PD-AAP-183

Best available copy -- pages 16 - 24 missing and several other parts missing

PD-AAF-183
ISN-38091

931-0227/52

CONTROL OF IRON DEFICIENCY
DSAN-C-0045

James D. Cook, M.D., University of Kansas Medical Center, Kansas City, Kansas

Contract Period: 1 October 1978 - 30 September 1981
Report Period: 1 October 1978 - 31 August 1979

Total Contract Funding: $648,856
Total Current Year Costs: 94,358 Estimated Second Year Costs: $210,126

Narrative Summary

The prime 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 bioavailability of food, 2) iron availability from LDC diets, 3) development of iron fortification systems, 4) food technology, and 5) pilot fortification trials. With the exception of food technology, all of these areas involve measurements of iron absorption in man using radioisotopes. A total of 752 iron absorption tests have been performed in 188 volunteer subjects during the first 11 months of this three-year contract. The salient features of these studies are as follows.

About one-third of the studies have been performed to characterize factors affecting iron bioavailability of food. A study of bran demonstrated that the strong inhibitory effect of this food on iron absorption is not due to its phytate content but more probably to a high fiber content. A study of semipurified proteins identified a strong inhibitory effect of isolated soy protein on iron absorption. A similar but less dramatic inhibition was observed with egg albumin that may have been due to its iron-binding protein, conalbumin. Coffee was also shown to inhibit iron absorption, a finding that may relate to the use of sugar for iron fortification.

Efforts to develop iron fortification systems were initially focused on substances which could be added to food to improve iron assimilability. Vitamin E had surprisingly little effect on iron absorption, suggesting that the facilitating effect of ascorbic acid is due to its iron chelating activity rather than its reducing activity. Five different infant protein supplements were studied and all were found to impair iron absorption to about the same degree. Omitting calcium and phosphorus from the mineral mix added to infant supplements produced a twofold increase in iron absorption. An ascorbic acid analogue, ascorbyl palmitate, was found to facilitate iron absorption to the same extent as equimolar concentrations of ascorbic acid; the analogue has the advantage that it is not inactivated in the baking process. In a final study, vitamin A was shown to inhibit iron absorption indicating that the improvement in hematologic status that has been observed with vitamin A fortification is unrelated to the effect of the vitamin on iron absorption.

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 fully defined, 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 the effectiveness of these programs have 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 that 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 may depend on the level of dietary iron and the degree of its availability. At one extreme, diets with low-iron content but high availability would be best suited to iron fortification. In contrast, a diet with high content but low assimilation

would be more suitable for enhancement fortification. The best strategy may be to employ both of 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 influence 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 added form of 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 in 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 efficacy of the intervention strategy.

These basic objectives as outlined in the original contract remain unaltered. Isotopic measurements of food iron absorption have been and

will continue to be the major activity in this contract. Note in relation to item (4) above, support for food technology studies was deleted from the final contract. Also, in relation to item (2) above, a large study of dietary iron availability from different geographic regions in the world was published shortly after the start of this contract and lessens the need for this information. Moreover, it is cumbersome to study diets from different LDC until an effective collaboration has been established with nutritionists in the region. Thus, efforts under activity (2) above, will be limited to those countries where there is a strong potential for developing an effective system of fortification (item (3) above), and performing pilot fortification trials to measure its efficacy ((~~4~~ 5) above).

C. Continued Relevance of Objectives

The original objectives of this contract are still relevant and there is no need to modify these objectives at the present time with the exception of a more circumscribed evaluation of iron availability in LDC diets.

D. Accomplishments to Date

A detailed review of the accomplishments in this contract to date has been appended. That section of the report includes a detailed presentation and discussion of ten separate iron absorption studies which are summarized on the following page.

No.	Page	No. Subjects	Title
1	7	15	The Effect of Tocopherol on Absorption of Nonheme Dietary Iron
2	16	13	The Effect of Calcium and Phosphate on Iron Absorption from Corn-Soya-Milk
3	25	10	The Effect of Tocopherol on Absorption of Inorganic Iron
4	32	15	A Comparison of the Effect of Purified Protein Sources on Iron Absorption
5	41	13	The Effect of Ascorbyl Palmitate on Food Iron Absorption
6	49	13	Iron Bioavailability of Infant Food Supplements. I.
7	59	14	Iron Bioavailability of Infant Food Supplements. II.
8	67	12	Iron Absorption from Bread Baked with Ascorbyl Palmitate
9	75	11	The Effect of Coffee and Tea on Iron Absorption
10	82	12	The Effect of Vitamin A on Iron Absorption

About one-third of the total effort during the first year has been to identify dietary factors affecting iron bioavailability. Study 4 involved a comparison of the effect on iron absorption of different semipurified proteins. Egg albumin, heated egg albumin, isolated soy protein, and casein were evaluated by substituting protein equivalent amounts in a semisynthetic meal (SS). An increase in iron absorption was observed when egg albumin was heated and this effect may have been due to destruction by heat of the iron-binding protein, conablumin. A more important finding was a striking inhibition in iron absorption by isolated soy protein and additional studies are presently underway to further characterize this inhibitory effect.

In Study 9, the effect of coffee on iron absorption was evaluated because of recent data indicating that tea severely inhibits iron absorption. One cup of coffee was found to have approximately one-half the inhibiting effect on iron absorption as a similar volume of tea. This finding may be relevant to the use of sugar as a vehicle for iron fortification.

Study 10 was performed to examine the possible effect of vitamin A on food iron absorption. Recent studies have shown an improvement in hematologic status following vitamin A fortification. Such an effect might be explained by a direct stimulation of iron absorption by vitamin A. However, no such effect could be demonstrated in this study.

Four additional studies relating to factors affecting food iron availability were performed but have not been included in the appendix. A preprint of these studies will be forwarded within the next few weeks. Briefly summarized, the studies were designed to investigate the role of wheat bran on iron absorption. It was observed that when whole wheat bran was added to a meal, iron absorption was sharply reduced even in the presence of enhancing agents such as meat and ascorbic acid. Monoferric phytate, the predominant iron containing compound in wheat bran, was absorbed to the same extent as other dietary iron. Moreover, dephytinized bran produced the same degree of inhibition as whole bran indicating that phytate content is not the factor responsible for inhibition. In a final study, bran was separated into an insoluble fiber-rich component and a soluble phosphate-rich component in an attempt to localize the inhibiting effect. Both these components appeared to inhibit iron absorption to a similar extent.

The remaining iron absorption studies were directed at the development of effective systems of iron or enhancement fortification. Because iron

deficiency is very prevalent in infants and pregnant women, attention was initially focused on infant protein supplements which can serve as an effective vehicle for iron fortification. Study 2 was designed to determine whether high levels of calcium and phosphate that are added to CSM may influence the absorption of the ferrous fumarate it contains. Two levels of calcium and phosphate were compared with absorption from CSM containing no mineral supplement. No difference in iron availability was observed between moderate and high levels of calcium and phosphate but absorption increased more than twofold when these minerals were omitted.

Iron absorption from four other infant protein supplements was evaluated in studies 6 and 7. None of these supplements differed greatly in their effect on iron absorption but all were relatively inhibitory. It is interesting, in this regard, that all of these supplements contain soy protein which was shown in study 4 to markedly inhibit iron absorption. Infant supplements appear to be a poor vehicle for iron fortification but might be improved by deleting soy, omitting the calcium and phosphate, or perhaps by adding an enhancing substance such as ascorbic acid.

Additional studies were performed to identify compounds which might facilitate the absorption of dietary iron. The prototype is ascorbic acid but its enhancing activity is largely inactivated by heat during preparation of the food. In studies 1 and 3, vitamin E was tested as an alternative to ascorbic acid because it is more heat resistant. However, a surprisingly low level of enhancement was observed with vitamin E which suggests that the major affect of ascorbic acid is iron chelation rather than its reducing properties.

Other alternatives to ascorbic acid are analogues of the vitamin C. In one such compound, ascorbyl palmitate, was evaluated in study 5. When added to the SS meal, the enhancing effect of ascorbyl palmitate was

similar to that of ascorbic acid. In study 8, it was shown that ascorbyl palmitate retained its enhancing activity when added to flour prior to baking. These studies will hopefully serve as a stimulus to the development of more effective and less expensive vitamin C analogues.

In regard to research design, no major modifications are planned. It has proven cumbersome to study iron bioavailability from a wide range of LDC diets and efforts will be directed to a smaller number of LDC where a working collaboration has been established. Such collaboration is greatly facilitated by an interchange of investigators and for this reason, the Philippines has the highest priority for studies of LDC diet bioavailability at the present time. Ms. Anita Marzan from the Philippines is currently completing a 2-month training program at Kansas University supported by funds from the International Atomic Energy Agency (IAEA). Another nutritionist, Ms. Juanita Regacho, will begin a 6-month fellowship at Kansas University in November of this year with support provided by WHO.

E. Dissemination and Utilization of Research Results

This aspect of the contract will assume increasing importance as we move into the second year of the program. The following vehicles will serve as the major pathways of communication.

1. International Nutritional Anemia Consultative Group (INACG). This group, formed through support by USAID, is comprised of investigators in iron nutrition, representatives of LDC, and various agencies with major interest in the problem of nutritional anemia. The annual meeting of INACG will be held shortly in Cairo, Egypt (October 23-25, 1979) and the results of studies performed under this contract will be reviewed in detail at that session.

2. Scientific Publications. Publication in high quality journals will serve as the major avenue of communication. From the studies performed

to date and summarized in the appendix, the following publications are in preparation:

(a) 'Absorption of a Sustained-Release Form of Oral Iron' (preprint enclosed).

(b) 'The Inhibitory Effect of Bran on Iron Absorption in Man' (preprint will be forwarded).

(c) The Effect of Tocopherol on Iron Absorption.

(d) Infant Protein Supplements as a Vehicle for Iron Fortification.

(e) The Use of Ascorbyl Palmitate for Enhancement Fortification.

(f) The Effect of Vitamin A on Iron Absorption.

Articles (c) through (f) are in preparation.

3. Scientific Interchange. An effective means of communicating the results of ongoing studies has been through visits of nutritionists and hematologists to the iron research laboratory at Kansas University. The following individuals have visited the laboratory since the contract was initiated in October, 1978:

(a) Henryk Kirschner, M.D. and Janina Woroszylska, Ph.D., Institute of Social Medicine, Warsaw, Poland, Oct. 1, 1978.

(b) K. Siguard Olsson, M.D., Ostersund Hospital, Ostersund, Sweden, October 9, 1978.

(c) Keizo Shiraki, M.D., Professor of Physiology, Japan School of Medicine, Kitakyushu, Japan, October 18-19, 1978.

(d) Fernando Viteri, M.D., Chief, Human Nutrition and Biology Division, INCAP, Guatemala City, Guatemala, October 28-29, 1978.

(e) Luis Mejia, Ph.D., Associate Scientific Investigator, INCAP, Guatemala, Central America, November 13-17, 1978.

(f) O. A. Ajayi, M.D., Professor of Nutrition, University of Ibadan, Ibadan, Nigeria, December 6-12, 1978.

(g) Henryk Rafalski, M.D., Sc.D., Department of Human Nutrition, Medical Academy, Lodz, Poland, February 27-March 1, 1979.

(h) Uma Shankar Rai, M.D., Associate Professor of Pediatrics, Texas Tech University Health Sciences Center, Lubbock, Texas, April 8-10, 1979.

(i) William Simmons, Ph.D., Caribbean Food & Nutrition Institute, Kingston, Jamaica, September 9-15, 1979.

(j) Benjamin L. de la Cruz, M.D., Biomedical Research Division, Philippine Atomic Energy Commission, Quezon City, Philippines, September 15-30, 1979.

(k) Pensri Pootrakul, M.D., Associate Professor of Hematology, Siriraj Hospital, Bangkok, Thailand, September 1-October 30, 1979 (WHO Fellowship).

(l) Anita Marzan, Nuclear Research Associate, Philippine Atomic Energy Commission, Quezon City, Philippines (IAEA Fellowship), September 1-October 30, 1979.

F. Statement of Expenditures and Obligations

The expenditures and obligations for the first 11 months of this contract are as follows:

	Amount Awarded*	Amount Used**	% Used
Personnel	83,780	54,173	62
Foreign Collaboration			
Foreign Travel	13,000	1,414	11
Shipping	8,000	0	-
Equipment	20,000	18,202	91
Supplies	16,000	8,662	54
Other			
Volunteer Subjects	22,500	11,660	52
Maintenance Contracts	4,100	646	16
Publications	500	342	68
Indirect Costs	50,268	31,304	62
TOTAL	$218,148	$124,403	57%

* Amount awarded for the first 12 months of the contract.
** Amount used in the first 11 months of the contract (10/01/78-8/31/79).

As discussed previously, approximately one-third of the total effort was invested in factors affecting iron bioavailability and the remaining two-thirds in the development of fortification systems. These proportions can be applied directly to the total figure of $124,403 to determine the amount actually expended in these two categories of work activity. Thus, $41,000 was expanded for factors affecting iron bioavailability as compared to a projected expenditure of $64,000. Similarly, $83,000 was expended for the development of fortification systems as compared with a proposed expenditure of $110,000. No funds were expended for LDC diet iron bioavailability during the first year nor in pilot fortification trials.

The expenditures during the first 11 months of this contract totalled $124,403 as compared with the budgeted amount of $218,148. There are several reasons for this disparity. First, the listed expenditures are for 11 rather than 12 months. Second, recruitment and training of personnel was delayed because of the short interval between notification of the contract and the starting date. Thus, the personnel costs represent only about 9 months rather than 12 months of activity. Third, minimal funds were used in the category of foreign collaboration because some travel to LDCs was supported by other funds (WHO and UNICEF), and studies of LDC diet iron bioavailability were not undertaken in this initial year of the contract. Four, recruitment of volunteer subjects has been more difficult than had been anticipated. It was assumed that both male and female subjects could participate in these studies but final approval by AID stipulated that only male volunteer subjects could be used. This greatly reduces the number of potential volunteers; women have been more willing to participate because they are aware of the high prevalence of

iron deficiency in women. Moreover, the publicity given to the nuclear accident at Three-Mile Island raised many questions concerning hazards of participating in radioisotopic studies and made recruiting particularly difficult in the spring of this year.

Recruitment of volunteers continues to place some constraints on the rate at which this program can proceed. However, with the start of the fall semester, recruiting is progressing adequately. If this continues, expenditures during the second year will be much closer to the amount originally awarded.

G. Work Plan and Budget Forecast for the Coming Year

The work plan on this contract for the coming year will not deviate significantly from the scheme finally approved in the original contract application. The following specific studies are planned in the area of activity outlined in the original report.

Studies of factors affecting iron bioavailability will continue at about the same level of activity that was proposed in the original contract. About one-third of the total effort during the second year will be invested in this area--approximately 6-7 studies or a total of 80-90 subjects. These studies will attempt to characterize the nature of the inhibitory effect of isolated soy protein by studying the effect of iron absorption of various fractions obtained during its commercial preparation. Hopefully, some alteration in the method for preparing isolated soy protein may eliminate its inhibitory effect on iron absorption. Other semipurified proteins will also be evaluated for their effect on iron assimilation and additional studies of iron-binding protein such as lactoferrin and conalbumin will be performed. The effect of dietary fiber on iron absorption will be measured. Fiber is believed to be a major inhibitory substance in the diet. One study will be to compare meals closely matched in regard to the total content of fat, protein,

and carbohydrate but differing maximally in their content of naturally-occurring fiber. Purified fiber components may be evaluated by adding them to a normal diet. Additional studies of coffee may be performed to determine the effect of the method of preparation, dose response, etc.

Studies of LDC diet iron bioavailability are planned for the coming year but for the reasons stated previously, may not reach the scope originally proposed. These studies must await development of effective collaboration with various LDCs. Such a collaboration has been established in the Philippines and it is planned to undertake a series of studies at Kansas University within the next few weeks to characterize iron absorption from a Philippine diet. It is hoped that in early 1980, the same type of meals could be administered in the Philippines with food items purchased locally rather than in the U.S. This study will establish the validity of assessing iron bioavailability in LDC diets at Kansas University using foods purchased in the U.S.A.

During the second year, at least one additional LDC will be selected for studies of LDC diet iron bioavailability. As in the collaboration with the Philippines, it will be important to have investigators from the LDC spend a period of time in the Kansas laboratory. Hopefully, other agencies such as WHO or UNICEF will support this training. Other countries that will be considered are Egypt, Guyana, Caribbean Islands, Columbia and other South American countries.

The major emphasis in the coming year will be the development of fortification systems. Whereas enhancement fortification was the prime target during the first year, efforts in the second year will be directed to methods of iron delivery. There is increasing interest in using NaFeEDTA in fortification programs because absorption of this iron may be higher than the common pool of dietary nonheme iron. However, additional studies of absorption from NaFeEDTA are needed. For example, because of the high stability of this iron complex,

its absorption may not be facilitated by the presence of dietary ascorbic acid or animal tissue. If this proves to be the case, the potential advantage of using this compound may be negated.

Depending on the availability of technology studies, efforts during the second year will be focused on two iron delivery systems. The first is a suitable system for coating rice with iron. Preliminary studies have shown that when 50 mg iron as either $FeSo_4$ or NaFeEDTA are mixed with 1 pound rice, the presence of iron cannot be seen or tasted. However, equipment commonly employed for rice fortification mixes in a ratio of one part coated:99 parts uncoated rice. This will require that the coated rice contain about 5 gm iron/pound rice. If pilot studies show this to be feasible with either ferrous sulfate or NaFeEDTA, iron absorption studies will be performed in man to establish iron availability.

Another delivery system that will be examined during the second year is coated ferrous sulfate. The problem here has been to obtain radio-labelled material for studies of iron absorption in man since industrial manufacturers have been understandably reluctant to contaminate their equipment with radioisotopes. However, a small-scale laboratory method has been developed at Hoffman-La Roche that could be used to prepare radioactive material and we hope to conduct these studies during the second year. When lipid coated ferrous sulfate is added to monosodium glutamate (MSG) in a concentration of 50 mg iron/gm MSG, the iron cannot be detected. Coated ferrous sulfate could be used not only for MSG but possibly other food vehicles such as sugar or salt. Poor grades of salt and sugar are available in LDC which result in major problems when used for fortification because of high moisture content and impurities. Coated ferrous sulfate may prevent such reactions and yet be dissolved

in gastric secretions with sufficient speed that iron absorption is adequate. It will be important to establish this fact by radioisotopic studies.

Another area relating to iron fortification is the development of an *in vitro* system for measuring bioavailability of commercial iron sources. A study is presently being designed that will assess the bioavailability of three commerical iron sources (ferrous sulfate, ferric orthophosphate, and reduced iron). The availability of this iron will be measured simultaneously by *in vitro* systems currently used by industry to measure iron availability, by measurement in rats using the hemoglobin repletion model, and finally by radioisotopic studies in man. It is hoped that these studies will be conducted as a collaborative effort with individuals from FDA, USDA, and AID.

A final phase of this contract that was deleted from the original proposal is studies of *food technology*. Following a preliminary meeting in June of this year, members of CODOT are currently drafting proposed studies that could be submitted to AID for funding. Preliminary discussions will be made with USAID to solicit their assistance. Some support has been obtained from industry but goodwill efforts are unlikely to provide the level of committed effort that this project requires. Support in this area will be of critical importance to progress in this contract.

APPENDIX I

AID Contract Progress Report

GENERAL METHODOLOGY

The following is an interim report of investigations of food iron availability performed under USAID Contract No. DSAN-C-0045 from December 1, 1978 to September 1, 1979. The results are presented as a series of ten consecutively-numbered absorption studies performed in groups of 10-15 subjects each. The following aspects of methodology and data analysis are common to all of these studies.

Experimental Subjects. Each study was performed in normal male volunteer subjects between the ages of 18 and 45 years. Fully informed consent was obtained from the subjects, all of whom were remunerated for their participation in the studies. The majority of subjects were enrolled as medical or dental students at the University of Missouri, Kansas City. None of the subjects gave a history of hematological abnormalities or diseases known to influence the gastrointestinal absorption of iron. The majority of volunteers had normal iron status as gauged by serum ferritin levels; an occasional subject had mild iron deficiency due to either participation in other experimental studies or frequent blood donations.

Test Meals. Except for absorption studies from a reference dose of inorganic iron which is described below, the test meals contained either a mixture of food items or a combination of semi-purified dietary ingredients. The methodology used to measure the absorption of nonheme dietary iron was based on the prinicple of extrinsic radioiron tagging. With this technique it is possible to measure absorption from the common pool of nonheme iron that is formed when several food items are ingested in the same meal. Extrinsic tagging is performed by adding 0.1 mg

elemental iron as labelled $FeCl_3$ to the meal. This radioiron label is either mixed with the entire meal when studying liquid diets or is added to one of the liquid items in a conventional meal.

Composition of the test meal differed in each study depending on the nature of the experiment; specific meals are described under each study. Many studies were performed with one of two standardized meals that have been used extensively in published studies from this laboratory. The first meal was designed to represent a typical American meal. Originally this meal consisted of beef, corn, potatoes, bread, margarine, peaches,and ice milk; it contained a total of 696 kcalories, 29.4 gm protein, 67.6 gm carbohydrate, 34.9 gm fat, 202 gm calcium, 414 mg phosphorus, and 4.1 mg iron. To facilitate the preparation of large numbers of test meals for the AID studies, a similar type of meal was purchased on the morning of each study from a local McDonald's Restaurant. The meal consisted of a quarter-pound hamburger, regular size order of french fries, and one-half of a vanilla milkshake. The french fries and hamburger were reheated in a microwave oven immediately prior to administration. The milkshake served as a convenient vehicle for introduction of the extrinsic tag or other test materials. The composition of this standard meal (STD) as determined by analysis at WARF Institute is as follows: 791 kcal, 33.7 gm protein, 85 gm carbohydate, 35.3 gm fat, 662 mg calcium, 361 mg phosphorus, and 5.7 mg iron. In a preliminary study performed in 10 male volunteer subjects, absorption from the original standard meal and the McDonald's meal averaged 4.0 and 3.6%, respectively, a difference that was not statistically significant.

A second test meal that has been used extensively in published studies of food iron absorption from this laboratory was designed to match identically the biochemical composition of the original standard meal. The meal consists of semi-purified ingredients, the amount of which can be altered in each experiment so as to standardize total content of the major dietary ingredients in each test meal. This semi-synthetic meal (SS) contains 37.4 gm egg albumin as protein, 66.8 gm dextrimaltose as carbohydrate, 34.8 gm corn oil as fat, and sufficient dibasic calcium phosphate, dibasic potassium phosphate, and ferric chloride to bring the calcium, phosphorus, and iron content to 262, 361, and 4.1 mg, respectively. The composition of the SS meal was not revised to match the biochemical composition of the STD meal obtained from McDonald's.

A final test dose that was used in many studies consisted of a solution of inorganic iron and is referred to as reference absorption (REF). This test dose contained 3 mg iron as ferrous sulfate and 2 moles freshly prepared ascorbic acid/mole of iron in 50 ml iron-free water. The purpose of the REF test is to provide a measure of the intestinal avidity for iron in each subject and thereby permit standardization of absorption measurements performed in groups of subjects that differ in iron status. By expressing absorption from a given test meal as a ratio of REF absorption, the effect of differences in iron status between subjects or groups of subjects is eliminated.

Iron Absorption Measurements. In all studies four iron absorption tests were performed in each subject as two sets of parallel double isotope measurements. The day before the first meal the subjects reported

to the laboratory, were told the nature of the study, and gave their informed consent. Age, height, and weight of each subject was recorded, and 30 ml whole blood was obtained for measurements of hematocrit, serum iron, total iron-binding capacity, serum ferritin, and background blood radioactivity.

On the following day, the first test meal tagged with either ^{59}Fe or ^{55}Fe was administered to fasting subjects between 8 and 10 a.m.; nothing further was allowed by mouth for 4 hours. A second meal tagged with the alternate form of radioiron was fed under identical conditions the following day. Fourteen days later the subjects returned to the laboratory and 10 ml whole blood was obtained for duplicate measurements of ^{55}Fe and ^{59}Fe. A second pair of absorption tests was again performed with ^{59}Fe and ^{55}Fe on two successive days, and a final blood sample was obtained 14 days later to measure the rise in ^{55}Fe and ^{59}Fe blood radioactivity. The total dose of radioactivity administered to each subject was 4 uCi ^{59}Fe and 10 uCi ^{55}Fe.

Simultaneous measurement of ^{55}Fe and ^{59}Fe activity in 10 ml blood (day 0 x 1, day 14 x 2, day 28 x 2) was performed by liquid scintillation counting using a modification method of Eakins and Brown. Standards representative of each test dose were processed along with the blood samples. Percentage absorption was calculated from whole blood radioactivity and total blood volume, the latter estimated on the basis of height and weight. In these iron replete volunteers it was assumed that 80% of the absorbed iron was incorporated into circulating red cells 14 days after administering each test meal.

Statistical Analysis and Data Presentation. Iron absorption measurements expressed as a percent of the administered dose are highly skewed and the degree of skewing is particularly marked at low levels of absorption which are frequently encountered in studies of food iron availability. Previous studies of this problem have established that log transformation of percentage absorption will produce a Gaussian distribution. Statistical analysis was performed on logarithms of percentage absorption and the results transformed as antilogs to recover the original units of percent absorption.

When testing the significance of differences in mean absorption between any two test meals in a given study, analysis was performed on logarithms. Student's t was calculated to determine whether the mean difference in log absorption between any pair of meals was significantly different than zero; this is equivalent to testing in original units for a ratio different than unity.

In the main table prepared for each study, percent absorption from each meal is listed individually in each subject and the most important ratios are similarly tabulated. Also included for each study is a table which lists mean absorption ±1SE for all possible meal combinations. The reciprocal mean ratio (1/ratio) is also listed for each meal but not the associated ±1SE which can be calculated as reciprocals of ±1SE for the original ratios. Values for Student't t and the associated P values are also listed for each meal. In addition, correlation coefficients for absorption from each pair of meals (performed on logarithms) are listed together with the associated P value. These correlation coefficients reflect the precision of each paired comparison. Lower correlation

coefficients are usually obtained when comparing absorption from test meals administered 14 days apart rather than 24 hours apart--that is, when comparing absorption from either Meal A or B with either Meal C or D. Note that the meal designations (A,B,C,D) do not necessarily indicate the order in which the meals were actually administered; this order was sometimes altered in the tables to emphasize the most important comparisons in each study. Graphs are also included for each study showing percentage absorption and the important absorption ratios, both plotted on a logarithmic scale.

Study 1

THE EFFECT OF TOCOPHEROL ON ABSORPTION OF NONHEME DIETARY IRON

SUMMARY

This study was performed to determine whether tocopherol can enhance absorption of dietary nonheme iron in man. When 1 gm tocopherol was added to a test meal of low iron availability, mean absorption in 15 male volunteers increased from 1.59 to 2.28%; this 43% increase in absorption was statistically significant at the 0.2% level. However, when the same amount of tocopherol was added to a meal of high iron availability (containing meat), iron absorption decreased from 6.98 to 6.02%, a decrease that was not statistically significant. The anti-oxidant properties of vitamin E have a minimal effect on the assimilation of food iron.

RATIONALE

In considering manipulations of the diet that may reduce the prevalence of iron deficiency anemia, the accepted approach is to add more iron to the diet (iron fortification). An alternative approach is to add a substance to the diet which enhances the absorption of naturally-occurring food iron (enhancement fortification). Because of the marked increase in iron absorption that occurs when ascorbic acid is added to the diet, it is the only enhancing agent which has been given serious consideration. However, a major disadvantage of ascorbic acid is that it is not heat-stable and much of its reducing activity is lost during preparation of the meal.

The present study concerns the effect of tocopherol on absorption of dietary iron. The study was prompted by preliminary observations from Hoffman-La Roche showing that vitamin E added to flour is little altered by the baking process. Although there was a slight loss of vitamin E activity in bread baked with high levels of potassium bromate, minimal inactivation was seen when various flours were enriched with 15 mg/lb of dl-α-tocopherol (33% vitamin E spray-dried powder). Because the stability and retention of tocopherol is far superior to anything that could be accomplished when ascorbic acid is added to flour, absorption studies were performed to measure the effect of vitamin E on absorption of dietary nonheme iron.

DESIGN

Four separate iron absorption tests were performed in a total of 15 normal male volunteers. All meals consisted of the STD meal,as described under Methodology (hamburger, french fries, and milkshake),or some variation thereof. Meals C and D consisted of the STD meal, whereas meals A and B were the STD meal in which the meat pattie was removed to reduce

percentage absorption. To maintain a constant level of iron in all meals, ferric chloride was added to meals A and B to replace the nonheme iron content of the excluded meat pattie. Meals A and C were administered without tocopherol while meals B and D contained 3 gm spray dried α-tocopherol which provided the equivalent of 1 gm vitamin E; the vitamin was added as a 3 ml aqueous suspension.

Two different methods of adding the tocopherol and radioiron to the test meal were used. In the first method, designated in the table as "separate addition", the tocopherol and radiorion (0.1 mg iron as $FeCl_3$) were added separately to the milkshake, the tocopherol first and then the radioiron. In the second method, designated in the table as "premix", the radioiron was added to the tocopherol suspension and this mixture was then allowed to stand at room temperature for 15 minutes before it was added to the milkshake. The latter method was used to examine the possibility that with "separate addition," tocopherol dissolves in the lipid fraction of the milkshake, thereby preventing any interaction with radioiron added subsequently.

RESULTS

Results are listed for each subject individually in the accompanying table. Serum ferritin levels indicate that all subjects had normal iron status with the exception of subject 7 who had a ferritin value of 2ng/ml. Absorption in this iron deficient subject was much higher than in the other subjects. Mean values in the first table are listed individually for "separate addition" and "premix" but the statistical analysis listed in the second table is based on the composite group of 15 subjects.

In the study in which radioiron and tocopherol were added separately, mean absorption from the STD meal containing no meat increased from 2.84 to 5.3% when 1 gm tocopherol was added. The mean absorption ratio of 1.24 indicated a relatively slight effect of tocopherol on iron absorption. A larger effect was observed when radioiron and tocopherol were combined before adding them to the milkshake; mean absorption increased from .96 to 1.55%, representing an increase of 62%. However, the mean ratios of 1.24 and 1.62 observed with separate and premix conditions, respectively, were not statistically significant and results of both studies were therefore pooled. In the composite group of 15 subjects, tocopherol increased absorption from 1.59 to 2.28% and this 43% increase was statistically significant at the 0.2% level.

When meat was included with the STD meal (meal C) mean absorption increased to 3.91%. No effect was seen when tocopherol was added to this meal of high iron availability (meal D) and, in fact, absorption actually decreased slightly to 3.57%. The mean ratio of 0.91 for meal D/C was not significantly different from unity ($t = .99$, $P > .20$).

COMMENTS

The quantity of tocopherol added to these studies was deliberately chosen in large excess of the amount that would be economically feasible in a program of enhancement fortification because it was anticipated that the enhancing effect of vitamin E on iron absorption may be minimal. Although statistically significant, the relatively small increase of 43% observed when tocopherol was added to a meatless meal would have little impact on the iron status of a population. The enhancing effect of 1 gm tocopherol could be obtained by adding only 25 mg ascorbic acid.

Furthermore, the effect of tocopherol disappeared when it was added to a meal of high iron availability whereas the facilitation of iron absorption by vitamin C persists at higher levels of iron absorption.

The results of the present study may have some bearing on the mechanism by which ascorbic acid enhances food iron absorption. It was originally believed that vitamin C enhances iron absorption by virtue of its reducing activity. However, it has been shown that ascorbic acid forms a chelate with dietary iron in the stomach at low pH which remains soluble at the higher pH of the small intestine. The results of the present study would suggest that the chelating mechanism of ascorbic acid is much more important than its reducing property which was presumably mimicked by the anti-oxidant effect of vitamin E in this study.

STUDY 1. The effect of Vitamin E on iron absorption.

Subject	Age	Packed Cell Volume	Serum Iron	Tsf Sat	Serum Ferritin	IRON ABSORPTION Without Meat -Vit E(a)	Without Meat +Vit E(b)	With Meat -Vit E(c)	With Meat +Vit E(d)	Absorption Ratios b/a	d/c	c/a	d/b
		(%)	(ug/dl)	(%)	(ng/ml)	(% of dose)		(% of dose)					
Separate Addition													
1	18	39	98	21	23	1.00	0.65	2.87	5.43	0.65	1.89	2.87	8.35
2	23	44	118	39	86	1.62	2.02	2.71	1.36	1.24	0.50	1.67	0.67
3	31	44	80	24	51	1.73	3.85	13.20	7.36	2.22	0.55	7.63	1.91
4	25	49	134	31	134	1.81	2.53	4.01	2.71	1.39	0.67	2.21	1.07
5	35	45	157	34	42	2.22	2.35	1.51	1.31	1.05	0.86	0.68	0.55
6	18	46	115	26	24	7.41	6.60	27.70	38.02	0.89	1.37	3.73	5.76
7	26	39	69	15	2	18.27	34.97	47.30	39.33	1.91	0.83	2.58	1.12
MEAN	25	44	110	27	31*	2.84*	3.53*	6.98*	6.02*	1.24*	0.86*	2.45*	1.70*
Premix													
8	22	43	83	21	71	0.51	0.68	1.31	1.57	1.33	1.19	2.56	2.30
9	30	42	129	38	137	0.62	1.32	0.66	0.58	2.12	0.87	1.06	0.43
10	29	43	140	34	79	0.65	1.56	1.73	2.22	2.40	1.28	2.66	1.42
11	25	49	127	36	267	0.80	1.42	4.60	2.06	1.77	0.44	5.75	1.45
12	18	46	120	28	106	0.85	1.38	1.05	1.10	1.62	1.04	1.23	0.79
13	18	44	140	34	37	1.42	2.86	4.27	4.20	2.01	0.78	3.00	1.46
14	19	48	90	19	56	1.88	1.70	9.96	8.86	0.90	0.88	5.29	5.21
15	44	47	100	22	124	1.98	2.58	3.11	4.05	1.30	1.30	1.57	1.56
MEAN	26	45	116	29	93*	0.96*	1.55*	2.35*	2.26*	1.62*	0.96*	2.45*	1.45*
COMPOSITE MEAN	25	44	113	28	56*	1.59*	2.28*	3.91*	3.57*	1.43*	0.91*	2.45*	1.56*

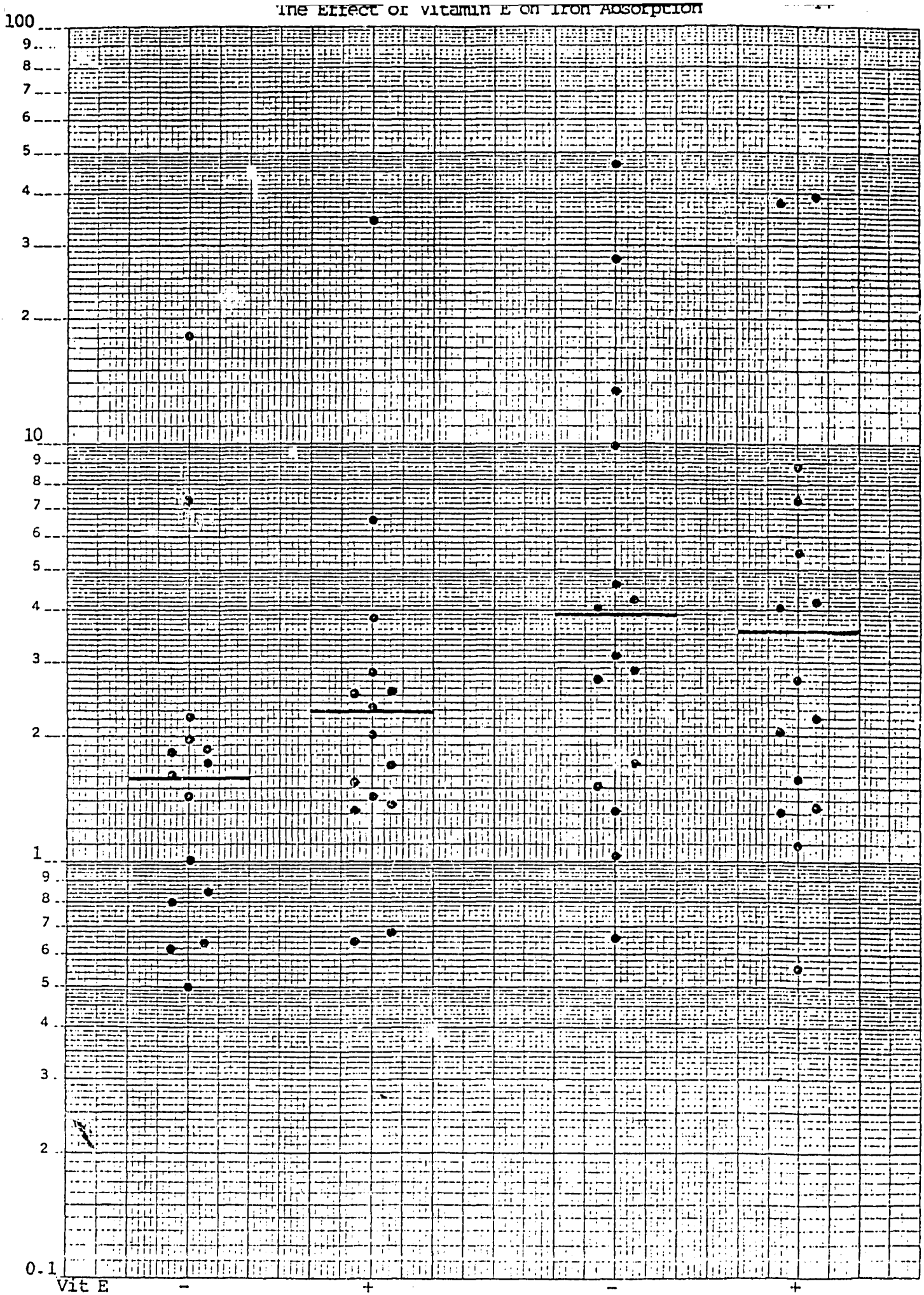
* geometric mean

Study 1. The effect of vitamin E on iron absorption.

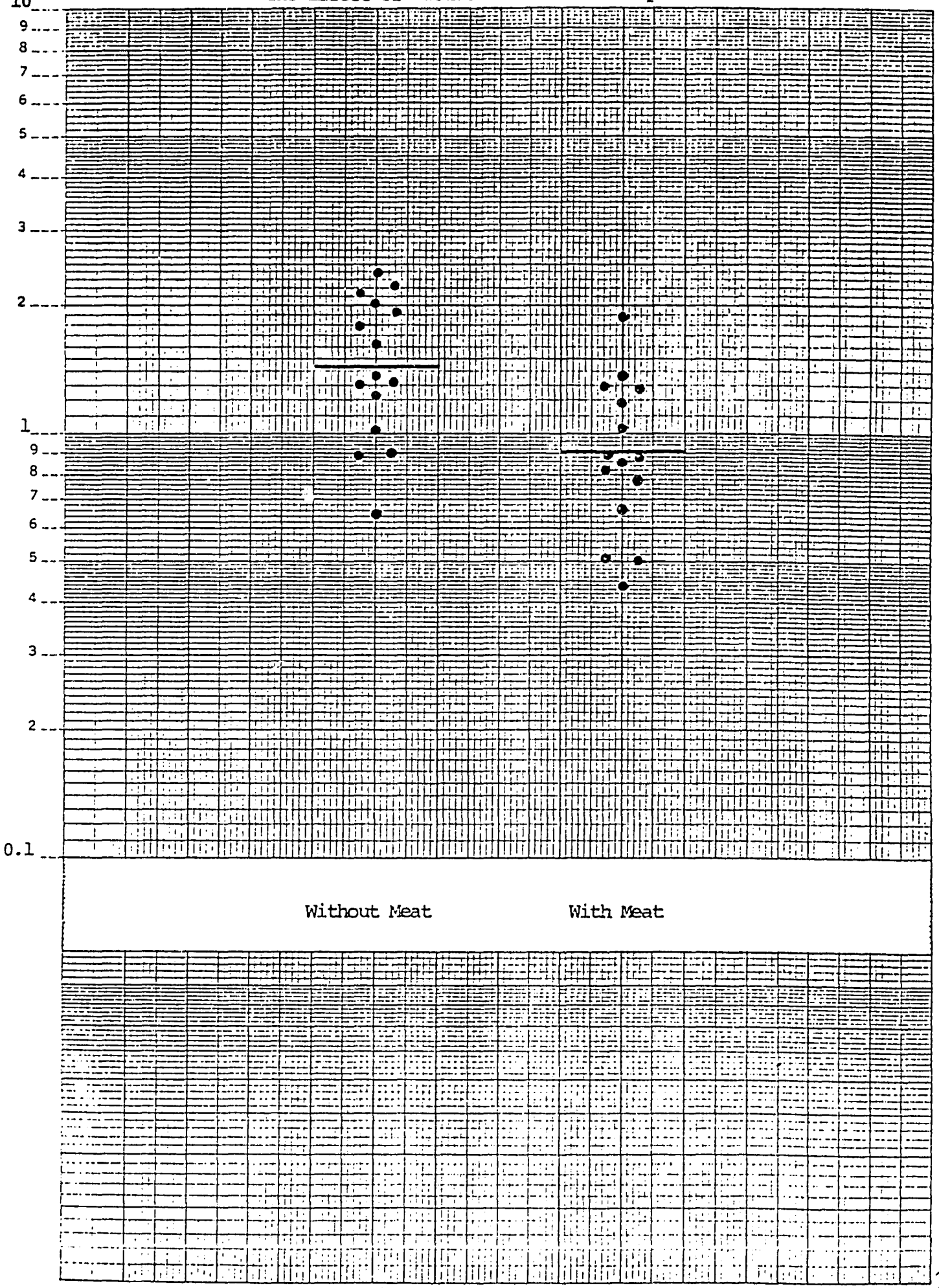
	ABSORPTION RATIOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	.70	.41	.45	.58	.64	1.10
-1SE	.63	.34	.37	.48	.51	.99
+1SE	.77	.48	.53	.71	.80	1.21
1/Ratio	1.43	2.45	2.24	1.71	1.57	.91
Student's t	3.60	5.29	4.48	2.82	2.03	.88
P Value	.0014	.00006	.0003	.007	.031	.20
Correlation*(r)	.919	.840	.822	.788	.716	.945
P Value	$<10^{6}$	.00004	.00008	.0002	.0013	$<10^{6}$

*Log Scale

The Effect of Vitamin E on Iron Absorption



The Effect of Vitamin E on Iron Absorption



Study 3

THE EFFECT OF TOCOPHEROL ON ABSORPTION OF INORGANIC IRON

SUMMARY

The addition of 1 gm α-tocopherol to an aqueous solution containing 3 mg iron as ferric chloride increased absorption significantly from 6.5 to 11.4%. However, when a buttered roll was ingested with the iron solution, tocopherol decreased absorption from 3.4 to 2.5%. Vitamin E has a small but significant enhancing effect on the absorption of inorganic iron.

RATIONALE

The possibility of using tocopherol as a food additive to enhance absorption of dietary iron was evaluated in Study 1. One gm tocopherol had only a minimal effect on iron absorption from the STD meal. However, the high lipid content of the milkshake may have impeded an interaction between the fat soluble vitamin and dietary iron. To further assess the effect of tocopherol on iron assimilation, 1 gm vitamin E was added to a solution of inorganic iron that was then administered either alone or with a dinner roll.

DESIGN

Four iron absorption tests were performed in each of 10 male volunteer subjects. All four meals contained 3 mg iron as labelled ferric chloride ($FeCl_3 \cdot 6H_2O$) in 100 ml water. Five percent cherry flavoring was added to disguise the metallic taste of iron. Meals A and B contained only the solution of inorganic iron. With meals C and D, the same iron solution was administered with a buttered dinner roll. Meals A and C contained no tocopherol whereas meals B and D contained 2.8 gm spray-dried α-tocopherol equivalent to 1 gm vitamin E. The tocopherol was added to the ferric chloride and allowed to stand at room temperature for 15 minutes prior to administration.

RESULTS

Iron absorption from the ferric chloride solution alone averaged 6.53% (meal A) and increased to 11.42% when taken with tocopherol (meal B). Mean absorption ratio (B/A) of 1.75 was statistically significant at the 2% level.

When labelled ferric chloride was ingested with a dinner roll, mean absorption fell by approximately 50% to a mean of 3.43%, a fall that is

consistent with the known inhibitory effect of food on iron absorption. When tocopherol was taken with ferric chloride and a dinner roll (meal D), there was a further decrease in iron absorption from 3.43 to 2.49%, although the difference was not statistically significant.

COMMENTS

This study provides additional evidence that the anti-oxidant properties of tocopherol have remarkably little influence on iron absorption. With meals A and B containing no food, 1 gm tocopherol increased absorption about twofold, similar to the effect seen in Study 1. This excludes the possibility that the limited effect of tocopherol on iron absorption observed in that study was due to sequestration of the vitamin in the lipid phase of gastrointestinal secretions.

In Study 1, tocopherol increased absorption from a meal of low iron availability although this enhancement was not observed in the present study with a small meal consisting of only a dinner roll. It is likely that the high correlation between meals A and B in Study 1 ($r = .92$) permitted the detection of a relatively small difference of 43%, whereas the lower correlation between meals C and D in the present study ($r = .58$) masked a significant difference. Nevertheless, it is clear that tocopherol has no potential for use as an enhancing agent in an intervention program to combat iron deficiency anemia.

STUDY 3. The effect of Vitamin E on iron absorption.

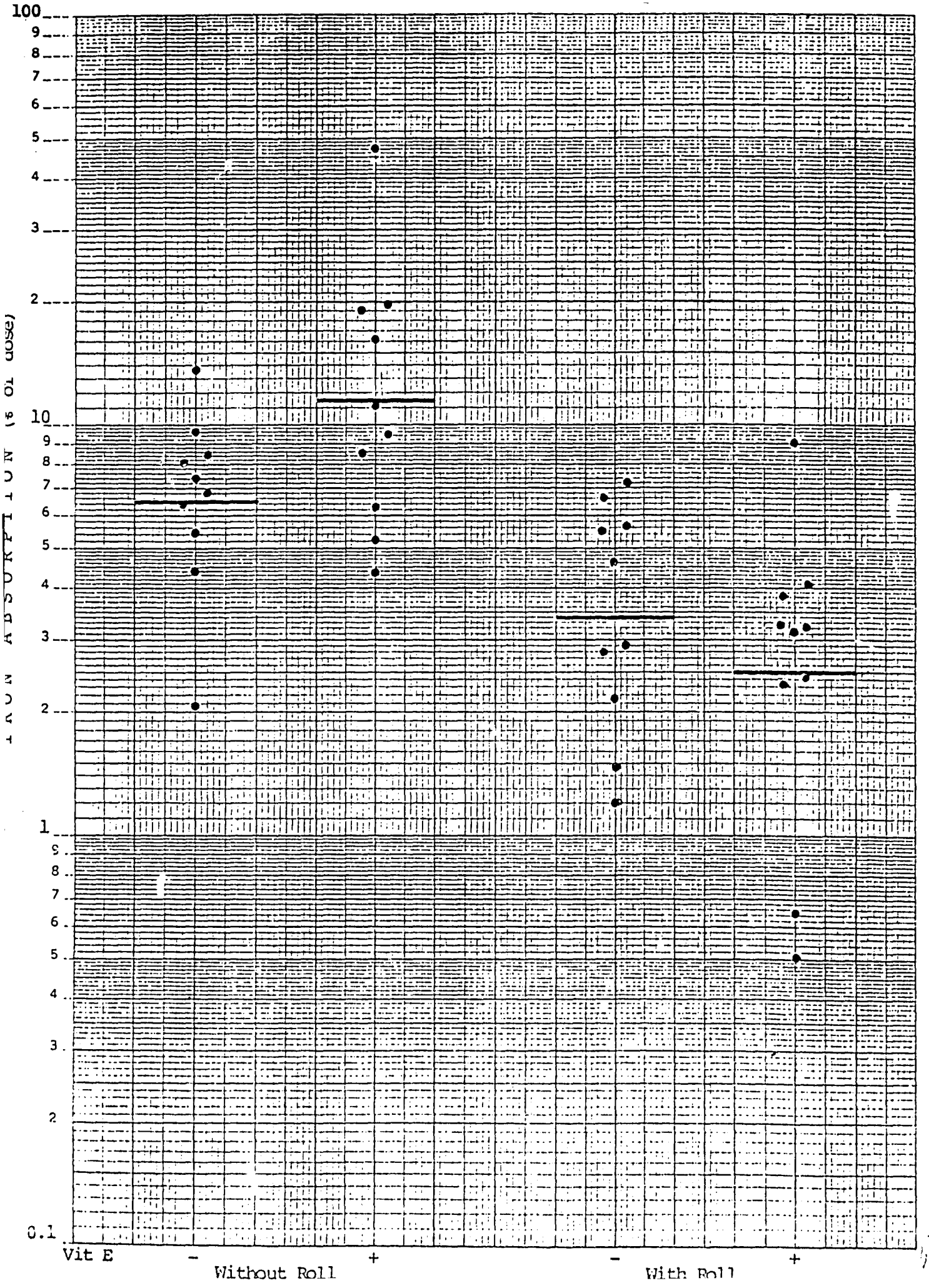
Subject	Age	Packed Cell Volume (%)	Serum Iron (ug/dl)	Tsf Sat (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) Without Roll -Vit E(a)	Without Roll +Vit E(b)	With Roll -Vit E(c)	With Roll +Vit E(d)	Absorption Ratios b/a	d/c	c/a	d/b
1	26	45	67	20	62	2.06	5.21	2.90	0.65	2.52	0.22	1.40	0.12
2	26	40	104	28	103	4.43	19.22	7.32	2.48	4.33	0.33	1.65	0.12
3	25	43	118	36	84	5.47	6.31	2.17	2.38	1.15	1.09	0.39	0.37
4	24	46	120	31	79	6.22	11.20	1.48	3.22	1.80	2.17	0.23	0.28
5	26	45	80	23	102	6.71	9.53	1.20	0.52	1.42	0.43	0.17	0.05
6	24	46	132	36	48	7.42	4.35	4.62	4.01	0.58	0.86	0.62	0.92
7	35	46	115	33	104	8.03	8.43	2.85	3.26	1.04	1.14	0.35	0.38
8	25	44	148	40	62	8.52	19.92	5.55	3.17	2.33	0.57	0.65	0.15
9	25	45	76	19	15	9.75	47.31	5.72	9.05	4.85	1.58	0.58	0.19
10	24	41	154	48	386	13.86	16.23	6.67	3.88	1.17	0.58	0.48	0.23
MEAN	26	44	112	31	76*	6.53*	11.42*	3.43*	2.49*	1.75*	0.73*	0.53*	0.22*
					-1 SE	5.55	9.07	2.80	1.90	1.42	0.58	0.42	0.17
					+1 SE	7.69	14.37	4.20	3.26	2.15	0.91	0.66	0.28

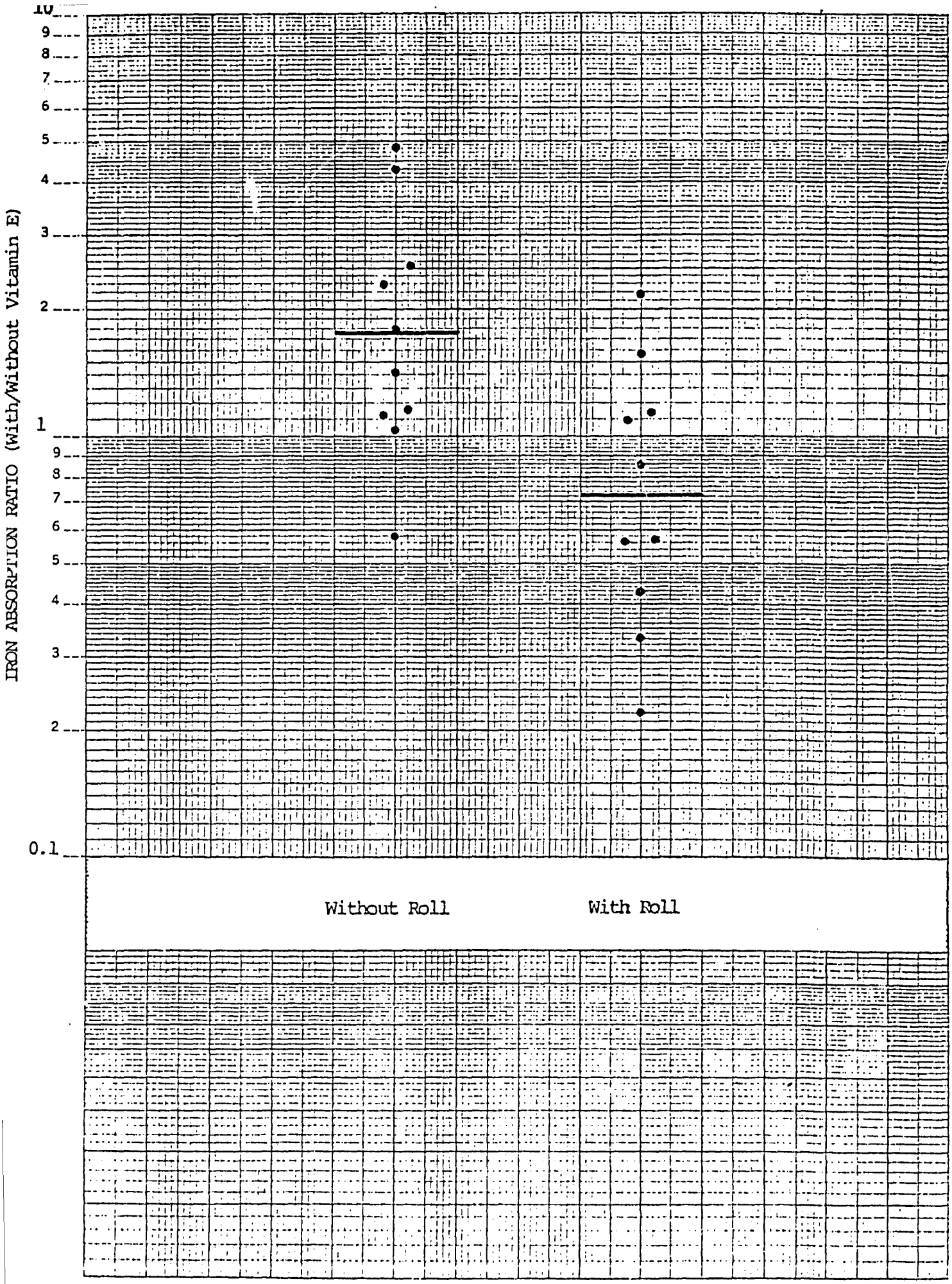
* geometric mean

Study 3. The effect of Vitamin E on iron absorption.

	ABSORPTION RATIOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	.57	1.90	2.62	3.33	4.58	1.38
-1SE	.46	1.53	2.13	2.66	3.58	1.10
+1SE	.71	2.38	2.22	4.15	5.85	1.72
1/Ratio	1.75	.53	.38	.30	.22	.73
Student's t	2.67	2.91	4.66	5.42	6.20	1.42
P Value	.013	.009	.0006	.0002	.0001	.095
Correlation*(r)	.476	.279	.643	.478	.527	.577
P Value	.082	.217	.022	.081	.059	.040

*Log Scale


TRUN ABSORPTION (% of dose)
100
9
8
7
6
5
4
3
2
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
0.1
Vit E
-
+
-
+
Without Roll
With Roll


IRON ABSORPTION RATIO (With/Without Vitamin E)
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
0.1
Without Roll
With Roll

Study 4

A COMPARISON OF THE EFFECT OF PURIFIED PROTEIN SOURCES ON IRON ABSORPTION

SUMMARY

Protein equivalent substitutions were made in the SS meal to compare the effect on iron absorption of egg albumin, heated egg albumin, isolated soy protein, and sodium caseinate. Similar mean absorption values of 2.45 and 2.74% were observed with egg albumin and casein, respectively. A significant increase in absorption to 6.21% was observed with heated albumin, a finding which may be explained by heat inactivation of conablumin, the iron-binding protein in egg white. Isolated soy protein (ISP) markedly inhibited the iron absorption (mean 0.46%) and there was no immediate explanation for this inhibitory effect.

RATIONALE

Published studies of iron absorption in both animals and man suggest that protein has a more significant effect on the assimilation of food iron than fat or carbohydrate. For example, animal protein has long been recognized by WHO as an important determinant of food iron availability, second only perhaps to ascorbic acid. Other studies have indicated that certain amino acids such as cysteine or methionine can facilitate iron absorption from the gastrointestinal lumen. In contrast, fat and carbohydrate are believed to be relatively passive in respect to food iron absorption with a possible exception of fructose which in high doses can facilitate iron absorption.

Although most reports suggest that protein enhances iron absorption, there is also evidence that it may impair iron absorption. In a recent study of the effect of various animal proteins on iron absorption, it was observed that iron absorption was inhibited by certain dairy products such as cheese, egg, and milk. In another study from this laboratory, the relative importance of the main dietary constituents in the SS meal was examined. There was relatively little effect on iron absorption when either the carbohydrate (dextrimaltose) or fat (corn oil) was either doubled or deleted. However, a significant decrease in absorption occurred when the quantity of egg albumin was doubled, and a comparable increase was observed when the protein was deleted from the SS meal. Other protein substitution studies using the SS and STD meal provided additional evidence that egg albumin impairs rather than facilitates iron absorption.

The present study was undertaken to compare the effect of semi-purified proteins on iron assimilation. One of the proteins was egg

albumin which has been studied extensively because of its use in the SS meal. It should be noted that egg albumin, ovalbumin, and albumin all refer to the product used as the protein source in the SS meal. This product is simply spray-dried egg white which is actually comprised of at least 12 different proteins. One of the proteins is conalbumin or ovatransferrin which is identical in the amino acid portion to serum transferrin in the hen; there may be small differences between conalbumin and transferrin in the carbohydrate moiety. Conalbumin constitutes 12% of egg white protein and binds the same amount of iron as transferrin--2 atoms iron/mole protein. Normally, conalbumin is less than 1% saturated with iron. The amount of egg white used in the SS meal contains enough conalbumin to bind approximately the 4.4 mg of iron contained in the SS meal.

The effect on iron absorption of egg albumin, isolated soy protein, and sodium caseinate was evaluated in the present study. The effect of heating albumin prior to addition to the SS meal was also evaluated.

DESIGN

Four iron absorption tests were performed in each of 15 male volunteers. The SS meal was used for all four test meals which contained the same quantity of corn oil (34.8 gm), dextrimaltose (66.8 gm), and vanilla flavoring. No calcium or phosphate was added to these meals.

The four test meals differed only in respect to the source of protein. Meal A was identical to the usual SS meal and contained 36.7 gm egg albumin equivalent to 29.4 gm protein. Meal B was identical to meal A except that egg albumin was heated to 70°C for 5 minutes before adding it to the other components of the SS meal.

The remaining two meals contained two other protein sources to provide a comparison of egg albumin. Meal C contained 33.9 gm isolated soy protein (Ralston Purina Company, St. Louis, Missouri) and meal D contained 32.6 gm sodium caseinate (National Casein, Chicago, Illinois). Sufficient ferric chloride was added to each of the test meals to bring the total iron content to 4.1 mg. The protein source and iron content of the four meals are summarized as follows:

	A	B	C	D
Protein source	Egg albumin	Heated Egg albumin	Soy	Casein
Protein %	80.0	80.0	86.8	90.2
Protein added (gm)	36.7	36.7	33.9	32.6
Iron content (μg/gm)	4.5	4.5	117.4	8.4
Intrinsic iron/meal (mg)	0.4	0.4	4.0	0.3
Added iron (mg)	3.7	3.7	0.1	3.8

All test meals were prepared on the day before administration by mixing dextrimaltose, corn oil, and protein source in a Waring blender in a total volume of 200 ml deionized water/test meal. Individual meals were kept at 4°C overnight in plastic cups and resuspended on the following morning. Radiolabelled ferric chloride in a volume of 1 ml .01 normal HCl was added to each meal immediately prior to serving.

RESULTS

Highly significant differences in iron absorption were observed from the four test meals. Absorption with the SS meal (A) ranged from 1.0 to 7.5% with a geometric mean of 2.49%. An unexpected increase in iron absorption from 2.49 to 6.2% was observed when egg albumin was heated before adding it to the SS meal (B). The mean absorption ratio of

2.50 (meal B/A) was highly significant ($t = 4.86$, $P < .0001$). Absorption with ISP (meal C) fell sharply to a mean of 0.46%. The fivefold difference in absorption between soy and egg albumin was significant at the 0.001% level. Absorption of the meal containing casein (meal D) averaged 2.74% which was nearly identical to absorption with uncooked albumin.

COMMENTS

There were two major findings in this study. The first, and least suspected, was a marked decrease in absorption from the soy-based meal as compared with ovalbumin and sodium caseinate meals. This is in sharp contrast to published studies suggesting that soy may be one of the most available sources of non-animal dietary iron. For example, when whole soy was tagged hydroponically with radioiron and fed to human subjects, mean absorption was 6.9% or at least 10 times greater than the level observed in the present study. It is possible that the commercial method used for preparation of ISP may render iron less available or produce a substance that inhibits iron assimilation. Because soy protein is used extensively in infant food supplements and as a meat extender, the inhibition in iron absorption caused by ISP deserves further study.

A second interesting finding in this study is the 2.5-fold increase in absorption observed when the egg albumin was heated prior to administration. One explanation for this is that the iron-binding capacity of conalbumin was destroyed by the heating process. The finding is of particular interest because of recent animal studies suggesting that a transferrin-like protein may be secreted by the intestinal mucosal cell and complex with iron to facilitate iron uptake by the mucosa. It is also possible that the lactoferrin contained in human breast milk may have a similar influence on iron absorption in infants.

STUDY 4. The effect of protein concentrates on iron absorption.

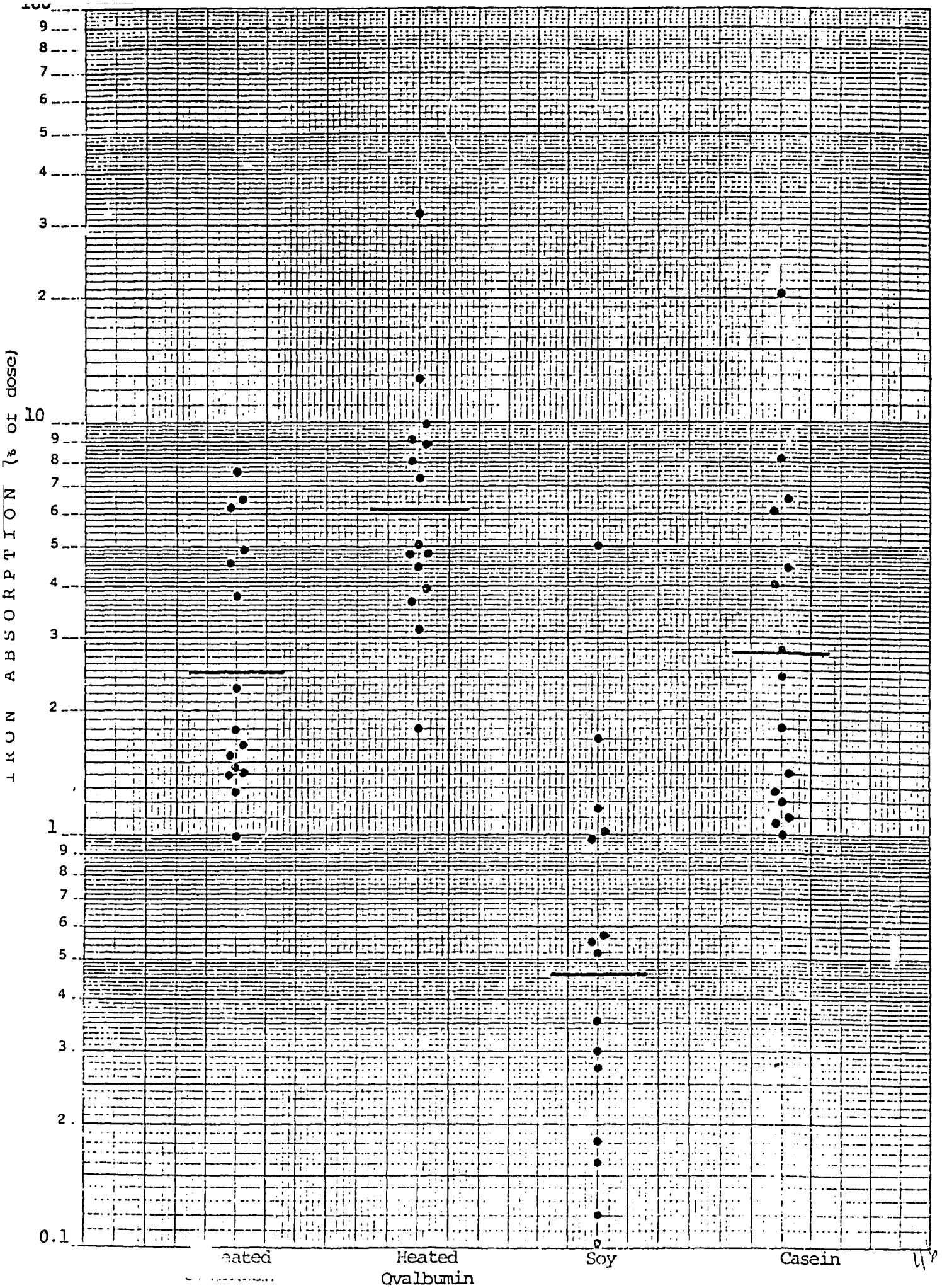
Subject	Age	Packed Cell Volume	Serum Iron	Tsf Sat	Serum Ferritin	IRON ABSORPTION Ovalbumin Unheated(a)	Ovalbumin Heated(b)	Soy(c)	Casein(d)	Absorption Ratios b/a	c/a	d/a
		(%)	(ug/dl)	(%)	(ng/ml)	(% of dose)		(% of dose)				
1	29	47	153	46	61	0.97	3.98	0.16	1.00	4.10	0.17	1.03
2	26	50	178	44	34	1.26	4.57	0.07	1.11	3.62	0.06	0.88
3	27	46	138	39	59	1.40	9.05	1.70	1.40	6.46	1.22	1.00
4	25	45	92	28	164	1.41	3.68	0.56	1.21	2.60	0.40	0.86
5	26	43	119	32	31	1.43	8.82	0.36	4.12	6.16	0.25	2.94
6	25	46	95	28	46	1.55	4.83	0.18	1.83	3.11	0.12	1.19
7	25	44	106	30	94	1.63	8.01	0.30	6.43	4.71	0.18	4.00
8	25	43	120	36	51	1.80	4.77	0.27	1.06	2.65	0.15	0.59
9	25	49	159	43	88	2.26	1.82	0.95	2.41	0.80	0.42	1.08
10	28	50	206	54	48	3.78	3.18	0.52	1.26	0.84	0.14	0.33
11	30	43	158	46	55	4.51	9.98	0.56	6.03	2.21	0.12	1.35
12	24	44	136	36	37	4.82	12.60	1.16	8.37	2.61	0.24	1.75
13	25	46	142	39	45	6.25	7.41	0.12	4.43	1.18	0.02	0.71
14	26	48	162	49	114	6.50	4.76	1.03	2.78	0.73	0.16	0.43
15	26	44	68	19	12	7.51	31.75	4.97	20.45	4.22	0.66	2.78
MEAN	26	46	136	38	53*	2.49*	6.21*	0.46*	2.74*	2.50*	0.19*	1.10*
					-1 SE	2.08	5.21	0.35	2.16	2.07	0.15	0.92
					+1 SE	2.98	7.41	0.62	3.47	3.01	0.24	1.31

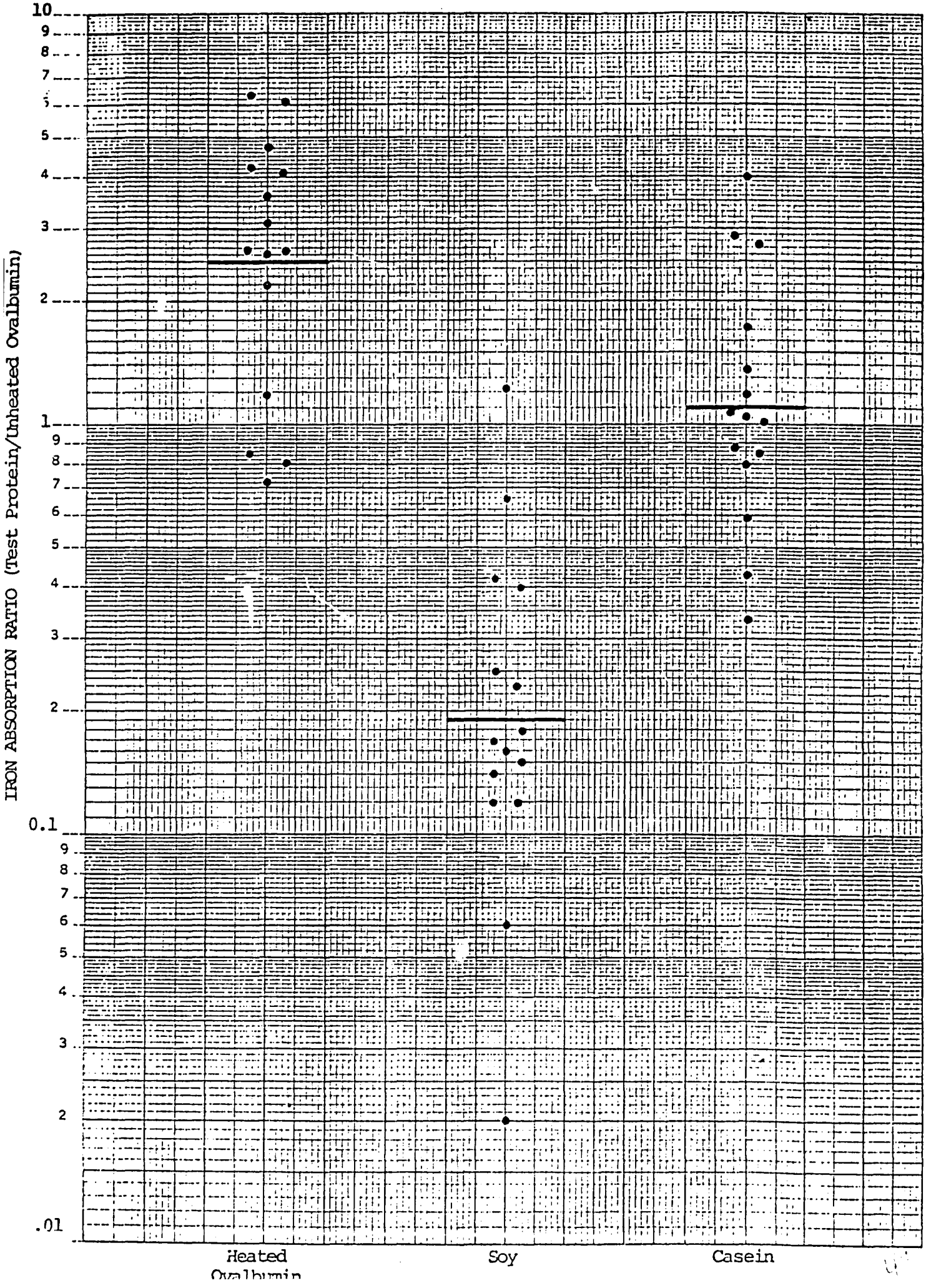
* geometric mean

Study 4. The effect of protein concentrates on iron absorption.

	ABSORPTION RATIOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	.40	5.33	.91	13.31	2.27	.17
-1SE	.33	4.13	.76	10.26	1.96	.13
+1SE	.48	6.87	1.09	17.26	2.63	.22
1/Ratio	2.50	.19	1.10	.08	.44	5.87
Student's t	4.86	6.55	.54	9.96	5.58	6.75
P Value	.0001	.000006	.300	$< 10^{6}$	.00003	.000004
Correlation*(r)	.432	.481	.662	.455	.784	.512
P Value	.050	.035	.004	.044	.0003	.025

*Log Scale


IRON ABSORPTION (% of dose)
100
9
8
7
6
5
4
3
2
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
0.1
eated
Heated
Ovalbumin
Soy
Casein


IRON ABSORPTION RATIO (Test Protein/Unheated Ovalbumin)
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
0.1
9
8
7
6
5
4
3
2
.01
Heated Ovalbumin
Soy
Casein

Study 5

THE EFFECT OF ASCORBYL PALMITATE ON FOOD IRON ABSORPTION

SUMMARY

Ascorbyl palmitate (AP), a synthetic analogue of ascorbic acid, was examined in this study as a substance which might be used to facilitate the absorption of native dietary iron. When added to the SS meal, AP produced an increase in absorption that was similar in magnitude to equimolar concentrations of ascorbic acid. Because of greater resistance of AP to oxidation as compared with ascorbic acid, AP might be suitable for enhancement fortification.

RATIONALE

The enhancing effect of ascorbic acid on the absorption of nonheme iron is well documented. However, vitamin C is readily inactivated under conditions that hasten the oxidation such as exposure to air, heat, or sunlight; this instability lessens its value for enhancement fortification. Ascorbic acid analogues exist that are more stable than ascorbic acid. Two such compounds, ascorbic-2-sulphate and ascorbic-2-phosphate, have not been approved for human administration and therefore were not evaluated. However, a third compound, ascorbyl palmitate (AP), is used in the food industry as an emulsifier in colored food drinks to keep the carotenoid pigments in solution. AP is a white, fluffy compound that is poorly soluble in either water or fat. However, it is moderately soluble in ethanol and its solubility is doubled if the temperature is raised to 60-70°C. Studies by Dr. Paul Seib at Kansas State University, have indicated that AP may retain much of its reducing activity when baked into bread, a process that largely inactivates ascorbic acid.

The present study was performed to assess the potential of AP for enhancement fortification. The effect of adding either AP or equimolar concentrations of ascorbic acid to the SS meal was evaluated. A final absorption test was performed to determine whether deletion of the corn oil in the SS meal altered the influence of AP on iron absorption.

DESIGN

All four test meals consisted of the SS meal containing no additional calcium or phosphate salts. Meal A was the SS meal alone. Meal B was the SS meal to which 100 mg ascorbic acid was added immediately prior to administration. Meal C contained 236 mg AP which is equivalent in molar

concentrations to 100 mg ascorbic acid. AP was added to meal C by first dissolving in absolute ethanol and dispensing in a volume of 1 ml. The final test dose, meal D, was identical to meal C except that corn oil was deleted from the SS meal. Sufficient labelled ferric chloride was then added to each test meal to bring the total iron content to 4.1 mg. To eliminate any effect of adding 1 ml alcohol to test meals containing AP, the same quantity of ethanol was added to meals A and B.

RESULTS

Mean absorption from the SS meal in this study was 4.47%, somewhat higher than the value of 2.49% observed in Study 4. When 100 mg ascorbic acid was added, absorption increased sharply to 7.26%, an increase that was significant at the .01% level. Moreover, mean absorption with meals containing AP was nearly identical; values of 7.5% were obtained in meal C and 7.91% in meal D from which corn oil had been omitted. Thus, the degree of enhancement in iron absorption was remarkably similar regardless of the form of ascorbate added.

COMMENTS

This preliminary study with ascorbyl palmitate indicates that it is as effective as ascorbic acid in promoting the absorption of dietary nonheme iron. In a previous study, the addition of 100 mg ascorbic acid to the SS meal increased absorption from 2.08% to 7.86%. The absorption ratio in that study (with/without ascorbic acid) of 3.77 was substantially higher than the value of 1.68 observed in this study. Nevertheless, the mean absorption from the SS meal containing ascorbic acid in that study (7.86%) is very similar to values of 7.26, 7.50, and 7.91% observed here. The possibility that AP might enter the lipid phase of the SS meal and not interact with inorganic iron was excluded in this study; absorption with meals C and D was nearly identical.

The facilitation of iron absorption by AP is presumably related to its content of ascorbate although the exact mechanism of the enhancement is unknown. It is possible that lipase in the pancreatic secretions cleaves the palmatic acid from the ascorbate ring and thereby releases ascorbate for reduction and chelation. Because AP is poorly soluble in an aqueous medium, it is unlikely that iron reacts with AP prior to this cleavage. Even though AP was solubilized in alcohol before mixing with the SS meal, a precipitate formed immediately when it was added.

This preliminary study of ascorbyl palmitate indicates that it may be as effective as ascorbic acid when administered in equimolar amounts to a meal containing purified sources of protein, carbohydrate, and fat. An additional study is planned to determine whether ascorbyl palmitate is more stable than ascorbic acid when baked into bread.

STUDY 5. The effect of ascorbic acid and ascorbyl palmitate on iron absorption.

Subject	Age	Packed Cell Volume	Serum Iron	Tsf Sat	Serum Ferritin	IRON ABSORPTION -AA/-AP (a)	+AA (b)	+AP (c)	+AP/-Oil (d)	Absorption Ratios b/a	c/a	d/c	b/c
		(%)	(ug/dl)	(%)	(ng/ml)	(% of dose)							
1	24	44	216	58	101	1.16	2.62	1.25	2.70	2.25	1.07	2.16	2.09
2	27	45	95	32	320	1.80	2.82	2.22	4.60	1.56	1.23	2.07	1.27
3	34	44	128	41	128	2.18	6.93	4.27	3.62	3.17	1.95	0.84	1.62
4	28	45	123	35	127	2.48	1.91	2.27	5.95	0.77	0.91	2.62	0.84
5	25	43	136	45	294	2.96	3.31	2.88	7.38	1.11	0.77	2.56	1.14
6	28	44	120	35	80	3.53	4.67	14.48	7.55	1.32	4.10	0.52	0.32
7	21	48	159	45	160	4.80	11.83	8.17	9.38	2.46	1.70	1.14	1.44
8	29	45	170	49	30	5.97	14.92	12.83	17.66	2.49	2.14	1.37	1.16
9	31	47	123	27	20	6.06	9.76	9.23	7.45	1.61	1.52	0.80	1.05
10	22	45	182	52	57	6.80	9.42	9.11	5.15	1.38	1.33	0.56	1.03
11	21	48	102	23	32	7.07	15.25	24.13	10.61	2.15	3.41	0.43	0.63
12	22	45	114	31	54	8.68	7.72	17.36	9.66	0.88	2.00	0.55	0.44
13	22	40	46	9	10	33.38	54.17	58.28	49.53	1.62	1.74	0.84	0.92
MEAN	26	45	132	37	85*	4.47*	7.26*	7.50*	7.91*	1.62*	1.68*	1.05*	0.97*
					-1 SE	3.52	5.63	5.53	6.45	1.45	1.48	0.88	0.84
					+1 SE	5.66	9.35	10.17	9.71	1.83	1.90	1.26	1.12

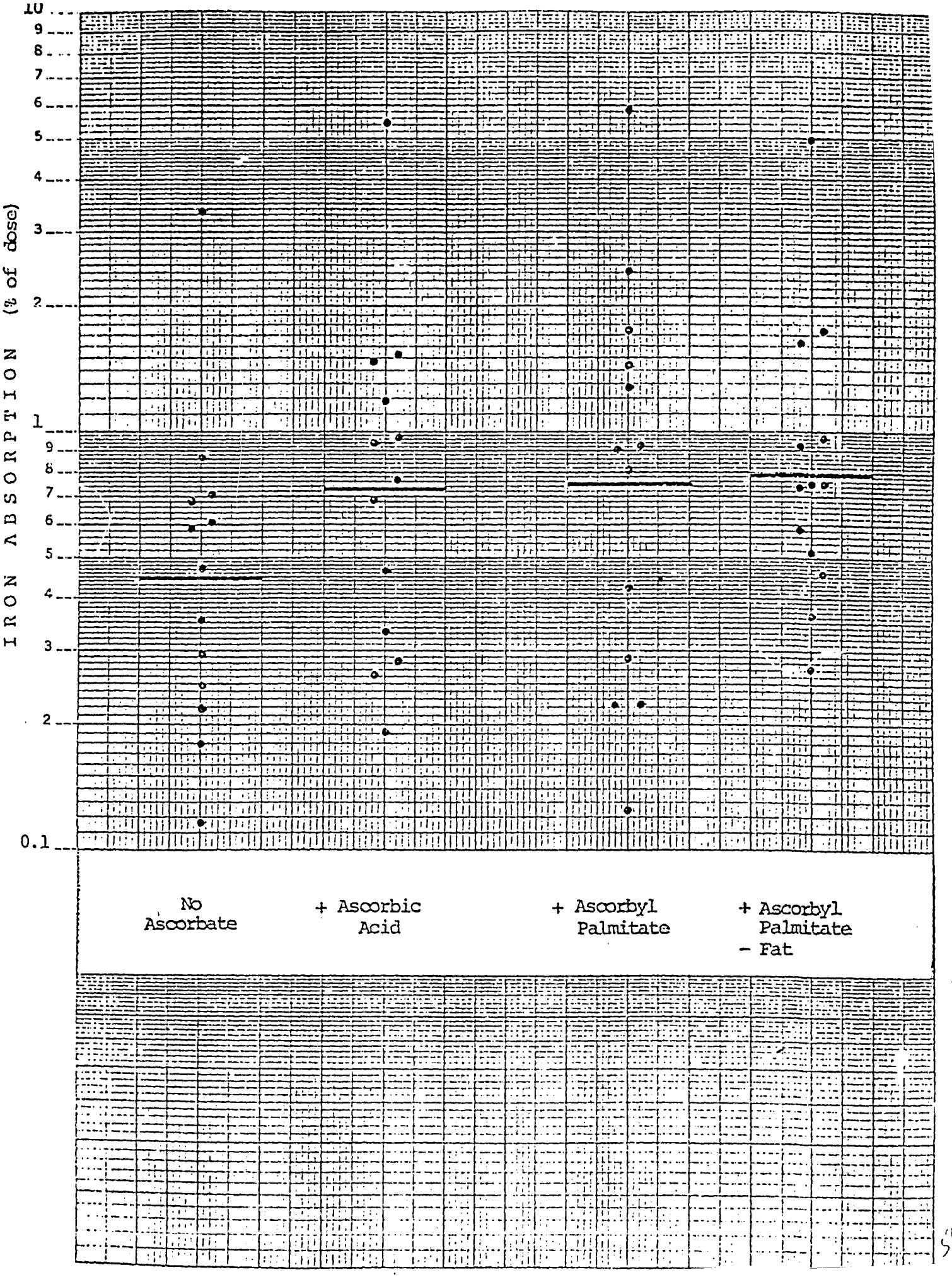
* geometric mean

Study 5. The effect of ascorbic acid and ascorbyl palmitate on iron absorption.

	ABSORPTION RATIOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	.62	.60	.56	.97	.92	.95
-1SE	.55	.53	.51	.84	.79	.79
+1SE	.69	.67	.63	1.12	1.06	1.13
1/Ratio	1.62	1.68	1.77	1.03	1.09	1.05
Student's t	4.15	4.15	5.23	.23	.58	.300
P Value	.00007	.0007	.0001	.40	.286	.38
Correlation*(r)	.888	.922	.888	.884	.810	.828
P Value	.00002	.000003	.00002	.00003	.0004	.0002

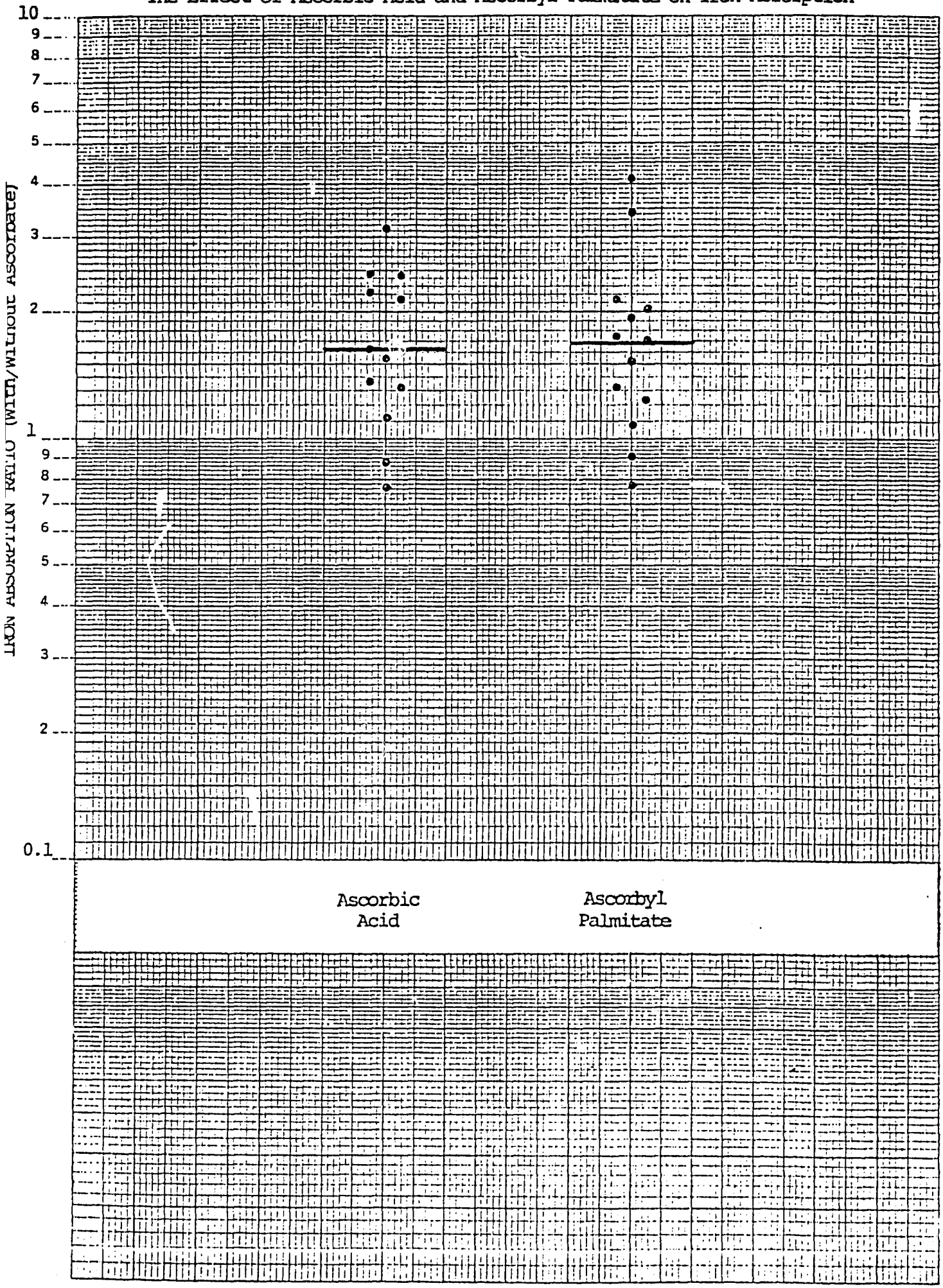
*Log Scale

... Ascorbic Acid and Ascorbyl Palmitate on Iron Absorption



54

The Effect of Ascorbic Acid and Ascorbyl Palmitate on Iron Absorption



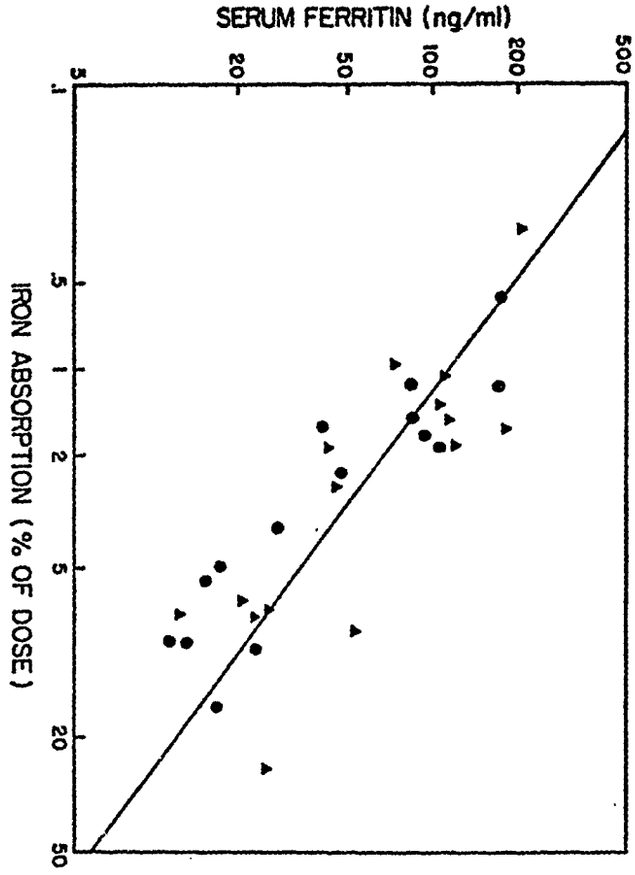

SERUM FERRITIN (ng/ml)
500
200
100
50
20
IRON ABSORPTION (% OF DOSE)
50
20
5
2
1
.5
.1

Figure II

56

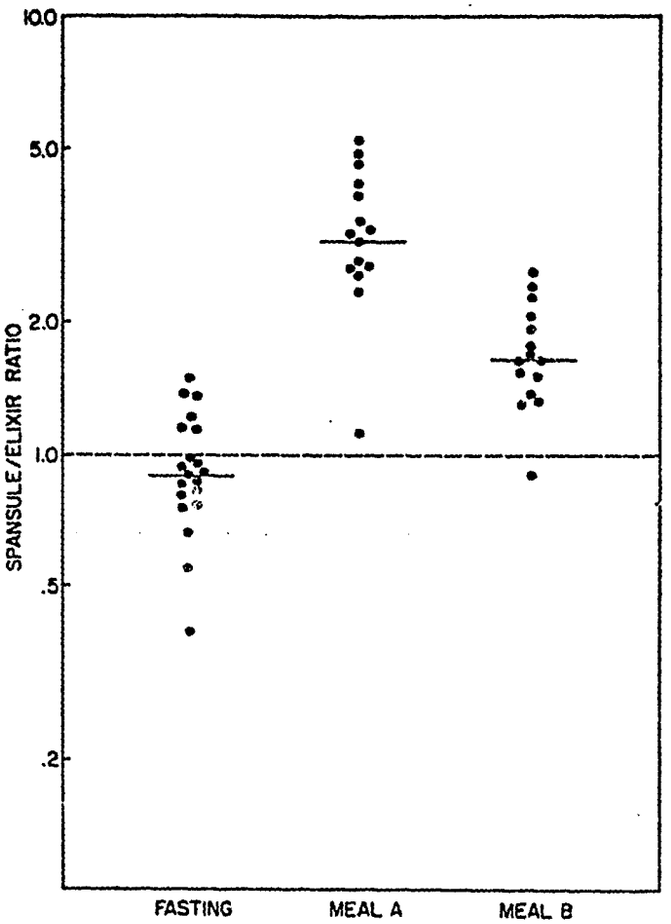


Figure I

LEGENDS

Figure I

Absorption of iron administered in a delayed-release spansule expressed as a ratio of absorption from ferrous sulphate elixir. The horizontal lines represent geometric means. When administered with food, absorption from the delayed-release spansule was significantly higher.

Figure II

The correlation of iron absorption from a delayed release spansule and serum ferritin measurements. Measurements with both Meal A (o) and Meal B (▲) are included.

TABLE 2. Iron absorption from Feosol spansules and elixir.

Subject		Sex/Age	Packed Cell Volume	Serum Ferritin	IRON ABSORPTION Blood Spansule	Blood Elixir	Whole-body Counter	Absorption (Spansule/t
			(%)	(ng/ml)	(% of dose)		(% of dose)	
FASTING								
1	JM	M 24	47	71	1.05	1.96		.53
2	DC	M 25	49	142	2.26	3.03		.75
3	CR	M 24	46	138	3.50	3.08		1.14
4	SW	M 22	51	162	2.60	3.11		.84
5	MF	M 23	48	109	3.01	3.93		.77
6	RB	M 24	47	80	4.99	4.04		1.23
7	TR	M 26	48	52	1.58	4.08		.39
8	TS	M 27	44	29	7.39	4.94		1.50
9	SH	M 26	49	18	12.79	13.25		.97
10	MW	F28	40	113	2.19	2.70		.81
11	DR	F37	44	82	2.80	3.10		.91
12	ZA	F27	41	59	4.23	3.13		1.35
13	EW	F22	45	101	3.66	3.23		1.12
14	BN	F29	43	103	3.49	4.35		.80
15	CC	F23	40	35	4.49	4.74		.95
16	PC	F34	48	106	5.64	6.80		.83
17	DW	F26	44	33	5.31	8.23		.65
18	RR	F35	45	59	14.74	10.84		1.36
19	LM	F28	39	44	13.53	15.18		.89
20	MH	F29	43	14	17.06	18.45		.92
	Mean	27	45	64*	4.38*	4.92*		.89*
MEAL A								
1	TK	M20	43	176	1.55	.37	1.26	4.18
2	LB	M29	49	170	.56	.50		1.12
3	MT	M22	42	103	2.35	.50	.89	4.70
4	DA	M27	41	84	1.41	.51	.54	2.76
5	RG	M31	52	95	1.73	.55		3.14
6	RM	M20	51	28	3.65	1.36		2.68
7	JR	F27	41	85	1.13	.22	.65	5.13
8	MO	F20	39	41	1.61	.33	.73	4.87

TABLE 1. Composition of standardized meals.

	Weight (gm)	Energy (kcal)	Protein (gm)	Iron Content (mg)
MEAL A				
Egg	54	117	8	1.3
Muffin w/dressing	78	233	10	1.9
Bran flakes, 40%	28	86	3	1.3
Sugar	5	19	--	--
Milk, 2% lowfat	227	131	10	--
Coffee	170	2	--	0.2
TOTAL	562	588	31	4.7
MEAL B				
Hamburger	94	269	23	3.0
Bun w/dressing	70	151	3	2.1
Potatoes, french-fried	69	210	3	0.5
Milkshake, vanilla	145	162	5	0.1
TOTAL	378	792	34	5.7

BEST AVAILABLE COPY

1. Webster JJ: Treatment of iron deficiency anemia in patients with iron intol erance: Clinical evaluation of a controlled-release form of ferrous sulfate Curr. Ther. Res. 4:130-134, 1962

2. Posner LB, Wilson F: An evaluation of sustained-release molybdenized ferrous sulphate in iron-deficiency anemia of pregnancy. J. New Drugs 3:155-160, 19

3. Rybo G, Solvell L: Side-effect studies on a new sustained release iron prepa Scand. J. Haematol. 8:257-264, 1971

4. Mitchell ABS: Oral iron tolerance. Postgrad. Med. J. 50:702-706, 1974

5. Crosland-Taylor P, Keeling DH, Cromie BW: A trial of slow-release tablets of ferrous sulphate. Curr. Ther. Res. 7:244-248, 1965

6. Middleton EJ, Nagy E, Morrison AB: Studies on the absorption of orally adminis iron from sustained-release preparations. N. Engl. J. Med. 274:136-139, 1966

7. Dagg JH: Treatment of iron deficiency and iron overload. Brit. Med. J. 2:494-4 1974

8. Fairbanks VF, Fahey JL, Beutler E: Clinical Disorders of Iron Metabolism. New York, Grune and Stratton, 1971: 297-304

9. Crosby WH: Prescribing iron? Think safely. Arch. Intern. Med. 138:766-767, 1978

10. Stevenson TD: Therapeutic assay of a sustained-release form of oral iron. Curr. Ther. Res. 4:107-110, 1962

11. Layrisse M: The utilization of iron from a controlled-release dose form. Curr. Ther. Res. 5:232-237, 1963

12. Israels MCG, Cook TA: New preparations for oral iron-therapy. Lancet 2:654-657, 1965

13. Norrby A, Solvell L: Iron absorption and haemoglobin regeneration studies on a new sustained release iron preparation. Scand. J. Haematol. 8:231-240, 1971

14. Norrby A, Arvidsson B, Solvell L: Total absorption of iron and haemoglobin regeneration during 4 weeks of oral iron therapy--a comparative study of 4 different iron doses. Scand. J. Haematol. 20:33-55, 1974

15. Bothwell TH, Pirzio-Biroli G, Finch CA: Iron absorption. I. Factors influencing absorption. J. Lab. Clin. Med. 51:24-36, 1958

16. Callender ST: Quick and slow-release iron: A double-blind trial with a single daily dose regimen. Br. Med. J. 4:531-532, 1969

17. Parker AC, Simpson JD, Richmond J: Comparison of absorption of "slow iron" and ferrous sulphate tablets BP in patients following partial gastrectomy. Scot. Med. J. 17:314-315, 1972

18. Boye Nielsen JB, Ikkala E, Solvell L, Bjorn-Rasmussen E, Ekenved G: Absorption of iron from sustained release and rapidly disintegrating tablets. Acta. Med. Scand. 194:123-127, 1973

BEST AVAILABLE COPY

19. Baird IM, Walters RL, Sutton DR: Absorption of slow-release iron and ef of ascorbic acid in normal subjects and after partial gastrectomy. Bri 4:505-508, 1974

20. Boye Nielsen JB, Ikkala E, Solvell L, Bjorn-Rasmussen E, Ekenved G: Abso of iron from slow-release and rapidly-disintegrating tablets--a comparati in normal subjects, blood donors and subjects with iron deficiency anaemi Scand. J. Haematol. Suppl. 28:89-97, 1976

21. Ekenved G, Arvidsson B, Solvell L: Influence of food on the absorption of types of iron tablets. Scand. J. Haematol. Suppl. 28:79-88, 1976

22. World Health Organization Technical Report Series No. 405. Nutritional Ana Report of a WHO Scientific Group, 1968

23. Miles LEM, Lipschitz DA, Bieber CP, Cook JD: Measurement of serum ferritin b 2-site immunoradiometric assay. Anal. Biochem. 61:209-224, 1974

24. Unpublished observations.

25. Brise H, Hallberg L: A method for comparative studies on iron absorption in m using two radioiron isotopes. Acta. Med. Scand. Suppl. 376:7-22, 1962

26. Cook JD, Monsen ER: Food iron absorption. I. Use of a semisynthetic diet to study absorption of nonheme iron. Amer. J. Clin. Nutr. 28:1289-1295, 1975

27. Watt BK, Merrill AL: Composition of foods--raw, processed, prepared. In: Agri. Res. Hdbk. No. 8, Washington, D.C., U.S. Gov. Print. Ofc., 1963

28. Eakins JD, Brown DA: An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Intern. J. Appl. Radiation Isotopes 17:391-397, 1966

29. Brown E, Hopper J, Hodges JL, Bradley B, Wennesland R, Yamaushi M: Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. J. Clin. Invest. 41:2182-2190, 1962

30. Cook JD, Palmer HE, Pailthorp KG, Finch CA: The measurement of iron absorption by whole-body counting. Phys. Med. Biol. 15:467-473, 1970

31. Cook JD, Layrisse M, Finch CA: The measurement of iron absorption. Blood 33: 421-429, 1969

32. Layrisse M, Cook JD, Martinez C, Roche M, Kuhn IN, Walker RB, Finch CA: Food iron absorption: a comparison of vegetable and animal foods. Blood 33:430-443, 1969

33. Cook, JD, Lipschitz DA, Miles LEM, Finch CA: Serum ferritin as a measure of iron stores in normal subjects. Amer. J. Clin. Nutr. 27:681-687, 1974

34. Hallberg L, Ryttinger L, Solvell L: Side effects of oral iron therapy. Acta. Med. Scand. Suppl. 459:3-10, 1966

35. Levy F, Andersen P, Ekren T: Absorption and side-effects after peroral administration of sustained release iron tablets. Acta. Med. Scand. 204:303-310, 1978

36. Mouratoff GJ, Batterman RC: Serum iron absorption tests of a sustaine form of oral iron. J. New Drugs 1:157-159, 1961

37. Ekenved G: Absorption of different types of iron tablets--correlation serum iron increase and total absorption of iron. Scand. J. Haematol. 28:51-63, 1976

38. Hallberg L, Bjorn-Rassmussen E, Ekenved G, Garby L, Rossander L, Pleeha Suwanik R, Arvidsson B: Absorption from iron tablets given with differe of meals. Scand. J. Haematol. 21:215-224, 1978

BEST AVAILABLE COPY

ABSORPTION OF A SUSTAINED-RELEASE FORM OF ORAL IRON

Cook, J.D., Lipschitz, D.A., Skikne, B.S.

*From Division of Hematology
Department of Medicine
Kansas University Medical Center
39th & Rainbow Boulevard
Kansas City, Kansas 66103

**Supported in part by grant AM 19011 from National Institutes of Health and contract DSAN-C-0045 from U.S. Agency for International Development

64

BEST AVAILABLE COPY

ABSTRACT

A multiple-dose double radioiron technic has been used to compare absorption of iron administered as an elixir and a controlled-release spansule; both formulations contained 50 mg elemental iron as ferrous sulphate. When administered in the fasting state to normal volunteer subjects, mean absorption from the elixir and spansule averaged 4.9 and 4.4% respectively, a difference that was not statistically significant. However, when administered with meals that either enhance or inhibit the absorption of dietary iron, the absorption ratio of spansule:elixir averaged 1.70 and 3.13 respectively and with both meals, the difference was highly significant. It is concluded that controlled-release iron formulations may offer a therapeutic advantage in patients who develop gastrointestinal side effects with conventional oral iron therapy.

INTRODUCTION

Gastrointestinal side effects such as nausea and epigastric discomfort occur commonly in patients receiving conventional oral iron therapy. Because these symptoms are believed to correlate with the peak concentration of administered iron in the gastrointestinal lumen, a common pharmaceutical approach has been to administer iron in a form which retards its rate of dissolution. Previous studies have shown that such controlled-release preparations do reduce gastrointestinal side effects such as nausea or vomiting (1-4). Nevertheless, the use of these formulations has been frequently criticized on the basis that any reduction in gastrointestinal side effects is due to lower solubility and reduced absorption (5-9).

The efficacy of delayed-release iron preparations has been examined in a number of published reports. The majority of clinical studies have shown an adequate hemoglobin response in patients with iron deficiency anemia (2, 10-14), but this is not a sensitive method for assessing iron availability. Radioisotopic measurements have afforded greater precision in assessing iron absorption but studies of slow-release preparations have given conflicting results (13, 15-21). These discrepancies may be accounted for by differences in the nature of the delayed-release formulation and perhaps also by differences between batches of the same formulation. The study reported here was undertaken to compare the absorption of iron from a ferrous sulphate elixir with that of a commercial delayed-release formulation of ferrous sulphate having an *in vitro* release rate of 80% or more at 1 hour.

BEST AVAILABLE COPY

66

METHODS

Subjects. Three separate iron absorption studies were performed in a total of 19 male and 31 female volunteers having a median age of 26 years (range, 20-55 years). All subjects were in good health and gave no history of recent infections or of disorders known to impair absorption from the gastrointestinal tract. None of the subjects were anemic using WHO criteria of a hematocrit above 36% in women and 39% in men (22). Iron status was assessed by serum ferritin determinations using the method of Miles, et al (23). Fully informed consent was obtained in each subject prior to study.

Preparation of labelled dosage forms. A double radioiron technic was used to compare iron absorption in each subject from ferrous sulphate administered either as ^{59}Fe-labelled elixir or as ^{55}Fe-labelled delayed-release capsules*. The method used for preparing and analyzing the radiolabelled delayed-release spansule will be reported separately (24) and described here only briefly. Two-hundred fifty grams of commercial ferrous sulphate pellets were irradiated with thermal neutrons for 423 hours at 40°C and a neutron flux of approximately 5 x 10^{7} n/cm^{2}/sec. The irradiated pellets were then aged to decay undesirable radionuclides, pan-coated with lipid material, and encapsulated. The preparation was analyzed chemically as well as for radioactivity. A Ge(Li) detector coupled to a multi-channel analyzer was used for the analysis of gamma emitters, and a Si(Li) was used with the same analyzer for the x-ray analysis of ^{55}Fe. Each capsule contained 167 mg of dried ferrous sulphate USP, equivalent to 50 mg of elemental iron, and 0.5 uCi ^{55}Fe. The elixir was

'Feosol' Spansule Capsule, Smith, Kline and French Laboratories

61

BEST AVAILABLE COPY

'Feosol' Elixir containing 44 mg elemental iron/5 ml to which ferrous sulphate had been added to yield a dose of 50 mg elemental iron/5 ml. Immediately prior to administering the elixir, $^{59}FeSO_4$ (New England Nuclear) was added to give 0.2 μCi ^{59}Fe/5 ml dose.

Administration of test doses. Both iron preparations were administered between 7 and 9 a.m. following an overnight fast. To reduce the effect of day-to-day variability in iron absorption, a multiple dose administration technic (25) was employed as follows. On the first morning of each study, one-half of the subjects were given 5 ml ferrous sulphate elixir while the remaining subjects were given the delayed-release iron spansule. The two formulations were then alternated on successive days for a total of 10 days so that each volunteer received 5 doses of each preparation. The total dose of radioactivity administered to each subject was 2.5 μCi ^{55}Fe and 1 μCi ^{59}Fe.

Three separate studies were performed which differed in regard to whether iron was administered alone or with food. In the first study performed in 20 volunteer subjects, the iron was administered to fasting individuals with a small volume of water and nothing further was allowed by mouth for 3 hours. In the remaining two studies, the labelled iron was taken with one of two standardized meals which were selected as meals that would either inhibit (meal A) or enhance (meal B) iron assimilation. Most of the food items were purchased on the morning of the study from a local McDonald's restaurant. Meal A, considered a typical breakfast, consisted of an Egg McMuffin, bran flakes, and milk; all of these items are believed to inhibit the assimilation of dietary iron. Meal B consisted of a hamburger, french fries, and a milkshake;

BEST AVAILABLE COPY

the meat contained in this meal is known to facilitate iron absorption (26).

The chemical compositions of meals A and B that are listed in Table 1 were obtained from food composition tables (27) or by chemical analyses performed by WARF Institute, Inc., Madison, Wisconsin. It was assumed that the small difference in energy and iron content of the two meals would not affect iron assimilation. The ^{59}Fe elixir or ^{55}Fe capsule was ingested midway through the two meals and no additional food was allowed for 3 hours.

Determination of iron absorption. The subjects returned 14 days following administration of the final test dose at which time sufficient blood was obtained to permit duplicate measurements of ^{55}Fe and ^{59}Fe radioactivity in 10 ml whole blood. Standards representative of each test dose were processed simultaneously. Radioactivity was measured by liquid scintillation counting on samples prepared by a modification of the method of Eakins and Brown (28). Sufficient counts were obtained to reduce the error of the net counting rate to less than $\pm$ 2% in subjects absorbing more than 1% of the administered test dose. Percentage absorption of both isotopes was calculated on the assumption that 80% of the absorbed dose was incorporated into circulating red cells 14 days following the final test dose. Total blood volume was calculated from the sex, height, and weight of each subject (29).

In the two studies in which the iron was administered with food, retention of whole-body ^{59}Fe was measured on the final day of the test immediately prior to obtaining the blood sample. These measurements

 BEST AVAILABLE COPY

were obtained with a whole-body counter consisting of four six-inch NaI (Tl) crystals and a mobile cot. Total body ^{59}Fe radioactivity was measured during a single 10-minute traverse of the patient as described previously (30).

Statistical analysis. Because of skewed distribution of iron absorption data, mean values and standard deviations were calculated on a logarithmic scale and the results retransformed as logarithms to recover the original units (31,32). Statistical comparison of absorption from the elixir and spansule was based on a Student's t-test to determine whether the mean logarithm of $^{55}Fe/^{59}Fe$ absorption ratio differed significantly from 0.

RESULTS

Iron absorption data are listed for all three studies in Table 2. When the two iron preparations were administered without food, similar iron absorption means of 4.4 and 4.9% were observed with delayed-release and elixir, respectively. Although absorption ranged widely, from 1 to 18.5%, the absorption ratio in individual subjects varied from only .53 to 1.50 (Figure 1). The mean spansule/elixir ratio of 0.89 ($\pm$ 1 SE, .83-.96) did not reflect a significant difference in absorption ($t = 1.58$, $p > .05$).

In contrast with studies in fasting subjects, significant differences in absorption from the two preparations were observed when administered with food. For meal A of low iron availability, mean absorption from delayed-release iron decreased from the fasting mean of 4.4% to 3.3%

BEST AVAILABLE COPY

while absorption fell from 4.9% to 1.0% when administered as elixir. The absorption ratio for spansule/elixir averaged 3.13 (± 1SE 2.84-3.44) and was greater than unity in all subjects; the difference in absorption was highly significant ($t = 11.85$, $p < .0001$).

When the two iron preparations were administered with meal B containin meat, the mean absorption from elixir of 1.69% was somewhat higher than with meal B, whereas the mean absorption of 2.87% from the delayed-release spansule was very similar to the mean of 3.3% with meal A. With meal B the mean absorption ratio of spansule/elixir was 1.70 (± 1SE, 1.59-182) which again reflected a highly significant difference in absorption ($t = 7.76$, $p < .0001$).

In the second and third studies in which absorption was also determined by whole-body counting, there was satisfactory agreement between the latter and values based on measurements of incorporated ^{59}Fe red cell radioactivity. In the total group of 30 subjects, identical mean absorption values of 1.41% were obtained by the two technics. When absorption was low, however, there were substantial differences between the two methods. At these low absorption values, the net whole-body counting rate was frequently only 5-10% above background radioactivity, whereas duplicate blood determinations were highly consistent. Thus, the major discrepancy at low absorption levels was attributed to whole-body counting values, and measurements of blood radioactivity were therefore used to calculate percentage absorption of ^{59}Fe.

The wide range in iron absorption between different subjects is typical of that observed in previous studies (31, 32). Despite this high subject-to-subject variability, however, an excellent corres- was observed between ^{59}Fe and ^{55}Fe absorpt-

BEST AVAILABLE COPY

largely reflects the advantage of using multiple dose administration. The correlation coefficient between absorption with the elixir and spansule was .945 in fasting subjects and .977 and .988 with meal A and meal B, respectively. These high correlations between ^{59}Fe and ^{55}Fe absorption values is further evidenced by the narrow range of absorption ratios in the 3 studies (Figure 1). In the study in fasting subjects for example, the 95% confidence limit for the mean ratio of .89 is .77-1.03 indicating that a difference in absorption of more than 15% would have been detected in this study as statistically significant.

Excellent correlations were also observed between absorption from either dose formulation and body iron stores as measured by serum ferritin. In the 3 separate studies, the absolute correlation coefficient for the relationship between log serum ferritin and iron absorption was greater than .8. The correlation between serum ferritin and iron absorption from the delayed-release spansules administered with food is shown in figure 2.

DISCUSSION

Gastrointestinal side effects occur in 10-25% of patients on oral iron therapy. The most common symptoms are epigastric pain, nausea, and vomiting, and these upper gastrointestinal symptoms increase progressively with increasing doses of administered iron. Lower gastrointestinal symptoms such as diarrhea and constipation occur less commonly and are unrelated to the dose of oral iron. There is also a significant psychogenic component to these symptoms since side effect studies have demonstrated 5-15% of patients receiving a placebo will develop these same gastrointestinal symptoms (4,34,35).

BEST AVAILABLE COPY

In an effort to circumvent gastrointestinal side effects of ir a variety of pharmaceutical preparations have been marketed during th past decade in which the solublization of iron in the gastrointestinal lumen is purposely delayed. It is assumed with this approach that gastric irritation is decreased by reducing the peak concentration of ionized iron in the stomach or small intestine. These controlled-release preparations are of 3 types. With the erosion type, the iron salt is mixed with an excipient that is designed to dissolve more slowly in the gastrointestinal tract. The elution type consists of a physiologi inert plastic matrix containing minute interstices into which the iron is dispersed; this iron is then eluted in the gastrointestinal lumen as secretions gradually diffuse into the central core of the tablet. In the pelleted controlled-release formulation, iron is contained in numerous pellets which are administered in a gelatin capsule. The pellets are coated with blends of selected waxes and fats that dissolve in the gastrointestinal lumen at differing rates. By varying both the amount and chemical property of the pellet coating, iron release can be varied from a few minutes to several hours.

A number of studies have demonstrated that gastrointestinal side effects occur less commonly with delayed-release iron than with conventional iron therapy. The most complete study of this type was performed by Rybo and Sovell (3) in 1,166 blood donors. Ferrous sulphate containing 100 mg elemental iron was administered twice daily either as rapidly-disintegrating tablets or an an elution-type sustained-release formulation while a third group was given a placebo. Although no difference was observed between the two iron formulations in the frequency of constipation or diarrhea, a significantly higher frequency of nausea and epigastric

 BEST AVAILABLE COPY

pain occurred with conventional ferrous sulphate as compared with ei sustained-release iron or placebo. Similar results have been reporte in earlier studies (1,2).

Because the gastrointestinal absorption of iron is confined to the early segment of the small intestine, it is generally assumed that any delay in the release of administered iron would carry it beyond the site of maximal absorption. Numerous studies have been performed to determine the absorption of sustained-release iron formulations but the findings have been quite variable. Some of these disparities can be attributed to a lack of precision in technics for measuring iron absorption. For example, a common approach in earlier studies was to measure the hemoglobin response in patients with iron-deficiency anemia. Isolated studies of sustained-release iron have demonstrated therapeutic efficacy (10,11), and comparative studies have shown that delayed-release iron gives a therapeutic response that is equal to or better than conventional iron tablets (2,12). However, hemoglobin response curves are not a sensitive index of iron absorption and this is particularly so when the dose of oral iron is in excess of that needed to obtain an optimal response.

Serum iron tolerance curves have also been used to assess the absorption of therapeutic iron, and have provided evidence that absorption of sustained-release iron is equal to (36) or less than (6) conventional iron tablets. It should be noted that if the dissolution of iron in the gastrointestinal tract is delayed, the serum iron response may not be a suitable index of total absorption. However, in a recent study it was shown that rapidly disintegrating and delayed-release iron produced very similar serum iron responses, a finding that was confirmed by isotopic measurements (37).

BEST AVAILABLE COPY

Interestingly, the same serum iron response was observed with the two formulations despite the fact that there were major differences in their dissolution rate in vitro.

Much greater precision in studies of iron absorption is afforded by the use of radioisotopic technics. Several studies employing isotopes have been performed to measure absorption of sustained-release iron but the findings have again been conflicting. Bothwell, et al (15) observed lower absorption from a 'slow' release spansule as compared with a 'quick' release preparation. In a later study, absorption of 100 mg delayed-release iron given once daily was compared with the absorption of 33 mg iron as ferrous sulphate given three times daily. Only 2 of the 11 subjects absorbed more iron from the slow release than from the conventional iron preparation but the absorption ratios in this study were highly variable. Subsequent studies employing a whole-body counter have demonstrated no differences in the absorption of conventional iron and sustained-release formulations in normal subjects (16), iron deficient patients (19), or patients with a partial gastrectomy (17).

A number of important studies have recently been performed using a multiple-dose double isotope technic to assess absorption from an elution-type sustained-release iron preparation. In an initial report, 100 mg iron was administered as ferrous sulphate twice daily with meals, either as sustained-release or as conventional ferrous sulphate tablets. In regular blood donors, absorption of the sustained-release iron averaged 29% higher than the conventional iron tablet; the difference was highly significant (13). In later studies performed in blood donors and iron

BEST AVAILABLE COPY

deficient subjects, the absorption of 100 mg iron in the sustain release formulation given twice daily was compared with the absor of 50 mg ferrous sulphate administered in 4 daily doses. Absorpt: the two preparations was similar in blood donors but in iron defic subjects absorption of the delayed-release iron was 24% higher tha that with conventional ferrous sulphate (18).

In a more recent Scandinavian study, the relative absorption of sustained-release and conventional iron was shown to depend not only c iron status but also on whether the preparations were administered wit food (21). Thus, in 16 normal subjects given 100 mg iron as ferrous sulphate, mean absorption was 7.3% in fasting subjects and fell to 0.9% when administered with food. In these same subjects, absorption of sustained-release iron in fasting subjects averaged 5.6% but with food a lesser decrease to 1.69% was observed. These findings are almost identical to those in the present study where higher absorption was obtained when controlled-release iron was given with a meal of either high or low iron availability.

Because of the higher cost of sustained-release preparations, their use cannot be advocated in all patients requiring oral iron therapy. However, previous studies have consistently demonstrated a lower frequency of gastrointestinal side effects with controlled-release iron as compared with ferrous sulphate tablets, and the present report has established that this can be achieved without a reduction in the quantity of iron absorbed. Delayed-release preparations therefore provide a much safer and less costly alternative to parenteral iron therapy in patients who cannot tolerate conventional iron therapy because of

symptoms. Moreover, the frequent doses required with delayed-release iron will alleviate to a certain extent the poor compliance which is frequently encountered with oral iron therapy (4) and which is believed to be a major problem in iron supplementation programs in developing countries.

Study 8

IRON ABSORPTION FROM BREAD BAKED WITH ASCORBYL PALMITATE

SUMMARY

Iron absorption from extrinsically tagged bread was significantly increased when ingested with 100 mg ascorbic acid or a molar equivalent amount of ascorbyl palmitate. More importantly, a significant increase in absorption was also observed when the same quantity of ascorbyl palmitate was added to the flour before baking. It is concluded that, unlike ascorbic acid, the enhancing effect of ascorbyl palmitate on iron absorption is little altered by the baking process.

BEST AVAILABLE COPY

RATIONALE

It was demonstrated in Study 5 that ascorbyl palmitate (AP) produced the same increase in iron absorption from the SS meal as did ascorbic acid. However, unlike ascorbic acid which is inactivated at baking temperatures, AP forms a complex with starch in bread that protects the ascorbate portion from oxidation. Preliminary studies by Dr. Paul Seib at Kansas State University indicated that approximately 80% of ascorbic acid activity is retained when AP is baked into the bread.

In this study, bread prepared from iron-fortified flour (ferrous sulphate) was tagged extrinsically with radioiron and administered to volunteer subjects. Iron absorption was measured from test meals containing bread alone, bread with ascorbic acid, and bread with AP added either to the dough before baking or to the bread at the time of administration.

DESIGN

All four test meals consisted of 100 gm of bread containing the following ingredients:

flour	100 g
sugar	6.0 g
nonfat dry milk	4.0 g
shortening	3.0 g
water	66.0 ml
salt	1.5 g
yeast	2.0 g
potassium bromate	1.0 mg
ferrous sulfate	3.2 mg

Loaves of bread weighing 140-150 gm were baked in individual pans. The ends of each loaf were trimmed until each weighed 100 gm. The loaf was then sliced into four portions and radioactive ferric chloride containing .01 mg iron in 1 ml .01 N HCl was pipetted evenly over the face of each slice. Two pats of soft margarine were spread on the slices and administered to volunteer subjects with deionized water.

BEST AVAILABLE COPY

Each test meal contained approximately 2.2 mg iron as ferrous sulfate/100 gm of trimmed bread, which is equivalent to the present level of iron fortification in the U.S.A. The bread alone was administered in meal A as a control. Meal B differed in that sufficient AP was added to the flour before baking to provide 236 mg AP/100 gm trimmed bread. For meals C and D, the enhancing agent was simply sprinkled on the bread after dispensing the radio-iron solution on the bread slices; the margarine was then spread on the slices taking care not to disrupt the distribution of the enhancing agent. With meal C, the enhancing agent was 236 mg AP and for meal D, 100 mg ascorbic acid. In summary, the four test meals were as follows:

Meal A: Control.

Meal B: AP baked into bread.

Meal C: AP after baking.

Meal D: Ascorbic acid after baking.

RESULTS

Twelve male volunteers were fed the test meals. Two of these subjects had reduced iron stores as evidenced by serum ferritin levels of 4 and 11 ng/ml. Much higher absorption from all four test meals was observed in these two volunteers.

Absorption from the control meal (A) averaged 5.67% with a range from 1.86 to 33%. When AP was baked into the bread (meal B), a slight but significant increase in absorption to 6.96% was observed. A similar increase to a mean of 7.60% was observed when AP was sprinkled on the bread (meal C) and to 9.15% when ascorbic acid was administered with the bread (meal D). Relative to the control meal (A), absorption ratios for the three meals containing enhancing agents (B,C,D) indicated increases of 23, 34, and 61%, respectively. This increase was statistically significant with meals B and D ($P < .004$ and .003, respectively) but not with meal C ($P = .066$).

The most important statistical analysis in this study is the comparison between meals B, C, and D, all of which contained an enhancing agent. Absorption from meal D was significantly higher than either of the meals containing AP but the difference between the two meals containing AP (B and C) was not statistically significant. These results indicate that little, if any, of the enhancing activity of ascorbyl palmitate is destroyed by the baking process.

COMMENTS

AP increases iron absorption from bread whether it is baked into the loaf or added just prior to administration. The magnitude of this effect, however, is modest (23 and 34%, respectively) and significantly lower than equimolar concentrations of ascorbic acid. However, the enhancement due to 100 mg ascorbic acid in this study is very similar to that observed with the SS meal in Study 5; mean absorption ratios (+/- ascorbic acid) were 1.68 and 1.61, respectively.

The important observation here is that an ascorbic acid analogue retains its enhancing effect on absorption when added to flour before baking. The cost of AP will preclude its wide spread use as a food additive to combat iron deficiency. Nevertheless, the feasibility of developing an enhancing agent more stable than vitamin C has been demonstrated and will hopefully stimulate efforts to develop similar, less expensive, enhancing compounds.

BEST AVAILABLE COPY

STUDY 8. The effect of ascorbyl palmitate on iron absorption from fortified bread.

Subject	Age	Packed Cell Volume (%)	Serum Iron (ug/dl)	Tsf Sat (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) Control (a)	AP Added To Dough (b)	AP Added To Bread (c)	AA Added To Bread (d)	Absorption Ratios b/a	c/a	d/a
1	27	43	168	46	55	1.86	2.21	7.40	6.88	1.18	3.97	3.69
2	28	40	115	34	130	3.37	4.43	1.80	5.37	1.31	0.53	1.59
3	21	39	115	40	78	3.67	2.87	1.93	2.15	0.78	0.52	0.58
4	27	44	98	27	44	3.77	4.08	6.66	5.35	1.08	1.76	1.41
5	19	40	188	53	115	3.80	5.20	10.25	9.53	1.36	2.69	2.50
6	20	48	64	16	64	3.96	4.45	4.22	5.50	1.12	1.06	1.38
7	22	43	90	28	47	4.03	4.22	5.16	5.42	1.04	1.28	1.34
8	19	42	140	42	61	4.37	4.81	3.52	5.00	1.10	0.80	1.14
9	27	46	57	17	32	5.06	6.85	6.06	8.86	1.35	1.19	1.75
10	18	38	82	29	27	9.81	15.82	20.37	25.91	1.61	2.07	2.64
11	21	40	273	68	4	27.30	50.55	57.51	59.35	1.85	2.10	2.17
12	21	40	126	33	11	36.00	43.85	39.10	42.40	1.21	1.08	1.17
MEAN	23	42	126	39	41	5.67*	6.96*	7.60*	9.15*	1.23*	1.34*	1.61*
					-1 SE	4.40	5.19	5.55	6.90	1.15	1.12	1.40
					+1 SE	7.32	9.32	10.40	12.15	1.31	1.60	1.85

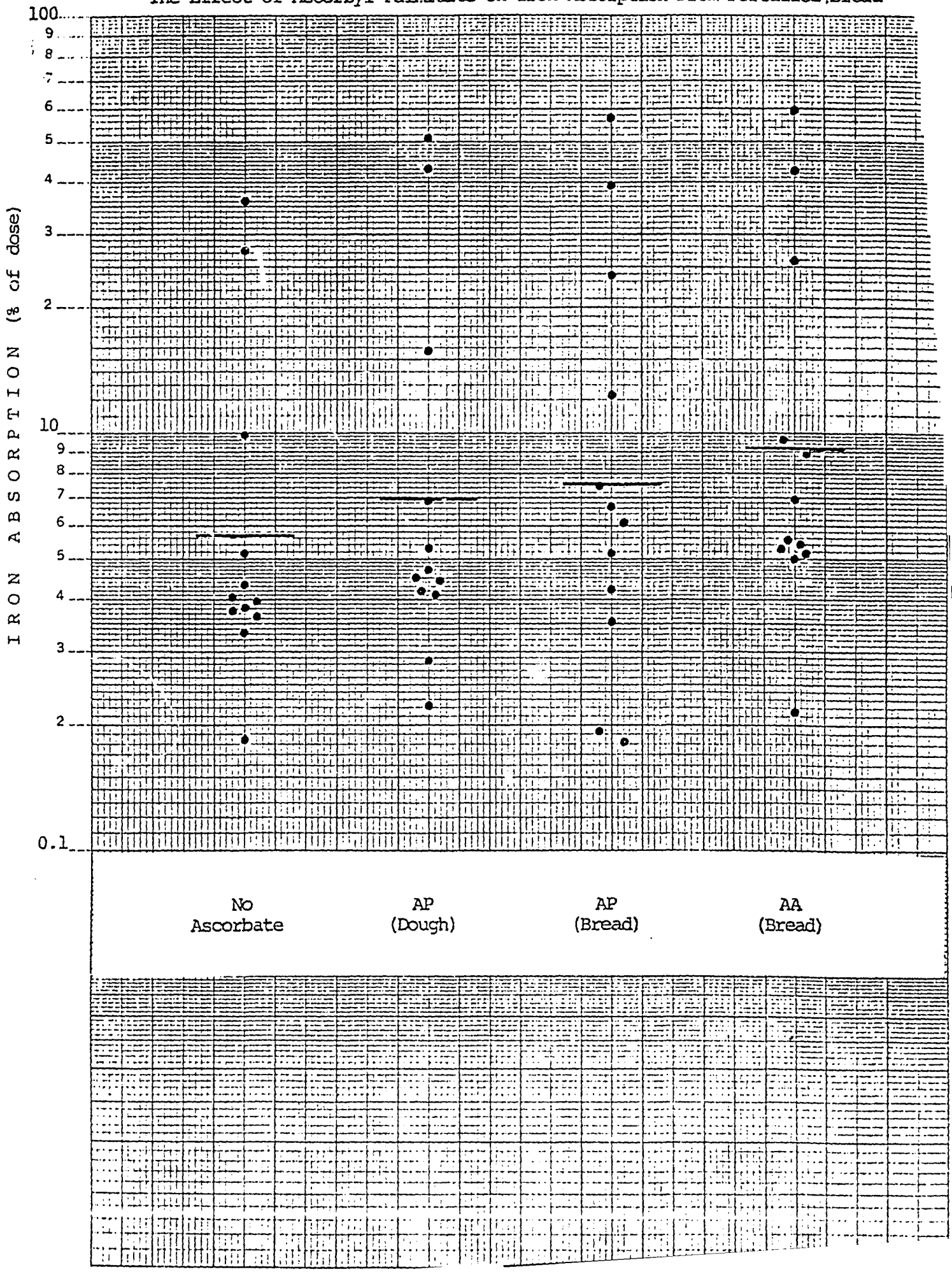
*geometric mean

Study 8. The effect of ascorbyl palmitate on iron absorption from fortified bread.

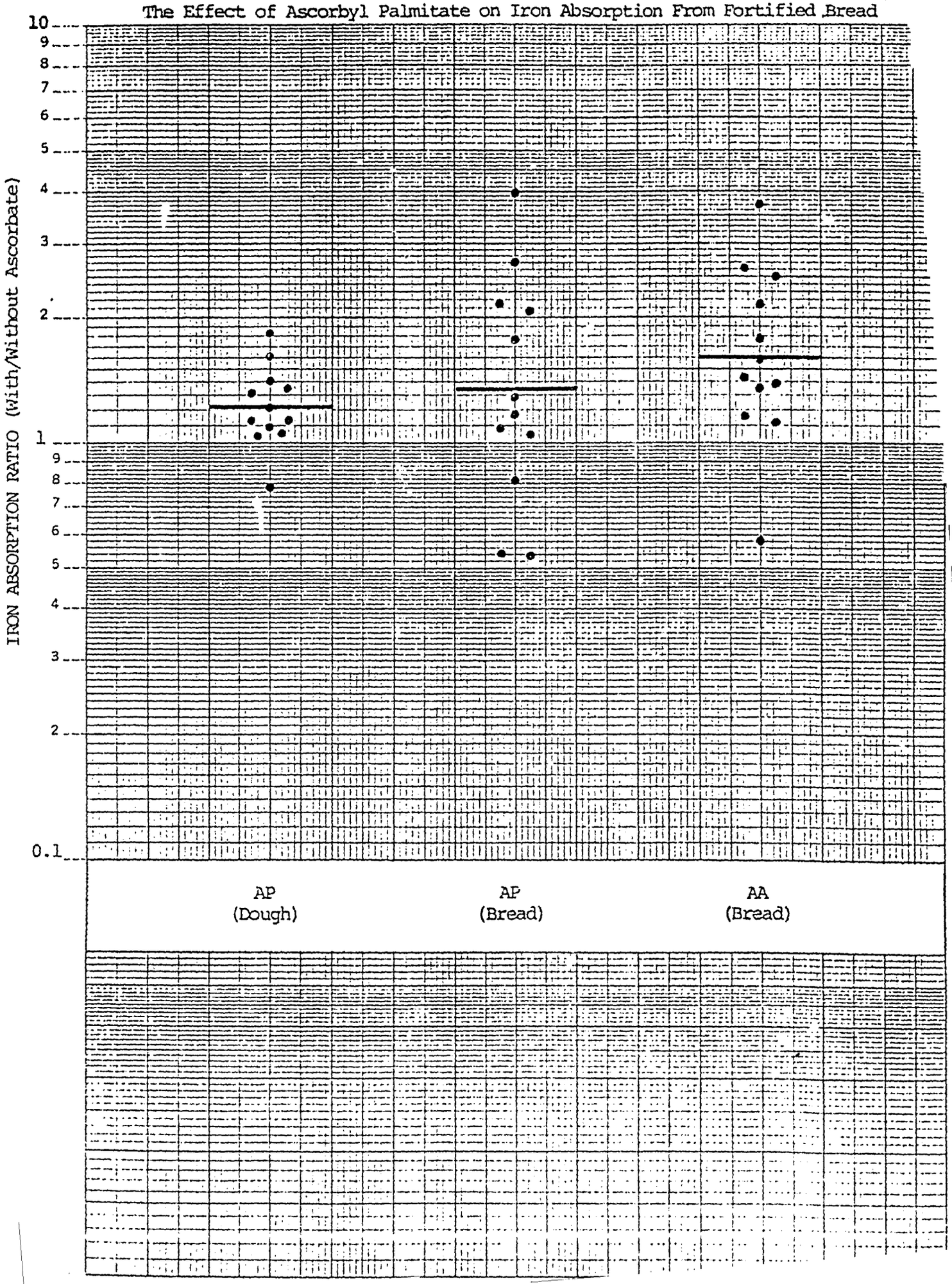
	ABSORPTION RATIOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	.82	.75	.62	.92	.76	.83
-1SE	.77	.62	.54	.78	.69	.75
+1SE	.87	.89	.71	1.07	.84	.92
1/Ratio	1.23	1.34	1.61	1.09	1.32	1.20
Student's t	3.20	1.63	3.45	.560	2.67	1.91
P Value	.004	.066	.003	.293	.011	.041
Correlation*(r)	.982	.820	.872	.866	.936	.951
P Value	$<10^{6}$	.0005	.0001	.0001	.000004	$<10^{6}$

*Log Scale

The Effect of Ascorbyl Palmitate on Iron Absorption From Fortified Bread



The Effect of Ascorbyl Palmitate on Iron Absorption From Fortified Bread



Study 9

EFFECT OF COFFEE AND TEA ON IRON ABSORPTION

SUMMARY

A study was performed to assess the effect of coffee on the assimilation of dietary nonheme iron and to compare this effect with the inhibiting activity of tea. When a cup of tea was ingested with a hamburger meal, absorption decreased from 3.71 to 1.32%; this 64% decrease is significant at the 0.1% level. Absorption averaged 2.25% when a cup of coffee was ingested with the same meal and this 39% decrease is significant at the 5% level. Thus, coffee impairs absorption of food iron but to a lesser extent than tea.

RATIONALE

Recent interest in the use of sugar as a vehicle for food iron fortification has made it important to determine the effect on iron absorption of popular sweetened beverages such as tea and coffee. The inhibitory effect on iron absorption of brewed tea has been the subject of several recent articles. Indeed, the impairment is so striking that it has been recommended as a means of reducing absorption of dietary iron in patients with iron-loading diseases such as thalassemia major. There are statements in the literature that coffee has a similar effect but there is no supporting data. Because coffee is a popular beverage both in this country and in LDCs, a study was undertaken to define the effect of coffee on food iron absorption.

DESIGN

Four iron absorption tests were performed in each of 11 male volunteer subjects. The first three meals consisted of a quarter-pound hamburger with bun and differed only in respect to the beverage consumed with the hamburger. A meat-containing meal was chosen for the study so that any inhibiting effect of a beverage would be more readily detected. Meal A was administered with 200 ml water, meal B with the same volume of tea, and meal C with the same volume of coffee. Meal D consisted of the reference dose of inorganic iron.

The tea administered with meal C was prepared using proportions recommended by Lipton's commercial product (Orange Pekoe and Pekoe Cut Black). Each cup of tea was prepared by pouring 250 ml boiling water over 1.75 gm tea, brewing for 4 minutes, and then pouring the supernatant through a sieve into the flask from which the beverage was consumed.

Coffee was prepared using an automatic drip coffee maker. Each cup of coffee was prepared by adding 250 ml water to 7 gm coffee. All test meals

(except meal D) were labelled by pipetting 0.1 mg iron as radioactive ferric chloride in .1 N HCl over the hamburger pattie just prior to administration.

RESULTS

Absorption from the control meal taken with water averaged 3.71% with a range of 0.96 to 11.21%. When tea was consumed with the meal (B) absorption fell to 1.32% and this 64% decrease was significant at the 0.1% level. Absorption with coffee (meal C) averaged 2.25% and this 39% decrease from the control meal was also significant (P = .034). The difference in absorption with tea and coffee was also significant; the absorption ratio (B/C) averaged .59 (P = 0.03).

The depression in iron absorption due to tea observed here is very similar to data published previously by Disler et al., in 1975. A larger quantity of tea was used in that study (5 gm). Absorption ratios (+/- tea) averaged .26, .32, and .23 when tea was ingested with inorganic iron, bread, or a complete meal, respectively. The mean ratio of 0.36 observed in this study is in close agreement.

The effect of coffee observed here was less dramatic but still statistically significant. If the effect of coffee is dose-related, then 1 cup of tea is comparable in its inhibiting effect to 2 cups of coffee. An even greater inhibiting effect of coffee may have been observed if the radioiron tag were mixed with coffee rather than added to the hamburger pattie; the former method would be more relevant in regard to iron fortification of sugar.

No effort was made in this study to characterize the inhibitory effect of coffee. It is likely that tannins which are known to be responsible for impairing iron absorption with tea are also the factor in coffee that inhibits iron absorption. It will be important to conduct additional studies with coffee to determine, for example, the effect of the method of preparation (perked versus instant) and the effect of adding radioiron to the beverage

STUDY 9. The effect of tea and coffee on iron absorption.

Subject	Age	Packed Cell Volume (%)	Serum Iron (ug/dl)	Tsf Sat (%)	Serum Ferritin (ug/ml)	IRON ABSORPTION Control (a) (% of dose)	+Tea (b) (% of dose)	+Coffee (c) (% of dose)	Reference (d) (% of dose)	Absorption Ratios b/a	c/a	a/d
1	29	48	117	34	65	0.96	0.48	1.08	10.85	0.50	1.12	0.08
2	26	44	245	66	68	1.76	0.56	0.86	11.63	0.31	0.48	0.15
3	25	45	124	32	67	1.78	1.47	4.30	4.33	0.82	2.41	0.41
4	26	46	140	37	97	2.53	1.68	2.02	19.06	0.66	0.79	0.13
5	30	44	141	41	156	2.71	1.82	2.47	10.96	0.67	0.91	0.24
6	22	46	180	48	102	4.16	2.18	2.22	14.37	0.52	0.53	0.28
7	26	43	188	46	47	5.55	0.87	2.05	19.55	0.15	0.36	0.28
8	23	43	266	68	34	5.81	0.30	0.51	23.21	0.05	0.08	0.25
9	28	43	189	57	58	6.91	1.70	4.91	20.55	0.24	0.71	0.33
10	22	45	113	30	34	8.75	3.43	4.21	26.02	0.39	0.48	0.33
11	19	44	125	33	27	11.21	5.32	7.93	36.81	0.47	0.70	0.30
MEAN	25	45	166	45	60*	3.71*	1.32*	2.25*	15.73*	0.36*	0.61*	0.24*
					-1 SE	2.94	1.01	1.75	13.22	0.28	0.47	0.21
					+1 SE	4.68	1.71	2.88	18.71	0.45	0.78	0.27

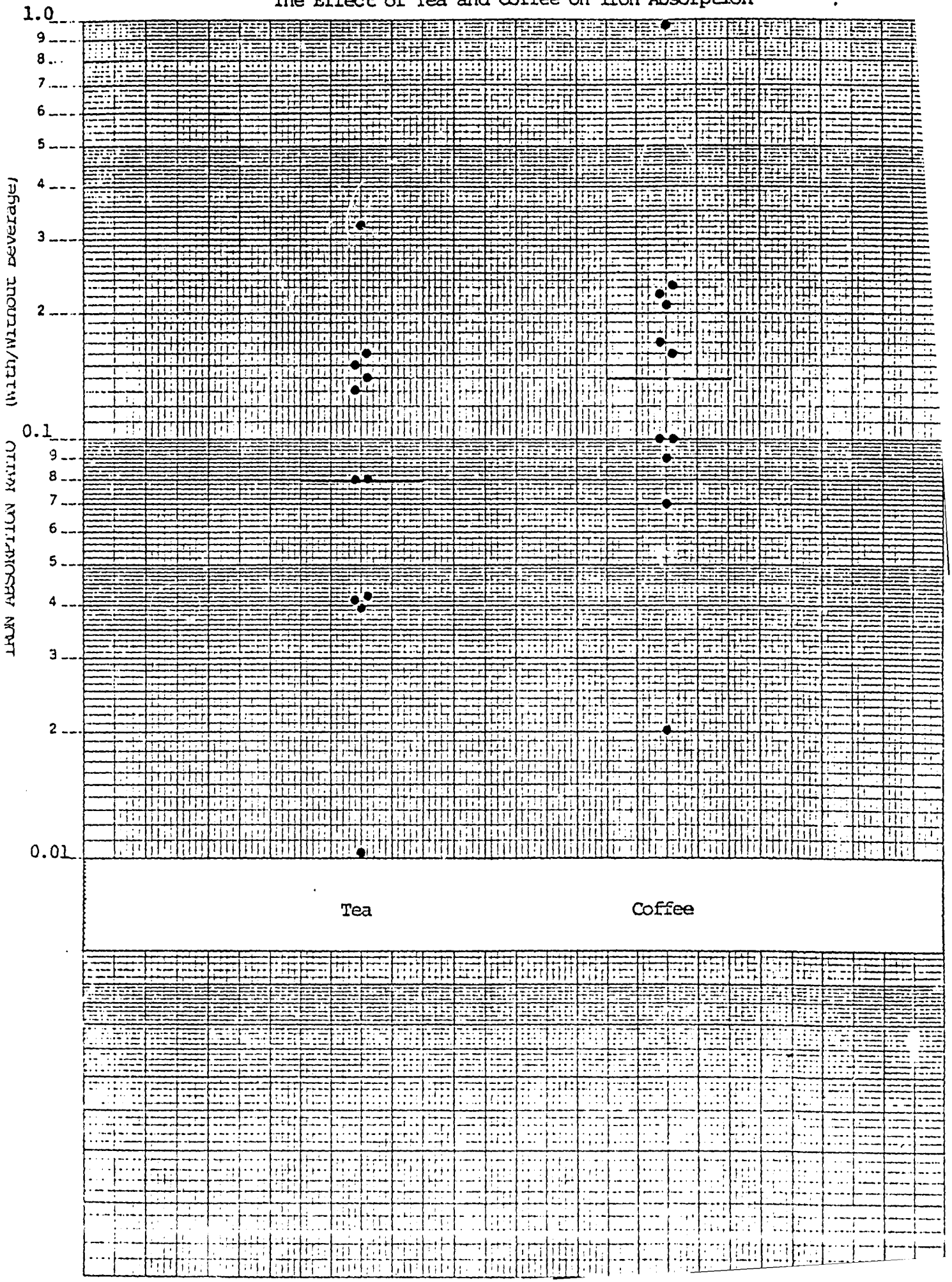
* geometric mean

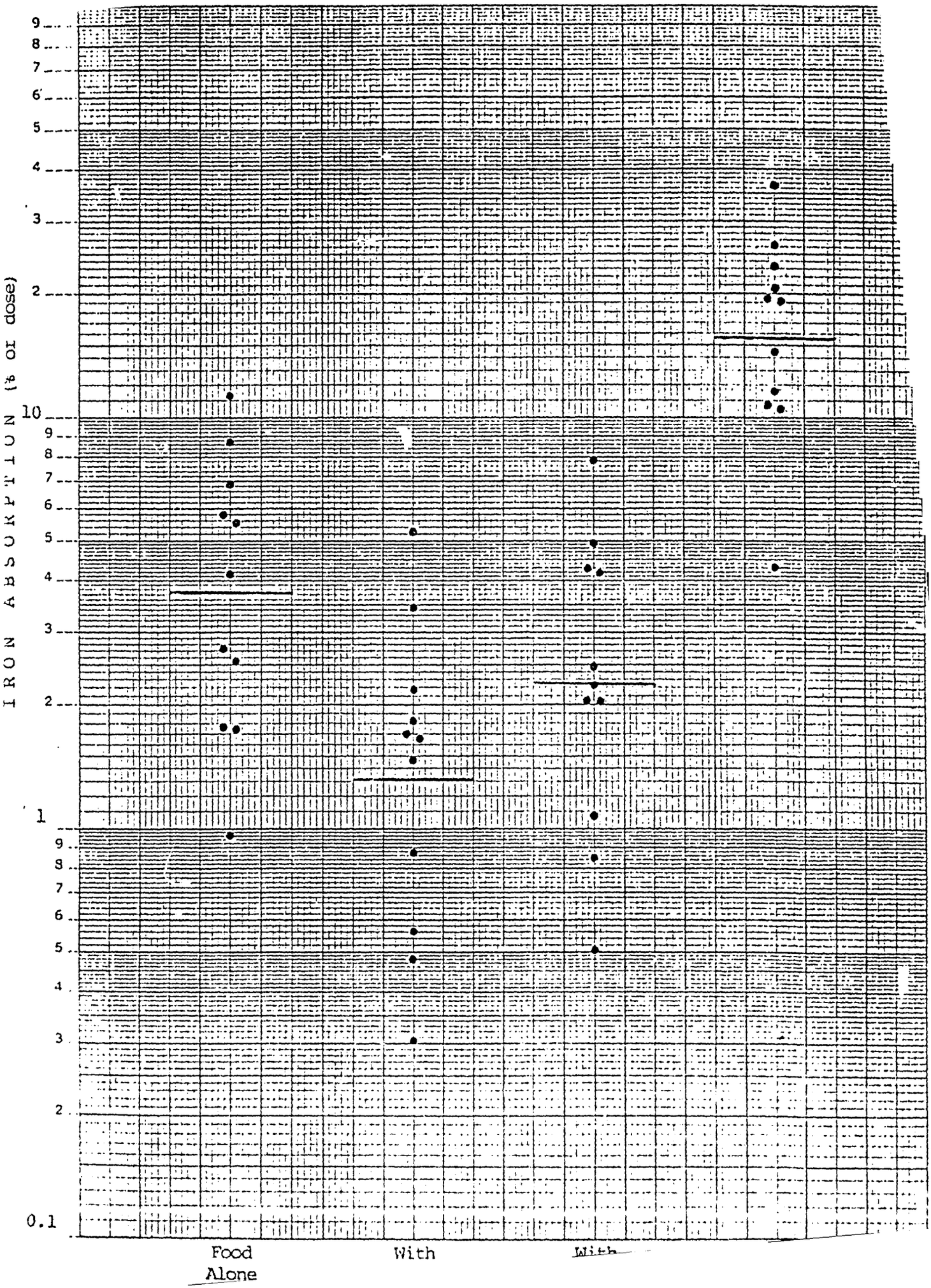
Study 9. The effect of tea and coffee on iron absorption.

	ABSORPTION RATIOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	2.81	1.65	0.24	0.59	0.08	0.14
-1SE	2.21	1.29	0.21	0.53	0.06	5.27
+1SE	3.58	2.11	0.27	0.65	0.11	9.25
1/Ratio	0.36	0.61	4.23	1.71	11.91	6.99
Student's t	4.29	2.03	10.21	4.86	9.25	6.92
P Value	0.0008	0.034	$<10^{6}$	0.0003	$<10^{5}$	0.00002
Correlation*(r)	0.524	0.470	0.793	0.907	0.290	0.142
P Value	0.049	0.072	0.0018	0.00006	0.193	0.338

*Log Scale

The Effect of Tea and Coffee on Iron Absorption




IRON ABSORPTION (% of dose)
9
8
7
6
5
4
3
2
10
9
8
7
6
5
4
3
2
1
9
8
7
6
5
4
3
2
0.1
Food Alone
With
With

Study 10

THE EFFECT OF VITAMIN A ON IRON ABSORPTION

SUMMARY

This study was prompted by recent evidence suggesting that iron status of a population may improve following vitamin A fortification. When 25,000 units of vitamin A were added to a solution of inorganic radiorion (ferric chloride) mean absorption fell from 5.81 to 4.22%; the mean absorption ratio of .73 (+/- vitamin A) was marginally significant (P = .052). When the same test doses were administered with a dinner roll, mean absorption fell from 3.67 to 2.80%; the mean absorption ratio of .76 (+/- vitamin A) was marginally significant (P < .01). It is concluded that vitamin A has a modest inhibitory effect on iron absorption in man.

RATIONALE

Recent prevalence studies have suggested an inter-relationship between vitamin A and hematologic status. Anemia occurs more frequently in children with vitamin A deficiency and an increase in hemoglobin levels has been observed following vitamin A fortification. Recent studies in small laboratory animals have provided additional evidence of an association between vitamin A and iron nutrition although they suggest that this relationship is due to a disturbance in internal iron kinetics in vitamin A deficient animals.

One explanation for an association between iron and vitamin A nutrition is a direct facilitation of iron absorption by the vitamin. Subjects with vitamin A deficiency may develop a defect in iron absorption or the vitamin may facilitate iron absorption in vitamin A replete individuals. This latter possibility was explored in this study by determining the effect of 25,000 IU vitamin A on the absorption of inorganic iron administered either with or without food.

DESIGN

Four iron absorption tests were performed in each of 12 volunteer subjects. All test meals contained 3 mg radioiron as ferric chloride ($FeCl_3 \cdot 6H_2O$) diluted in 50 ml water flavored with cherry syrup. A suspension of vitamin A was made by dispersing sufficient spray-dried vitamin A palmitate (Hoffman-La Roche) in 1 ml water containing 25,000 IU. Meals B and D were ingested with vitamin A which was allowed to incubate for 15 minutes at room temperature before ingestion.

The first pair of meals (A and B) contained only ferric chloride whereas with meals C and D, ferric chloride was administered with a dinner roll and margarine. In summary, the four test meals were as follows:

Meal	Roll	Vit. A
A	-	-
B	-	+
C	+	-
D	+	+

RESULTS

Absorption from ferric chloride administered alone averaged 5.81% with a range from 1.55 to 20.43%. Absorption fell moderately to a mean of 4.22% when vitamin A was ingested with the iron. The absorption ratio (+/- vitamin A) averaged .73 which was barely significant at the 5% level ($t = 1.78$, $P = .052$). When the same test meal was ingested with a dinner roll (meal C), mean absorption fell to 3.67% and to 2.80% when vitamin A was included (meal D). The mean absorption of .66 (+/- vitamin A) was significant at the 1% level. Thus, the inhibiting effect of vitamin A was remarkably similar whether or not bread was included with the test meal.

COMMENTS

The present study has excluded any enhancing effect of vitamin A on iron absorption whether administered as a solution of inorganic iron or ingested with a small meal. Rather, a slight inhibition in iron assimilation was observed when a large quantity of vitamin A was added. It is unlikely that any effect of vitamin A on iron absorption would occur at the lower levels used for vitamin A fortification. Obviously, the study does not exclude an interaction between vitamin A and iron nutrition but does exclude iron absorption as a basis for such an interaction.

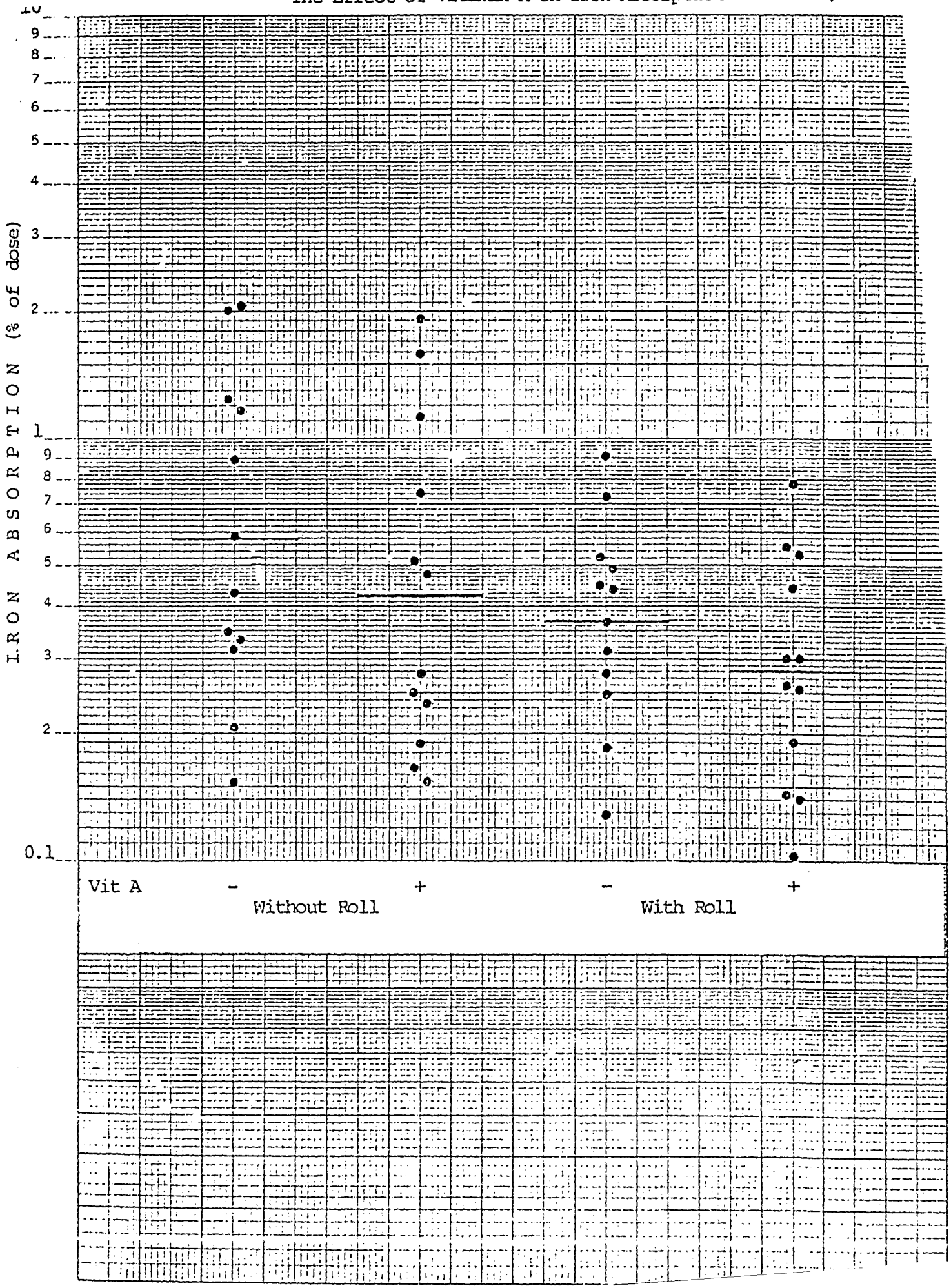
STUDY 10. The effect of Vitamin A on iron absorption.

Subject	Age	Packed Cell Volume (%)	Serum Iron (ug/dl)	Tsf Sat (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) Without Roll -Vit A(a)	Without Roll +Vit A(b)	With Roll -Vit A(c)	With Roll +Vit A(d)	Absorption Ratios b/a	d/c	c/a	d/b
1	36	44	77	18	120	1.55	1.66	1.80	1.91	1.07	1.06	1.16	1.15
2	29	44	175	57	78	2.02	2.35	5.20	5.83	1.16	1.12	2.57	2.48
3	44	45	139	38	108	3.18	2.50	1.28	0.96	0.78	0.75	0.40	0.38
4	24	43	103	29	79	3.30	2.78	2.80	1.38	0.84	0.49	0.84	0.49
5	25	44	132	45	155	3.43	1.56	7.38	4.41	0.45	0.59	2.15	2.82
6	21	44	144	43	40	4.38	4.75	3.70	2.60	1.08	0.70	0.84	0.54
7	24	46	194	49	73	5.92	7.53	2.45	2.56	1.27	1.04	0.41	0.33
8	21	48	194	45	32	8.80	11.27	4.87	5.55	1.28	1.13	0.55	0.49
9	23	45	64	17	155	11.58	5.01	3.12	1.43	0.43	0.45	0.26	0.28
10	25	45	103	26	71	12.28	1.88	4.40	3.00	0.15	0.68	0.35	1.59
11	24	45	171	40	36	20.06	10.58	4.43	3.01	0.52	0.67	0.22	0.28
12	24	44	100	24	35	20.43	19.07	9.11	7.77	0.93	0.85	0.44	0.40
MEAN	25	45	133	36	75*	5.81*	4.22*	3.67*	2.80*	0.73*	0.76*	0.63*	0.66*
					-1 SE	4.53	3.31	3.12	2.33	0.61	0.70	0.50	0.52
					+1 SE	7.46	5.38	4.32	3.37	0.87	0.84	0.79	0.84

*geometric mean

Study 10. The effect of vitamin A on iron absorption.

	ABSORPTION RATOS					
	a/b	a/c	a/d	b/c	b/d	c/d
Mean Ratio	1.38	1.58	2.07	1.15	1.50	1.31
-1SE	1.15	1.26	1.59	0.91	1.18	1.20
+1SE	1.65	1.98	2.69	1.46	1.91	1.43
1/Ratio	0.73	0.63	0.48	0.87	0.66	0.76
Student's t	1.78	2.04	2.77	0.58	1.71	2.98
P Value	0.052	0.033	0.009	0.285	0.058	0.006
Correlation*(r)	0.733	0.468	0.295	0.355	0.401	0.873
P Value	0.003	0.062	0.176	0.128	0.098	0.0001

*Log Scale

The Effect of Vitamin A on Iron Absorption