorough homogenization, absorption of the extrinsic tag is nearly identical to absorption of a food item in the meal that has been biosynthetically labeled (Cook et al., 1972). These observations provide convincing evidence that a common pool of absorbable nonheme iron is formed with foods ingested in the same meal and that iron absorption from this pool can be measured by extrinsic tagging.

Although most studies have shown that extrinsic labeling is a valid means of measuring nonheme iron absorption, there have been a few exceptions. When biosynthetically tagged whole grain rice was tagged extrinsically with a second form of radioiron, absorption of the two labeled foods differed markedly whereas this difference was not seen when rice was administered as flour; apparently the extrinsic tag does not permeate the whole rice kernel (Björn-Rasmussen et al., 1973). Some forms of fortification iron, such as ferric orthophosphate and sodium iron pyrophosphate, do not exchange completely with dietary iron because of their high degree of insolubility (Cook et al., 1973) and it is likely that iron contamination of food by dirt and/or food handling processes also resul in an insoluble form of dietary iron that does not exchange with native food iron. Iron in ferritin and hemosiderin have been shown to exchang incompletely with an extrinsic tag, presumably because the protein coat of these storage iron proteins inhibits complete isotopic exchange. (Layrisse et al., 1975; Martinez-Torres et al., 1976). Nevertheless,

with these few exceptions, extrinsic tagging has proven to be a valid method of measuring dietary nonheme iron absorption.

D. Dietary Heme Iron

It is well known that iron enters the mucosal cell from the intestinal lumen not only as ionic iron but also as an intact porphyrin complex. This heme iron is rapidly degraded within the mucosal cell by heme oxygenase and the liberated iron enters the same pathway as absorbed ionic iron. Thus, it is either transported to the serosal surface where it enters the circulation or it is incorporated into ferritin as storage iron within the mucosal cell. It is important to distinguish between heme and nonheme iron because they are affected quite differently by the composition of the meal. Absorption of nonheme iron is markedly affected by substances that enhance iron absorption, such as ascorbic acid, and by inhibitors that bind nonheme iron and render it unavailable to the mucosal cell, such as tea or fiber. The absorption of heme iron, on the other hand, is little affected by the composition of the meal because this iron remains sequestered within the porphyrin complex until it enters the mucosal cell.

The same principle of extrinsic tagging for measurement of nonheme iron absorption can also be used to measure heme iron absorption; heme iron is labeled by adding a small quantity of radioactive hemoglobin to the test meal. Because there are two separate isotopes of iron available, ^{55}Fe and ^{59}Fe, it is possible to separately tag heme and nonheme iron in the same meal and thereby provide a measure of total iron absorption from a complete meal. This two-pool model is particularly relevant in studies of animal foods since animal tissue contains both forms of iron.

The validity of the two-pool model for studying iron absorption has been evaluated by comparing iron absorption measurements with predicted values of absorption based on measurements of body iron loss. In one study, a meal composed of aliquots of all foods contained in a typical 6-week diet was tagged with heme and nonheme radioiron and administered to 32 normal male subjects (Björn-Rasmussen et al., 1974). Dietary iron intake based on this composite meal was estimated at 17.4 mg iron daily of which 1 mg was in the form of heme iron. Absorption of nonheme iron averaged 5.3% or 0.8 mg/day while absorption of heme iron averaged 37% or 0.37 mg/day. The combined absorption of 1.2 mg iron daily is in close agreement with estimates of iron loss in normal men. Similar absorption data have been obtained by Layrisse and Martínez-Torres (1972) who studied a meal of meat, black beans, maize, and rice containing a total of 4.5 mg iron of which 1.5 mg was in the form of heme. In normal subjects, absorption of heme iron averaged 27% or 0.34 mg as compared with a value for nonheme iron of 6% or 0.12 mg; total absorption was 0.46 mg iron. In iron deficient subjects, absorption of heme and nonheme iron increased to 37% (0.52 mg) and 14% (0.43 mg), respectively, to give a total absorption of 0.95 mg. These two studies not only validate double extrinsic tagging, but also underscore the importance of heme as a source of dietary iron.

III BIOAVAILABILITY OF IRON IN ANIMAL FOODS

A. Meat

The use of biosynthetically tagged animal products in human iron absorption studies provided the first clear evidence of the beneficial

effect of these foods on iron balance. Early studies were performed with chicken (Moore, 1964) and rabbit muscle (Heinrich et al., 1969), but the most extensive observations have been made using veal (Layrisse et al., 1969). Radioactive iron was injected into the jugular veins of 3-month old calves; two to four months later, the animals were sacrificed, and the biosynthetically labeled muscle and liver were removed and kept frozen until administered in test meals to human subjects.

Veal muscle was cooked and served as hamburger to 107 fasting adult subjects, many of whom were iron deficient (Martinez-Torres and Layrisse, 1971). Geometric mean absorption in the composite group was 21.5%. In 58 of these subjects, absorption was also measured from a reference dose of ferrous sulfate; absorption averaged 23.8% giving a mean absorption ratio veal:reference of 1.12. The high bioavailability of veal muscle in this study was due at least in part to the high content of heme iron. Eighty-eight to ninety-three percent of the radioactivity was recovered in the supernatant of thoroughly hemogenized meat and 93-97% of this radioactivity was present as hemoglobin or myoglobin. Further evidence of the high heme iron content of veal was that substances that are known to alter the absorption of nonheme iron had little effect on the absorption of iron from veal muscle. For example, when 500 mg ascorbic acid was added to veal muscle, a slight increase (16%) was observed but this rise was not statistically significant. Similarly, the addition of 500 mg desferrioxamine to the labeled food produced a relatively modest decrease (27%) in absorption. These results are in striking contrast to the profound effect that ascorbic acid and desferrioxamine have on the absorption of nonheme iron.

B. Liver

Similar studies were performed with labeled veal liver (Martinez-Torres et al., 1974). When cooked veal liver was fed to 74 adult subjects, absorption averaged 19.8% with a typically broad range of 2.8 to 55.4%. In 27 of these subjects, reference dose absorption was measured simultaneously and averaged 16.8%; the liver:reference absorption ratio averaged 1.00. Analysis of the labeled veal showed that 78% of the radioactivity was in the form of hemoglobin while the remaining 22% was contained in either hemosiderin or ferritin. As with veal muscle, the addition of compounds such as ascorbic acid or desferrioxamine, which markedly influence nonheme iron absorption, had little or no effect on the assimilation of labeled liver. When 500 mg ascorbic acid was added to the labeled liver, iron absorption in 10 subjects actually decreased from a mean of 15.3 to 13.8%. When 500 mg desferrioxamine was added to the same food, mean absorption decreased from 20.6 to 8.6%. Although this difference was statistically significant, the effect was far less striking than the effect of this chelate on nonheme iron absorption.

C. Other Foods

Apart from veal muscle and liver, fish is the only other food that has been studied extensively by biosynthetic labeling (Martinez-Torres and Layrisse, 1970). Radiolabeled fish muscle was fed to 19 adult subjects who absorbed a mean of 18.3%; reference dose absorption averaged 44.8%. The iron in fish muscle is apparently less available than the iron in either veal muscle or liver. Iron absorption from eggs has also been studied by biosynthetic labeling (Moore, 1964; Chodas et al., 1957; Callendar et al., 1970). Mean absorption from iron in tagged eggs

was consistently less than 5%; this low iron availability is in striking contrast to results obtained with animal tissues.

IV EFFECT OF ANIMAL PROTEIN ON FOOD IRON ABSORPTION

A. Effect on Nonheme Iron Absorption

Animal foods are an important constituent of our diet, not only because of high intrinsic iron availability but also because of their ability to increase the absorption of all nonheme iron ingested simultaneously. This enhancing effect was first demonstrated in a series of studies by Layrisse et al., (1968). Vegetable and animal foods biosynthetically tagged with different radioiron labels were fed both separately and simultaneously to volunteer subjects to determine the extent to which the absorption of one food was affected by the other. Because both foods were tagged with separate radioiron labels, it was possible to detect any increase in iron absorption from the staple food as well a any decrease from the animal food.

Representative mixing studies with biosynthetically tagged foods are listed in Table I. Maize or black beans were used as the staple food and veal muscle, veal liver, or fish as the animal food. In the first study iron absorption from a meal containing only maize (4 mg iron) averaged 4.1% but when maize (1 mg iron) was fed together with veal muscle (3 mg iron), absorption of maize iron increased to 7.6%; the absorption ratio with:without animal protein averaged 1.87, an effect that was highly significant. Indeed, in all six studies shown in Table I, the increase in iron absorption from staple foods when taken with

veal muscle (3 mg iron), veal liver (2 mg iron), or fish (1 mg iron) was highly significant and veal liver was at least as effective as veal muscle.

While these studies identify the enhancing effect of animal tissue on nonheme iron absorption, it is difficult to estimate their quantitative effect on iron absorption from a normal diet. In order to obtain a constant total iron content in all meals in the studies listed in Table I, a smaller quantity of the staple food was given with animal tissue than when it was given alone. Since the quantity of food in a meal may have a more important effect on absorption than its iron content, it may be preferable to make substitutions on the basis of protein or caloric content rather than on iron content.

A final point in these mixing studies is the relatively minor effect of maize and black beans on the absorption of iron from veal muscle, veal liver, or fish. For example, absorption of veal muscle fell by only 14% and 17% when eaten with maize and black beans, respectively. Absorption of veal liver was reduced by 25 and 44% when consumed with 1 and 2 mg maize iron, respectively. The enhancing effect of animal tissue is much greater than the inhibitory effect of vegetal foods.

In a more recent study, several animal foods were evaluated with regard to their effect on nonheme iron absorption by substituting them in two types of test meals (Cook and Monsen, 1975). The first, considered to be a <u>standard</u> meal, was a meal containing beef, potatoes, corn, bread,

margarine, ice milk, and peaches. This meal contained 696 calories and 4.1 mg total iron; beef provided most of the protein. The second was a semisynthetic meal containing semipurified ingredients in which ovalbumin was the only source of protein. This meal was designed to match exactly the composition of the standard meal in respect to food energy, fat, protein, carbohydrate, calcium, phosphorus, and iron. Several animal foods were substituted in both meals including pork, lamb, beef, chicken, fish, whole milk, cheese, and powdered egg. These foods were substituted for beef in the standard meal and for ovalbumin in the semisynthetic meal in amounts that would provide the same quantity of protein. When the animal foods were substituted for beef in the standard meal, no adjustments were made for differences in nutrient content. With the semisynthetic meal, however, the other constituents were varied so that the substituted meal had the same total nutrient content as the original meals. Four iron absorption test meals were given to each volunteer subject: standard, semisynthetic, substituted standard, and substituted semisynthetic meals.

The results of the study are summarized in Table II. When pork, lamb, liver, chicken, and fish were substituted for beef in the standard meal, absorption ratios (substitute:beef) ranged from 0.82-1.03; in no case did the ratio reflect a significant difference. However, with dairy products, which contain no animal tissue, absorption ratios ranged from 0.22-0.39, all of which were statistically significant. Comparable results were obtained with the semisynthetic meal. Thus, when animal tissue was substituted for ovalbumin, the increase in absorptio ranged from 2.11 to 3.77. Some enhancement was also observed with chicken

and fish (2.43 and 2.11, respectively), although the increase was less pronounced than with other animal tissues. On the other hand, milk, cheese, and eggs were all similar to ovalbumin in their effect on the absorption of nonheme iron; ratios ranged from 0.88 to 1.22. These results indicate that dairy products have little or no enhancing effect on the absorption of dietary iron, and should not be considered as animal products with respect to food iron availability.

B. Effect on Ferritin and Hemosiderin Iron Absorption

Nonheme iron in animal tissue is comprised of ferritin and hemosiderin, the two major forms of storage iron in tissue. Nonheme iron in animal foods averages between 20 and 60% depending in part on the body organ; muscle, for example, has a much higher nonheme iron content than liver. Early studies in which purified ^{59}Fe labeled ferritin alone was administered to human subjects indicated an absorption of about 7% in normal subjects, values that were distinctly lower than absorption from other animal foods (Hussain et al., 1965). More extensive studies with radiolabeled ferritin and hemosiderin were reported subsequently by Layrisse et al., (1975), and Martinez-Torres, et al., (1976). Iron absorption of purified ferritin in 108 subjects ranged from 0.1 to 13.5% with a geometric mean of 1.9%, a much lower absorption than was observed in earlier studies. When purified ferritin was administered with biosynthetically tagged maize, iron absorption from maize was actually higher than from ferritin (2.0 and 0.9%, respectively); similar results were obtained with wheat and soybeans. However, when purified labeled ferritin was fed with animal tissue, a striking increase in absorption occurred. Absorption

increased from 1.3 to 12% when 2 mg ferritin iron was administered with veal muscle containing the same quantity of iron, and in a second study using veal liver, absorption increased from 1.7 to 6.6%. These absorption levels were comparable to those observed in meals containing both vegetable and animal foods. Since low absorption of ferritin iron may be an artifact related to purification of the protein, it seems unnecessary to regard ferritin and hemosiderin as separate pools of dietary iron.

C. Effect on Heme Iron Absorption

Animal tissue consists predominantly of heme iron and therefore is little affected by factors such as ascorbic acid or desferrioxamine which have a profound effect on nonheme absorption. It would seem unlikely that other foods would have any effect on the absorption of purified hemoglobin. Surprisingly, however, iron absorption from labeled hemoglobin is much less when the hemoglobin is given alone than when given with meat. Martinez-Torres and Layrisse (1971) observed an increase in absorption from 12 to 22% when labeled hemoglobin was mixed with meat prior to administration and this finding was recently confirmed by Hallberg et al., (1979). The enhancing effect of meat on hemoglobin iron absorption is more important from a physiologic than from a nutritional standpoint because, with the exception of heme iron fortification, dietary heme iron is consumed as a component of animal tissue.

D. Mechanism of Meat Effect

One of the vexing questions in iron nutrition is what mechanism accounts for the enhancing effect of meat and liver on nonheme iron absorption. Some inferences can be drawn from studies of the enhancing

effect of ascorbic acid, the only other dietary substance that is known to be as effective as meat in increasing iron absorption. There is evidence that the enhancing effects of meat and ascorbic acid are not additive. When 100 mg ascorbic acid was added to a semisynthetic meal (Cook and Monsen, 1977), the absorption ratio with:without ascorbic acid was 3.19. When the same quantity of vitamin C was added to a standard meal, a lower ratio of 1.67 was observed. In another study, the addition of 100 mg fish to a meal containing fresh papaya (70 mg ascorbic acid), produced no further enhancement in iron absorption (Layrisse et al., 1974).

A series of iron absorption studies designed to identify the effect of animal protein on iron absorption were recently reported by Björn-Rasmussen and Hallberg (1979). The possibility that meat acts by stimulating gastric secretion was ruled out when it was shown that meat increased iron absorption of maize from 0.7 to 2.1% in patients with histamine-fast achlorhydria. Furthermore, when betazole, a drug that stimulates gastric secretion, was administered to normal subjects, absorption did not increase significantly. A modest increase in absorption was observed when a mixture of amino acids in amounts corresponding to that contained in beef muscle was added to maize, but contrary to previous observations (Martinez-Torres and Layrisse, 1970), no increase was observed when cystine alone was added to maize. When beef was fractionated into a water extract (broth) and an insoluble residue and fed with a meal of maize, the residue increased iron absorption from 1.4 to 2.4% whereas the broth had no enhancing effect; the meat factor therefore does not appear to be water soluble.

The possibility that meat acts by stimulating mucosal uptake, or iron transport, would explain the similar enhancing effect on nonheme iron, ferritin iron, and heme iron. However, a mucosal cell effect should also exist with inorganic iron and meat does not increase the absorption of ferrous chloride (Layrisse and Martinez-Torres, 1972). Meat might act by stimulating gastric secretion or altering gastrointestinal motility in favor of iron absorption but it seems more likely that animal tissue promotes the solubility of both heme and nonheme iron, presumably by separate biochemical mechanisms. Whether a "meat factor" is present in meat when administered or only formed during digestion of the food in the gastrointestinal tract is still a matter of speculation.

V EFFECT OF INTAKE OF ANIMAL FOODS ON IRON STATUS

A. Estimates of Food Iron Availability

Little or no association has been found in various nutritional surveys between total iron intake and iron status. This may indicate that blood loss due to menstruation or pregnancy are more important determinants of iron nutrition than is the diet. Another possible explanation is that the total intake of dietary iron is less important nutritionally than the type of diet consumed. A model has recently been developed for estimating food iron availability and predicting absorption from the composition of the meal (Monsen et al., 1978). It is of interest to review this model since the content of animal food is an important parameter in estimating iron availability.

The first step in estimating iron absorption from a given meal is to calculate the total content of heme and nonheme iron. It is assumed for this purpose that pork, liver, fish, beef, lamb, and chicken all

contain 40% heme iron although, as noted earlier, this percentage may vary appreciably. Because heme iron absorption is not affected by the nature of the meal, iron absorption from heme is calculated by multiplying heme iron content by 25%, the estimated level of percentage absorption of heme iron in an individual with 500 mg body iron stores.

Estimating nonheme iron absorption from a meal is more complex because of food interactions. Two enhancing substances in the diet were considered to have overriding importance: ascorbic acid and animal foods including fish and poultry. One gram animal food was assumed to be roughly equivalent in its enhancing effect to 1 mg ascorbic acid. Meals were divided on this basis into those of low, medium, and high iron availability (Table III). Absorption of nonheme iron from these meals was estimated at 3, 5, and 8%, respectively, in a subject with 500 mg iron stores.

This model for estimating absorption of dietary iron is undoubtedly oversimplified and is a preliminary step at best in determining the importance of the diet in iron nutrition. Other dietary factors that are known to influence iron absorption, such as tea or EDTA, were not included in the estimates because precise quantitative data on their effect is not available. It is also not clear what factor should be used when the meal contains both ascorbic acid and animal food. Even with these limitations, however, this model will come much closer to determining the importance of diet in maintaining iron balance.

B. Epidemiologic Studies

Many attempts have been made to establish a correlation between iron intake and iron status of a population, but the correlation has been poor when based on total iron intake without regard to food iron availability. However, a recent study of 7,000 people in Finland showed that the intake of meat products does indeed correlate with iron status (Tukkanen and Seppänen, 1975). The intake of animal products was found to be significantly lower in both male and female anemic subjects as compared with those having a normal hemoglobin level. A correlation was also observed in this study between meat consumption and iron status as measured by either the serum iron or total iron-binding capacity (Figure 1). A much clearer picture of the importance of meat products should emerge when the model described above is used in future studies of dietary iron intake and its relation to iron status.

VI IMPORTANCE OF ANIMAL FOOD IN IRON FORTIFICATION

A. Nonheme Iron Fortification

The only practical long-term approach for combating iron deficiency anemia in a population is to increase dietary iron intake by fortifying with iron a common item in the diet such as bread, sugar, or rice. However, recent studies suggest that the success of iron fortification will largely depend on the intake of meat, fish, and poultry, and that without these animal foods, even heavy fortification is unlikely to be effective. For example, Layrisse et al., (1973) added increasing amounts of ferric chloride to meals containing either maize (1 mg iron), meat alone (3 mg iron), or a mixture the two foods in the ratio 2 mg meat iron:1 mg maize iron. Ferric chloride was chosen as the iron salt

because it undergoes complete exchange with dietary nonheme iron. Absorption values when 5, 20, and 60 mg iron were added are shown in Figure 2. When 60 mg iron, a level far above that which would be technically feasible on a national scale, was added to the maize meal, only 0.3 mg iron was absorbed. When this same amount of iron was added to a meal containing either meat alone or a mixture of meat and maize, an average of 3.27 mg and 1.22 mg, respectively, was absorbed.

Similar findings were observed in a later study by Hallberg et al., (1978). When 5 mg fortification iron as ferrous sulfate was added to a rice-based meal containing only vegetables, absorption in an iron-deficient individual was estimated at 0.03 mg iron. When 60 gm fish was added to the same meal, net absorption of fortification iron increased to 0.32 mg. The authors concluded that iron fortification may be feasible with a rice-based diet, but that a certain minimal threshhold level of fortification iron would be required to achieve a reasonable level of absorption. The relatively high level of 5 mg fortification iron per meal was considered realistic even with diets containing only negligible amounts of fish or animal foods.

B. Heme Iron Fortification

The low bioavailability of inorganic iron added to meals containing little or no animal products has prompted the suggestion that heme iron in animal blood recovered from the slaughterhouse could be employed for iron fortification (Reizenstein, 1975). Hemoglobin iron has the advantage that it is less inhibited by dietary substances that impair the absorption of nonheme iron. The dose response curve also may be more favorable with hemoglobin iron than with inorganic iron, at least in patients with

latent iron deficiency. Vehicles that have been suggested for heme iron fortification include blood sausage, pudding, meat products, and baking products.

The major limitation of fortification with animal blood is the risk of iron overload in iron replete individuals. Because heme iron absorption is less affected by iron status than nonheme iron absorption, heme iron might be well absorbed in iron replete individuals which could conceivably create a risk of iron excess in susceptible segments of the population. In addition, the dark color of blood greatly limits the number of food items that could serve as vehicles for fortification. The costs of recovering hemoglobin and processing it to avoid bacterial contamination are undoubtedly high. It is therefore unlikely that slaughterhouse blood will be extensively used for iron fortification.

VII SUMMARY

Studies employing double extrinsic tags of heme and nonheme iron to measure absorption of dietary iron have clearly established the importance of animal foods in iron nutrition. The major factor that accounts for high assimilation of iron from animal foods is their content of myoglobin and hemoglobin. These iron complexes enter the mucosal cell of the intestinal tract as intact molecules and therefore are little affected by dietary substances that impair the absorption of nonheme iron. Animal foods also play an essential role in iron nutrition because of their ability to enhance assimilation of all forms of dietary nonheme iron ingested simultaneously. It is unlikely that iron fortification programs would be effective in populations consuming only minimal quantitites of meat, fish, or poultry.

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Table I. Effect of animal tissue on the absorption of nonheme iron from a staple food.

Animal Food	Staple Food	No. of Subjects	Iron Content Of Staple Food (mg)		Iron Absorption From Staple Food (% of dose)		Mean Absorption Ratio (with:without animal food)
			Fed Alone	Fed With Animal Food	Fed Alone	Fed With Animal Food	
Veal Muscle [a] (3mg iron)							
	Maize	15	4	1	4.1	7.6	1.87
	Black beans	11	4	1	4.8	10.7	2.21
Veal Liver [b] (2mg iron)							
	Maize	12	2	2	4.9	10.9	2.22
	Maize	14	3	1	2.2	7.0	3.17
Fish [c] (1mg iron)							
	Maize	6	3	2	4.2	7.5	1.78
	Black beans	8	3	2	1.0	1.7	1.70

[a] Martinez-Torres and Layrisse, 1971
[b] Martinez-Torres *et al.*, 1974
[c] Layrisse *et al.*, 1968

TABLE II. Effect on nonheme iron absorption of substituting various animal products in meals containing either albumin (semisynthetic) or beef (standard) as the source of protein.†

SUBSTITUTE		SEMISYNTHETIC MEAL			STANDARD MEAL		
		Mean Iron Absorption (% of dose)		Absorption Ratio (SUB:ALB)	Mean Iron Absorption (% of dose)		Absorption Ratio (SUB:BEEF)
		ALB	SUB		BEEF	SUB	
Beef	(7)*	1.7	5.1^{+++}	2.96^{a}	--	--	--
Pork	(7)	1.6	5.2^{+++}	3.21^{a}	9.7	9.3	0.95^{a}
Lamb	(7)	1.6	5.2^{+++}	3.23^{a}	7.6	7.8	1.03^{a}
Liver	(8)	1.4	5.4^{+++}	3.77^{a}	3.8	3.9	1.01^{a}
Chicken	(7)	1.4	3.4^{++}	2.43^{ac}	11.5	10.4	0.90^{a}
Fish	(8)	1.9	3.9^{+}	2.11^{ac}	14.8	12.2^{+}	0.82^{a}
Milk	(8)	0.7	0.9	1.22^{bc}	5.5	1.6^{++}	0.29^{b}
Cheese	(8)	2.0	2.4	1.20^{bc}	9.4	3.6^{++}	0.38^{b}
Egg	(10)	0.8	0.7	0.88^{b}	8.2	1.8^{++}	0.22^{b}
Albumin	(7)	--	--	--	10.0	3.9^{++}	0.39^{b}

† Cook and Monsen, 1976

* number of subjects

$^{+}$ significant difference, $P < .05$; $^{++}$ significant difference, $P < .01$; $^{+++}$ significant difference, $P < .001$.

^{abc} mean ratios with differing superscripts are significantly different by Duncan's multiple range test.

TABLE III. Dietary Iron Availability.†

Meal Iron Availability	Meat, Fish Poultry (gm)		Ascorbic Acid (mg)	Nonheme Iron Absorption (%)
Low	<30	and	<25	3
Medium	30-90	or	25-75	5
High	>90	or	>75	8

† Monsen et al., 1978

FIGURE LEGENDS

Figure 1. Relationship between percent of population with TIBC greater than 400 ug/dl and daily intakes of meat in women (○) and men (●). (Takkunen and Seppänen, 1975)

Figure 2. Absorption of fortification iron (ferric chloride) added to meals containing maize alone (100 gm), meat alone (150 gm), and maize plus meat (50 and 100 gm, respectively). The enhancing effect of meat on nonheme iron absorption is apparent. (Layrisse et al., 1973)

Figure 1

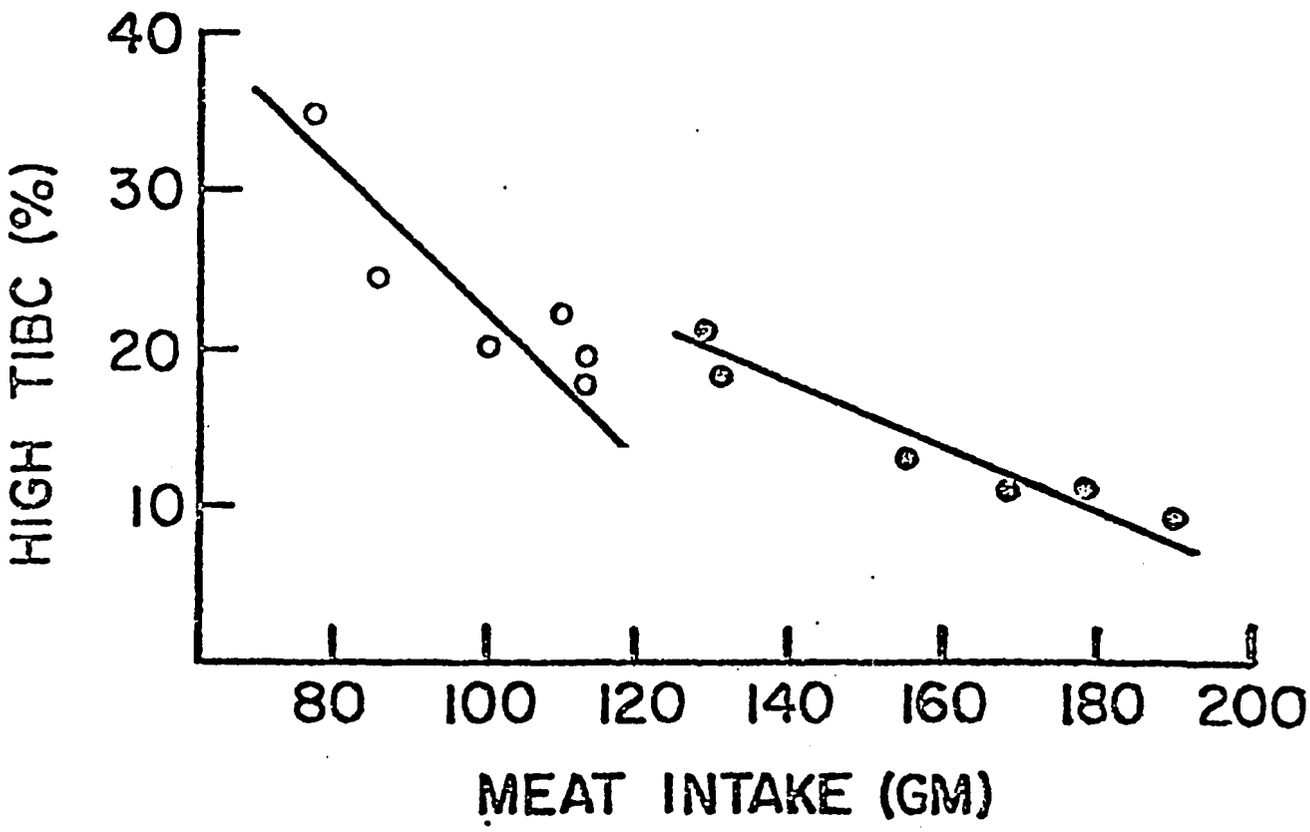
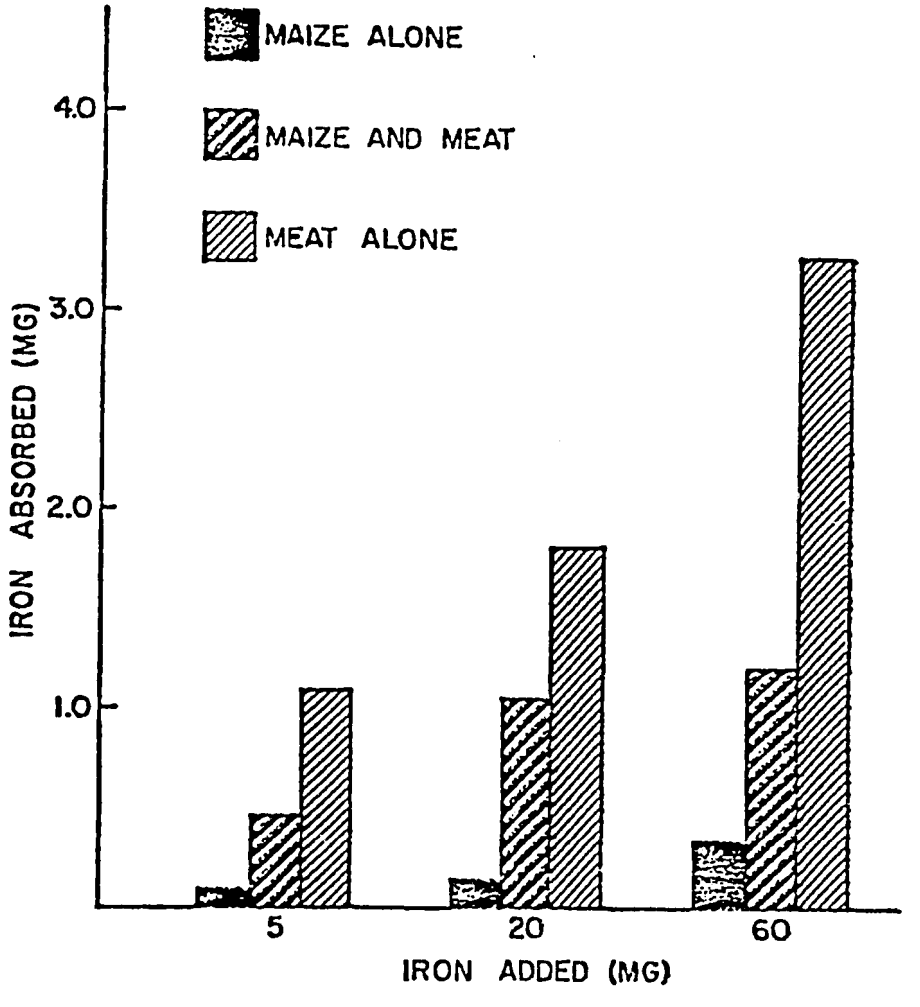


Figure 2


MAIZE ALONE
MAIZE AND MEAT
MEAT ALONE
IRON ABSORBED (MG)
4.0
3.0
2.0
1.0
5
20
60
IRON ADDED (MG)

Clinical Evaluation of Iron Deficiency

James D. Cook

SINCE IRON deficiency was first recognized as a cause of anemia at the turn of the century, its prevalence has not changed dramatically. There has, however, been continuing refinement of the laboratory methods to identify iron lack. The most important recent development is the measurement of serum ferritin which has provided a new dimension in assessing iron status. With the iron measurements now available, it is possible not only to identify iron deficiency readily but also to estimate the magnitude of the deficit and to monitor its correction with iron therapy.

A wide spectrum of laboratory measurements of iron status will be discussed in this review. It should be noted at the outset that there is no single test or combination of tests that is optimal in all clinical settings. The optimal set of methods is different for the infant, pregnant woman, and elderly patient. Similarly, the detection of mild iron deficiency in an otherwise healthy individual requires a different approach than the identification of iron deficiency in the hospitalized patient with severe anemia or advanced malignancy. A degree of flexibility is required in the diagnostic approach to iron deficiency.

LABORATORY PARAMETERS OF IRON STATUS

Iron deficiency usually evolves slowly and progresses through several stages before it develops into frank anemia. The sensitivity of various iron measurements varies with the severity of iron lack and on this basis iron deficiency is commonly divided into three stages. The earliest stage is *storage iron depletion* where iron reserves are lost but there is not yet a decrease in iron supply to the developing red cell. The second stage is *iron deficient erythropoiesis* in which erythroid iron supply is diminished but the circulating hemoglobin is not significantly decreased. The third and final stage is overt *iron deficiency anemia*. These three stages are referred to in the European literature as prelatent, latent, and manifest iron deficiency. The key laboratory measurements for each stage are listed in Table 1.

The only strictly quantitative method for measuring body iron is to phlebotomize the subject at weekly intervals until significant anemia develops. By determining the total amount of hemoglobin iron removed and the induced deficit in circulating hemoglobin iron, the iron stores originally present can be calculated by subtraction. While this approach is very tedious and rarely used today, it is still the standard by which other iron measurements are calibrated.

Bone Marrow Iron

This technique is the time honored method for assessing storage iron. Since the reticuloendothelial (RE) cell is the immediate source of plasma iron destined for erythroid marrow and since the bone marrow is the most accessible site for examining the RE cell, histologic evaluation of marrow iron provides a direct measure of iron stores.[16] The value of the marrow examination is that the presence of marrow iron in a patient with significant anemia excludes iron deficiency with two possible exceptions. In patients with a recent massive hemorrhage, large particles of iron may occasionally persist during the rapid mobilization of iron reserves.[66] The other exception is in patients recently given parenteral iron which may be seen histologically for weeks or even months in the face of continued iron deficiency.[56] The evaluation of marrow iron is used extensively in hospitalized patients because a marrow examination is often performed as part of the hematologic evaluation and because the results can be obtained in a matter of hours, faster than other iron measurements.

There is recent evidence that the examination of marrow iron may not be as infallible as generally believed, especially when using aspirated marrow rather than a needle biopsy. Fong et al. observed a significant disparity in stainable

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Table 1. Laboratory Measurements of Iron Status

I. Storage Iron Depletion
1. RE marrow iron
*2. Serum ferritin
3. Serum transferrin
II. Iron Deficiant Erythropoiesis
*1. Transferrin saturation
*2. Free erythrocyte protoporphyrin
3. Red cell indices
4. Sideroblast count
III. Iron Deficiency Anemia
*1. Hemoglobin or hematocrit
2. Therapeutic trial

*Basis for estimating body iron (Fig. 1).

iron in 251 needle biopsy sections and simultaneously obtained aspirated smears.[35] In most instances, stainable iron was more obvious in the aspirate than in the biopsy specimen. However, in another study comparing stainable iron in aspirates and biopsy specimens from 1,000 patients, the opposite disparity was noted.[46] Iron was detected in the biopsy sample in more than one-third of the marrows considered to have absent iron on an aspirated specimen. Thus, examination of marrow smears resulted in a significant overdiagnosis of iron deficiency. In the same study, a much better correlation with marrow biopsies was observed when iron stores were considered present or increased on aspirated samples. The disparity between marrow aspirates and biopsies was probably the result of assessing marrow aspirates containing insufficient marrow stroma. The absence of marrow iron on an aspirated specimen is not unequivocal evidence of absent stores. When evaluating marrow iron it is important to examine both unstained and stained aspirates, to obtain generous amounts of stroma, and to confirm the findings on marrow biopsy when possible.

Serum Ferritin

Body iron in excess of that required for hemoglobin formation is stored in tissues as ferritin, a soluble protein designed specifically for this function. Using a sensitive immunoradiometric assay, workers in Cardiff were the first to demonstrate that tiny quantities of ferritin were invariably present in human sera and that the concentration of the protein varied with body iron stores.[1] This report has been followed by a flood of literature, almost all of which has confirmed the value of serum ferritin as a measure of iron status.

There is ample evidence that in normal subjects, serum ferritin is directly proportional to body iron stores. During infancy and childhood, serum ferritin closely parallels the developmental changes in iron status.[62,63] Several studies have shown that the mean serum ferritin in adult men is approximately three times higher than in menstruating women, a difference that is consistent with the known sex difference in iron status. In men, the serum ferritin level rises slowly throughout life beginning in the late teens, whereas in women this gradual increase does not begin until the time of menopause.[20,34,53,61] Several workers have demonstrated a high correlation between serum ferritin and the number or frequency of blood donations[13,34,58] and others have demonstrated a strong inverse correlation between serum ferritin and iron absorption in normal subjects.[17,19,27,38,69] In a recent study by Bezwoda et al., serum ferritin, iron absorption, and chemical measurements of marrow non-heme iron were compared in 50 patients with a wide range of iron stores;[20] remarkably high correlations were observed between all three of these measurements. Studies in which iron stores have been measured quantitatively by phlebotomy[17,42,69] have provided the most convincing evidence of the close relationship between serum ferritin and iron stores. Weighted means from these studies indicated that within the range of 20–300 μg/l, 1 μg/l serum ferritin was equivalent to 10 mg storage iron.

The 'normal range' of serum ferritin has been the subject of much controversy. Since serum ferritin reflects iron status, substantial differences might be anticipated depending on age, sex, socioeconomic status, or geographic area of the survey. Surprisingly, there has been excellent agreement in mean serum ferritin levels in different reports. In six out of eight surveys performed in groups of 90 or more adult males, the median or geometric mean ferritin was between 92 and 94 μg/l, while in 4 of these series that reported 95% confidence limits, the lower cutoff point varied from 10–27 μg/l.[72] In five out of eight surveys in normal adult women, the median or geometric mean ferritin varied from 23–34 μg/l and from 50–55 in the remaining

three series. As noted previously, age was an important variable. In one study of men and women aged 73 years, geometric means of 166 and 161 μg/l were observed in 31 men and 22 women, respectively.[61]

One drawback of serum ferritin measurements is the complex methodology. Many laboratory methods are available, including immunoradiometric assays (IRMA) using labeled antibody, radioimmunoassays (RIA) using labeled antigen, and enzyme-linked immunoassays (EIA) which eliminate the need for radioisotopes. Despite the wide range of techniques, interlaboratory comparisons have shown that differences between laboratories are not substantially higher than batch-to-batch differences within the same laboratory. The increasing number of commercially available ferritin assay kits has made the cost of serum ferritin measurements comparable to other iron measurements such as transferritin saturation or free erythrocyte protoporphyrin.

An important advantage of the serum ferritin is its relative stability with repeated measurements in the same subject. When serum iron and serum ferritin measurements were performed 2 wk apart, correlation coefficients of 0.31 and 0.88 respectively were observed.[19] In a more recent study in normal subjects, the day-to-day variability in serum ferritin measurements in the same subject was 15% as compared to 28% for serum iron and transferrin saturation, despite the fact that the variability between assays was higher for serum ferritin than for serum iron.[59] Thus, most of the variability in serum ferritin measurements relates to the assay rather than to physiologic factors. Studies in normal subjects have failed to show a significant circadian rhythm[14,25] although in one study, a variation of 10% was recorded with troughs in the early morning and late afternoon.[51]

Total Iron-Binding Capacity (TIBC)

The iron transport parameters (serum iron, TIBC, transferrin saturation) usually do not change until storage iron is completely exhausted. An exception is the TIBC which may begin to increase as iron stores are depleted.[6,70] In one study performed in 326 male and female subjects between 20 and 50 yr of age, a significant inverse correlation between serum ferritin and TIBC was observed despite the fact that iron deficient erythropoiesis was carefully excluded on the basis of normal hemoglobin, free erythrocyte protoporphyrin, and transferrin saturation.[19] While the TIBC may reflect storage iron depletion, it is less sensitive to changes in iron stores than the serum ferritin.

Transferrin Saturation (TS)

When iron stores have been exhausted, the serum iron and TS decline. Of the various iron transport measurements, a TS below 16% is the best single criterion of impairment in iron supply to the developing red cell or iron deficient erythropoiesis.[4,40] Laboratory errors in measuring the serum iron and TS were common in the past because of iron contamination, but this factor has been largely eliminated by the use of disposable equipment and automated laboratory techniques. The serum ferritin and free erythrocyte protoporphyrin (FEP) will probably replace the serum iron and TIBC eventually, but since the latter are now an established part of the automated serum chemistry profile, this will likely occur very slowly.

The main clinical disadvantage of the TS is that it is highly labile. Because the plasma iron compartment is small in relation to the quantity of iron passing through it each day, even small changes in RE cell output produce marked changes in TS. There is a pronounced diurnal variation in serum iron and TS which can result in marked differences within the same individual over 24 hours. Even when measurements are performed at the same time each day, day-to-day variability is of the order of 30%–40%.[65] The serum iron in women usually falls 2–3 days prior to the onset of menstruation and may not return to normal for several days.[73] Moreover, a precipitous fall in serum iron can occur in a matter of hours following the onset of inflammation.[29]

Another disadvantage of TS common to all measurements of iron deficient erythropoiesis is that abnormal values are not specific for iron deficiency. A decrease in TS occurs in a wide range of clinical disorders and, as a result, the measurement is more useful in assessing iron status in a normal population than in a hospital-

ized patient. In a recent survey in over 1,400 women between 38 and 60 yr of age, 80% of women with a TS below 16% had absent iron on bone marrow examination compared to 25% of all the women.[50] In a hospital setting, the TS is useful for identifying iron deficiency only if it is associated with an increase in TIBC. Iron deficiency cannot be excluded if the TIBC is normal or reduced and, unfortunately, this applies to the majority of hospitalized patients. However, a TS above 20%–25% is helpful in excluding iron deficiency; marked elevations are the rule in disorders such as thalassemia major or sideroblastic anemia that are often confused with iron deficiency because of hypochromia and microcytosis.

Free Erythrocyte Protoporphyrin (FEP)

A lack of iron supply to the developing red cell impairs heme synthesis and results in an accumulation of protoporphyrin IX in the red cell. The introduction during the past decade of simple and rapid techniques for measuring FEP has resulted in an important new laboratory parameter of iron deficient erythropoiesis. FEP levels are expressed either as μg/dl whole blood or μg/dl red blood cells (RBC). Normal FEP is 35 ± 50 μg/dl RBC, levels greater than 100 μg/dl indicating overt iron deficient erythropoiesis.

Several modifications in FEP measurements have been described in recent years. The FEP:hemoglobin ratio (μg protoporphyrin/g hemoglobin) appears to be a useful clinical parameter[60,67] and measurement of the protoporphyrin:heme ratio has recently been described by Labbe et al. as a sensitive index of iron deficient erythropoiesis.[47] The protoporphyrin:hemoglobin ratio incorporates a correction for hemoglobin as does the FEP:hemoglobin ratio but since the metabolic ratio of protoporphyrin to heme is determined, the assay does not require accurate measurement of the volume of the blood sample. The method requires only 250 μl whole blood and the levels are stable at 4°C for at least 10 days. The mean protoporphyrin:hemoglobin ratio is 16.0 ± 3 with an upper cutoff level of 32. Finally, instruments are now available that can measure FEP directly by the fluorescence on a blood film.[15] This approach requires no technical training, is exceedingly fast, and is apparently sufficiently accurate. Even with standard micromethods for FEP, a single determination requires only about 10 minutes and 20 μl blood.

The FEP provides about the same information as the TS but is more stable since it increases only after several weeks of iron deficient erythropoiesis and does not return to a normal level for weeks or even months after iron therapy.[48] The FEP may be particularly useful in recognizing iron deficiency in infants. In a recent study by Thomas et al., TS, FEP, hemoglobin, and serum ferritin levels were measured in 20 normal infants and 20 infants with frank iron deficiency anemia as determined by microcytosis and a hemoglobin below 10 g/dl.[67] The two groups could be completely separated only with the FEP:hemoglobin ratio. Moreover, TS and serum ferritin became erratic following iron therapy and were affected occasionally by acute viral illness. On the other hand, the FEP:hemoglobin ratio declined steadily and showed none of the wide variations that occurred with TS or serum ferritin. One problem with the FEP is that the level is increased in lead poisoning which is often an important differential diagnosis in children. A careful history, measurement of blood lead levels, and demonstration of a fall in FEP to normal levels with iron therapy all serve to exclude lead exposure.

Red Cell Indices

In terms of sensitivity, red cell indices are intermediate between measurements of iron deficient erythropoiesis and frank iron deficiency anemia. Hypochromic and microcytic red cells will appear in the circulation before a significant decrease in hemoglobin concentration has occurred but if the change in red cell indices is pronounced, overt anemia is usually already present. Thus, while considered as a measurement of iron deficient erythropoiesis, red cell indices are most helpful in identifying iron deficiency in a patient with established anemia.

There is still some debate about which is the best red cell index for identifying iron deficiency, especially since their sensitivity has changed dramatically with the introduction of electronic counting equipment. The mean corpuscular volume (MCV), which is highly variable when

measured manually, is now regarded by most workers as the most sensitive, particularly in infants and children.[24] Moreover, because it is measured directly, the MCV is less subject to sampling errors. Other workers, however, have found the mean corpuscular hemoglobin (MCH) more reliable for identifying iron deficiency (Hershko unpublished data,[50]), while all agree that the mean corpuscular hemoglobin concentration (MCHC) is of least clinical help.

An additional advantage of the MCV is that it is relatively specific for iron deficiency if the change is pronounced. In adults, moderate decreases to 70–80 fl are often seen in patients with the anemia of chronic disease or malignancy but values below 70 fl occur only with iron deficiency anemia or thalassemia minor. In geographic areas where thalassemia is rarely seen, an MCV below 70 fl is strong evidence of iron deficiency anemia.

There is one final point about hypochromia and microcytosis as signs of iron deficiency. Examination of peripheral blood is often said to be the simplest and most direct approach to diagnosing iron deficiency anemia. In one study, however, when hematologists examined peripheral smears from normal and iron deficient patients, iron deficiency was incorrectly diagnosed in 6% of normal smears, and was not recognized in 51% of smears in patients with proven iron deficiency anemia.[33] Thus, while it is helpful to examine the peripheral blood smear, a firm diagnosis of iron deficiency requires additional laboratory measurements.

Sideroblast Count

In the classic study of laboratory measurements of iron deficiency by Bainton and Finch, the sideroblast count determined from an iron stain of aspirated marrow was identified as a useful measure of iron deficient erythropoiesis.[4] Duplicate sideroblast counts on 300 normoblasts had a counting error of ± 10%, although considerable experience was required to achieve this degree of reproducibility. As originally described, the sideroblast *count* was the percent of nucleated red cells that contained at least one sideroblast granule. The sideroblast *score*, which was recently introduced, is the total number of granules in 100 normoblasts.[8] Both of these measurements correlate closely with the TS but like other measurements of iron deficient erythropoiesis, they are not specific for iron deficiency; about one-half of patients with anemia of chronic disease will have values in the iron deficient range.[4]

Because sideroblast counts are very time consuming and relatively inaccurate, the preferred measure of iron supply to the developing red cell is still the TS. Nevertheless in patients who have had a bone marrow examination, a qualitative estimate of sideroblast granules is very useful in distinguishing iron deficient erythropoiesis from intracellular blocks in hemoglobin synthesis.

Hemoglobin/Hematocrit

The final stage of iron deficiency is associated with a significant decrease in circulating hemoglobin. One problem in identifying this stage has been in deciding what constitutes an abnormal hemoglobin. An exact definition of anemia is less important in hospitalized patients because the possibility of iron deficiency is only considered in those with significant anemia. It is in otherwise healthy individuals who would be included in prevalence studies of nutritional anemia that the criterion of anemia becomes an issue. The WHO criteria for anemia have become widely accepted in recent years: adult males, below 13 g/dl; menstruating women, below 12 g/dl; pregnant women, below 11 g/dl.

The main limitation of hemoglobin as a measure of iron deficiency is the marked overlap in frequency distribution curves in anemic and normal individuals. Because of this, use of any single criterion for anemia results in a large number of false negative and false positive results. The problem was first demonstrated by Garby et al. who defined anemia in the population by the response or lack of response to oral iron therapy.[16] Using a single cutoff point, they found that 17% of truly anemic women were classified as normal while 35% of normal women were classified as anemic. Similar results were obtained by frequency distribution analysis of hemoglobin levels in pregnant women.[18] One-quarter of both anemic and normal women were misclassified when a single cutoff hemoglobin of 11 g/dl was used and the number of normal women classified as anemic actually exceeded the number of truly anemic women.

The concept of broad distributions of hemoglobin levels in normal and iron deficient subjects has clinical relevance. A patient with a hemoglobin slightly below the cutoff level for anemia may actually be hematologically normal, while an individual with a hemoglobin level in the low normal range may have a 2–3 g/dl deficit in circulating hemoglobin. The hemoglobin concentration does not always identify anemia with precision, especially in the region between normal and anemic populations. It is better to interpret hemoglobin levels in this range with a certain probability that the patient is either normal or anemic.

Therapeutic Trial

The serum ferritin is to the detection of storage iron depletion what the therapeutic iron trial is to the detection of iron deficiency anemia. They are the most sensitive measures of early and late iron deficiency respectively and they also provide precise quantitative information on the deficit in body iron. The most conclusive evidence of iron deficiency anemia is a significant rise in hemoglobin following oral or parenteral iron therapy. An increase of 1 g/dl is often considered significant, but a 2 gm increase is much more reliable. The therapeutic trial has been very helpful in detecting subclinical iron deficiency in prevalence studies and in determining the relative importance of iron lack when the cause of anemia is multifactorial. In a clinical setting, its value depends on the type of patient. If anemia is detected in the office or clinic in a patient at high risk of developing iron deficiency, a practical inexpensive approach is to place the patient on oral iron and determine the hemoglobin response in 3–4 wk. The therapeutic trial is not helpful in hospitalized patients because of the time required to make a diagnosis but it can be advantageous in clinical investigation. In a patient with several possible causes of anemia, such as rheumatoid arthritis or chronic renal failure, a therapeutic iron trial provides concrete evidence of clinically significant iron deficiency anemia.

THE CLINICAL SPECTRUM OF IRON DEFICIENCY

Nutritional Iron Deficiency

Nutritional is defined here as iron deficiency resulting from an imbalance between dietary iron supply and physiologic iron requirements. It is by far the commonest cause of anemia in infants, children, and premenopausal women. Recognition of this relatively mild iron deficiency as it occurs in susceptible segments of our population is particularly relevant to outpatient practice.

Valuable information about the detection of iron deficiency has been obtained in recent nutritional anemia surveys by including specific measurements of iron status. In one study, measurements of TS, FEP, serum ferritin, and hemoglobin were performed in over 1,500 subjects living in the northwestern USA.[20] Based on WHO criteria, the prevalence of anemia in the total sample was 8.3%. When only one of the three iron measurements was abnormal, the prevalence of anemia increased to only 10.9% as compared to 28% when two or more measurements were abnormal and to 63% when all three were abnormal. Thus, the recognition of iron deficiency anemia was greatly facilitated by including more specific measurements of iron status.

Similar findings were recently reported in a study of South African children.[26] The prevalence of anemia in the total sample was 24.3%. When the serum ferritin was below 12 μg/l prevalence increased to 38.8% compared to 18.2% when the serum ferritin was above 12 μg/l. When both serum ferritin and TS were abnormal, the prevalence of anemia increased to 70.0% while only 10.2% of children with both measurements in the normal range were anemic. By using two specific iron measurements in tandem, the ability to detect significant iron deficiency was greatly improved.

There is recent evidence that the four key iron measurements (Table 1) can be used not only to define the three stages of iron lack but also to provide a quantitative estimate of storage iron in a population.[21] The results of a survey in 426 adult women are shown in Fig. 1. In iron replete individuals, iron stores can be calculated by multiplying the serum ferritin by 10. At the other extreme, iron deficiency anemia, the deficit in body iron can be calculated from the hemoglobin level as follows. In a 70 kg woman, 1 g/dl hemoglobin is equivalent to about 150 mg iron. Assuming a normal hemoglobin in women of 14 g/dl, a 300 mg iron deficit in circulating

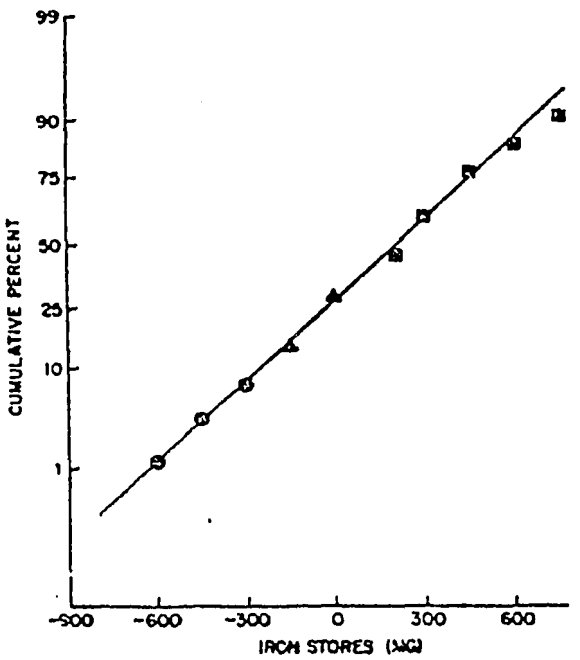

Fig. 1. Frequency distribution of iron stores in normal women as estimated from serum ferritin (▣), transferrin saturation and/or free erythrocyte protoporphyrin (▲), and hemoglobin concentration (●). Negative values represent the amount of iron that must be replaced in circulating red cells before iron reserves can reaccumulate.[21]

hemoglobin has already occurred when the hemoglobin has fallen below 12 g/dl; each additional 1-g decrease equals a deficit of 150 mg. Hemoglobin levels below 12 g/dl can be converted directly to iron deficit. For example, at a hemoglobin level of 10 g/dl, the deficit in circulating hemoglobin iron is about 600 mg. Note that the hemoglobin can be used for this calculation only when iron deficiency has been identified by abnormalities in at least two of the three specific iron measurements, TS FEP, and serum ferritin.

In the results depicted in Fig. 1, two additional points on the frequency distribution of iron stores were obtained from the TS and FEP. When only one of these was abnormal, iron stores were estimated at 0, while when both were abnormal, the predicted iron deficit was 150 mg. The fact that iron stores calculated in this manner followed a Gaussian distribution has provided some evidence of its validity. In an otherwise healthy individual, these calculations provide not only an estimate of the severity of iron lack, but a means of monitoring longitudinal changes in body iron status. It must be emphasized, however, that the calculation is only valid in patients with no evidence of chronic disease or malignancy.

Iron Deficiency Anemia

The laboratory approach to identifying iron deficiency in a patient with significant anemia is different than when the hemoglobin is normal or only slightly reduced. Measurements of iron deficient erythropoiesis, including TS, FEP, and MCV, may be helpful in ruling out iron deficiency when they are normal but will not distinguish the anemia of chronic disease from true iron deficiency. If moderately severe anemia is detected in an outpatient at risk of developing iron deficiency, a therapeutic trial of iron is the most economical approach, especially if a rise of more than 2 g/dl is observed. In a hospitalized patient, a firm diagnosis of iron deficiency requires a marrow examination or a serum ferritin determination.

The major value of the serum ferritin in an anemic patient is the fact that a level below 12 μg/l is absolutely diagnostic of iron deficiency. In a total of 154 cases of uncomplicated iron deficiency reported in the literature, values above the cutoff level of 12 μg/l have occurred in only 2.6% of cases (Table 2). Conversely, values below 12 μg/l are rarely if ever seen in a patient with stainable marrow iron.

Unfortunately, a serum ferritin value in the normal range does not exclude iron deficiency anemia because several disorders such as infection, chronic disease, and liver disease elevate the serum ferritin independently. The choice between the marrow iron examination and the serum ferritin in a hospitalized patient depends upon the urgency of the diagnosis, whether a bone marrow is indicated for other reasons, and if infection or liver disease is present which can

Table 2. Serum Ferritin Levels in Iron Deficiency Anemia

Author	Year	No. of Patients	Mean Serum Ferritin	No. Above 12 μg/ml
Addison et al.[1]	1972	15	5.3	1
Jacobs et al.[42]	1972	21	5.0	0
Siimes et al.[63]	1974	13	4.0	0
Lipschitz et al.[54]	1974	32	4.0*	2
Leyland et al.[51]	1975	28	6.1	1
Heinrich et al.[38]	1977	45	6.0	0
Total		154		4
		False positives = 2.6% (4/154)		

*geometric mean.

confuse the interpretation of the serum ferritin. In the absence of complicating disease, the latter is preferable because it is less expensive and less unpleasant for the patient.

The serum ferritin is useful not only in identifying iron deficiency, but also in monitoring its repair. In an initial study on the effect of iron therapy on serum ferritin, Siimes et al. reported a disproportionately abrupt rise within the first few days of placing infants on oral iron therapy.[63] Thomas et al. later reported that ferritin values were highly variable in iron deficient infants placed on oral iron therapy.[67] A more recent study in adults suggested that the serum ferritin remains a valid measure of body iron stores in the face of iron therapy.[71] Despite an adequate hemoglobin response, no elevation in serum ferritin was observed within the first 3 wk of placing patients with iron deficiency anemia on 180 mg elemental iron daily. With twice this level of iron, most patients did show increases in serum ferritin in 2–3 days but the values returned to subnormal a few days after discontinuing it. Presumably, patients given more than 300 mg elemental iron daily absorbed iron in excess of the needs of the developing red cell and some of this iron was diverted into stores temporarily. This study indicated that conventional doses of iron in patients with iron deficiency anemia do not elevate the serum ferritin until the hemoglobin has returned to normal. Serum ferritin measurements are therefore useful in monitoring the recovery from iron deficiency anemia and in determining the endpoint of iron therapy with precision.

Serum ferritin has also provided a valid measure of iron status following parenteral iron therapy, although some time should be allowed to elapse. Lappin et al. measured serum ferritin in 36 pregnant women 1 wk and 4 wk following injection of an average of 1,349 mg iron dextran by total dose infusion.[49] The mean rise 1 wk following injection was disproportionately high, 295 μg/l. At 4 wk, however, the mean rise of 138 μg/l was very close to a value of 130 ug/l predicted from the known correlation between serum ferritin and iron stores. Thus, the serum ferritin is useful in identifying the need for additional parenteral iron in patients with high sustained iron losses, as occurs with maintenance hemodialysis.

Secondary Iron Deficiency

The commonest cause of anemia in a hospitalized patient is the anemia of chronic disease (ACD). Disorders typically associated with ACD include rheumatoid arthritis, collagen vascular disease, tuberculosis, fungal infections, inflammatory bowel disease, lymphoma, and metastatic carcinoma. ACD is the main differential diagnosis of iron deficiency anemia not only because it is highly prevalent, but also because it produces iron deficient erythropoiesis by impairing iron release from the RE cell. In contrast to true iron deficiency, RE stores are normal or increased.

Much has been published recently on the use of serum ferritin in distinguishing iron deficiency from ACD. Because the serum ferritin behaves as an acute phase reactant, the level is disproportionately elevated for each grade of marrow iron in patients with active infection or inflammation. In 39 such patients reported by Lipschitz et al., serum ferritin averaged 305 μg/l with a range of 10–1650 μg/l.[52] Marrow iron in 25 of these patients was compared to 27 hospital controls who had no evidence of infection or inflammation. At each level of marrow iron, the serum ferritin was about three times higher in patients with infection than in controls (Fig. 2).

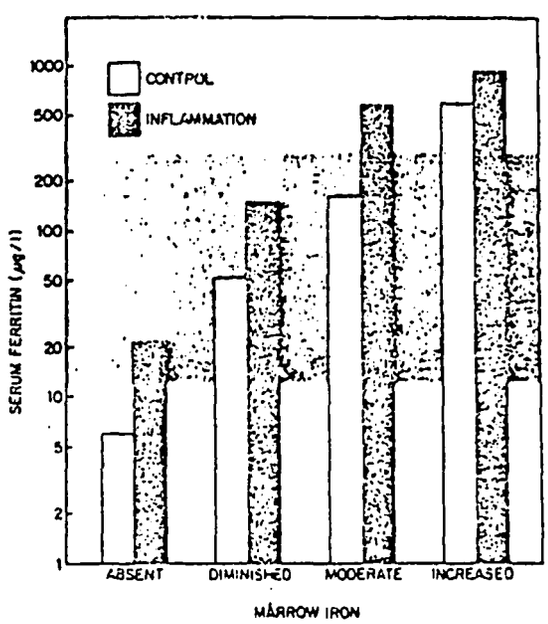

Fig. 2. Relationship between serum ferritin and marrow hemosiderin in patients with inflammation and/or infection. The shaded area depicts the normal range.[52]

Thus, the serum ferritin can reliably distinguish iron deficiency anemia from ACD.

A diagnostic problem arises when iron deficiency and ACD coexist in the same patient. One disorder in which this commonly occurs is *rheumatoid arthritis*. In 13 such patients with absent marrow iron, serum ferritin averaged 38 μg/l; diagnostic levels below 12 μg/l were observed in only three.[11] It was concluded that the serum ferritin was of diagnostic value only when the level was below 12 μg/l or greater than 200 μg/l. Subsequent studies, however, suggested that this diagnostic gray zone could be narrowed appreciably. In 21 anemic patients with rheumatoid arthritis who had reduced or absent marrow stores, the serum ferritin was invariably below 100 μg/l whereas in 14 patients with normal marrow iron stores, the level was always above 100 μg/l.[64] Koerper et al. administered iron therapy to 15 anemic patients with juvenile rheumatoid arthritis, 11 of whom showed significant hematologic response.[45] The pretreatment levels of hemoglobin, FEP, and MCV in these patients were indistinguishable from those who showed no therapeutic response. In six of the responders, the pretreatment serum ferritin was below 25 μg/l, while in the remaining five patients, it ranged from 40–200 μg/l. It was concluded that a serum ferritin below 25 μg/l predicted a response to oral iron but that a higher level did not preclude a response.

Another disorder associated with inflammation and a high incidence of iron deficiency is *chronic inflammatory bowel disease*. In 45 such patients, the serum ferritin ranged from 22–650 μg/l in those with stainable marrow iron as compared to 7–55 μg/l in those with absent marrow iron.[68] Based on a cutoff level of 18 μg/l, it was concluded that the serum ferritin had disappointingly low sensitivity although if values between 20 and 55 μg/l were considered uninterpretable, no diagnostic errors would have occurred in identifying patients with marrow iron by serum ferritin. Bartels et al. later reported that the serum ferritin level was invariably below 50 μg/l in patients with chronic inflammatory bowel disease and absent marrow iron, and in two-thirds of these cases the level was below 12 μg/l.[7] Surprisingly, a serum ferritin below 12 μg/l was observed in four patients with 1+ marrow iron, a finding that may be explained by previous parenteral iron therapy.

Nelson et al. recently studied 73 patients with *clinical disorders known to elevate serum ferritin*, including liver disease, alcoholism, chronic inflammatory disease, and malignancy.[55] A serum ferritin below 30 μg/l indicated absent marrow iron, while levels above 100 μg/l excluded iron deficiency. Kerlin et al. conducted a prospective study in 250 patients with a hemoglobin below 10.6 g/dl and an MCV below 86 fl.[43] In patients diagnosed as iron deficient by other criteria, the serum ferritin level was greater than 20 μg/l in 30% but in none was the value above 50 μg/l. Conversely, no patients in the iron replete group had a serum ferritin below 50 μg/l.

There is still some doubt as to the level of serum ferritin that identifies iron deficiency in patients with infection, inflammation, or malignancy. Most studies indicate that a value below 50 μg/l is consistent with iron deficiency and probably deserves a trial of oral iron therapy. On the other hand, most patients with a serum ferritin above 100 μg/l are iron replete and are unlikely to respond to iron therapy. Diagnostic uncertainty remains with levels between 50–100 μg/l, although most of these patients will have stainable marrow iron.

Chronic Renal Failure

A complex clinical situation where iron deficiency is commonly seen is in patients on chronic hemodialysis. In contrast to significant iron overload in the predialysis era, the typical picture is now the anemia of chronic renal failure and superimposed iron deficiency from blood loss associated with maintenance dialysis. Several reports have shown that the serum iron, TIBC, and TS are of little value in identifying iron deficiency in these patients.[3,9,30,57] Because of a reluctance to perform repeated bone marrow examinations to monitor iron status in these chronically ill patients, it has been common practice to administer parenteral iron regularly to avoid iron deficiency. There are now several reports of significant iron overload and associated tissue damage from excessive parenteral iron.[2,5,37] In one of these studies, serum ferritin levels above 1,000 μg/l were observed in 64 of 120 patients given 100 mg iron dextran intravenously at 2 wk intervals for several years, and retrospective post mortem study of 22 of these patients showed significant amounts of iron in

liver and spleen with occasional myocardial iron loading.[17]

Serial measurements of serum ferritin levels provide an acceptable alternative for monitoring iron balance in these patients. Several workers have shown a high correlation between marrow iron and serum ferritin.[3,23,41] In one study, for example, average serum ferritin levels of 42, 387, 1,200, and 3,224 μg/l were reported in patients with 0, 1+, 2+ and 3+ iron stores, respectively.[41] As in patients with ACD, the serum ferritin level below which iron deficiency can be diagnosed is clearly higher than 12 μg/l. Bell et al. analyzed bone marrow stores and serum ferritin levels in 55 patients on maintenance dialysis by discriminant function and concluded that serum ferritin levels below 82 μg/l indicated reduced or absent marrow iron.[10] The majority of studies, however, have suggested a lower level of 50–60 μg/l.[9,23] Lynn et al. gave 1 g parenteral iron to 31 patients on maintenance hemodialysis and observed that iron produced no hematologic improvement when the pretreatment serum ferritin was above 55 μg/l.[34] Thus, a serum ferritin below 50–55 μg/l is highly suggestive if not diagnostic of iron deficiency in patients with chronic renal failure.

In summary, the optimal approach to monitoring iron balance in these patients is to perform serum ferritin measurements at 4–6 wk intervals from the outset of maintenance hemodialysis. When the level falls below 100 μg/l, the patient should be placed on 60–100 mg elemental iron daily, and the dose can be gradually increased to maintain a serum ferritin level between 50–100 μg/l. Some patients might eventually require parenteral iron but this should not be continued once the serum ferritin level rises above 200 μg/l.

Thalassemia Minor

This disorder is the only hematologic abnormality other than iron deficiency that is associated with a marked decrease in the MCV to below 70 fl. Its detection is clinically important because vigorous and prolonged iron therapy can lead to iron overload and tissue damage in these patients. Several formulae have been proposed for distinguishing the microcytosis of iron deficiency from that of β-thalassemia trait.[44] One such discriminate function is MCV-RBC-5xHb-K where K is a constant used to calibrate the Coulter counter.[31] When this formula was recently applied to 193 adult subjects with iron deficiency and 240 subjects with thalassemia minor, 92.7% of patients with iron deficiency anemia were correctly diagnosed as compared to 95% of patients with thalassemia minor.[32] However, patients with complicating disorders including pregnancy were excluded from this study and the formula was only applied to adult patients when microcytosis was thought to be due to either iron deficiency or thalassemia minor. Other workers have reported that in anemic patients with microcytosis, the red cell count alone was as helpful as more complex formulae. None of the data calculated from electronic red cell counting, however, was sufficiently accurate to distinguish iron deficiency from thalassemia minor without additional laboratory measurements.

Hershko et al. reported that the combination of serum ferritin and electronic red cell counts could yield a very satisfactory discrimination between iron deficiency, thalassemia minor, and normal patients.[39] The study included 50 anemic patients with thalassemia trait, 39 patients with iron deficiency anemia, and 87 normal subjects. All patients with iron deficiency anemia had a serum ferritin below 16 μg/l as compared to none of the normal controls and only 2% of the thalassemic patients. On the other hand, all but three of the 50 thalassemic subjects had an MCV below 80 as compared to none of the controls. Using serum ferritin and MCV, patients with iron deficiency and β-thalassemia trait could be diagnosed with an accuracy of over 95%. Nevertheless, even with these screening methods, the positive diagnosis of β-thalassemia trait still requires demonstration of an elevated hemoglobin A_2 level since these patients can develop iron deficiency and a low serum ferritin with the same frequency as normal individuals.[28,41]

SUMMARY

While the prevalence of iron deficiency has remained relatively constant, there has been continuing refinement in its laboratory recognition, especially with the recent introduction of serum ferritin and FEP measurements. It is helpful to classify iron deficiency into three stages. *Storage iron depletion* is identified by

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INHIBITION OF FOOD IRON ABSORPTION BY COFFEE[1-3]

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Dual isotope studies were performed in iron replete human subjects to evaluate the effect of coffee on nonheme iron absorption. A cup of coffee reduced iron absorption from a hamburger meal by 39% as compared to a 64% decrease with tea, which is known to be a potent inhibitor of iron absorption. When a cup of drip coffee or instant coffee was ingested with a meal composed of semi-purified ingredients, absorption was reduced from 5.88% to 1.64 and 0.97% respectively and when the strength of the instant coffee was doubled, percentage iron absorption fell to 0.53%. No decrease in iron absorption occurred when coffee was drunk one hour before a meal, but the same degree of inhibition as with simultaneous ingestion was seen when coffee was taken one hour later. In tests containing no food items, iron absorption from NaFeEDTA was diminished to the same extent as that from ferric chloride when each was added to a cup of coffee. These studies demonstrate that coffee inhibits iron absorption in a concentration-dependent fashion.

Tea has a potent inhibitory effect on the absorption of nonheme iron (1-3). For example, Disler et al. (1) demonstrated that iron absorption from a meal was reduced by as much as 87% when tea was included. Coffee is another beverage that is often consumed with meals. Its effect on iron absorption has not been studied in detail although Derman et al. (4) observed a 37% reduction when coffee was added to a meal containing maize. Because coffee is commonly consumed in countries where iron deficiency anemia is prevalent, a significant inhibitory effect on dietary iron absorption has nutritional relevance. We report here the inhibitory effect of coffee on nonheme iron absorption in human subjects.

METHODS

Subjects. Thirty-seven volunteer subjects (thirty-six men and one postmenopausal woman) ranging in age from 18 to 50 years participated in four separate iron absorption studies. All were in good health and denied any history of gastrointestinal disorders that might affect iron absorption. The mean of serum ferritin measurements on days 1, 14, and 20 of the study show iron status to be uniformly normal (5). Written informed consent was obtained from each volunteer before the study and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Absorption Measurements. Each study included four test meals which were labeled alternately with either ^{55}Fe or ^{59}Fe and given to the subjects between 7 and 9 AM following a 10-hr fast. Only water was allowed for the subsequent 3 hr. Meals A and B were administered on consecutive days and meals C and D were likewise given two weeks later. Nonheme iron absorption was measured by incorporated red cell radioactivity in blood samples drawn two weeks after each pair of meals. Radioiron measurements were made on duplicate 10 ml blood samples (5). Sufficient counts were obtained on each sample to reduce the net counting error for each isotope to less than $\pm$ 2% in subjects absorbing more than 1% of the test dose. Absorption was calculated on the basis of blood volume, and erythrocyte incorporation was assumed to be 80% of absorbed iron (5).

Test Meals. Four studies were conducted in which two types of meals were served with different beverages (Table 1). In studies 1 and 3, the test meal was a beef patty (113 g uncooked weight), a bun, and a 200 ml drink of water, tea, or coffee. In study 2, a mixture of semipurified ingredients was used for all four test meals; each 340 ml serving contained dried egg albumen (Monark Egg Corp., Kansas City MO), corn syrup solids (Dri-Sweet 36, Hubinger Co., Keokuk IA), corn oil, and $FeCl_3 \cdot 6H_2O$ which provided 14.7 g protein, 68 g carbohydrate, 35 g fat, and 4.1 mg

iron. Coffee or water (200 ml) was served with each meal in study 2. The test dose in study 4 consisted only of 200 ml of either water or instant coffee to which 3 mg iron as either $FeCl_3 \cdot 6H_2O$ or NaFeEDTA was added.

The tea for study 1 was prepared by adding boiling water to dry tea (Orange Pekoe and Pekoe Cut Black Tea, Lipton Inc., Englewood Cliffs NJ; 1.75 g/200 ml serving) and steeping for four minutes. Drip coffee (studies 1 and 2) was made in an automatic drip coffee maker (Proctor-Silex) using 70 g Folger's drip grind coffee (Folger Coffee Co., Cincinnati OH) and 1,625 ml cold tap water. Instant coffee (studies 2, 3, and 4), was prepared by adding 200 ml boiling water to 1.5 g freeze-dried coffee (Taster's Choice, Nestle Company, White Plains NY) unless otherwise stated.

The extrinsic tag method was used throughout (6,7). The radioiron was mixed with 0.1 mg carrier iron as $FeCl_3 \cdot 6H_2O$ in 1 ml 0.01 N HCl. The radioiron label was sprinkled over the beef patty in meals containing a hamburger and was added directly to the semipurified liquid meal in study 2. In study 4, the radioiron tag was added directly to the beverage which contained 3 mg iron as either NaFeEDTA or $FeCl_3 \cdot 6H_2O$. A preliminary investigation demonstrated that there was complete exchange between NaFeEDTA and $FeCl_3 \cdot 6H_2O$.

Statistics. Because the distribution of iron absorption data expressed as percentage of administered dose is positively skewed, individual values were converted to logarithms for statistical analysis and the results reconverted to the anti-logarithm to recover the original units (8). All mean values for iron absorption and meal ratios are reported as geometric means. The paired *t*-test was used to compare iron absorption from any two meals within the same study.

RESULTS

Study 1 was performed to compare the effect of tea and coffee on iron absorption from a hamburger meal. When water was given with this meal, 3.71% of the nonheme iron was absorbed (meal A, Table 2). With tea as the beverage (meal B) absorption fell to 1.32%, representing a highly significant 64% inhibition ($p < 0.001$). When a cup of drip coffee was taken with the hamburger (meal C), a 39% decrease in iron absorption, to a mean of 2.25%, occurred ($p < 0.05$). A direct statistical comparison indicated that coffee was significantly less inhibitory than tea (B/C = 0.59, $p < 0.001$). Meal D consisted of a reference dose of ferrous ascorbate containing 3 mg iron as $FeSO_4$ and 18.9 mg ascorbic acid. Mean absorption in these iron replete subjects averaged 15.73%.

Study 2 was designed to compare the effect of drip and instant coffee and to determine whether the inhibitory effect of coffee is dose related. In general, coffee had a greater inhibitory effect on iron absorption from the semipurified meal (Table 3). Absorption from meal B containing drip coffee averaged 1.64% as compared to 5.88% from the control meal (A), a reduction of 72%. A somewhat greater decrease of 83% was observed when a cup of instant coffee was ingested with the meal (C). Doubling the concentration of the instant coffee caused a further 45% decrease in iron absorption which was also statistically significant ($D/C = 0.55$, $p < 0.01$).

Study 3 examined the effect of varying the time when coffee was ingested in relation to the hamburger meal (Table 4). When the coffee and hamburger were taken simultaneously (meal C), iron absorption fell from a control mean of 8.12 to 4.58% ($p < 0.05$). Coffee taken one hour prior to the hamburger (meal B) led to a small decrease in mean absorption that was not statistically significant. On the other hand, with coffee taken one hour after the hamburger (meal D), the degree of inhibition was similar to that observed when the beverage and meal were consumed simultaneously.

Study 4. In this final study, the effect of coffee on iron absorption from NaFeEDTA was compared with that from $FeCl_3$ in the absence of food (Table 5). Although percentage absorption from NaFeEDTA in water was slightly lower (3.83%) than from $FeCl_3$ (5.04%), the difference was not statistically significant. When the same forms of iron were given with coffee, 70% decreases in absorption were observed ($C/A = 0.30$, $D/B = 0.31$, $p < 0.001$). Thus, percentage absorption from $FeCl_3$ relative to NaFeEDTA was constant regardless of the beverage ($A/B = 0.76$, $C/D = 0.73$).

DISCUSSION

The studies reported here demonstrate that coffee, like tea, markedly reduces iron absorption. These results are in keeping with some previous observations by Layrisse et al. who studied the availability of ferrous sulfate-fortified sugar added to several beverages (9). Although a direct comparison between the effect of coffee and other drinks was confounded by the use of different groups of subjects, the bioavailability of the iron was considerably lower when added to coffee than when added to soft drinks or orange juice. Absorption from iron-fortified sugar was reduced by 44% in a meal containing a vegetable, meat, and coffee and by 75% when the sugar was used in coffee alone. Coffee with milk was twice as inhibitory as black coffee.

Tea is thought to influence iron absorption at a luminal level by the formation of insoluble iron tannates. Our study suggests that coffee may also exert its effect in the lumen since the reduction in absorption was most marked when the coffee was taken with the meal or an hour later. A significant fraction of the meal would still be in the stomach after one hour since the half time of gastric emptying for this hamburger meal is approximately 180 min (Skikne, Lynch, Cook, unpublished observations).

Recent in vitro experiments suggest that coffee may inhibit iron absorption by a chemical mechanism different from that of tea. Kojima et al. (10) compared the effect of different beverages on the amount and chemical form of soluble iron in pinto bean suspensions after incubations at pH 2 and 6. Whereas tea reduced the proportion of soluble iron to only 12% of the quantity in bean supensions without additions, drip and instant coffee had no significant effect on iron solubility. Nevertheless, all soluble iron in both beverages was oxidized to the ferric state. Our studies suggest that this form of iron has limited availability for absorption in humans.

These findings may have important implications for iron nutrition because coffee is a favored beverage in many parts of the world. However, the effect on iron balance is difficult to gauge

since the methods of preparation and patterns of consumption, both of which influence absorption, differ considerably within and between populations. The inhibitory effect of coffee may also be relevant to the choice of the vehicle and iron salt for fortification programs. Sugar, which is commonly used in coffee has been proposed as a vehicle for iron fortification (11). It has also been suggested that NaFeEDTA is the most suitable iron compound for fortifying sugar because of its greater chemical stability (11-15). A recent study demonstrating that maize meal porridge and bran reduce iron absorption from ferrous sulfate but have little effect on NaFeEDTA (15) supports the contention. On the other hand, tea is equally inhibitory to ferrous sulfate and NaFeEDTA and our findings suggest that this also is true of coffee consumed alone; 70% inhibition was observed for both ferrous sulfate and NaFeEDTA. Nevertheless, Layrisse et al. found that when a bulky breakfast meal (120 g black bean gruel, 4 tortillas, and a bread roll) was ingested with a cup of coffee, absorption from NaFeEDTA was significantly better than from ferrous sulfate (11). It would appear that the use of NaFeEDTA to fortify sugar used in beverages would be advantageous only if the beverages are taken with meals.

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Table 1. Composition of Test Meals[1]

Study	Meal Type	Beverage Added to Meals			
		A	B	C	D
1	STD	Water	Tea	Coffee[2]	REF
2	SP	Water	Coffee[2]	Coffee[3]	Coffee[3]
3	STD	Water	Coffee[3]	Coffee[3]	Coffee[3]
4	None	Water	Water	Coffee[3]	Coffee[3]

[1] Abbreviations: REF, reference dose without food; STD, standard meal; SP, semipurified meal.

[2] Drip coffee

[3] Freeze-dried instant coffee

Table 2. Effect of tea and coffee on iron absorption from a standard meal.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION Water (A)	Tea (B)	Coffee (C)	REF* (D)	Absorption Ratios B/A	C/A	B/C
				(% of dose)						
1	29	48	65	0.96	0.48	1.08	10.85	0.50	1.12	0.44
2	26	44	68	1.76	0.56	0.86	11.63	0.31	0.48	0.65
3	25	45	67	1.78	1.47	4.30	4.33	0.82	2.41	0.34
4	26	46	97	2.53	1.68	2.02	19.06	0.66	0.79	0.83
5	30	44	156	2.71	1.82	2.47	10.96	0.67	0.91	0.73
6	22	46	102	4.16	2.18	2.22	14.37	0.52	0.53	0.98
7	26	43	47	5.55	0.87	2.05	19.55	0.15	0.36	0.42
8	23	43	34	5.81	0.30	0.51	23.21	0.05	0.08	0.58
9	28	43	58	6.91	1.70	4.91	20.55	0.24	0.71	0.34
10	22	45	34	8.75	3.43	4.21	26.02	0.39	0.48	0.81
11	19	44	27	11.21	5.32	7.93	36.81	0.47	0.70	0.67
Mean†	25	45	60	3.71	1.32	2.25	15.73	0.36+	0.61‡	0.59+
			-1 SE	2.94	1.01	1.75	13.22	0.28	0.47	0.53
			+1 SE	4.68	1.71	2.88	18.71	0.45	0.78	0.65

*REF = Reference dose

†Geometric means except Age and Packed Cell Volume.

+$p < 0.001$; ‡$p < 0.05$

Table 3. Effect of type and strength of coffee on iron absorption from a semipurified meal.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Coffee: Water (A)	Drip (B)	FD*(1.5g) (C)	FD(3.0g) (D)	Absorption Ratio B/A	C/A	D/A	D/C
1	44	55	55	1.35	1.06	0.36	0.23	0.78	0.26	0.17	0.63
2	30	49	76	3.22	2.45	2.57	1.65	0.76	0.79	0.51	0.64
3	24	39	114	4.48	1.88	0.43	0.33	0.41	0.09	0.07	0.76
4	23	43	114	5.11	1.10	0.27	0.23	0.21	0.05	0.04	0.85
5	35	55	132	6.22	0.86	0.40	0.17	0.13	0.06	0.02	0.42
6	50	37	56	9.03	3.37	2.45	2.21	0.37	0.27	0.24	0.90
7	28	45	46	9.51	0.83	2.62	1.43	0.08	0.27	0.15	0.54
8	27	45	86	10.00	1.91	1.11	0.28	0.19	0.11	0.02	0.25
9	31	43	181	15.98	3.56	2.56	0.90	0.22	0.16	0.05	0.33
Mean†	31	44	87	5.88	1.64	0.97	0.53	0.28+	0.17+	0.09+	0.55‡
			-1 SE	4.60	1.36	0.70	0.38	0.22	0.12	0.07	0.48
			+1 SE	7.52	1.98	1.36	0.75	0.36	0.22	0.13	0.64

*FD = Freeze-dried instant.

†Geometric means except Age and Packed Cell Volume.

+$p < 0.001$; ‡$p < 0.01$

Table 4. Effect of time of consumption of coffee on iron absorption from a standard meal

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Water (A)	Coffee Added Before (B)	With (C)	After (D)	Absorption Ratios D/A	C/A	D/A	D/C
1	24	46	28	1.86	3.00	1.73	2.72	1.61	0.93	1.46	1.57
2	19	49	51	3.98	3.18	4.37	3.90	0.79	1.09	0.97	0.89
3	25	44	95	5.48	2.20	3.33	2.53	0.40	0.60	0.46	0.75
4	25	46	48	9.81	8.67	2.00	2.02	0.88	0.20	0.20	1.01
5	19	48	30	10.61	8.78	2.76	2.76	0.82	0.26	0.26	1.00
6	18	50	22	19.95	17.70	10.16	13.93	0.88	0.50	0.69	1.37
7	28	44	22	27.72	14.06	30.13	19.56	0.50	1.08	0.70	0.64
Mean†	23	47	37	8.12	6.30	4.58	4.55	0.78	0.56+	0.56+	0.99
			-1 SE	5.70	4.62	3.12	3.23	0.66	0.44	0.43	0.88
			+1 SE	11.57	8.60	6.72	6.41	0.92	0.73	0.73	1.12

†Geometric means except Age and Packed Cell Volume.

+$p < 0.05$

Table 5. Effect of coffee and water on absorption of iron from NaFeEDTA and $FeCl_3$.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	IRON ABSORPTION (% of dose) Water NaFeEDTA (A)	Water $FeCl_3$ (B)	Coffee NaFeEDTA (C)	Coffee $FeCl_3$ (D)	Absorption Ratios A/B	C/D	C/A	D/B
1	23	47	395	1.42	1.60	0.40	0.17	0.89	2.35	0.28	0.11
2	32	46	141	2.55	4.15	0.72	0.92	0.61	0.78	0.28	0.22
3	31	44	179	2.61	7.38	0.63	0.95	0.35	0.66	0.24	0.13
4	19	45	60	3.30	1.73	1.08	1.02	1.91	1.06	0.33	0.59
5	30	47	55	3.57	1.98	0.83	0.90	1.80	0.92	0.23	0.45
6	18	45	31	4.10	5.97	0.72	4.50	0.69	0.16	0.18	0.75
7	28	46	122	4.13	3.23	1.75	1.50	1.28	1.17	0.42	0.47
8	28	45	102	4.78	9.82	1.01	1.66	0.49	0.61	0.21	0.17
9	25	44	65	5.68	11.53	4.95	5.48	0.49	0.90	0.87	0.48
10	27	49	27	13.60	29.30	3.71	11.02	0.46	0.34	0.27	0.38
Mean†	26	46	86	3.83	5.04	1.14	1.57	0.76	0.73	0.30+	0.31+
			-1 SE	3.18	3.74	0.88	1.08	0.63	0.58	0.26	0.25
			+1 SE	4.62	6.79	1.47	2.28	0.92	0.92	0.34	0.39

†Geometric means except Age and Packed Cell Volume.

+$p < 0.001$

AJCN MS #7320(R)

THE USE OF CAPILLARY BLOOD FOR MEASUREMENTS OF CIRCULATING FERRITIN[1-3]

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ABSTRACT

The accuracy and reproducibility of using stored capillary blood for measurements of circulating ferritin were evaluated by comparison with simultaneous measurements on venous blood. In an initial study, the between and within-sample variability of capillary determinations was about three times higher than with venous measurements, but the difference was reduced considerably by using capillary serum rather than plasma. With both serum and plasma, the mean ferritin in capillary specimens was 3-5% higher than in venous blood. These minor drawbacks with capillary measurements are outweighed by the benefit of improved compliance in prevalence surveys when finger-stick sampling can be used in place of venipuncture.

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INTRODUCTION

When assessing the iron status of a population, it is preferable to perform a battery of laboratory measurements such as hemoglobin, hematocrit, red cell indices, transferrin saturation, free erythrocyte protoporphyrin, and serum ferritin (1-3). A major drawback of this approach, however, is a much lower compliance in prevalence studies when several milliliters of venous blood are required. This is especially true in the pediatric age group. One could circumvent this drawback by performing only those measurements which require a small sample of capillary blood. A useful pair of such measurements would be hemoglobin to detect iron deficiency anemia, and serum ferritin to measure iron stores. The validity of using capillary blood for hemoglobin measurements has been assessed in several published studies. In the present report the accuracy and reproducibility of ferritin measurements in capillary blood were evaluated by comparison with simultaneous venous determinations. To simulate conditions in a typical population study, both capillary and venous specimens were stored at -20^{C} for up to 2 weeks prior to assay.

METHODS

Two studies, A and B, were performed in 20 and 21 normal adult subjects respectively. The studies differed only in the technique used for processing and storing capillary specimens. Two venous and two capillary samples were obtained from the right and left arm of each subject. For venous specimens, 3 ml blood was drawn without venostasis from each forearm into a dry syringe and immediately transferred to a glass tube measuring 100 x 16 mm (Becton-Dickinson, Rutherford NJ). After allowing the blood to clot for 2 hr at room temperature, serum obtained by centrifugation at 900 x g for 20 min was transferred to a clean glass tube and stored at -20°C.

Capillary specimens were obtained from the middle finger of each hand using disposable blood lancets (Microlance, Becton-Dickinson, Rutherford NJ). After wiping away the first drop, free-flowing blood was obtained without finger compression. In study A, capillary plasma was obtained by collecting the sample into two heparinized microhematocrit tubes measuring 75 x 1 mm (Fisher Scientific, Pittsburg PA). The tubes were sealed with Critoseal (Lancer, St. Louis MO), centrifuged for 5 min, and broken just above the buffy coat. The plasma was transferred to 400 ul Eppendorf tubes (Brinkman Instruments, New York) and stored at -20°C.

In study B, capillary serum was obtained by collecting the sample in plain capillary glass tubes measuring 100 x 1.6 mm, (Kimble Products, Vineland NJ). The tubes were sealed with Critoseal, allowed to stand at room temperature for 1 hr, and centrifuged for 5 min. The serum was transferred to round-bottom vials (7 x 40 mm; Milian Instruments, Switzerland) of the type commonly used in WHO-sponsored studies of nutritional anemia.

Serum Ferritin. Measurements were performed in triplicate on 10 ul plasma or serum samples by the 2-site immunoradiometric assay described by Miles et al. (4), except that Sepharose 4B rather than aminocellulose was used for purification of antiferritin antibodies (5). All quantitative transfers in the assays were performed with an electronic pipettor (Micromedic, Philadelphia PA).

Statistical Analysis. Serum ferritin levels have been shown in prior studies to be log normally distributed (4). Variability estimates were therefore performed after log transformation. Within-sample variability was calculated from triplicates while between-sample variability was based on differences in values between the right and left arm (Appendix). The results are expressed as standard deviation (SD) of $\log_e$ (ferritin).

RESULTS

Variability. In study A, the SD for venous determination was similar within samples (0.053) and between samples (0.046) (Table 1). The SD for capillary measurements was sharply higher, 0.166 with separate finger punctures and 0.195 with assays on the same sample. The higher variability with capillary determinations is apparently not related entirely to the skin puncture per se because the SD within samples was just as high as that between samples. Since previous studies have shown no difference in variability between serum and plasma ferritin with larger volumes of venous blood (4), the higher variability of capillary measurements may relate to storage of small sample volumes.

To examine the effect of sample size on variability, multiple ferritin determinations were performed on a single pool of serum and plasma using the same volumes (100-200 ul) and equipment as used for capillary determinations in study A. When the samples were processed without interim storage, the variability for serum and plasma was similar. However, when the smaller sample volumes were stored at -20°C prior to ferritin determinations, the SD for 18 replicate determinations was much higher with plasma (0.359) than with serum (0.135). We suspect that minute fibrin clots in thawed plasma resulted in intermittent obstruction of the micropipettor when removing samples from the Eppendorf tubes.

In study B serum rather than plasma was used for capillary determinations. In this study a slightly larger non-heparinized capillary tube was used to obtain the sample, and the frozen sample was stored in a larger round-bottom tube. The variability with venous measurements was very similar to that observed in study A (Table 1). The within-sample SD of capillary determinations was significantly reduced from 0.195 to 0.091 ($p < 0.01$), and was only slightly higher than the SD of 0.072 obtained with venous determinations. The between-sample SD for capillary measurements (0.110) was also significantly reduced ($p < 0.01$) although it remained appreciably higher than the value of 0.040 observed with venous measurements.

Accuracy. In both studies, ferritin values were significantly higher with capillary specimens than with venous. The relative difference averaged 5.2% in study A (115.9 vs. 122.2 µg/l, $t = 2.01$, $p = 0.03$) and 7.2% in study B (61.8 vs. 66.6 µg/l, $t = 3.75$, $p < 0.001$). Because regression analysis showed no significant difference between studies A and B for either the slope or intercept, the data from the two studies were pooled (Fig. 1). It is apparent that the disparity between capillary and venous measurements is essentially constant within the range of 0-200 ug/l.

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To determine whether the higher ferritin observed with capillary measurements was related to differences in sample volume, several milliliters of serum were obtained from 10 normal subjects and placed in three types of containers (Table 2). When 1 ml was stored in a larger vial, the mean ferritin was 163.9 µg/l as compared to significantly higher means of 169.3 and 168.4 µg/l when 0.1-0.2 ml was stored in smaller vials. These represent relatively small differences of 3.3 and 2.7% respectively. It was concluded that the disparity was due to evaporation during freezing and thawing of small volumes.

DISCUSSION

The major difficulty in employing capillary samples for measurements of ferritin in this study was related not to the protein measurement per se but to the storage of minute volumes. High within-sample variability with capillary specimens was reduced appreciably by using serum rather than plasma. It should be noted that even this problem would not usually arise in a hospital setting but only when the sample must be frozen because of anticipated delay between collection of the sample and performance of the assay, a common practice in prevalence studies. Although between-sample variability with capillary serum was more than twice that of venous measurements, the modest gain in precision would not on its own justify venous sampling in a population study.

Since blood cells and most tissues contain a higher concentration of ferritin than is present in serum (6), we anticipated that admixture of capillary blood with cytosol released from tissue might result in substantially higher ferritin levels in specimens obtained by skin puncture. This possibility was also suggested by Segall et al. who observed a significantly higher ferritin with capillary than with venous samples in newborn infants (7). Although we also observed that ferritin measurements in capillary serum and plasma were 5-7% higher than in venous samples, at least half of the difference was explained by evaporation while freezing and thawing 100-200 ul sample volumes. In prevalence studies it would be advisable to measure this evaporation loss under local field conditions with particular attention to the technique used for sample processing and storage.

APPENDIX

The within-sample and between-sample variances in Study A were calculated as follows:

$$\text{Within-sample variance} = \frac{\sum_{i=1}^{(20)} \sum_{j=1}^{2} \sum_{k=1}^{3} (X_{ijk} - \bar{X}_{ij})^2}{(20)\ (2)\ (3)}$$

$$\text{Between-sample variance} = \frac{\sum_{i=1}^{20} \sum_{j=1}^{2} (\bar{X}_{i1} - \bar{X}_{i2})^2}{(20)\ (2)}$$

$i = 1,\ldots,20$ subjects

$j = 1,2$ samples

$k = 1,2,3$ replicates

X_{ijk} = ferritin measurement on kth replicate in jth sample of ith subject.

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LEGEND

Figure 1. Relationship between capillary and venous measurements of circulating ferritin. The interrupted line is the line of identity. The correlation coefficient is 0.9904 ($p < 0.01$). The slope and y intercept are 1.027 and 3.1 respectively.

Table 1 Comparison of ferritin measurements in venous and capillary blood.

	Venous	Capillary
Study A (capillary plasma)		
Mean ferritin (μg/l)	115.9	122.2
Standard deviation $\log_e$ (ferritin)		
Within	0.053	0.195
Between	0.046	0.166
Study B (capillary serum)		
Mean ferritin (μg/l)	61.8	66.6
Standard deviation $\log_e$ (ferritin)		
Within	0.072	0.091
Between	0.040	0.110

Table 2 Effect of storage container volume on serum ferritin values (μg/l)

Sample	Large Volume* (a)	Small Volume Study A (b)	Small Volume Study B (c)	Difference a-b	Difference a-c
1	25.0	27.0	23.4	-2.0	1.6
2	36.2	44.9	40.1	-8.9	-3.9
3	9.0	9.1	9.7	-0.1	-0.7
4	342.6	343.4	349.5	-0.8	-6.9
5	283.4	288.1	287.9	-4.7	-4.5
6	183.1	203.4	192.1	-20.3	-9.0
7	10.9	15.4	10.6	-4.5	0.3
8	363.9	360.6	370.0	3.3	-6.1
9	333.8	349.2	348.0	-15.4	-14.2
10	51.2	51.2	52.5	-0.3	-1.3
MEAN	163.9	169.3	168.4	-5.4	-4.5

*vial capacity, 2 ml

IRON STATUS OF ELDERLY AMERICANS

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Key Words: Age, iron nutrition, anemia.

Abstract

Studies of iron nutriture in the elderly are limited and very few include observations on individuals over the age of 75. The two Health and Nutrition Examination Surveys carried out by the United States Department of Health, Education and Welfare demonstrate that the mean iron intake of Americans is adequate until the age of 75. However with changes in the major food sources there is a decrease in iron derived from meat and a concomitant rise in the proportion supplied by breakfast cereals. Alterations in dietary iron bioavailability that may result from this have not been studied. Physiological data suggest that the elderly do not represent a target population for iron deficiency since iron requirements are no greater than those of adult men and lower than those of children and menstruating women. Furthermore, there is little direct evidence of a high prevalence of iron deficiency in the elderly, but the laboratory measurements that have proved useful in defining iron status in younger people have not been standardized for or extensively utilized in older people. Anemia is still the most important known consequence of significant iron deficiency. However, the application of hemoglobin or hematocrit standards used in younger people to the elderly as well as the assumption that anemia can be equated with iron deficiency invalidates the conclusions of many surveys. Hemoglobin and hematocrit measurements are not suitable screening tests for iron deficiency in the elderly and there is an urgent need for a clearer understanding of the physiological and nutritional factors responsible for lower hemoglobin values in older people, par-

The continued prevalence of iron deficiency in developing countries as well as in some segments of the industrialized societies of the Western world has made it the subject of extensive research, particularly during the last quarter of a century.[1] The attention of investigators has been focused on children and menstruating women in whom nutritional anemia is most commonly recognized. While a few comprehensive surveys provide information about iron intake in Americans up to the age of 75, very little data is available for people beyond this age. Several investigators have attempted to define iron status in older people, but conclusions have usually been based on hemoglobin or hematocrit values with at the most one other parameter of iron nutrition. Interpretation of such data is uncertain because of the potential etiological complexity of anemia in elderly populations. Despite these restrictions, the information available is relatively consistent allowing us to draw some general conclusions.

The special characteristics of iron balance in man limit the usefulness of animal models for determining the effect of age on iron status. There are species differences in the ability to extract iron from food and to absorb the various chemical forms of iron in the diet[2]. Furthermore, iron excretion, and therefore the iron requirement of most laboratory animals is different from that of man[3]. Even the study of population groups outside

the United States is of limited value because of significant variations in dietary patterns. Some information from such investigations may be useful, eg. comparisons between the iron status of elderly people and children or menstruating women in areas where iron deficiency is common, may help define the physiological requirements of the elderly.

For the reasons outlined above, we have based our evaluation of information relating to iron status in the elderly on measurements of dietary iron intake and iron stores in elderly Americans. Observations from other countries have been used only when relevant to the impact of aging on physiological aspects of iron nutrition. We have selected 65 years and above to define the elderly population although this has not been used as a restrictive criterion since valuable data from some studies in younger subjects would otherwise have been excluded.

Intake

The most complete recent information on iron intake among people living in the United States is derived from the two U.S. Department of Health, Education and Welfare Health and Nutrition Examination Surveys (HANES 1, 1971-1974[4] and HANES 2, 1976-1980[5]). The data from these more comprehensive surveys (Tables 1 and 2) is in agreement with information from a number of other studies from different parts of the country[6-12]. Patterns of

dietary intake recorded in HANES 1 and 2 are similar although there is a consistent increase in iron consumption for both males and females of all age groups in HANES 2. The HANES 2 survey indicates that mean dietary iron intake for older Americans (both men and women) meets the 1980 recommended dietary allowance (RDA) of 10 mg[13] and it is particularly noteworthy that the average female maintains an iron intake in excess of 10 mg from childhood to age 74, even though her average caloric intake peaks at 1857 kcal at age 9-11 years and then steadily declines by 43% to a caloric intake of only 1295 kcal at age 65-74 years. This is achieved by a steadily increasing iron nutrient density from less than 6 mg iron/1000 kcal to nearly 8 mg iron/1000 kcal for the older American women.

While the average intake of all older Americans sampled in HANES 2 meets the 1980 RDA for iron, it is apparent from Table 3 that average intakes of certain subgroups of women in this and earlier surveys fails to do so, the lowest value being found in low income Black women over 59 years of age in the Ten State Nutrition Survey. In all cases, lower mean iron intakes were associated with lower mean caloric intakes so that the nutrient density for iron was consistently equal to or higher than that computed from the 1980 RDA for iron and calories for the respective age groups. Therefore when the average iron intake fell below the RDA, it appeared to be associated with a reduced

caloric intake. In such a setting, the latter would be likely to be the more significant nutritional deficiency since iron depletion would occur at a very slow rate.

The wide range of individual iron consumption in the HANES 2 study is at least in part a reflection of day-to-day variability in iron intake since each value represents a single day's food. A United States Department of Agriculture Food Consumption Survey carried out in 48 states between April 1977 and March 1978 was based on the average intake over 3 days[14]. From 95 to 97% of men and 86 to 90% of women over the age of 50 consumed 70% or more of the RDA for iron.

These data suggest that average overall iron intake is satisfactory. However, the availability of dietary iron for absorption is as important a factor as overall total iron content in determining nutritional adequacy[15]. The two most important promoters of iron absorption yet identified are ascorbic acid which increases the availability of dietary nonheme iron and meat which provides readily available heme iron as well as improving the absorption of any nonheme iron present in the meal[16]. In a recent study from Finland, iron deficient members of an elderly population were found to eat less meat and consume more milk products than the others[17]. While it is not possible to draw any definite conclusions about iron availability in the

diets of the elderly in the United States, it is important to note that whereas men and women aged 35-44 derive 26.4 and 21.3% of their iron from meat, this is the source of only 18.7 and 14.3% in elderly men and women respectively (Fig. 1)[5]. A concomitant rise in the proportion of dietary iron supplied by breakfast cereals occurs; from 5.6% to 15.9% in men and 7.3% to 14.8% in women. Over the same period mean daily heme iron consumption falls from 2.5 mg to 1.8 mg in men and from 1.6 mg to 1.2 mg in women. Mean ascorbic acid intake is unchanged. Thus iron bioavailability may be altered but this has not been studied.

Some groups of the elderly may be at a special disadvantage in terms of both quantity and bioavailability of food iron. They include some nursing home patients[18] and those who take only a few hot meals, have a physical disability or live alone[19,20]. Food dislikes have also been suggested to be closely related to iron deficiency in post-menopausal women[21].

In summary, the available information indicates that average total iron intake is adequate in elderly Americans. However the bioavailability of food iron is undefined. Some segments of the population may not receive sufficient iron, but this is most likely to be associated with a reduced caloric intake. Its significance is therefore questionable.

Dietary Requirements

Iron status is determined by the balance achieved between absorption and the sum of excretion, the requirements of growth and development and menstrual losses or the demands of pregnancy and lactation in women[22]. Since excretion is relatively fixed and extremely limited, iron balance is achieved largely through the regulation of absorption. There is ample evidence that the intestinal mucosa modifies absorption as an inverse function of body iron stores so that any change in absorption must be interpreted in the context of iron balance.

Decreased iron absorption has been reported in old age[23-25]. Unfortunately, the earlier studies were not well controlled for iron status, and the presence of underlying gastrointestinal diseases. The most comprehensive, that of Jacobs and Owen[25], demonstrated a progressive fall in percentage absorption of nonheme iron from a mixed meal, but no difference in heme iron absorption. The study included women in whom frank iron deficiency was excluded only by the measurement of serum iron and transferrin saturation. Characterization of iron status was thus incomplete. Although decreasing nonheme iron absorption was attributed to hypochlorhydria, it is possible that the trend reflected gradually increasing iron stores. In a later study that was carefully controlled for iron status, Marx[26] found no

effect of age on the rate of iron absorption from ferrous ammonium sulfate taken in the fasting state. However, since hydrochloric acid is relatively unimportant for the adequate absorption of soluble ferrous salts[27], these observations do not refute the suggestion of Jacobs and Owen that hypochlorhydria may lead to impaired nonheme food iron absorption in older people.

In the elderly the body's need for iron is determined primarily by the rate of excretion since growth and menstrual losses no longer occur. It is difficult to measure total body iron excretion which involves losses from the bowel, through the skin and in the urine. Earlier studies were based on chemical balance techniques but formidable technical difficulties make the results suspect[22]. Valid results have been obtained using radioisotopic methods although the method is limited by the necessity of obtaining uniform labelling of body iron before the rate of decrease in radioiron can be used to calculate excretion. Complete mixing takes at least a year[28,29]. This factor limits the value of whole body counters because ^{59}Fe must be used, and it has a relatively short half-life (45 days). Measurements have usually been commenced soon after injection and the results have shown considerable variation[30-32]. The most reliable radioisotopic studies have been based on the measurement of the change in specific activity in an easily accessible major iron com-

partment--the blood stream. Using ^{55}Fe which has a half-life of 2.94 years it has been possible to make observations over a prolonged period. In the studies of Green et al.[29], initial blood samples were drawn at 90 days and thereafter at four- to eight-month intervals for a period varying between 17 and 54 months. The mean daily iron excretion rates for three groups of men obtained in this study were 0.95 mg ($\pm$ 0.30) for Seattle Whites, 0.90 ($\pm$ 0.31) for Venezuelan Mestizos and 1.02 ($\pm$ 0.22) for Durban Indians. Using the same technique, Finch[28] found values of 0.61 ($\pm$ 0.08) mg in men aged 57-84 years and 0.64 ($\pm$ 0.05) mg in nonmenstruating women between the ages of 59 and 77. The value for menstruating women aged 32-44 was 1.22 ($\pm$0.11) mg. While no direct comparisons have been made in a single study, these data taken together suggest that iron loss in older men and women is no greater than in younger men. If anything, a small decrease may occur.

Iron requirements of the elderly could be increased if aging was associated with the enlargement of a major physiological compartment or a change in the distribution of iron that led to the necessity for a bigger storage pool to supply the needs of metabolically active compartments. Sixty per cent of body iron is in the hemoglobin of circulating red cells. Hemoglobin concentration remains unchanged in healthy elderly men and women until the age of about 70. Thereafter there is a significant

fall in men. The effect of age on hemoglobin values is discussed in detail later. Furthermore, the ratio between blood volume and body weight[33-35] has been shown in different studies either to remain unchanged or to decrease slightly. However, one study does suggest that age may affect the distribution of absorbed iron. Marx[26] found that a mean value of 91.2% of absorbed iron was located in circulating red cells 14 days after administration in younger controls whereas in the elderly the average was only 66%. The mean in controls of 91.2% is somewhat higher than that observed by other workers[36] except where the population studied included a high proportion of young women[37]. Some of the discrepancy between the elderly and control groups in Marx's study may therefore reflect differing iron status. A more direct study of plasma iron kinetics would be required to interpret the difference he found and to distinguish between the effects of alterations in iron balance, erythropoiesis and blood volume.

The physiological studies discussed above suggest that iron requirements decrease with age particularly in women. Investigations of the effect of age on storage iron provide corroborative evidence for this conclusion. Three types of measurement may be used to determine the size of body iron stores. The most direct is the assay of the nonheme iron concentration in various

organs. The liver contains about one third of the body iron store, and a number of surveys have been based on liver iron concentrations at necropsy in victims of acute trauma or illness that are unlikely to affect iron metabolism[22]. Similar information can be obtained in the clinical setting by the microscopic evaluation of bone marrow iron stores since there is a semiquantitative relationship between stainable bone marrow hemosiderin and storage iron[38]. The most recent addition to our diagnostic armamentarium is the serum ferritin assay. In health and uncomplicated iron deficiency serum ferritin concentrations are directly correlated with the size of the storage iron compartment[39-41]. The iron store of a 70 kg adult can be calculated as follows:[42]

Storage iron (g) = Serum ferritin concentration (ug/l) x 10.

While the amount of storage iron in infants and children is also correlated with serum ferritin concentration, the actual quantity present is considerably less and proportional to body size.

Loh and Chang[43] measured hepatic nonheme iron concentrations in Malaysians and Singaporeans at various ages. After the neonatal period, liver iron concentrations remained low until puberty in the males. Following this there was a progressive rise until the age of 16-25. The level then remained essentially unchanged until the age of 79. Among women, the low levels persisted until

after the menopause. Then there was a gradual increase until levels similar to those in the men were reached in the 60-79 year old age group. Age was associated with an increase in liver weight. Thus when expressed as total liver iron content, the highest levels were encountered in subjects of both sexes aged between 60 and 79. A similar trend is seen in other populations (Fig. 2).

The effect of age on iron stores has been examined by histological evaluation of stainable bone marrow iron and serum ferritin assay in clinical studies. Both parameters have been shown to increase with age[44-50], the most complete data being obtained from serum ferritin levels (Fig. 3). Improvement in serum ferritin levels occurs even in populations where iron deficiency is prevalent. MacPhail et al studied a group of South African Indian women among whom iron deficiency is a common problem[51]. Serum ferritin concentrations rose two-fold after the age of 45 and were associated with an increase in mean hemoglobin level and transferrin saturation and a concomitant fall in iron absorption.

Therefore iron status measurements tend to confirm the physiological data and suggest that the body's iron requirements are lowest in old age. Bone marrow iron stores and serum ferritin concentrations increase with age. In societies whose dietary iron intake is suboptimal, older people are less often iron deficient than children and younger women.

Evidence for Iron Deficiency

Considerable refinement of the clinical laboratory methods for recognizing iron deficiency has occurred in recent years[42]. As outlined above, depletion of iron stores without loss of iron from the functional compartment may be recognized by a decrease in bone marrow iron, or a drop in the serum ferritin concentration. A fall in transferrin saturation, a rise in red cell free erythrocyte protoporphyrin (FEP) and a decrease in the mean red cell volume are evidence of more severe iron deficiency and reflect an iron supply that is insufficient to meet the needs of the erythroid marrow for hemoglobin formation. However, since these measurements are indices of the balance between iron supply and bone marrow demand, they are not specific for iron deficiency. Abnormalities also occur when iron transport or hemoglobin synthesis is interrupted. Nevertheless when used in combination with other indicators of iron deficiency, they are useful for evaluating iron status in nutritional surveys. Once iron deficiency is sufficiently severe a fall in the hemoglobin or hematocrit occurs.

Employing the serum ferritin, transferrin saturation and FEP, it is possible to identify individuals with depleted iron stores and those with iron deficient erythropoiesis, i.e., those in whom the iron supply to the erythroid marrow is inadequate. While a single measurement is usually adequate in the patient

with anemia, inconsistencies are seen in individuals with mild iron deficiency without anemia when one measurement is made. In a study of 1500 subjects living in the Northwestern United States, the measurement of the three were combined with hemoglobin estimation[46]. The overall prevalence of anemia in this survey was 8.3%. When one of the three iron status measurements was abnormal, the prevalence increased to 10.9%, as compared with 28% when at least two were abnormal and 63% when all three were abnormal. Similar findings have been reported in South African children[52] using transferrin saturation and serum ferritin, comparable values being 24.3% for the whole population and 70% when both serum ferritin and transferrin saturation were abnormal. In general, measurements of iron status indicate that the prevalence of iron deficiency is approximately twice that of iron deficiency anemia. This simply indicates that the cut-off point used to define anemia is relatively insensitive for the detection of iron deficiency, since many of the individuals with mild iron deficiency will have hemoglobin values in the normal range.

The improved diagnostic specificity that combinations of these assays provide has not been exploited in the elderly. Furthermore, while the significance of per cent transferrin saturation, serum ferritin and FEP have been documented in children and younger adults, their interpretation in the aged has not been

evaluated carefully. There are some reasons for believing that the results obtained may be less specific. Two studies suggest that the serum iron concentration decreases slightly with age[54,55] although the use of transferrin saturation as the critical measurement would appear to eliminate this objection. Loria et al.[56] have shown recently that some subjects with anemia and increased ferritin levels respond to iron, implying that the correlation between serum ferritin and iron stores seen in younger people may be altered in the old age. One reason for this could be the co-existence of inflammation which increases ferritin values and therefore changes the level of ferritin diagnostic of iron deficiency. The need for more careful standardization of normal values for older people is evident.

In most nutritional surveys, the prevalence of iron deficiency has been measured by determining the percentage of observations that fall outside the normal range for one or more of these parameters. Unfortunately as we have indicated the specificity and sensitivity of each varies widely in different settings. An alternative model that uses a combination of measurements to estimate the distribution of iron stores in a population has recently been described by Cook and Finch[53]. The approach may prove particularly valuable in the elderly. Where studies can be conducted over a long period of time, definitive information may also be obtained by measuring the response to iron therapy.

One of the most direct ways of evaluating iron status has been the measurement of liver iron concentration. Results available demonstrate that liver iron concentrations in the elderly are comparable with those of young men, and about two-fold greater than those of menstruating women (Fig. 2)[57-62]. While this method provides accurate data on storage iron concentration, two problems do exist. The first is the difficulty of ensuring that the group sampled is representative of the whole population, even if material is obtained only from cases of accidental death. The second is the shift of iron that might occur from red cells to tissue stores in conditions such as infective and neoplastic disorders. Despite these restrictions, several thousand measurements carried out by investigators do provide useful information about the variation in iron stores in different countries. Unfortunately, relatively few measurements have been made in the elderly living in the United States.

Health Implications of Iron Deficiency

Because of the important role of iron in oxygen transport by the blood, in energy generation by mitochondria and in many other critical enzymatic activities within the body, one might expect that iron deficiency would lead to widespread physiological effects. Indeed, structural and functional abnormalities of mitochondria have been demonstrated in experimental models in

animals in addition to the obvious consequences for oxygen transport in the blood[63]. However, while there have been speculations concerning various tissue effects of iron deficiency in man, the one clear-cut abnormality is that of anemia. There seems to be little question that the maximum work performance and productivity in humans is decreased in the presence of moderate to severe anemia[64,65]. Thus, the monitoring of hemoglobin concentration is the most practical way of assessing the morbidity associated with iron deficiency at the present time. Unfortunately in the past many researchers have assumed that the measurement of the hemoglobin concentration is also the best method for detecting nutritional iron deficiency and determining its prevalence.

The results of investigations prior to 1976 are summarized by Bowering and Sanchez[66]. Where the lower limit of normal for hemoglobin in men and women was set at 11 or 12 g/100 ml an average incidence of anemia for both sexes of between 4 and 4.4% was reported for subjects not receiving medical care and 10-41% for those seeking medical treatment. It is important to note the marked difference between studies involving hospital patients and those carried out in apparently healthy people. In many instances blood loss associated with a variety of diseases was established as the primary cause of anemia in the patient

population. The significance of etiological factors other than iron deficiency particularly in hospital patients was emphasized by the report of Cooper et al.[67] They found that 490 of 1500 hospital patients surveyed were anemic. Infection, renal disease and neoplasms ranked as the most frequent causes. The extrapolation of this type of information, which is often quoted as evidence of a high prevalence rate for iron deficiency in the elderly, to older Americans in general is unwarranted.

Limitations to the use of the hemoglobin concentration or hematocrit as a screening procedure for detecting iron deficiency exist even in apparently healthy individuals. There is a marked overlap in the frequency distribution curves of anemic and normal people [68,69]. Any single discriminant value may lead to a large number of false negative and false positive results. Using a single cutoff point in pregnant women Garby et al.[68] showed that 17% of women classified as normal responded to iron while 35% of those considered anemic failed to do so. The use of the hemoglobin concentration in the elderly is further complicated by changes that appear to be associated with aging. Shapleigh et al.[70] reviewed the early studies of the effect of age on hematological values concluding that hemoglobin levels fell slightly in the elderly and that the sex difference was diminished. More recent surveys carried out on either random

samples of natural populations or on elderly people screened to exclude disorders likely to affect the hemoglobin level support this conclusion[71-85]. The results of several studies that include measurements in older people are summarized in Fig. 4. The difference in hemoglobin concentration seen in younger men and women tends to diminish with age because of a decrease in the average value for men, the fall starting in the late 60's. There appears to be little change in mean values for women below the age of 85. The reason for the decrease in men has not been established and the role of undiagnosed disease is uncertain. Since women drawn from the same population groups appear to be unaffected, dietary factors seem unlikely. The withdrawal of the androgen effect may be important. The association of lower white cell counts with lower hematocrit values has been regarded as evidence for a decreasing hemopoietic reserve in both sexes[86].

The interpretation of surveys based on hemoglobin values in elderly Blacks is further complicated by the observation that Blacks have lower mean hemoglobin values than Whites even when matched for economic status making dietary factors less likely to be responsible. The difference which ranges between 0.3 and 1 g cannot be explained by the inclusion of subjects with inherited abnormalities of hemoglobin and persists in the elderly[5,87-94].

The difficulties encountered in using hemoglobin concentration to detect iron deficiency in the elderly is illustrated by the Ten-State Nutritional Survey, 1968-70[95] The criteria set for deficient and low hemoglobin levels were less than 12 and 12-13.9 and less than 10 and 10-11.9 for all adult men and women irrespective of age or ethnic origin. Low and deficient hemoglobin values were most common in Black men; 51.1 and 14.2% of Black males with a poverty income ratio of less than 1 who came from low income ratio states had low or deficient hemoglobin levels respectively. Values for women in the same group were 27.5 and 5.1%. It is not clear that the individuals identified as anemic were really physiologically abnormal. However even if this was so it seems unlikely that iron deficiency would be responsible for a prevalence rate that was so much higher in men than in women, a conclusion that is supported by the low level of correlation between both serum iron and percentage transferrin saturation and hemoglobin concentration. The Ten-State Nutrition Survey criteria were used for a study of the incidence of anemia in residents of Missouri[96] and revealed that 30% of White males over the age of 59 were classified as anemic, although only a small percentage had hemoglobin levels below 12g/dl. It would again seem unlikely that iron deficiency was an important factor since most individuals had a dietary intake considered adequate for iron.

Using the same hematological criteria, the first HANES survey recorded a high prevalence of low hemoglobin values in Blacks over the age of 60 years (29.6% in the low income group and 22.8% in the income group above poverty level)[97]. Comparable values for Whites were less than 10%. However, less than 5% of Blacks had low serum iron concentrations or percentage transferrin saturation levels and in general the percentage of abnormal values was higher in Whites.

Studies from other parts of the United States have yielded varying results depending to some extent on age distribution and racial composition of populations sampled as well as differing criteria for anemia. Nevertheless iron deficiency appears uncommon. Fourteen percent of men and 3% of women in rural Utah (mean age 69) had low hemoglobin levels and none were in the deficient range (men < 12 g/dl, women <10 g/dl)[98]. Only a small proportion had low serum iron levels. A survey among an elderly predominantly Black population in an urban poverty area of Dade County, Florida demonstrated a 14% incidence of anemia (hemoglobin < 12 g/dl)[99]. However, no subject had a serum iron level below 50 ug/dl or a transferrin saturation of less than 15% leading to the conclusion there was no evidence of iron deficiency in these elderly people. An assessment of the nutritional status of 100 elderly men and women living in Corvallis, Oregon[9] showed that while 20% of men had hemoglobin values below

14 g/dl, only 1% of women had levels below 12/dl. Serum iron values were low in 4% of men and none of the women and transferrin saturations in 1% of the women but no men, again suggesting that iron deficiency was rare. Finally, Gershoff et al.[100] examined the effect of both food iron fortification and oral ferrous sulphate in anemic elderly residents of the Boston area. After the fortification trial, both control (no fortification) and fortification groups showed a 0.4 g/dl rise in hemoglobin. Three months administration of ferrous sulphate to those whose hemoglobin levels remained below 13 g/dl was without effect.

Taken together these observations identify two major problems in the evaluation of iron nutrition in elderly Americans; the fact that anemia has been equated with iron deficiency in many studies and the lack of a clear understanding of other factors affecting hemoglobin concentration in the aged. Therefore it is our belief that any study of older Americans in which anemia is used as the initial screening procedure for iron nutrition, other parameters of iron status being evaluated only in anemic individuals, has introduced so great a degree of inaccuracy that it should be disregarded. The assumption that anemia is a satisfactory criterion for identifying iron deficiency in the elderly and that most anemic individuals are iron deficient is invalid and must be discarded.

Health Implications for Iron Excess

The ease with which iron intake can be increased markedly by the ingestion of pharmaceutical preparations makes a brief consideration of the possible hazards of excessive iron consumption necessary. Individuals who have a normal regulatory mechanism for absorption appear to be able to maintain iron stores in the physiological range despite high dietary iron or even iron supplementation. Failure of this homeostatic mechanism occurs in idiopathic (hereditary) hemochromatosis, certain iron loading anemias such as thalassemia and sideroblastic anemia and occasionally following portacaval anastomosis or in association with porphyria cutanea tarda[22]. Tissue iron deposition in these disorders is associated with significant morbidity and increased mortality that may be aggravated by a rise in dietary iron intake. They are relatively rare conditions and usually present before the age of 65 although the protective effect of menstrual blood loss may delay the onset of symptoms in some women with idiopathic hemochromatosis. However it has been demonstrated recently that idiopathic hemochromatosis is an autosomal recessive disorder genetically linked to the HLA locus and that the gene may be present in up to 2-10% of the population in some areas of the world including parts of the United States[101]. Male heterozygotes accumulate 4-5 g of iron by the age of 40 years. Although most of them appear to stabilize at this level, it is not clear whether a high iron intake might lead

to a progressive increase in body iron. These observations may have particular relevance to the elderly since studies carried out in the United States[12] and Sweden[102] suggest that some elderly people take large quantities of supplemental iron.

Important Issues Requiring Further Investigation

It is possible to draw some conclusions about iron nutrition in the elderly only because the small amount of information available is relatively consistent. The conclusions are necessarily tentative and based mainly on investigations involving people aged 65-75. There have been very few studies of any aspect of iron nutrition in individuals over the age of 75. The major conclusions may be summarized as follows:

1. While iron deficiency does not appear to be a general consequence of aging, its prevalence particularly in some disadvantaged population groups living in the United States is unknown.

2. Some information about overall iron intake has been gathered, but food iron bioavailability and the adequacy of food iron absorption in the elderly remains to be defined. Both factors may be particularly relevant to populations in whom economic circumstances dictate restricted food choices.

3. The characterization of the normal ranges for the more specific indicators of iron status including serum ferritin, transferrin saturation and free erythrocyte protoporphyrin is incomplete in the elderly. Their application to the determination of iron status will be essential in obtaining reliable information from epidemiological surveys. Hemoglobin measurements should not be considered a means of detecting iron deficiency but rather of evaluating its severity once established.

4. The most definitive evidence for dietary iron deficiency in population studies is the evaluation of the response to oral iron supplements. This approach has only been used in a few limited studies.

5. Our understanding of the physiology of human iron metabolism has been advanced by a wide variety of experimental techniques. However, the effect of aging on these processes is unknown.

6. It has recently been demonstrated that iron deficiency may impair energy generation in mitochondria and other important enzymatic processes. These studies should be extended to include older members of the population.

7. Perhaps the most urgent question from a practical point of view is the better definition of the effect of aging on hemoglobin concentration. A clearer understanding of the "anemia" of aging, the factors involved in its genesis as well as a more precise definition of physiological normality are essential to the selection of suitable hemoglobin standards for the elderly.

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TABLE 1

Comparison of mean energy and iron intakes for males reported in HANES 1[4] and 2.[5]

HANES 1 1971 - 1974				HANES 2 1976 - 1980			
Age	Energy	Iron		Age	Energy	Iron	
yr	kcal	mg	mg/1000 kcal	yr	kcal	mg	mg/1000 kcal
-				6-11mos.	1001	12.8	12.8
1	1316	7.5	5.7	-			
2-3	1563	8.2	5.3	1-2	1311	8.7	6.6
4-5	1826	9.4	5.2	3-5	1628	10.5	6.5
6-7	2061	11.2	5.4	6-8	1981	12.5	6.3
8-9	2173	11.3	5.2	-			
10-11	2261	12.7	5.6	9-11	2183	14.4	6.6
12-14	2519	13.6	5.4	12-14	2430	15.9	6.5
15-17	2981	16.3	5.5	15-17	2817	17.4	6.2
18-19	2949	16.4	5.6	-			
20-24	2888	16.5	5.7	18-24	3040	17.8	5.8
25-34	2739	16.7	6.1	25-34	2734	17.3	6.3
35-44	2554	15.9	6.2	35-44	2424	16.1	6.6
45-54	2301	14.5	6.4	45-54	2361	16.2	6.9
55-64	2076	13.7	6.6	55-64	2071	14.8	7.1
>64	1805	12.1	6.7	65-74	1828	14.1	7.7

TABLE 2

Comparison of mean energy and iron intakes for females reported in HANES 1[4] and 2.[5]

HANES 1 1971 - 1974				HANES 2 1976 - 1980			
Age	Energy	Iron		Age	Energy	Iron	
yr	kcal	mg	mg/1000 kcal	yr	kcal	mg	mg/1000 kcal
-				6-11mos.	991	12.9	13.0
1	1207	7.2	6.0	-			
2-3	1412	7.2	5.1	1-2	1262	8.5	6.7
4-5	1628	8.4	5.2	3-5	1508	9.5	6.3
6-7	1829	9.6	5.2	6-8	1807	10.8	6.0
8-9	1864	9.8	5.2	-			
10-11	2023	10.3	5.1	9-11	1857	11.4	6.2
12-14	1932	10.4	5.4	12-14	1813	10.8	5.9
15-17	1756	9.5	5.4	15-17	1731	9.9	5.7
18-19	1739	10.0	5.8	-			
20-24	1691	10.0	5.9	18-24	1687	10.6	6.3
25-34	1638	10.3	6.3	25-34	1643	10.9	6.6
35-44	1558	10.4	6.7	35-44	1579	11.2	7.1
45-54	1533	10.6	6.9	45-54	1439	10.4	7.3
55-64	1382	9.8	7.1	55-64	1401	10.7	7.6
>64	1307	9.2	7.0	65-74	1295	10.2	7.9

TABLE 3

Comparison of iron intakes in different ethnic and income groups.

Reference	Group	Age	Iron	
		yr	mg	mg/1000 kcal
HANES 1[4]	All Income Male	> 64	12.1	6.7
	All Income Female	> 64	9.2	7.0
HANES 2[5]	All Income Male	65-74	14.1	7.7
	All Income Female	65-74	10.2	7.9
	Low Income Male	65-74	10.4	7.1
	Low Income Female	65-74	9.6	7.8
Ten State[6]	All Male	> 59	13.1	6.7
	All Female	> 59	9.6	6.8
	Low Income White Male	> 59	12.1	6.3
	Low Income Black Male	> 59	10.6	7.1
	Low Income Sp.Amer.Male	> 59	13.7	7.6
	Low Income White Female	> 59	9.5	6.6
	Low Income Black Female	> 59	7.8	6.7
	Low Income Sp.Amer.Female	> 59	11.1	6.8
USDA[10]	Male	65-74	14.5	7.6
	Male	> 74	13.4	7.6
	Female	65-74	10.6	7.5
	Female	> 74	10.1	7.6
Recommended Dietary Allowance[13]	Male	65-74	10.0	4.2
	Male	> 74	10.0	4.9
	Female	65-74	10.0	5.6
	Female	> 74	10.0	6.3

Legends to Figures

Fig. 1. Proportion of daily intake drived from the major food sources for iron--meat, breakfast cereals, other grain products and vegetables. Data from HANES 2, 1976-80.[5]

Fig. 2. The relationship between age and liver iron concentration. Measurements from the United States indicated by open blocks. The age range for the elderly population was 61-93 yrs for males and 46-93 yrs for females. Data from references 57-62.

Fig. 3. The effect of age on serum ferritin concentration. Study a was carried out in the United States[46], b in Canada[47], c in the United Kingdom[48], and d in Italy.[50]

Fig. 4. Effect of age on hemoglobin concentration. Mean values from surveys of unselected elderly and elderly screened for hematological disorders[73,74,77-85]. Males: closed circles, Females: open circles.

Figure 1.

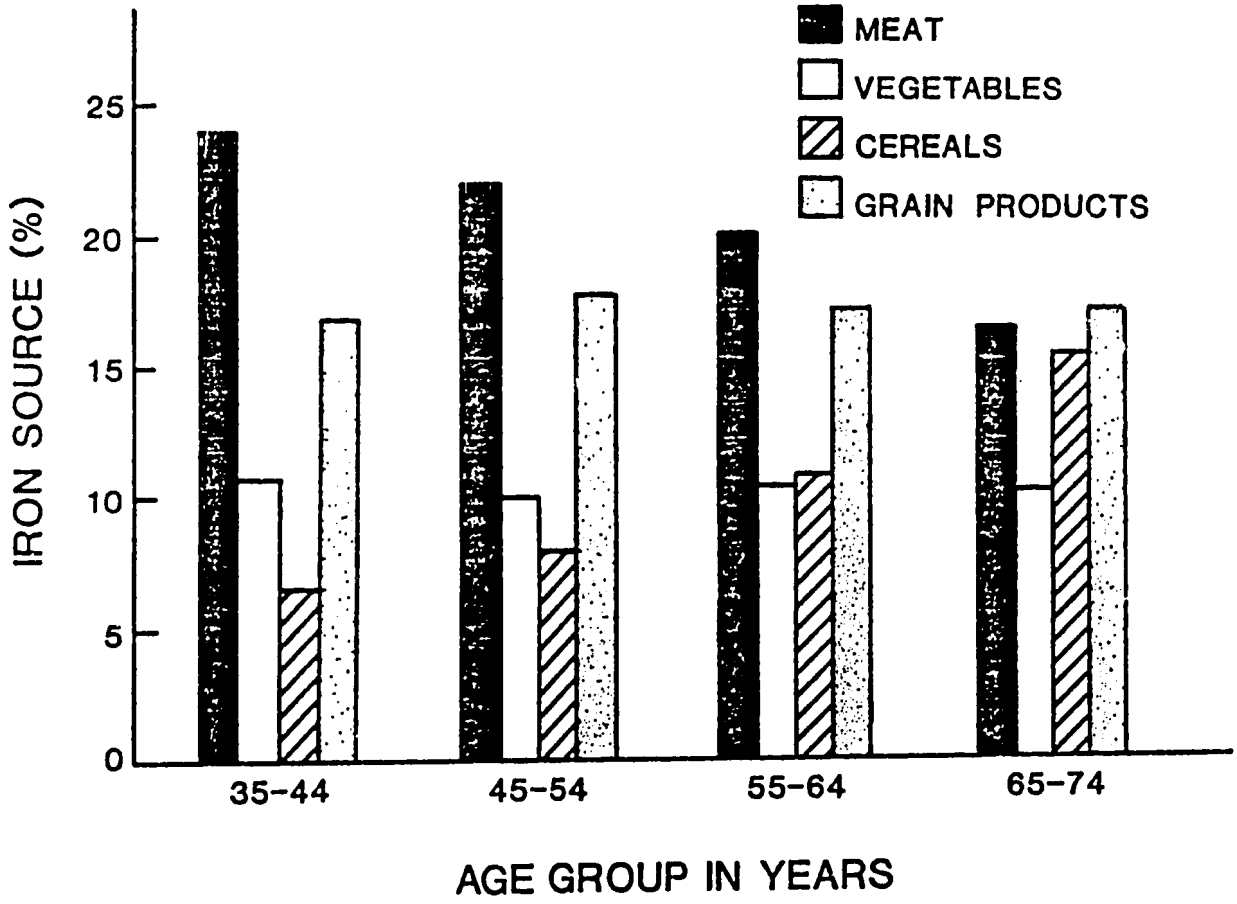

MEAT
VEGETABLES
CEREALS
GRAIN PRODUCTS
IRON SOURCE (%)
25
20
15
10
5
0
35-44
45-54
55-64
65-74
AGE GROUP IN YEARS

Figure 2.

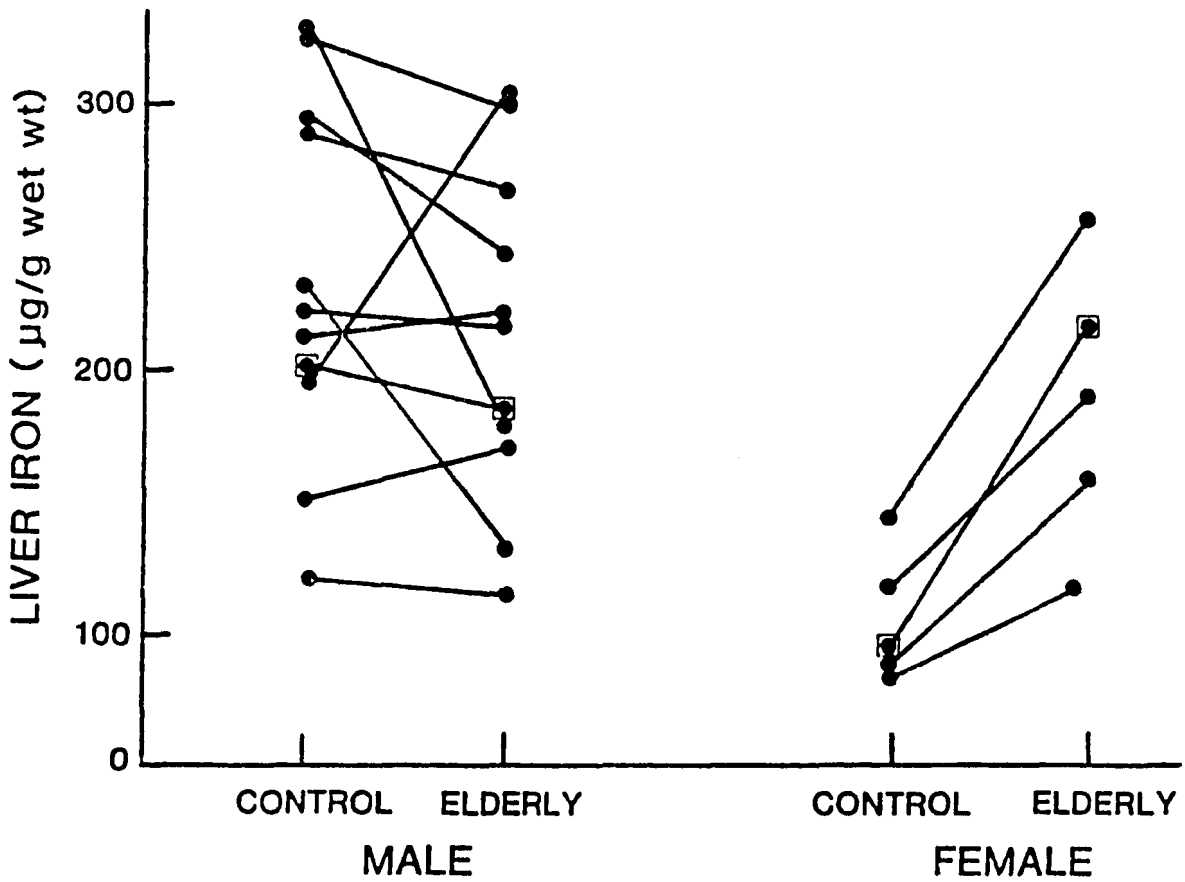

LIVER IRON (µg/g wet wt)
300
200
100
0
CONTROL
ELDERLY
MALE
CONTROL
ELDERLY
FEMALE

Figure 3.

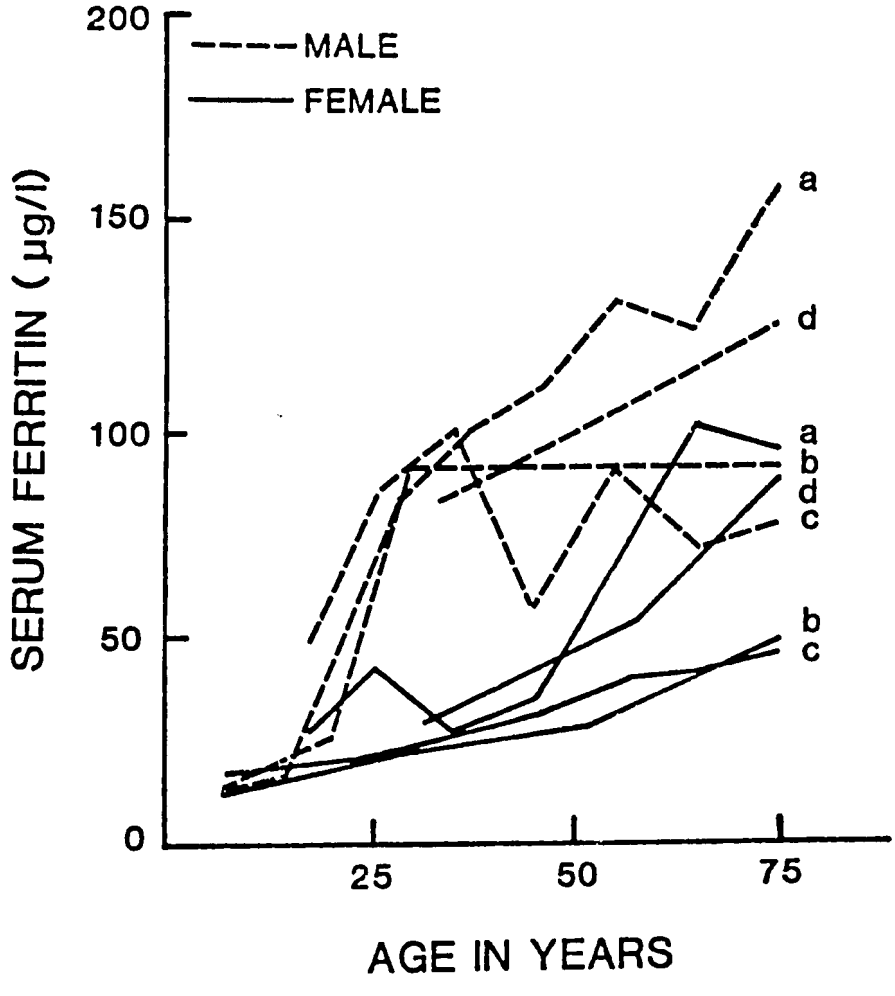
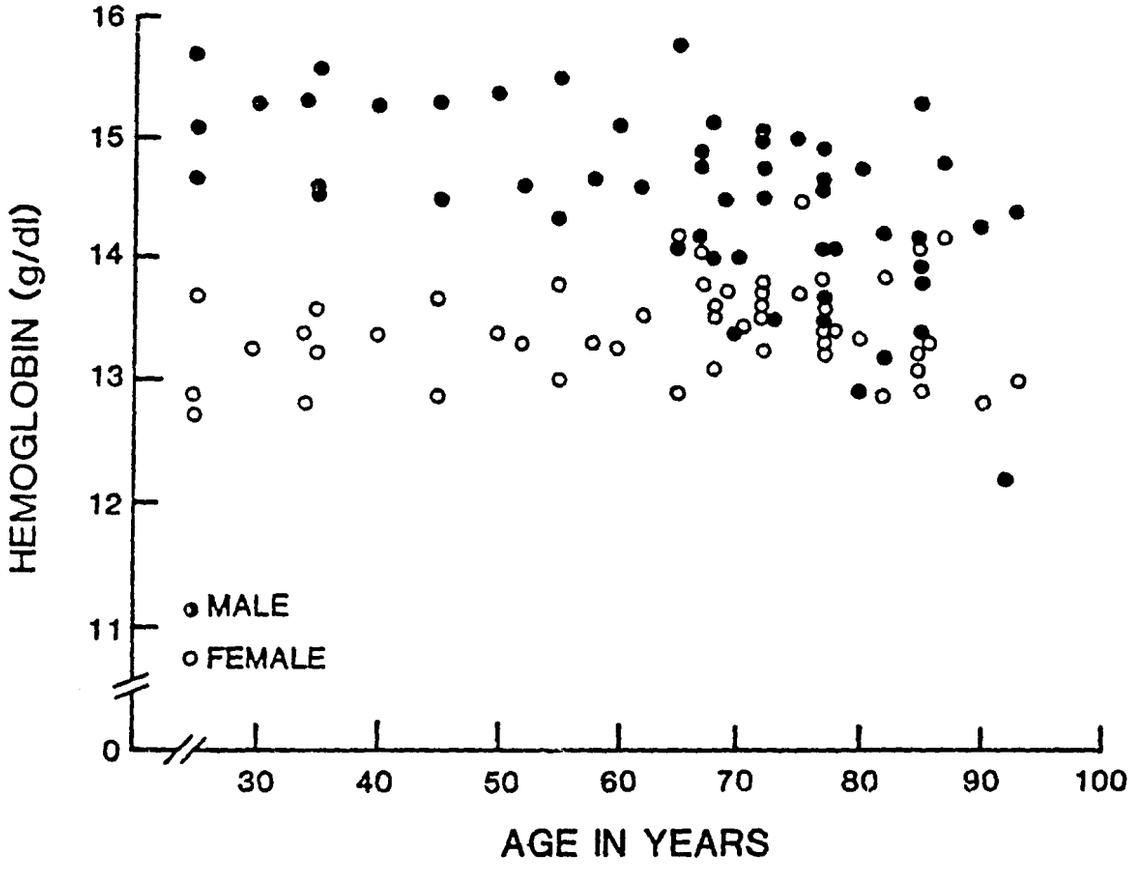

200
150
100
50
0
MALE
FEMALE
SERUM FERRITIN (µg/l)
a
d
a
b
d
c
b
c
25
50
75
AGE IN YEARS

Figure 4.


16
15
14
13
12
11
0
HEMOGLOBIN (g/dl)
MALE
FEMALE
30
40
50
60
70
80
90
100
AGE IN YEARS

IRON DEFICIENCY ANEMIA

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Iron deficiency is the commonest nutritional disorder in man and the commonest cause of anemia[1]. It is encountered most frequently in developing countries. The exact prevalence is difficult to define for several reasons. In many surveys, the finding of anemia has been equated with iron deficiency, yet other factors such as chronic infection, hemoglobinopathy, and folic acid deficiency may be primary causes of or associated factors in anemia. Moreover the hemoglobin concentration or hematocrit value, which are the most frequently employed measures of iron status in prevalence studies, are relatively insensitive because of the variation of values in normal subjects. Garby et al[2] using a response to 3 months iron therapy as evidence of anemia demonstrated that when a single hemocrit value was used as the cutoff point 17% of truly anemic women were wrongly classified as normal while 21% of normal women were misclassified as anemic. Therefore interpretation of some studies is limited by inadequate characterization of iron status. Other investigators have used specific methods to determine iron status (e.g. measurement of liver iron concentration in individuals dying suddenly), but have made only small numbers of observations that may not be representative of the whole population.[3]

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In this review we have not attempted to deal with iron deficiency in a comprehensive way but rather to emphasize some aspects where our understanding has been advanced by recent observations.

IRON BALANCE

The metabolic requirements of human beings for iron are met by a positive balance during the years of growth and a rate of absorption that is physiologically matched with a relatively fixed rate of loss in the adult. Full term infants have about 80 mg iron/kg body weight or an average of 250 mg at birth and require about 100 mg or 0.3 mg/day during the first year to maintain a hemoglobin level of about 11 g/dl. Premature infants have only about 160 mg iron at birth and must absorb about 1.0 mg/day to prevent the onset of anemia.[3]

From the age of 2 years until adolescence, the daily iron requirement increases by 0.5 to 0.8 mg because of growth, a higher hemoglobin level, and a rise in iron losses from 0.2 to 0.5 mg/day. In addition to increased body mass during the adolescent growth spurt, further increases in hemoglobin concentration of 2 gm/dl ~~in the male and 1 gm/dl in the female~~ combined with obligatory losses result in a total daily iron requirement of 1.6 mg for males. After the growth spurt, this

drops to 1.2 mg/day until the level of iron stores increases to the adult level of about 1,000 mg.[3] Normal men lose 0.1 mg in urine, 0.2-0.3 mg from skin, and 0.6 in feces each day.[4] Only about 0.14 mg of the fecal loss comes from bile or epithelial cells; the remainder reflects hemoglobin iron in approximately 0.5 ml blood normally lost by healthy individuals. Therefore total daily losses for adult men are 0.9-1.0 mg.

Nonmenstruating women lose 0.7-0.8 mg iron per day by the same routes. Menstrual iron loss remains fairly constant in the individual, but there is a marked variation among apparently healthy women. The median amount is about 30 ml (equivalent to an additional daily iron requirement of 0.45 mg).[3] However, it should be noted that over 10% of women lose more than 80 ml[5] significantly increasing their iron needs. Different methods of birth control can drastically affect menstrual blood loss and therefore iron requirements. Combined variety oral contraceptives tend to reduce menstrual flow[6], while intrauterine devices lead to increased bleeding.[7]

Although pregnancy eliminates menstrual loss, about 1200 mg iron are needed during this period to supply the requirements of the fetus, blood loss associated with delivery, expansion of the red cell mass, and continued obligatory excretion from the gut, skin and urine. During the three trimesters of pregnancy, daily iron

requirements are 0.8, 4, and 6 mg respectively. The net loss from a pregnancy is approximately 750 mg since about 450 mg is used for the increase in red cell mass that returns to normal after delivery.

ETIOLOGICAL FACTORS IN IRON DEFICIENCY

Iron balance is dependent on adaptations of iron absorption since the rate of excretion is controlled by factors unrelated to iron balance. It is evident from the preceeding discussion that requirements of different people may vary widely. Even moderate limitations to the rate of assimilation may cause iron deficiency in some of them.

Many factors may influence iron absorption. Only three appear to be important under most physiological circumstances. They are the amount of iron ingested, its bioavailability and the iron status of the individual. Significant increases in the rate of erythropoiesis promote iron absorption in conditions associated with ineffective erythropoiesis.[3] However, within the normal range the rate of erythropoiesis does not seem to play a major role since iron absorption is normal in patients with renal failure and erythroid hypoplasia.[8-10]

Absorption is controlled primarily by the mucosal cells of the proximal small intestine; the fraction of available iron absorbed is inversely proportional to the size of body iron

stores.[11] On the average, an American man has an iron store of 1 gm and absorbs 6% of the 15-20 mg iron in his daily diet. On the other hand, an American woman, consuming a smaller quantity of the same diet (12 mg iron), absorbs 15% of the iron and maintains an iron store of 300 mg[3]. The maximum rate at which iron can be absorbed from a typical "high availability" Western meal by an iron deficient individual is considered to be 3-4 mg/day.[12-15] In many parts of the world, the maximal rate is probably much lower due to the poor bioavailability of dietary iron in cereal-based meals.

Iron deficiency occurs when the dietary intake of bioavailable iron is inadequate, when absorption is impaired, or when bleeding occurs and causes iron losses that exceed the capacity of the gastrointestinal tract to extract iron from the diet.

Intake of Bioavailable Iron

Dietary iron intake greatly exceeds the body's requirements. Therefore, nutritional deficiency most often results from poor availability rather than insufficient quantity. The use of radioisotopes has made it possible to demonstrate that different sources of food iron show marked variation in their bioavailability.[16] Furthermore, when eaten together in a single meal, different foods interact with one another and iron availability becomes a characteristic of overall meal composition rather than a property specific to the food item. As a result all the iron

in a meal can be considered to enter one of two common pools in the lumen of the upper small bowel.[17,18] The smaller of the two, the heme pool, contains hemoglobin and myoglobin from meat and is almost always readily absorbed. All other dietary iron, collectively termed nonheme iron, forms the larger pool. It is absorbed less well. The bioavailability of the nonheme pool is markedly affected by other dietary components.[1] In a study using Swedish soldiers[19], 30% of the heme iron was absorbed as compared to only 5% of the nonheme iron. Therefore, 1 mg heme iron constituted only 6% of the total iron content of these meals, yet contributed 30% of the absorbed iron.

The uptake of heme as the intact metalloporphyrin by the mucosal cell provides one reason for its higher bioavailability.[20] Iron is separated from the porphyrin ring intracellularly. In contrast, nonheme iron is released from the parent compound before entering the mucosal cell and is then susceptible to interaction with other dietary constituents in the lumen of the gut. They may either increase or diminish its rate of absorption; bioavailability being determined by the balance between enhancers and inhibitors. All components of the nonheme iron pool are affected proportionately.

Only a small fraction of the many factors that may influence iron absorption have been studied (Table 1). While several substances are known to inhibit nonheme iron absorption, only two

nutritionally significant enhancers have been identified--ascorbic acid and animal tissue.[21] The increase in iron absorption with ascorbic acid is directly proportional to the amount present in the meal regardless of whether it is in a purified form or a component of food.[22] Besides contributing highly available heme iron, animal tissue in the diet produces a 2- to 4-fold rise in the absorption of nonheme iron. This property is found in a variety of animal products, exceptions being eggs, milk and cheese.[23]

In the last few years investigations have focused on dietary components that reduce the amount of absorbable iron in a meal. The most potent known inhibitor is tea, which decreases absorption by a factor of 4 when one cup is taken with a meal.[24] Inhibition appears to result from the formation of insoluble iron-tannate complexes. Polyphenolic compounds similar to those in tea are present in coffee and may account for its marked, but somewhat less potent inhibition.[25] Moreover, recent investigations carried out by Torrance et al.[26] suggest that the inhibitory effects of many vegetables and cereal foods may be due to the presence of tannins.

A variety of other foods reduce nonheme iron absorption. They include the whites[27] or yolks[28] of hen's eggs. The dominant inhibitory compound is considered to be vitellin, the major

phosphoprotein complex in egg yolk. Wheat bran consistently depresses iron absorption.[29,30] In the past, it has been assumed that phytate plays a key role in this effect. The recent observation that enzymatic hydrolysis of phytate in whole bran does not diminish the inhibitory effect suggests that other factors are responsible.[30] The fiber in bran and other vegetable food is moderately inhibitory to iron absorption[31] although studies using purified fiber sources have yielded variable results.[32]

Recent studies carried out by Cook et al.[33] have stimulated a number of investigators to further examine the potential effects of soy products on iron absorption. These processed food constituents are important because of their widespread use as meat extenders and as protein sources in food supplements. While some earlier studies had suggested that soybeans were a good source of available iron, Cook et al. demonstrated marked inhibition of nonheme iron absorption from semipurified meals in which soy flour or isolated soy protein were the major protein sources. It is unlikely that the considerably smaller proportions of soy products found in American meals will have as marked an influence. Nevertheless, their observations indicate that soy products are unlikely to provide as much available iron as meat does. Substitution of soy protein for meat may therefore have long-term effects on iron balance unless measures are taken to improve available iron content. Finally, it should

be noted that inorganic calcium and phosphate salts as well as ethylene diamine tetra-acetic acid which is present in the American diet may reduce nonheme iron availability.[3]

In summary, the bioavailability of dietary nonheme iron is modified by many food components and is an important determinant of iron status. With the two most potent enhancers of absorption, ascorbic and and animal tissue, unavailable to much of the world's population for economic reasons, poor iron bioavailability is the primary cause of nutritional anemia.

Malabsorption

Malabsorption of iron is rare in Western countries but may contribute to the high prevalence of iron deficiency anemia in the Indian subcontinent and Haiti where histological abnormalities of the intestinal mucosa are frequently present.[3] It only occurs in gastrointestinal disorders that affect the upper small bowel. The observations of Bezwoda et al.[34] suggest that the importance of moderate malabsorption may have received insufficient attention. They compared absorption from heme and nonheme food iron and ferrous sulphate in three groups of iron deficient volunteers, patients with idiopathic hemochromatosis who had undergone extensive phlebotomy, Indian women with iron deficiency who were otherwise apparently healthy, and white hospital patients with iron deficiency (Fig. 1). The three groups had comparable degrees of iron deficiency. Heme iron was

equally well absorbed by all groups, but nonheme iron was less well assimilated by both the white patients and the Indian women. However, the most striking finding was very poor absorption of nonheme iron from the meal by the Indian women. Malabsorption of this degree could be a major factor in the genesis of anemia in these people who eat very little if any meat.

Gastric surgery is the commonest cause of impaired iron absorption in Western countries. Malabsorption which affects both heme and nonheme food iron is probably a consequence of the loss of acid secretion as well as the reservoir function of the stomach. In some operations, the diversion of gastric contents away from the area of maximal iron absorption in the duodenum is an additional factor.[3] The more recent surgical procedures that consist of vagotomy combined with drainage of the stomach lead to a lesser degree of impairment of absorption.[35] Bleeding is an important contributing factor in most patients.

The relationship between chronic gastritis and iron deficiency has not been defined clearly. The frequency of the association varies in different countries suggesting that etiological factors distinct from those primarily responsible for iron deficiency may be present. Iron deficiency may well be a consequence of reduced iron absorption with intermittent bleeding from the atrophic mucosa and possibly increased free iron loss from the gastric membrane being contributory factors in most

patients[3]. However, improvement with iron therapy in some patients suggests the possibility of an etiological role for iron lack.

Finally, the absorption of iron may be inhibited by drugs or other substances taken with the food. Pica, the eating of nonfood material, may lead to iron deficiency if the material eaten binds iron, e.g. some clays have been reported to adsorb iron making it unavailable.[37] Antacids have a similar effect.[38] Tetracycline forms a strong complex with iron yet iron balance has not been impaired in patients on long-term tetracycline therapy for acne.[39] Recently cimetidine has been shown to cause modest inhibition of nonheme iron absorption presumably by reducing gastric acid secretion.[40]

Blood Loss

Increased menstrual loss which has already been discussed under the heading of "Iron Balance", and gastrointestinal bleeding are the commonest reasons for increased iron requirements leading to negative iron balance. On a worldwide basis, hookworm infestation is the most important cause of bleeding from the gut. Hookworm loads showing over 5,000 ova/gm feces are almost always associated with anemia.[41] Other parasitic infections such as schistosomiasis[42] and Trichuris trichuria[43] may play a role although available evidence is less definitive.

Infective causes of gastrointestinal blood loss are rare in industrialized countries. Nevertheless, iron deficiency in men and postmenopausal women is usually a consequence of blood loss from the gastrointestinal tract. Common causes in adults include peptic ulceration, hiatal hernia, gastritis, drug ingestion (alcohol, nonsteroidal anti-inflammatory drugs and anticoagulants), and neoplasms.[44] Occult blood loss from the bowel is also important in infancy.[45] A thermo-labile factor in cow's milk may be responsible both for bleeding and for the associated protein-losing enteropathy.[46]

Anemia owing to undetected bleeding from other sites is much less frequently encountered. Bleeding into the urinary tract is easily noticed and usually alerts the patient to the problem before iron deficiency occurs. Although iron stores are depleted in regular blood donors, anemia seldom results except among female donors from poorer communities.[47]

LABORATORY EVALUATION OF IRON DEFICIENCY

The accurate evaluation of iron status depends upon laboratory investigations since there are no specific clinical features of iron lack. Different tests provide information about various parts of the iron cycle (Fig. 2) and no single investigation or

combination of tests is optimal for the diagnosis of iron deficiency in all situations. A clear understanding of the significance and limitations of each diagnostic procedure is therefore essential to the selection of appropriate tests for evaluating iron status in any particular setting. Currently available laboratory tests provide information about one or more of the following aspects of iron metabolism: 1) the size of body iron stores; 2) the adequacy of the iron supply to the erythron; and 3) the size of the erythron itself.

Evaluation of Iron Stores

Serum Ferritin

Iron is stored primarily in the reticuloendothelial cells of the spleen, liver, and bone marrow, and in the parenchymal cells of the liver, as ferritin. Sensitive immunoradiometric assays have demonstrated that minute quantities of this protein are present in the plasma[48,49] and that the serum ferritin concentration is directly proportional to the size of the body iron store. Thus the serum ferritin concentration parallels the developmental changes in iron status during infancy and childhood[50,51]. In men there is a progressive rise in the concentration after the teenage years while in women it remains at a relatively constant level until the menopause and then undergoes a gradual increase[52]. Direct evidence relating serum ferritin concentration to the size of body iron stores has been obtained by comparisons with chemical measurements of nonheme iron con-

centration in the bone marrow[11] and studies in which the body iron store was measured by phlebotomy[53-55]. The phlebotomy studies have demonstrated that the relationship is quantitative in health and uncomplicated iron depletion. Within the range 20-300 ug/l, the size of iron stores in an adult can be calculated as follows:

Iron store (mg) = serum ferritin (ug/l) x 10

Cook et al.[49] found mean serum ferritin concentrations of 94 and 34 ug/l in 174 normal men and 152 normal women between the ages of 20 and 50 years in whom anemia and iron deficient erythropoiesis had been excluded on the basis of a hemoglobin concentration greater than 13 and 12 gm/dl in men and women respectively, a protoporphyrin concentration below 95 ug/dl red blood cells and a transferrin saturation greater than 20%. Very similar mean values have been obtained in most subsequent surveys with large sample sizes.

A serum ferritin value of less than 12 ug/l is virtually always indicative of absent marrow iron stores. Furthermore, in uncomplicated iron deficiency values are rarely greater than 12 ug/l.[56] Therefore the serum ferritin assay is relatively sensitive and specific in uncomplicated iron deficiency. It can be performed on a small blood sample and measurements in the same individual at different times of the day or on different days vary very little. While most assays are still performed by immunoradiometric technics, methods for enzyme-linked immuno-

assays that eliminate the need for radioisotopes have recently become available.

Unfortunately, the usefulness of the serum ferritin is limited in many clinical situations where iron deficiency coexists with infection, other inflammatory disorders, neoplasms, liver disease, or chronic renal disease. Under these circumstances, the usual relationship between iron stores and serum ferritin is disturbed. Ferritin levels tend to be higher and more variable. Nevertheless direct comparisons between serum ferritin concentrations and bone marrow iron stores have demonstrated that iron status remains an important determinant of serum ferritin in patients with inflammatory disorders or chronic renal failure (Fig. 3). While the data for Fig. 3 summarize the observations of different investigators[57-61] thereby raising some questions about the comparability of the assessment of marrow iron stores, it appears that the greatest discrepancies in serum ferritin concentration are encountered when stores are decreased. This type of analysis demonstrates that the serum ferritin is less specific in inflammatory disorders and chronic renal diseases but may still prove a useful guide to the need for further evaluation of iron status in such patients. A value of less than 50 ug/l is suggestive evidence of iron deficiency while a value above 100 ug/l makes iron deficiency unlikely[56]. The usefulness

of the serum ferritin assay in patients with liver disease and neoplasms is less certain; Prieto et al.[62] found no relationship between serum ferritin and liver iron content in patients with liver disease unless a correction was made using the serum aspartate transaminase level.

Bone Marrow Iron Stores and Sideroblast Iron

The most reliable method for assessing body iron stores in patients with complex anemias is the semiquantitative histological estimation of the hemosiderin content of bone marrow.[3] All of the iron normally present in reticuloendothelial stores is available for hemoglobin synthesis. In the presence of anemia, the finding of hemosiderin in the bone marrow excludes iron deficiency as the factor limiting hemoglobin synthesis with two exceptions. Soon after a severe hemorrhage, iron mobilization from large particles may be too slow to meet the erythroid marrow's demands, leading to the persistence of stainable iron despite an inadequate iron supply to the red cell precursors.[63] A fraction of parenterally-administered therapeutic iron dextran may be found in macrophages for some months after administration despite continued iron deficiency.[64]

Evaluations are usually made on either aspirate or biopsy samples. Disparities have been noted between the two technics carried out simultaneously, iron being more obvious in either the aspirate[65] or the biopsy.[66] At least part of this dis-

crepancy may be explained on technical grounds such as the examination of marrow smears containing an insufficient number of particles or the use of acid to decalcify bone biopsies resulting in the leaching of iron from the specimen. Ideally both types of preparation should be studied.

The second important component of the bone marrow examination is the sideroblast count. Both the number of sideroblasts[67] and the total number of iron granules in 100 normoblasts[68] are measures of the adequacy of the iron supply to developing red blood cells. A sideroblast count of less than 10% (normal 35%) is indicative of an inadequate supply and iron deficient erythropoiesis. It is found in iron deficiency as well as in patients with the anemia of chronic disease where iron supply is restricted despite plentiful storage iron.

Iron Supply

Serum Iron and Iron Binding Capacity

Once iron stores are sufficiently depleted, the rate of delivery of iron to the bone marrow is limited and the plasma iron concentration falls. A serum iron below 40 ug/dl probably indicates that insufficient iron is available for the needs of the erythron, but the most reliable criterion for the onset of iron deficient erythropoiesis is a percentage saturation of transferrin with iron of less than 15%.[67,69] Unlike the serum iron and percentage saturation of transferrin, the total iron

binding capacity (TIBC) is affected before the iron supply falls. The TIBC rises as storage iron decreases.[70,71]

Measurements of serum iron and TIBC have long been standard procedures in the evaluation of iron deficiency. Laboratory errors in serum iron assays due to iron contamination, although common in the past, have largely been eliminated by the use of disposable equipment and automated methods, yet the reliability of transferrin saturation as an index of iron deficiency is still limited by its lability. There is a marked diurnal fluctuation and even when specimens are collected at the same time of day, variability in the same person on different days may be as much as 30-40%.[72] Furthermore, reduced transferrin saturation is not specific for iron deficiency and reflects only a discrepancy between supply and demand. When supply is interrupted despite adequate iron stores, transferrin saturation falls. This may occur rapidly after the onset of inflammation[73]. In such disorders the TIBC is usually reduced as well. Therefore a low transferrin saturation associated with and increased TIBC remains a useful indicator of the presence of iron deficiency.

Free Erythrocyte Protoporphyrin

Rapid simple technics for measuring free erythrocyte proto porphyrin (FEP) have become available recently and sufficient accuracy can be achieved by measurement of fluorescence on a

blood film without preliminary processing.[74] Protoporphyrin IX accumulates in developing red cells when heme synthesis is impaired and a rise in the FEP level is a sensitive indicator of an inadequate iron supply.[75] Although FEP provides the same information as percentage transferrin saturation, it is a more stable measurement because the content of each red cell remains unaltered throughout its lifespan.[75,76] While plasma iron may fall within a few hours of infection or within a few days after bleeding, increases in protoporphyrin can only be detected a week after the iron supply becomes deficient.[75] During treatment for iron deficiency FEP:Hemoglobin ratios decline steadily but remain elevated until hemoglobin levels return to normal.[76] Measurements of FEP do not distinguish between the anemia of iron deficiency and that due to chronic inflammatory disorders. It may also be increased in other conditions that interfere with heme synthesis e.g., lead poisoning, chronic inflammatory disease, erythropoietic protoporphyria, acute myelogenous leukemia.

Iron Deficiency Anemia

Red Cell Indices

Iron deficiency anemia is characteristically hypochromic and microcytic. As the iron supply is interrupted, normal red cells are replaced by a deficient population. Consequently, these cells are present before anemia is manifest, but it is difficult

to recognize them with certainty and mild iron deficiency may not be diagnosed on morphological grounds in 50% of patients.[77] Once a sufficient proportion of the circulating cells have been produced under iron deficient conditions, the red cell indices become abnormal. The degree of change depends both on the duration of iron deficient erythropoiesis and on the discrepancy between iron supply and marrow demands.[3] Values close to normal may be encountered if the marrow is relatively hypoplastic. The advent of electronic counting equipment has revolutionized the reliability and sensitivity of the red cell indices. Mean corpuscular volume and mean corpuscular hemoglobin are the most reliable measures of iron deficiency.

Hemoglobin and Hematocrit

The final stage of gradually developing iron deficiency is anemia. As we have already mentioned, mild anemia may be difficult to recognize because of the significant overlap between normal values and those of patients who will respond to iron therapy.[2] Nevertheless, WHO criteria for anemia have become widely accepted: adult men <13 g/dl, menstruating women <12 g/dl, pregnant women <11 g/dl. The use of percentile curves instead of single values is more satisfactory in children whose levels undergo developmental changes.[78]

The best approach to diagnosing iron deficiency anemia depends upon the setting in which it is being studied. The accuracy of

prevalence studies may be improved if measurements of transferrin saturation, free erythrocyte protoporphyrin, serum ferritin, and hemoglobin concentration are combined.[52,79,80] Two of the most important conditions that need to be distinguished from iron deficiency are anemia associated with inflammatory disorders and neoplasms ("anemia of chronic disease"), and thalassemia minor. Since the serum ferritin is normal or raised in both conditions, it is the most reliable test for differentiating them from pure iron deficiency. Hersko et al.[81] have reported that the combination of serum ferritin estimation and electronic red cell counts satisfactorily discriminates between normality, iron deficiency, and thalassemia. Iron deficiency may be a component of many complicated anemias among hospital patients. Evaluation of bone marrow iron stores is then essential for an accurate diagnosis. For clinic or office patients in whom iron deficiency is considered likely, a therapeutic trial of oral iron is often the least expensive and most definitive approach.

EFFECTS OF IRON DEFICIENCY

Related to Anemia

The clinical impact of iron deficiency is clearest once there has been a significant fall in the hemoglobin concentration. Mild anemia may have little effect because various compensatory adjustments including increased cardiac output and reduced hemoglobin oxygen affinity due to increased red cell 2,3-diphosphoglycerate, improve oxygen delivery.[82] Severe anemia

limits work capacity.[83-86] This is in part due to the fact that the increment in cardiac output and respiratory minute volume per unit increase in oxygen consumption is elevated in iron deficiency anemia[84,87,88] while the maximum cardiac output and respiratory minute volume remain fixed.[83,88,89] The precise hemoglobin level at which exercise capacity is restricted because of anemia is not clear. In some studies a direct correlation has been found between exercise capacity and hemoglobin concentration (Fig. 4).[85,90] On the other hand, Davies et al.[88] could not detect a decrease in aerobic work capacity until the hemoglobin concentration fell below 12 g/dl.

The very real nature of the disabilities caused by severe iron deficiency is most evident in developing countries where a significant reduction in the productivity of laborers[91,92], and an increased maternal and perinatal mortality[3] have been reported.

Unrelated to Anemia

While the consequences of significant iron deficiency anemia have been recognized for some time, recent investigations have revealed a variety of disabilities that result from tissue iron lack and are independent of the degree of anemia. Concentrations of a number of metabolically active tissue iron compounds are consistently reduced in the iron deficient state. These include myoglobin; enzymes with heme as the prosthetic group such as the

cytochromes, catalase and peroxidase; and the metalloflavoprotein enzymes such as NADH, succinic, xanthine, aldehyde, and alpha-glycerophosphate dehydrogenases.[93] In addition, iron depletion may inhibit processes where iron is present as an essential cofactor, e.g. aconitase, tyrosine, and trytophan hydroxylases.

Many clinical abnormalities have been attributed directly to iron deficiency with or without anemia. They include symptoms such as fatigue and malaise, and various morphological abnormalities of epithelial structures. Although careful study has failed to demonstrate an association between nonspecific symptoms and iron deficiency in the absence of anemia, perversions of taste in the form of pica or a craving for ice do appear to be occasional manifestations of iron lack. Its exact relationship with mucosal and epithelial disorders such as angular stomatitis, glossitis, webbing of the esophagus, atrophic gastritis, and koilonychia is uncertain. The frequency of such findings seems to be decreasing and there is a marked geographic variation in prevalence suggesting that other factors may play an important role.

Abnormalities of Gastrointestinal Function

As discussed under the section on malabsorption of iron, gastric mucosal atrophy may be responsive to iron therapy in some patients. In addition, flattening of the duodenal villi with

cellular infiltration and malabsorption have been described in iron deficient infants.[94,95] Lactose intolerance associated with reduced disaccharidase levels may be present.[96] Furthermore, impaired assimilation of iron itself in iron deficient infants has been reported[97,98] with reversion to normal following iron therapy[94,95]. Others[3] have not been able to confirm these observations, and it is noteworthy that impaired iron absorption has not been observed in older children[99] or in adults.[100] There may be rare instances in which iron deficiency impairs iron absorption, but increased absorption is far more characteristic. It is associated with this excessive uptake of other metals such as lead and cadmium.[101-103] Poisoning may result. Furthermore, the synthesis of mucosal enzymes responsible for the metabolism of drugs and various toxins is critically dependent on the absorption from the intestinal lumen.[104]

Skeletal Muscle Dysfunction

Iron deficiency reduces work capacity. In the past it has been assumed that this is due entirely to the limiting effect of anemia on oxygen delivery. However Finch et al.[105] have demonstrated recently that iron deficient rats show a marked reduction in running ability compared to control animals even when the hemoglobin concentrations of both groups have been adjusted to the same level. In their studies the disability

could be corrected in 4 days by iron therapy providing clear evidence of impaired exercise tolerance attributable directly to tissue deficiency. A subsequent study led them to the conclusion that a reduction in the mitochondrial enzyme alpha-glycerophosphate dehydrogenase causes accumulation of lactic acid and that this impairs work capacity [106] Koziol et al.[107] and McLane et al.[108] confirmed the decrease in mitochondrial iron-containing enzymes in skeletal muscle and the concomitant decline in muscle respiratory capacity. However, the latter investigators dispute the primary importance of alpha-glycerophosphate dehydrogenase depletion. Whatever the underlying biochemical mechanism abnormalities appear to be limited to skeletal muscle. Myocardial function is not impaired.[109]

Behavioral and Neurological Abnormalities

Iron deficiency may affect behavior and learning in experimental animals[110] and man.[111] Iron deficient children have lower scores in tests of learning performance and attentiveness when compared with their iron replete peers.[112] Progressive deterioration in school achievement tests has been observed in iron deficient adolescent boys[113]. Moreover, treatment of iron deficiency in infancy produces a significant increase in scores obtained using the Bayley scales of infant development.[114]

The physiological basis for these abnormalities has not been elucidated but appears to be independent of the degree of anemia since improvements following iron therapy have been noted before any change in hemoglobin has occurred. Iron is normally present in the brain in relatively large amounts being concentrated in the basal ganglia. Its concentration is not directly related to body iron stores but brain iron depletion can be induced in experimental animals raised under iron deficient conditions.

Alterations in a number of putative neurotransmitters have been described. Youdim and Green[115] showed that after the administration of the monoamine oxidase inhibitor tranylcypromine followed by L-tryptophan iron deficient rats were much less responsive than controls. Symes et al.[116] found reduced brain monoamine oxidase. However this has not been confirmed by other investigators.[115,117] Mackler et al.[117] have demonstrated that iron deficient rats have reduced concentrations of another key enzyme involved in serotonin degradation in the brain-aldehyde oxidase. Nonheme iron is a catalytically necessary component of this enzyme and reduced enzyme activity was associated with elevated concentrations of serotonin and total 5-hydroxyindole compounds. All of these abnormalities returned to normal one

week after therapy with parenteral iron. Whatever the underlying biochemical basis may ultimately prove to be, it seems likely that there is a link between alterations in neurotransmitters and the behavioral abnormalities reported in children with iron deficiency.

Catecholamine Excretion and Temperature Regulation

Urinary catecholamine and adrenal tyrosine hydroxylase levels are increased in iron deficiency.[118,119] Dillman et al.[120] have shown that excessive urinary catecholamine excretion results from an increased production rate that is not independent on the presence of anemia. Moreover the difference in catecholamine response between iron deficient and control animals is related to ambient temperature being far greater at 4°C than at 30°C. Their studies suggest that it represents a compensatory mechanism for an impaired ability to maintain body temperature, the primary abnormality being decreased conversion of thyroxin to triiodothyrine.[121]

Resistance to Infection

The relationship between iron deficiency and host resistance to infection is the subject of a recent extensive review.[122] It will therefore be dealt with only briefly. Impairment of the body's defense mechanisms including abnormalities of lymphocyte function and delayed hypersensitivity have been described.[123,124] The clinical consequences of these findings

are undefined. With few exceptions, epidemiological studies have yielded contradictory results[122] although iron deficiency does appear to have a role in the pathogenesis of chronic muco-cutaneous candidiasis.[125]

On the other hand, it has been suggested that iron deficiency may have a protective effect and that therapy particularly with parenteral iron may increase the risk for and morbidity of infections. Excess iron clearly facilitates the growth of a number of microorganisms in biological fluids in vitro and the inhibitory effects of the iron binding proteins transferrin and lactoferrin have been well established. However, it is noteworthy that in one study the growth of E. coli in a series of human sera with percentage transferrin saturations ranging from less than 15% to 100% did not differ.[126] Extrapolation of in vitro observations to clinical situations is uncertain. Pearson and Robinson[122] point out that some of the apparent associations between infection and iron loading states do not stand up to careful scrutiny, e.g. it has been suggested that overwhelming sepsis and salmonellosis results from hyperferremia in patients with sickle cell anemia yet these infections are most common in the first year of life before significant iron accumulation has occurred. Patients with thalassemia major do not have increased infections despite progressive iron overload unless splenectomy

has been performed. It is also difficult to define the possible role of iron therapy. Barry and Reeve[127] reported an increase in the frequency and mortality of gram negative septicemia when a policy of giving prophylatic iron dextran injections to Polynesian infants was instituted. The incidence decreased when the practice was discontinued. Masawe et al.[128] and Murray et al.[129] described an increased frequency of infections including the reactivation of pre-existing malaria, brucellosis, and tuberculosis in iron deficient patients given oral ferrous sulphate or parenteral iron dextran. However, similar results have not been reported from other parts of the world where iron dextran has been used.[122]

CONCLUSION

Our knowledge of the pathogenesis of iron deficiency and of the potentially far-reaching effects on metabolic processes has advanced considerably in recent years. We also have sophisticated technics for detecting its presence and severity. In contrast there have been fewer changes in our approach to prevention and treatment; nevertheless this aspect of iron deficiency anemia merits a few comments.

Iron deficiency anemia is never a complete diagnosis. It is merely a symptom of disturbed iron balance. Although iron therapy may produce a gratifying immediate response, a satisfactory long term solution to the problem depends on a clear definition of the reason for reduced absorption or increased loss and specific treatment of the underlying disorder where necessary.

In many parts of the world more available iron must be provided. The wide variations in requirements during pregnancy and childhood make prophylactic supplementation or targeted fortification the most practical solution in many circumstances. However, its impact is dependent on adequate health services and patient compliance. The effectiveness, potential danger and most suitable methods of implementation of fortification for the whole population remain areas of controversy. The general improvement in iron nutrition in Sweden cannot be attributed solely to iron fortification.[130] The widespread use of oral contraceptives leading to a reduction in both the average menstrual loss and its range in different women and increased intake of iron tablets are important factors.[131] Iron fortification should be most valuable in countries where deficiency is evident in all segments of the population. However, insufficient attention to bioavailability, the use of inappro-

priate iron compounds and difficulties in identifying suitable vehicles, have been major obstacles to the success of such programs.[3] Finally, the possible danger of iron overload as a result of fortification particularly in men in countries where their diet already contains sufficient iron further complicates the problem of designing optimal fortification policies.[131] Similar concerns have been expressed about the potential deleterious effects for patients with iron loading disorders such as idiopathic hemochromatosis and thalassemia.

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Table I: Food Components that Affect Dietary Iron Bioavailability

Enhancers

Ascorbic Acid
Animal Tissue (Meat and Fish)

Inhibitors

Tea
Coffee
Eggs (Yolk and White)
Wheat Bran
Soy Products
Calcium/Phosphate Salts
EDTA*

Possible Inhibitors

Fiber
Food Phytates

*The effect of EDTA depends on the quantity present and the bioavailability of iron in the meal. It may improve absorption from some low availability meals.

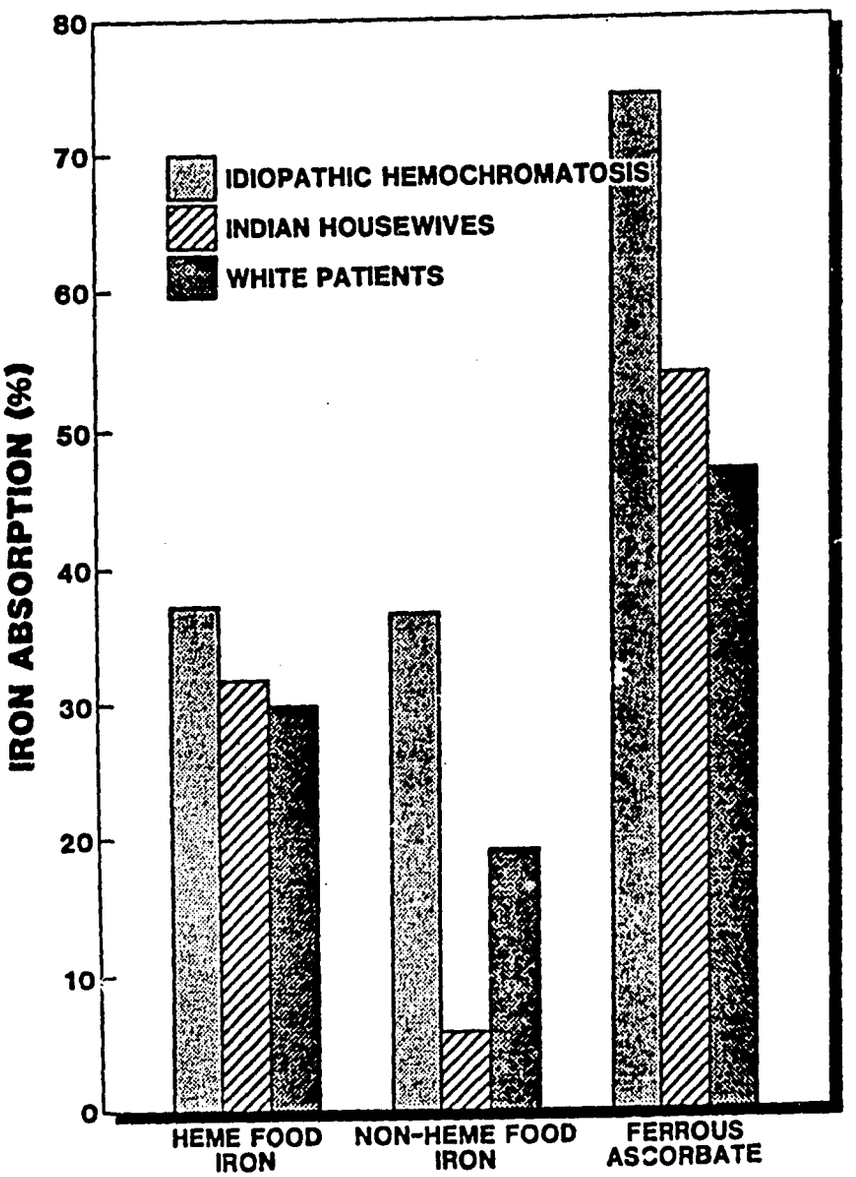
LEGENDS TO FIGURES

Fig. 1 Absorption of heme and nonheme iron from a meal containing bread and meat in patients with idiopathic hemochromatosis who had been phlebotomized extensively, iron deficient Indian housewives, and iron deficient white hospital patients. (Data from Bezwoda et al.[34])

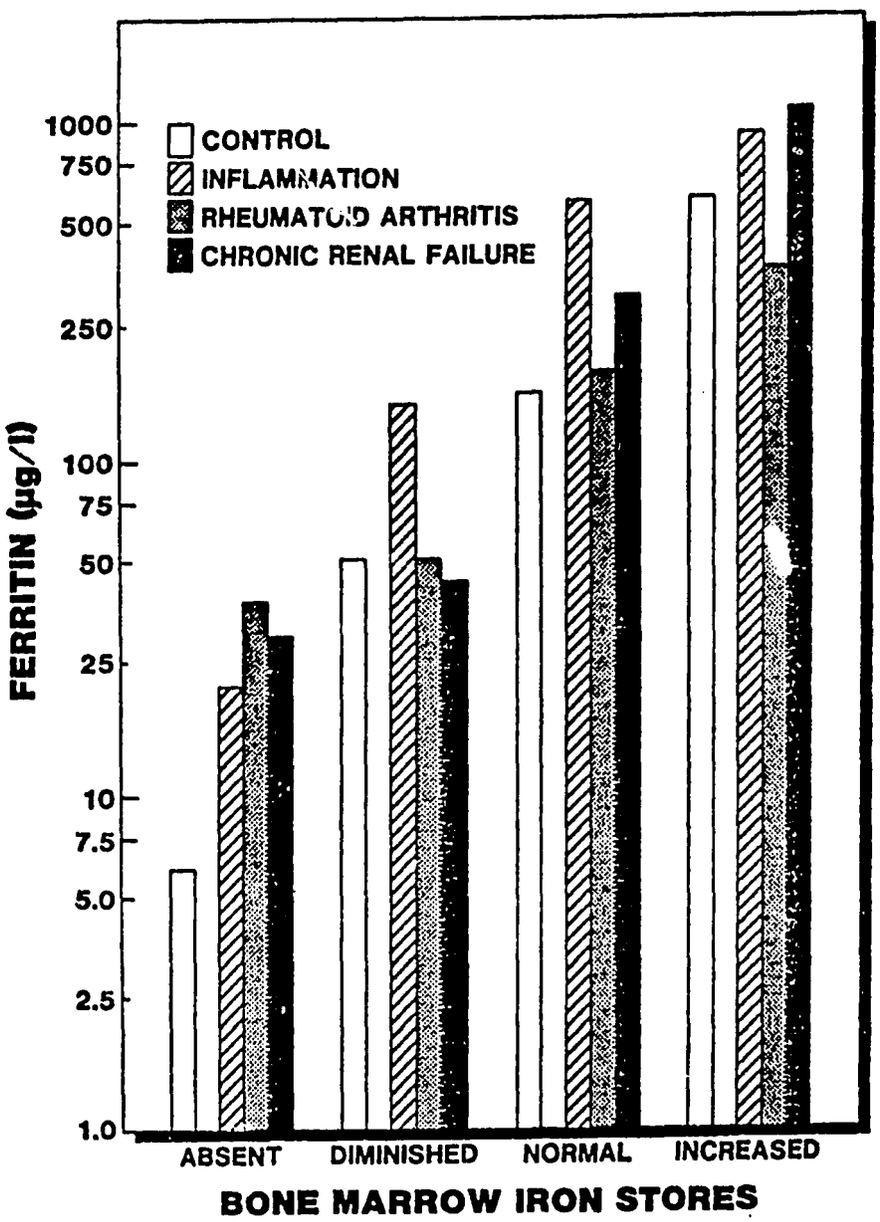
Fig. 2 The sequence of changes in laboratory tests used to evaluate iron stores caused by the gradual evolution of iron deficiency. (Modified from Bothwell et al.[3])

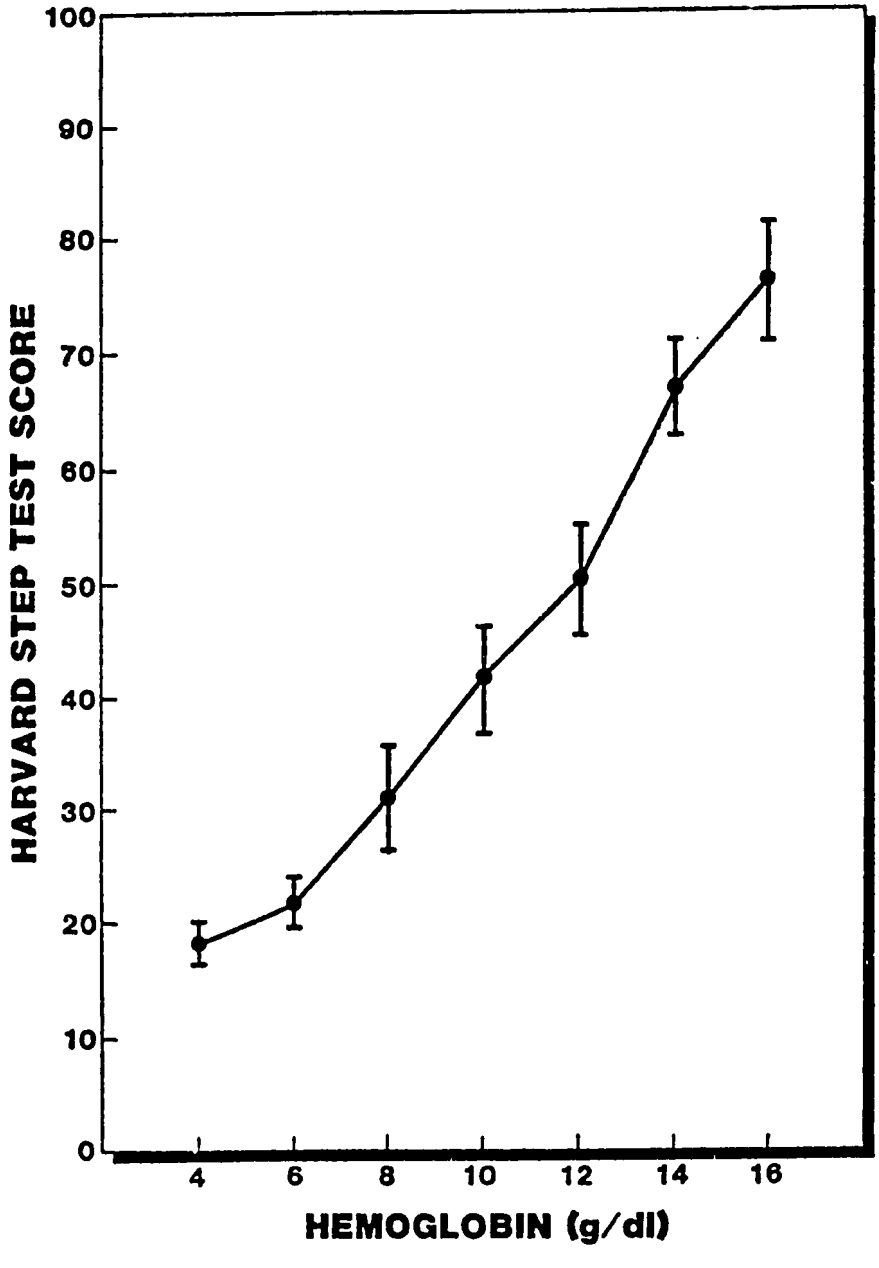
Fig. 3 Relationship between serum ferritin concentration and bone marrow iron stores in patients with inflammatory disorders, rheumatoid arthritis and chronic renal failure undergoing chronic hemodialysis. (Data from references 57-61.)

Fig. 4 The Harvard Step Test Score in Guatemalan agricultural laborers at different hemoglobin levels. (Vertical bars represent mean $\pm$ SE, adapted from Viteri and Torun.[85])


IRON ABSORPTION (%)
80
70
60
50
40
30
20
10
0
IDIOPATHIC HEMOCHROMATOSIS
INDIAN HOUSEWIVES
WHITE PATIENTS
HEME FOOD IRON
NON-HEME FOOD IRON
FERROUS ASCORBATE

	Normal	Iron Depletion	Iron Deficient Erythropoiesis	Iron Deficiency Anemia
Iron Stores →				
Erythron Iron →				
RE Marrow Fe (0–6)	2–3+	0–1+	0	0
Transferrin IBC (µg/dl)	330±30	360	390	410
Plasma Ferritin (µg/l)	100±60	20	10	<10
Plasma Iron (µg/dl)	115±50	115	<60	<40
Transferrin Saturation (%)	35±15	30	<15	<10
Sideroblasts (%)	40–60	40–60	<10	<10
RBC Protoporphyrin (µg/dl RBC)	30	30	100	200
Erythrocytes	normal	normal	normal	microcytic and hypochromic


CONTROL
INFLAMMATION
RHEUMATOID ARTHRITIS
CHRONIC RENAL FAILURE
FERRITIN (µg/l)
1000
750
500
250
100
75
50
25
10
7.5
5.0
2.5
1.0
ABSENT
DIMINISHED
NORMAL
INCREASED
BONE MARROW IRON STORES


HARVARD STEP TEST SCORE
100
90
80
70
60
50
40
30
20
10
0
4
6
8
10
12
14
16
HEMOGLOBIN (g/dl)