ICCNA PROGRESS REPORT AND WORK PLAN

IRON DEFICIENCY SUPPORT PROGRAM

AID COOPERATIVE AGREEMENT DAN-5115-A-00-7098-00

1991 - 1992

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ICCNA PROGRESS REPORT

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September 1991 - August 1992

The following report summarizes activities performed under the AID Cooperative Agreement from September 1, 1991 to August 31, 1992. The purpose of this program is to assist developing countries in assessing the prevalence of iron deficiency in different segments of the population and developing new approaches to combat nutritional iron deficiency anemia. The program at the International Center for the Control of Nutritional Anemia (ICCNA) is divided into intramural and extramural activities. The former is to develop more effective techniques for assessing the prevalence of iron deficiency in population studies and to develop more effective methods for fortifying food vehicles in populations at increased risk of nutritional anemia.

I. PUBLICATIONS

1. **Hurrell RF, Juillerat M, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. Am J Clin Nutr (In press).**

   Soy is a significant protein source in both industrialized and developing countries. Soy products provide a high quality protein of relatively low cost

and abundant supply and are used increasingly in infant formulas. One major disadvantage of this protein source, however, is its inhibiting effect on iron absorption. This property is shared by a wide range of soy products and has been studied extensively in this laboratory during the past decade.

The present study was undertaken to determine the extent to which the inhibiting effect of soy products is explained by its content of phytate. It was found that absorption from a liquid formula meal containing various soy isolates increased roughly 2-fold when the phytic acid content of the native product (500-1000 mg/100 g) was reduced below 20 mg/100 g by acid salt washing and ultracentrifugation. Restoration of the phytate level with phytic acid returned absorption to the original level. It was possible to achieve a phytate level of less than 1 mg/100 g using enzymatic treatment (phytase) and this resulted in a more dramatic 5-fold increase in iron absorption. These studies demonstrated that a significant increase in iron absorption occurs only after phytic acid is reduced to less than 10 mg/meal. It was also shown that even after removal of virtually all of the phytic acid, iron absorption from a soy containing meal was still only half that observed from an egg white control meal. These studies indicate that phytic acid is the major inhibitor of iron absorption in soy protein isolates but that other factors contribute to the poor bioavailability of soy containing products. These studies also indicate that the bioavailability of soy containing products can be substantially increased using enzymatic treatment. This is

of particular relevance to infant foods or infant formulas containing a high content of soy protein.

2. **Reddy MB, Cook JD. Assessment of dietary determinants in nonheme-iron absorption in humans and rats. Am J Clin Nutr 1991;54:723-728.**

Rats have been used extensively to evaluate the absorption of different forms of fortification iron but it has been much more difficult to predict dietary factors influencing nonheme iron availability in rats. In the present study, parallel measurements of iron absorption in humans and rats were performed using identical methodology and test meals to provide a direct assessment of the potential species differences in the response to dietary factors influencing nonheme iron absorption. Striking differences in the sensitivity of humans and rats to these effects were observed. A 2-fold enhancing effect of meat was seen in humans but no effect in rats. The addition of 100 mg of ascorbic acid increased absorption nearly 4-fold in humans as compared to only a 23% increase in rats. Similarly, tea, bran, and soy protein produced a dramatic inhibition of iron absorption in humans but had minimal effect on iron absorption in rats. Tea, bran, and soy protein produced a 20-30% decline in rats as compared to a 5- to 10-fold decrease in humans. The sensitivity of studies in rats was not enhanced by prior oral or parenteral iron loading to reduce the basal level of iron absorption from control meals. By eliminating any methodologic factors that might account for differences in the effect of dietary factors on iron absorption in rats and

humans, this study clearly demonstrated that the rat is not an adequate model for estimating bioavailability in humans. This finding is in keeping with prior evidence that rat absorption is qualitative and quantitatively different than humans. Unlike humans, rats absorb heme iron poorly, increase the concentration of mucosal transferrin with iron deficiency, and do not absorb ferrous iron preferentially. The results of this investigation show that although rats are often used to estimate the bioavailability of dietary nonheme iron, the results are not reliable. The absence of an effect of a specific dietary factor in rats cannot be taken as evidence against such an effect in humans. *In vitro* digestion methods may offer more promise as a screening method to identify factors affecting nonheme iron absorption in humans.

3. **Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 1991;54:717-722.**

A wide range of dietary factors are known to influence nonheme iron absorption including meat, ascorbic acid, polyphenols, phytate, fiber, and calcium. Depending on the meal content of these factors, percentage absorption can vary as much as 20-fold in the same individual. It has been assumed from studies using isolated meals that the type of diet consumed by adults has a significant influence on iron status. The present study was undertaken to explore the reason why epidemiologic studies of dietary

factors have generally failed to show an influence of the diet on iron status. It is possible that the methods for assessing dietary intake are too crude or that the number of determinants in the highly varied diet is too numerous to demonstrate a specific effect. Another possibility is that methods currently used to assess iron absorption exaggerate the effect of dietary factors affecting iron absorption. This latter possibility was examined in the present study by developing a technique for labelling the entire diet and comparing the results with single meal studies. The entire diet was labelled by having each subject consume a small radioactively tagged bread roll with each of the two main meals of the day over a 14 day period. Because it was necessary to eliminate any effect of iron status between three study groups consuming a self-selected, enhancing, or inhibiting diet, three different methods of correcting iron absorption were evaluated including serum ferritin measurements, absorption from a reference dose of inorganic iron, and absorption from a standard meal.

The results with single meal and dietary absorption were similar in subjects consuming a self-selected diet. However, single meal absorption overestimated the effect of enhancing or inhibiting factors as compared with complete dietary labelling. Thus, with single meal measurements, mean absorption ranged from 2.3 to 13.5% as compared to only 3.2 to 8.0% from the complete diet. It was also observed that the main factor affecting iron absorption was iron status as measured by the serum ferritin, not the diet. Thus, in this group of well-nourished, iron-replete subjects consuming an

optimal diet, no clear influence of the nature of the diet on iron absorption could be demonstrated. Caution should be used in extrapolating results of iron absorption studies from a diet typical for industrialized countries to regions where iron deficiency is highly prevalent. Differences in dietary composition undoubtedly contribute to geographic variations in the prevalence of iron deficiency in developing countries.

4. **Cook JD, Skikne BS, Baynes RD. Screening strategies for nutritional iron deficiency. In: Foman SJ, Zlotkin S, eds. Nutritional Anemias, Nestlé Nutrition Workshop Series, Vol. 30 New York:Raven Press, Ltd., 1992 (In press).**

In certain settings where the prevalence of nutritional iron deficiency is high, it may be more cost effective to screen the population at risk and supply iron only to those shown to be iron deficient. Whether screening is better than supplying iron to the entire population depends on several considerations but an important one is the availability of a sensitive and specific screening method at reasonable cost. In this overview of screening strategies, various laboratory approaches to screening for iron deficiency are reviewed, giving preference to those that can be performed by capillary sampling rather than venous sampling. When a single measurement is used, the hemoglobin concentration has been traditionally the method of choice but other more specific iron related measures such as serum ferritin, erythrocyte protoporphyrin, and serum transferrin receptor offer certain

advantages in respect to ease of performance and especially specificity. Of these, the serum transferrin receptor may be particularly useful because it only detects significant tissue iron deficiency and is not influenced by chronic inflammation. An even more reliable approach is to use two or more laboratory indices in tandem. The serum ferritin and hemoglobin combination is highly effective because if the serum ferritin is low in an anemic patient, iron deficiency anemia is unequivocally identified. The serum ferritin and serum receptor are also a powerful combination because they monitor the entire spectrum of iron status ranging from the iron replete individual to advanced iron deficiency.

5. **Cook JD, Baynes RD, Skikne BS. Iron deficiency and the measurement of iron status. Nutr Res Rev 1992 (In press).**

After reviewing the consequences of both a deficiency and excess in body iron, the focus of this extended review is on the choice of methods to assess iron status in population studies. The available techniques are discussed under the heading Storage Iron, Iron Transport, Red Cell Parameters, and Tissue Iron Need. The value of serum ferritin measurements as an index of iron stores is emphasized although it should be noted that the serum ferritin is a better measure of iron sufficiency than iron deficiency and is therefore particularly useful for examining changes in the iron replete segment of a population. Measurements of iron transport such as plasma iron, total iron binding capacity, and transferrin saturation

are too cumbersome and variable to be of value in large population studies. Various red cell parameters such as mean corpuscular volume and red cell distribution width are useful electronically generated determinations but the higher cost entailed make them less suitable in developing countries. The free erythrocyte protoporphyrin is a more cost effective approach and has certain advantages. The potential of the serum transferrin receptor as a survey measurement is extensively reviewed in this report including our experience with the assay technique, the effect of chronic infection, and its use in assessing iron status in pregnancy.

6. **Skikne BS, Cook JD. The effect of enhanced erythropoiesis on iron absorption. <u>J Lab Clin Med</u> (In press).**

A question that is often raised for individuals living at higher altitudes is whether their enhanced erythropoiesis has an influence on iron absorption and therefore iron status. The recent availability of recombinant erythropoietin provided an opportunity to examine the effect of enhanced erythropoiesis on iron absorption and thereby address this important question. In a group of normal subjects, the absorption of heme and nonheme iron was measured from a standard meal before and following a course of recombinant erythropoietin. The effect on absorption from a therapeutic dose of ferrous sulfate was also measured. Enhanced erythropoiesis had only a modest effect on the absorption of heme iron but a dramatic 5-fold increase on the absorption of nonheme dietary iron from

6 to 32%. The absorption of a therapeutic dose of ferrous sulfate (50 mg iron), a dose commonly used for iron supplementation in pregnancy, was increased from 2 to 18% when given with food and from 7 to 25% when given without food. The marked increase in absorption could be due to increased erythropoiesis or decreased iron stores because enhanced erythropoiesis was associated with a sharp drop in storage iron levels as measured by the serum ferritin. It was therefore necessary to introduce a correction for differences in iron status. When this was done it was shown that enhanced erythropoiesis did increase iron absorption independently of iron status, a 2.5-fold increase for dietary nonheme iron and a 3.0-fold increase with ferrous sulfate. This study provides clear evidence that iron absorption is enhanced by both iron deficiency and increased erythropoiesis. It also suggests that iron status will be more favorable in those living at higher elevations.

II. PRESENTATIONS

The following presentations and/or meetings heightened awareness of the importance of iron deficiency and served to convey the findings of recent studies at ICCNA on techniques to assess iron status and methods for alleviating iron deficiency.

1. Dr. Cook attended a workshop at Nestlé in Vevey, Switzerland from September 30 to October 4, 1991. In meetings attended by Drs. Hurrell,

Juillerat, Burri, and other collaborators at Nestlé, a review of iron absorption studies performed over the past year at ICCNA was made and the provisional program for the following six months was developed. An extended discussion was held in regard to collaborative work to identify the factor or factors in meat that promote the absorption of nonheme iron.

2. Dr. Cook attended a policy conference on micronutrient malnutrition entitled "Ending Hidden Hunger" held in Montréal, Québec, Canada on October 10-12, 1991. This large conference was co-sponsored by WHO, UNICEF, World Bank, Canadian International Development Agency, FAO, UNDP, and USAID. This international meeting was held to pursue the goals of the World Summit for Children held in New York in September 1990. The conference was held to emphasize the importance of all three micronutrients as a global health problem and did much to emphasize the importance of iron as one of the three major micronutrient deficiencies. An address by Dr. Ramalingaswami from the All India Institute of Medical Sciences was particularly important in this regard. The importance of iron deficiency was repeatedly stressed in this address and was similarly emphasized by other keynote speakers. This conference provided an opportunity to convey to health workers from many countries the activities of ICCNA and INACG in addressing the problem of global iron deficiency. The deliberations and major addresses in this conference are outlined in Appendix B-1.

3. Dr. Cook attended a research coordination meeting sponsored by the International Atomic Energy Agency (IAEA) in Vienna, Austria, October 29-31, 1991. The purpose of this conference was to coordinate global efforts at defining food iron bioavailability using isotopic methods. The meeting was attended by representatives from Chile, India, Myanmar, Pakistan, Peru, Philippines, Poland, Sri Lanka, and Venezuela. The conference provided an opportunity for an extended discussion of methods presently available to assess bioavailability and a summary of findings with these techniques. This meeting was the first of a series of meetings that will be held to coordinate methods to assess iron bioavailability in developing countries with emphasis on radioisotopic studies in humans using incorporated red cell radioactivity and whole body counting.

4. Dr. Cook attended a meeting at Stone Mountain, Georgia to discuss the Coordinated Strategies for Controlling Micronutrient Malnutrition, November 7-9, 1991. This conference was jointly sponsored by the International Life Science Institute (ILSI) and the Program Against Micronutrient Malnutrition (PAMM). Dr. Cook presented an overview of key developments of ICCNA in recent years with emphasis on the assessment of iron status using the serum transferrin receptor and the use of the gastric delivery system for iron supplementation in pregnancy. A monograph outlining the deliberations of this conference is included in Appendix B-2 and will be submitted in the coming year for publication.

5. Dr. Cook attended a conference in Washington, DC on January 15-17, 1992 at ILSI to discuss the preparation of a monograph on NaFeEDTA and its use for iron fortification. Collaborative studies of iron absorption with Professor T.C. Lee at Rutgers State University were also reviewed. The major focus of this collaboration was to develop a suitable method for fortifying rice with iron. Attendees at the conference included Drs. Sean Lynch, Richard Hurrell, T.C. Lee, Sam Kahn, and Suzanne Harris.

6. Dr. Cook was invited to attend a series of iron seminars in New Zealand from April 13-30, 1992. The purpose of these seminars was to draw attention to the liabilities of iron deficiency in a developed country. The conference was sponsored by the New Zealand Meat and Lamb Board who believe that the recent decline in meat consumption is resulting in a significant increase in the prevalence of iron deficiency. Seminars outlining the importance of iron deficiency were held in Auckland, Christchurch, Wellington, and Dunedin. Two separate videos outlining the impact of iron deficiency in children and young athletes was developed which provided convincing evidence for modifying the diet to improve iron balance.

7. Dr. Cook attended the FASEB Summer Research Conference on Trace Elements held in Copper Mt., Colorado, June 21-26, 1992. As an invited speaker, he presented a paper entitled "The Regulation of Iron Balance" outlining the differences in results obtained by single meal studies and by labelling the entire diet. This conference provided an opportunity to identify

interactions in trace element nutrition and will provide an important future vehicle to discuss the possible impact of iron fortification on other trace elements, particularly zinc and copper.

8. Dr. Cook was invited to present a paper at a conference on Nutrient Regulation During Pregnancy, Lactation, and Infant Growth held in Stockholm and Helsinki on August 9-12, 1992. Dr. Cook presented his recent findings on the use of serum transferrin receptor to assess iron status in pregnancy.

9. Drs. Cook and Skikne attended the 24th Congress of the International Society for Hematology held in London, England on August 23-27, 1992. Dr. Skikne presented a paper outlining the effect of enhanced erythropoiesis on the absorption of dietary heme and nonheme iron and on iron supplements.

III. FIELD PROGRAMS

1. Gastric Delivery System Evaluation

INVESTIGATOR: William Simmons, Ph.D.

LOCATION: Kingston, Jamaica

Work is now concluding in a large field trial in pregnant women to assess the hematological efficacy of a gastric delivery system (GDS) for iron

supplementation in pregnancy. The purpose of this survey was to determine whether a single tablet of GDS daily differed in hematological efficacy with two tablets of ferrous sulfate daily. The latter is the program for iron supplementation in pregnancy recommended in Jamaica. The participants in the study were randomized to a control group given folic acid alone, an $FeSO_4$ group given 50 mg elemental iron twice daily and a GDS group given a single capsule of 50 mg iron daily. A preliminary draft of the manuscript outlining results of the study was included in the 1990-1991 progress report. The analysis has been totally redone and the completed manuscript has now been submitted for publication (Appendix B-3).

The final analysis was performed in 376 pregnant women between 16 and 35 years of age and 14 to 22 weeks gestation. All women who agreed to participate in the trial were included if the hemoglobin concentration at the first clinic visit was between 80 and 110 g/L. Blood was obtained initially and after 6 and 12 weeks supplementation for measurements of red cell indices and iron parameters including the serum transferrin receptor.

The three study groups were remarkably similar with regard to numbers, drop-out rate, age, weight, and parity. However, in the placebo group, the gestational age was an average of one week earlier than the groups receiving iron. The groups were comparable in regard to baseline laboratory measurements except that the hemoglobin concentration in the no iron group was significantly higher than in the groups given iron (Table 2). The

transferrin saturation, serum ferritin, mean cell volume (MCV), and transferrin receptor, also indicated a better status at baseline in the placebo group; except for the difference in gestational age, this difference remains unexplained. Overall, the results showed a dramatic improvement in iron status in the groups given either ferrous sulfate or GDS, whereas iron status progressively deteriorated in women given no iron supplement. At the 12 week follow-up the only difference between the $FeSO_4$ and GDS groups was a slightly higher transferrin saturation in those given ferrous sulfate, presumably due to the more immediate release of iron to the circulation from a conventional iron tablet than from the GDS preparation. In sharp contrast, all data obtained in the no iron group showed a much higher prevalence of iron deficiency by any of several measurements. Measurements of the transferrin receptor were particularly useful in reflecting a progressive rise in the group given no iron, whereas a constant or slight fall occurred in the groups receiving iron. The change in hemoglobin was comparable in the two groups receiving iron although a slightly greater rise in hemoglobin was observed at 12 weeks in the women given GDS. It can be concluded that half the amount of iron given as GDS has equal or better efficacy than conventional ferrous sulfate tablets. Although this study was not designed to evaluate compliance (the iron tablets could be readily identified by both the participants and the investigator) the results do suggest that GDS will offer significant advantages for iron supplementation of pregnant women by eliminating

gastrointestinal side effects and reducing the frequency of administration of an iron supplement to once daily.

2. Serum Transferrin Receptor Measurements as an Index of Iron Status in Infants and Children

INVESTIGATOR: Tomas Walter, M.D.

LOCATION: Santiago, Chile

There is no available information at the present time concerning the utility of serum transferrin receptor measurements in infants, children, or adolescents. Consequently, during the past year we have entered a large collaborative project with Dr. Tomas Walter. Samples were obtained in several hundred preschool and school-aged children residing in Santiago, Chile. These samples have been collected over the past 3-5 years for a variety of investigations and have been stored at -20°C in the interim. A large battery of iron-related measurements have already been performed on these samples including red cell indices (MCV, red cell distribution width (RDW)), hemoglobin, serum iron, total iron binding capacity (TIBC), erythrocyte protoporphyrin, and serum ferritin. These samples were transferred to our laboratory in the spring of 1992 and analyses of both serum transferrin receptor and serum ferritin (repeat) are underway. One batch of these samples was obtained prior to measles immunization, and at 9 and 30 days follow-up. These samples were obtained in 12 month old infants and are designed to assess the possible effect of the inflammation

following routine immunization on serum receptor measurements. It is hoped that in contrast to an elevation seen in serum ferritin, the serum receptor will be unaffected by inflammation. The second study was performed in children 9 months to 5 years of age who were brought to the clinic with an acute febrile infection. Two samples were obtained, one at the time of admission to the clinic and a 30 day follow-up sample, the latter assumed to represent baseline after eradication of the infection. In addition to these two separate studies, a total of 1126 samples were obtained in infants at 6, 12, 15, and 18 months. This study will provide the first information about developmental changes of the serum transferrin receptor during infancy. It will also establish the utility of serum receptor measurements in children with inflammation. Finally, vitamin A determinations are being performed on many of these samples (CDC) and should provide some indication of the interaction between vitamin A and iron deficiency in infancy.

3. Efficacy of Iron Fortification of Wheat

INVESTIGATOR: William Simmons, Ph.D.

LOCATION: Grenada, W.I.

Substantial progress has been made during the past year in a study designed to evaluate the efficacy of fortifying baking flour in Grenada. Prior anemia surveys in this country in the mid-1980's demonstrated that iron deficiency was a serious nutritional problem with a prevalence ranging from

14% in young men to as high as 74% in pregnant women. As many as two-thirds of people in certain segments of the population have absent iron stores based on serum ferritin determinations. The staple foods in this population are wheat flour and wheat products. About one-half of the wheat consumed in the country is imported and milled locally but the fortification of this wheat depends on its distribution. All flour that is used commercially for baking is fortified to a level of 37 mg/kg flour whereas so-called "counter" flour, sold for home consumption is not fortified. The study outlined in Appendix B-4 is designed to measure the impact on iron status of the population of fortifying all counter flour with ferrous sulfate to a level of 44 mg/kg flour.

Beginning in April 1991, roughly 200 families from randomly selected enumeration districts were surveyed. A questionnaire was given that identified the age, sex, employment, income, and flour consumption of each participant. A finger-stick sample was obtained to assess iron status. A hemoglobin determination was performed with a Hemocue (Decentech, Inc., West St. Paul, MN) and the remainder of the sample was refrigerated for later harvesting of the serum for serum ferritin and serum transferrin receptor measurements. In a subset of this sample, a pilot study of the use of blood spot technology for sample storage and transfer was performed. These samples were stored and will be assayed later at ICCNA to evaluate the feasibility of using paper spot samples of serum or blood for analysis of ferritin and serum receptor.

Approximately 700 samples have been obtained as of late July, 1992. Unfortunately, a large proportion of these samples are hemolyzed apparently due to some delay in the harvesting the serum. This may necessitate repeating some, or all, of the initial survey.

4. Iron and Vitamin A Status of Preschoolers

INVESTIGATOR: Noel Solomons, M.D.

LOCATION: Guatemala City, Guatemala

In collaboration with Noel Solomons and his co-investigators as CeSSIAM in Guatemala and Kathryn Dewey, Department of Nutrition, University of California, Davis, an effort is being made to examine the correspondence in school-aged children between vitamin A and iron status. Prior studies have shown that as vitamin A deficiency is corrected, there is a significant improvement in hemoglobin levels suggesting a strong interaction between these two micronutrients. Samples are therefore being obtained in surveys in school-aged children in rural regions of Guatemala to assess vitamin A and iron status. In a preliminary trial performed in the fall of 1991, approximately 400 samples were assayed at ICCNA for serum ferritin and serum transferrin receptor. The serum ferritin measurements were uniformly low but within keeping with the degree of anemia in these children. However, serum receptor measurements were much lower than the normal range of measurements in our laboratory. The only reasonable explanation is that the samples had deteriorated at some stage during storage or

transport. Apparently, the samples had been thawed and refrozen several times in the process of removing serum for other studies including vitamin A measurements. This study demonstrates that the serum receptor is less durable with repeated freeze-thaw cycles than the serum ferritin and indicates the need for further studies of the optimal conditions for transporting samples in a field setting. As a follow-up investigation, a large number of samples are currently being obtained in Guatemala and will be hand carried to the United States in late August, 1992. Separate samples are being obtained for serum receptor measurements obviating the need for thawing and refreezing of serum prior to analysis. This second study will assist in identifying the optimal conditions for sample storage for serum receptor measurements.

5. Adherence to Iron Supplementation During Pregnancy

INVESTIGATOR: Eva-Charlotte Ekström

LOCATION: Dar es Salaam, Tanzania

In the Jamaican trial of GDS, emphasis was placed on hematologic efficacy of this new iron formulation. It remains to be established that the advantage of once-a-day administration and absence of gastrointestinal side effects is translated to a true difference in the adherence to iron supplementation by pregnant women. The ongoing trial in Tanzania is designed to address this question. The details of the investigation are outlined in Appendix B-5. All women registering at a maternal-child health (MCH) clinic at Ilula with a

gestational age less than 27 weeks will be given an opportunity to participate in this trial if their baseline hemoglobin is greater than 85 g/L. It is planned to enter 25 women each month for a total of 250 women. This study was initiated in late-spring 1992 and will run to the fall of 1993 if the accrual rate meets expectations. All women will be assigned randomly to receive either conventional iron or the GDS preparation. The GDS tablets are being supplied by Hoffman-La Roche, Basel, Switzerland, and have been specially prepared for this trial. The conventional ferrous sulfate preparation will provide 120 mg of elemental iron daily as recommended by WHO. The novel approach to this study is that adherence will be monitored by a system referred to as a "medication event monitor system" (MEMS). This consists of a microprocessor contained in the pill bottle cap that registers the time and date that each pill bottle is opened. Up to 1000 medication events can be registered with this computer providing information on tablet usage of which the participants are not aware. Preliminary data on just a few women suggests that adherence rate is surprisingly low for iron supplements. At intervals during the conduct of this study, samples will be obtained for measurements of serum ferritin and serum transferrin receptor that will be performed at ICCNA. Additional measurements of hemoglobin, hematocrit, and erythrocyte protoporphyrin are being performed in Tanzania. In addition, a large number of samples will be obtained on specialized filter paper for later analysis using "blood spot" technology. A measured portion of whole blood and plasma (30 µl) will be placed on the specially designed filter paper, stored at 4°C, and

transported to ICCNA for parallel measurements of serum ferritin and serum receptor. ICCNA has provided continuous technical support for this project.

6. Serum Transferrin Receptor in Adolescents

INVESTIGATOR: Leif Hallberg, M.D.

LOCATION: Göteborg, Sweden

A large trial has just been completed to assess the value of serum transferrin receptor in teenagers aged 15 to 16 living in Sweden where there is a high level of iron fortification of wheat products. Measurements of receptor and several additional iron related measurements have been performed in approximately 450 girls and boys. The purpose of the study is to assess the potential value of serum receptor in defining iron status at this susceptible age. The preliminary findings are outlined in Appendix B-6. Measurements of serum ferritin, hemoglobin, MCV, mean cell hemoglobin, transferrin saturation, and TIBC have been performed to permit comparisons with the serum receptor. One of the novel findings in this investigation is that the transferrin receptor was significantly higher in boys than girls at this particular age. This contrasts with results in adults that have repeatedly failed to show a sex difference in receptor measurements. The difference appears to be related to the higher androgenic stimulus of erythropoiesis in boys and provides further evidence that the transferrin receptor reflects the rate of erythropoiesis in addition to iron status. The results of this investigation are now being prepared for publication.

IV. INTRAMURAL PROGRAM

1. Assessment of Iron Status

An increasing proportion of the work conducted at ICCNA during the past year has entailed the preparation of standards and immunologic reagents for serum transferrin receptor measurements. This has been necessitated by a sharp increase in the number of samples being obtained in field studies and an increasing demand for these reagents in local laboratories. For example, the Caribbean Food and Nutrition Institute will require a large number of reagents for measurements of serum ferritin and serum receptor in conjunction with the wheat fortification project in Grenada. Beginning in the fall of 1991, work was initiated in our laboratory with a view to developing a filter paper method or "blood spot" technique for assessing iron status. The principle is that a small sample of either whole blood or plasma (e.g. 30 µl) can be placed on a specialized filter paper of fixed thickness and allowed to dry. These papers are stored at ambient temperature, and forwarded to a central laboratory where the blood spot is then eluted in a buffer. Measurements of serum ferritin and serum transferrin receptor are then performed for assessment of iron status. When coupled with a hemoglobin determination performed in the field using the Hemocue apparatus, a complete and precise quantitative evaluation of body iron status can be obtained.

In the past several months, we have undertaken studies to determine whether this blood spot technology is feasible. The work was initiated by obtaining a number of filter papers, available commercially for this purpose, to compare the reproducability of the elution procedure in recovering a spotted blood sample. A series of studies were then performed to determine the relative advantages of eluting the total sample as opposed to cutting out a disc within the spotted sample as is currently used for TSH measurements in evaluating iodine status. Disc sampling from a large blood spot showed a high variability of eluted hemoglobin and/or serum proteins indicating variation in the diffusion rate of hemoglobin and serum. Based on these studies it was concluded that it was necessary to elute the total sample spotted on the paper in a constant volume of 20-30 µl.

Because of the potential advantage of using whole blood for the spot determination rather than plasma, an extensive study was undertaken to compare measurements of serum ferritin and serum transferrin receptor in whole blood, serum, and washed red cells before and after hemolysis. It was found that serum transferrin receptor measurements on whole blood gave a reliable indication of the concentration in serum when corrected for hematocrit values. That is, the hemolyzed red cells contributed no additional transferrin receptor to serum. However, major difficulties were encountered in attempting to use whole blood measurements of ferritin to predict serum ferritin values. Complete hemolysis of red cells occurred when the blood sample was allowed to dry on the filter paper and in contrast to receptor

measurements, large amounts of intracellular red cell ferritin were released to the serum. A series of studies was next performed to determine whether a distinction between red cell ferritin and serum ferritin could be made on the basis of differences in the concentration of heart and liver ferritin which can be measured separately in our laboratory using specific monoclonal antibodies. This calculation is of potential use because most of the red cell ferritin is in the acidic or H form whereas most of the serum is in the basic or L form of ferritin. A total of 60-80 samples obtained clinically were then processed using both serum and paper analyses and measurements of transferrin receptor, L-ferritin, and H-ferritin concentrations. These studies indicated that a reasonable estimate of serum ferritin levels can be derived from measurements on a whole blood sample but that a substantial degree of precision is lost in this derived estimate. Consequently, although the use of whole blood for filter paper samples has not been excluded, our emphasis will be placed on eluting a fixed volume of 30 µl of plasma obtained by capillary sampling.

A series of studies was next undertaken to determine the effect of different storage conditions on measurements recovered from a blood spot sample. Spots of whole blood and serum were placed on filter paper and stored under varying conditions of temperature and humidity. It was found that measurements of ferritin and receptor remained stable for up to six weeks when stored at 4°C or at ambient temperature (approximately 75°C) or at a higher temperature (90°C) in the presence of a desiccant. However, the

combination of high humidity and elevated temperature led to rapid deterioration of the sample with regard to ferritin and receptor assays. These storage measurements are continuing but our preliminary studies suggest that filter paper specimens can be stored for prolonged periods at room temperature, if placed in a bag containing a desiccant to keep the humidity reduced, or for much longer periods at 4°C.

2. Iron Fortification Strategies

We continue our highly effective collaboration with Nestlé, Vevey, Switzerland in a program designed to develop newer methods of fortifying infant foods and food vehicles suitable for delivering added iron to children and adolescents. These studies are conducted in human volunteer subjects using test meals labelled with radioactive iron. The method has been described in several of the appended publications. During the past year, pilot studies were performed in conjunction with Professor T.C. Lee at Rutgers State University to determine the bioavailability of an iron fortified simulated rice grain. In a proposed program of **rice fortification**, this simulated rice granule will be diluted 1 to 100 with unfortified rice to deliver up to several milligrams of added iron daily. Studies in the Rutgers laboratory during the past year have suggested that the combined use of iron pyrophosphate, which is poorly available but causes no color or taste changes when added to rice, and EDTA, to promote absorption, might be a suitable strategy. Pilot studies with this double fortification strategy of iron

pyrophosphate and EDTA appeared very encouraging with regard to the customary problems of discoloration and unacceptable taste. Pilot absorption studies were therefore undertaken in this laboratory using radiolabelled iron pyrophosphate with and without added EDTA. In the first study, done with wheat cereal, it was found that the availability of iron pyrophosphate was exceedingly low (mean absorption <0.5%) and was not significantly improved by adding iron EDTA to the meal. A second absorption study in which extrinsically labelled tags were used was performed with rice separately fortified with either non-radioactive iron pyrophosphate or EDTA supplied from the Rutgers laboratory. Once again, no evidence was obtained that iron was released from iron pyrophosphate in a bioavailable form. It was decided to discontinue further bioavailability studies using this fortification strategy.

Additional studies were performed during the past year to identify a more effective means of **fortifying a chocolate drink powder** with iron. Previous studies have shown that ferrous fumarate can be used for this purpose but organoleptic problems developed when the chocolate powder was dissolved in boiling water. During the past year, a chocolate drink powder was fortified with either ferrous succinate or ferrous saccharate, which are known to be highly bioavailable and do not cause untoward changes in color or taste. Absorption studies were performed with a chocolate drink powder which had been fortified before and after processing with these two forms of iron. It was found that the availability of ferrous succinate was at least as good as

ferrous sulfate and we therefore identified this iron compound as a highly effective means of supplying fortification iron to children and adolescents.

Studies have also continued to better define the potential value of NaFeEDTA when used to fortify infant cereals. Because of a continuing concern that EDTA may have some adverse effects on the nutrition of other trace metals, we undertook studies to determined whether a reduction in the molar ratio of EDTA:iron would maintain the same absorptive advantage of EDTA while reducing the quantity of chelate used. We were able to show that a 30-50% reduction in molar ratio maintains the same level of elevated absorption as a 1:1 molar ratio. This observation is now being extended to two other food vehicles with similar findings although the ability to use a lower molar ratio of EDTA:iron is affected substantially by the food vehicle. These studies, in which varying combinations of EDTA and iron are used for fortification, are now being prepared for publication.

A final area that appears very promising with regard to fortification is processing cereals in a manner that reduces their inhibiting effect on iron absorption. In a series of studies over the past two years, we have identified phytic acid as a key inhibitor in many infant cereals and particularly soy products. Using standard chemical techniques, it has not been possible to reduce the level of cereal phytate to the point that a significant increase in iron absorption occurs. However, we have found that commercially available phytases are able to reduce the phytate content to

<1% of the native level and produce a striking enhancement of iron availability. We have now confirmed this improvement with infant cereals prepared from oats and soy. Additional studies will continue in this area during the coming year.

ICCNA WORK PLAN

IRON DEFICIENCY SUPPORT PROGRAM

AID COOPERATIVE AGREEMENT DAN-5115-A-00-7098-00

SEPTEMBER 1992 - AUGUST 1993

The following is an outline of the work proposed for the following 12 months under the Iron Deficiency Support Project beginning in September 1992. The proposed studies are divided into Extramural Programs or field studies, and Intramural Programs designed to refine methods for assessing iron status and developing more effective strategies for fortifying food vehicles.

I. EXTRAMURAL PROGRAMS (Field Studies)

1. Iron Status in Infants and Preschoolers - Chile

As discussed in Section III,2, we have developed a highly effective collaboration with Tomas Walter and his colleagues at INTA, Santiago, Chile. The purpose of these studies is to define the value of serum receptor measurements in assessing iron status in infancy and determining the extent to which this new measurement is able to separate infants with anemia secondary to chronic infection from those with true iron deficiency anemia. Samples are being collected in this collaborative study in children presenting with acute febrile illness and follow-up samples are being

obtained several weeks later when the infection has been fully resolved. Based on our published experience in adult patients with the anemia of chronic disease, we anticipate that the serum transferrin receptor will not be influenced by acute infection and will therefore remain a valid measure of iron status in infancy. A further goal of this collaborative effort is to establish the developmental changes in transferrin receptor during the first two years of life. A large number of samples are being collected in infants at birth and at 3, 6, 9, 12, 18, and 24 months of age. Several iron-related measurements are being performed in Chile and will be compared with serum receptor measurements performed at ICCNA. Over 1000 samples have now been collected in this collaborative study that will constitute a major portion of laboratory work at ICCNA during the coming 12 months.

2. Effect of Coffee Consumption on Iron Status - Guatemala City

Work is continuing on a project designed to evaluate the potential adverse affects of coffee consumption in preschool children. This is a collaborative program between the University of California, Davis (Kathryn Dewey) and CeSSIAM, Guatemala (Noel Solomons) and ICCNA with the objective to determine whether coffee consumption during infancy impairs iron status. Coffee is often given as early as two months of age either with foods or by bottle or cup. A total of 100 mother-infant pairs are presently being recruited in outpatient clinics at three hospitals serving low-income families in Guatemala City. To determine the effect of coffee consumption, blood

samples will be collected by venipuncture for subsequent measurements of hematocrit, erythrocyte protoporphyrin, and plasma ferritin. Serum transferrin receptor measurements will be performed at ICCNA and, in addition, it is likely that our laboratory will perform plasma ferritin measurements. Funding for this collaborative effort has been slow in developing but the study is now initiated and it is anticipated that most of the work in this trial will be completed during the following 12 months.

3. Effect of Iron Status and Placental and Infant Size - Jamaica

Convincing evidence has been obtained in recent years that iron deficiency anemia in the pregnant mother leads to a sharp increase in the rate of prematurity. There is also some preliminary evidence that anemia during pregnancy leads to an increase in placental size and a consequent reduction in fetal size. In a planned collaborative program with Terrance Forrester at the Tropical Metabolism Research Unit (TMRU) at the University of the West Indies, Kingston, Jamaica, it is planned to undertake sonographic measurements of placental size during gestation and correlate the findings with measurements of iron status including the serum transferrin receptor level. It is planned to perform serum receptor measurements and serum ferritin determinations at three points during the second and third trimester of pregnancy in an attempt to correlate placental size as measured by sonography and iron status. The study is still in the

planning stages but it is hoped that sampling will begin in the late fall of 1992.

4. Iron Supplementation of School Children in Jamaica

The objective of this proposed trial is to reduce the prevalence of iron deficiency anemia in school-aged children in Jamaica by supplementing the diet with fortified food products. Baseline studies will be performed to determine the nutrient intake of school-aged children living in Kingston, Jamaica to determine the typical diet and the iron intake. In a school feeding program currently in operation in Kingston, Jamaica, approximately 25,000 children attending primary schools receive a snack at lunch time consisting of a bun and a half pint of milk. The proposed study will determine the impact of using an iron fortified chocolate milk powder in combination with this lunch program. The details of this proposed study, to be funded by Nestlé, are outlined in Appendix B-8. Measurements of iron status, including serum ferritin and serum transferrin receptor, will be performed in collaboration with ICCNA. A random sample of school-aged children will be chosen for the food consumption study and dietary data collected by 24 hour recall. A finger-stick sample of blood will be obtained for hemoglobin determination. It is planned to enroll 240 children between the ages of 6 and 14 years from four separate schools. A control group will be fed one glass of skim milk containing unfortified milo, a second group milk with iron fortified milo, a third group the traditional school feeding, and

a fourth group a school lunch consisting of milk and a bun. The feeding trial will continue for 10 months and the impact of this fortification strategy will be determined by laboratory measurements as described in the proposed study.

5. Gastric Delivery System Trial - Indonesia

It is proposed to determine, in a field trial, whether the gastric delivery system (GDS) for iron supplementation is effective in improving hemoglobin levels in a population in Indramayu, West Java, and to determine whether the GDS improves the compliance rate as compared with the standard ferrous sulfate tablet. This trial will be conducted in Surabaya, Indonesia under support from the MotherCare Program, AID. The first trial will be conducted in anemic pregnant women who will be randomized among a group given GDS (50 mg iron daily) for 120 days during the last four months of pregnancy and a second group given standard ferrous sulfate therapy (60 mg elemental iron daily) for the same period of time. This trial differs from that recently concluded in Jamaica in that the total amount of iron consumed in the two groups will be similar. Both groups of women will be seen every 30 days. Hemoglobin, ferritin, and serum transferrin measurements will be performed on blood samples obtained at each visit and these analyses will be performed at ICCNA. The details of this first trial are outlined in Appendix B-9. A second trial will be performed in anemic non-pregnant women and will involve three groups. The first will be given

GDS iron for 90 days, the second, ferrous sulfate for the same period of time, and the third group will receive an identical appearing placebo capsule. The study will be double-blind in that neither the women nor the researcher will know which iron preparation is supplied. At the end of the trial, measurements of hemoglobin, ferritin, and serum transferrin receptor will be performed to establish hematologic efficacy. As in the first trial, all samples will be sent to ICCNA to perform the immunologic assays (ferritin and transferrin). The total number of samples is as follows. In the first trial, 300 pregnant women are divided into two study groups and examined at five separate times to provide 1500 blood samples. The infants of these women will be studied on two occasions to provide an additional 600 samples or a total of 2100 samples for serum transferrin receptor and an equal number for serum ferritin. In the second trial, 300 women divided into three study groups and tested on three separate occasions will provide a total of 900 determinations for serum and equal number of serum ferritin determinations. Funding for this project will be provided by the MotherCare Program.

II. INTRAMURAL PROGRAMS

1. Assessment of Iron Status

As discussed previously in the progress report for 1991-1992, provisional technology for the filter paper technique of sample storage and transport

has been established. During the coming year we will evaluate this technology in two large field studies, the details of which are included in Appendix B-4 and B-5. The first evaluation will be performed in conjunction with the wheat fortification project in Grenada. As outlined in that protocol, between 700-1000 samples will be obtained during the baseline study of nutritional iron status. In approximately one-third of the sampled individuals, an additional sample of both blood and serum will be obtained by capillary sampling for measurement in whole blood of H-ferritin, L-ferritin, and transferrin receptor and these same measurements will also be performed on serum. Three additional spots will be obtained for the filter paper at the time of this sampling; one unmeasured sample of whole blood, a 30 µl sample of whole blood, and a 30 µl sample of plasma. The unmeasured blood sample will be used to obtain duplicate disc samples with a paper punch while the total sample will be eluted from the measured volume of whole blood and plasma sample. These three measurements on three spots will therefore involve nine assays or a total of 15 assays per sample. Approximately 300 individuals will be surveyed with this technology, as many as 5000 immunoassays will be performed at ICCNA as part of this evaluation of the filter paper technique. This is a larger number of determinations than can reasonably be performed at the current funding level during a 12 month period and as a result, this evaluation will be done in stages. From 30-40 samples will be used to obtain the complete set of measurements and it is hoped that depending on the findings, some of these measurements can be curtailed after the preliminary results have

permitted exclusion of blood spots that do not give a reliable index of serum measurements.

A similar study is planned in conjunction with the GDS trial in Tanzania. In that trial, a measured volume of whole blood and of plasma is spotted on filter paper together with samples of whole blood and serum obtained by venous sampling. As described in the above study in Grenada, three measurements on blood, serum, spotted blood, and spotted serum, will be performed giving a total of approximately 12 assays per surveyed individual. It is hoped that the findings in the Grenada study will permit some reduction in the number of assays in this study which could total 2000 given the planned number of 200 surveyed pregnant women. This study, however, will serve to further validate the filter paper technology because of differences in the population, technique for acquiring the sample, and conditions for the storage and transport of the filter paper sample. The experience obtained in the Grenada and Tanzania studies should fully define the potential of filter paper sampling to assess iron status in the field.

The final area of potential activity during the coming year is to further define the normal range of serum transferrin levels in varying age and sex groups within an iron replete population. It is essential to acquire more data in a normal population to provide a means of evaluating changes in these measurements when performed in iron deficient populations. With this in mind, we have undertaken collaborative arrangements with CDC to perform

serum transferrin receptor measurements on sera collected as part of Health and Nutrition Examination Survey III (HANES III). Several thousand samples collected from all age groups are available in this collaborative study. The significance of serum transferrin receptor levels can be assessed by the large battery of iron related measurements that are being performed at CDC in connection with HANES III. It is unlikely that there is sufficient laboratory help available to make a significant impact on this task but given the importance of defining the normal range of serum transferrin receptor in various age and sex groups, we will make an effort to do this.

2. Fortification and Food Iron Availability

Several new collaborative undertakings with Nestlé are planned in the coming year in addition to concluding our current studies of the effect of phytate removal on iron availability from infant cereals and the use of diminished molar ratios of EDTA:iron for fortification.

One of the new areas is to assess the iron bioavailability of **sorghum** that is used as a food staple in large regions of Africa. We anticipate that the iron availability from sorghum will be much reduced as compared to other staple foods because of its content of phytate and polyphenols, both potent inhibitors of nonheme iron absorption. However, because of the widespread consumption of this staple food, we have imported several varieties of sorghum from Central Africa and are currently performing measurements

of phytate and polyphenol content on these samples. We will select those that have the broadest range of phytate and polyphenol content and do baseline studies of food iron absorption in human subjects using an extrinsic radioiron tag. The major thrust of this project, however, will be to determine whether various techniques for fermenting sorghum in its preparation as a food item influences nonheme iron absorption. The notion that fermentation may sharply improve the bioavailability of sorghum comes from *in vitro* studies reported within the last two years. Fermentation will have to be used to reduce the phytate content rather than phytase because processing large quantities of sorghum for population consumption is not technically feasible. However, if we can define a technique of fermentation that reduces phytate content and thereby enhances bioavailability, it could have a major impact on the iron status in regions where this food is widely consumed.

Another new activity that will be undertaken in the coming year is an attempt to identify the component in animal tissue that enhances the bioavailability of nonheme iron. It has been known for many years that certain animal foods such as red meat strongly promote the absorption of nonheme iron but the nature of this enhancing effect remains undefined. Many believe that it due to the chelating action of peptides released in the gastrointestinal tract following the digestion of meat protein. It is known that cysteine can form complexes with iron that promote iron bioavailability and it is reasonable to suspect that peptides containing three to ten amino acid

residues, including cysteine, may account for the promoting effect of meat. In the first phase of this study, various animal tissues have been obtained by Nestlé and lyophilized in large quantities to serve as a baseline for studies of iron availability in humans. These tissues include beef muscle, beef liver, turkey, and chicken. By comparing the promoting effect of these lyophilized sources of animal tissue, it should be possible to determine whether the promoting effect is due to a specific protein, for example actinomycin in muscle, or whether it is a common property of all animal tissues. Depending on the relative promoting effect of different whole animal tissues, various fractionation procedures will be performed at Nestlé to provide a range of animal tissue components in an attempt to define that fraction that is most active in promoting iron absorption. This project will be coupled with measurements using high performance liquid chromatography (HPLC) to define those proteins and/or peptides that are most active in binding iron. In addition, we will attempt to establish a reliable screening method, in the laboratory, using either guinea pigs, cannulated domestic swine, or variations of *in vitro* techniques. This is a large undertaking but the potential of defining a peptide which can then be synthesized and added to a food to promote its availability has enormous potential for alleviating iron deficiency in the developing world.

Studies are continuing in the Rutgers laboratory to search for alternate methods of fortifying rice with iron. As outlined earlier in this report, double fortification with iron pyrophosphate and EDTA did not appear promising

with regard to bioavailability. Presently, Professor Lee is working on a technique of fortifying rice with highly available ferrous sulfate. While this strategy in the past has provided a simulated rice granule that is unacceptable because of color changes, he has found that by reducing the pH of the added ferrous sulfate, a colorless simulated rice granule can be prepared. If these findings can be confirmed in the next few months, we will undertake a series of human studies to examine the bioavailability of the simulated rice granule in the same manner as was performed with iron pyrophosphate during the past year.

A final area of potential activity is to develop a set of chemical measurements to define the bioavailability of regional diets. Studies during the past few years have suggested that there are a limited number of specific biochemical determinants of nonheme iron availability in food of which ascorbic acid, the meat factor, polyphenol content, and phytate content are the most important. In recent months, we have been performing human absorption studies with a series of complex meals. Large quantities of these foods have been lyophilized for parallel studies of *in vitro* iron availability and planned studies of iron absorption in small laboratory animals. We have now established reliable methods for assaying the food content of ascorbic acid, iron binding polyphenolic complexes, phytate, and heme and nonheme iron. It is planned to develop algorithms by which the nonheme iron bioavailability in human subjects can be predicted by these chemical determinations. Although this is also a long-term project, it is an

essential part of a global program to develop more effective interventions to combat iron deficiency. It is believed that the choice between iron supplementation and fortification will depend, to a large extent, on the inhibiting properties of the local diet. While it is impractical to perform radioisotopic measurements of iron absorption in all regional diets, it is entirely feasible to perform a series of biochemical determinations on samples of a local diet to determine the degree of its inhibiting effect. Given the experience with measurements of food iron bioavailability developed in this laboratory during the past two decades, we are cautiously optimistic that we can develop a set of laboratory measurements to reliably predict nonheme bioavailability of different diets.

Soy protein, phytate, and iron absorption in humans[1-3]

Richard F Hurrell, Marcel-A Juillerat, Manju B Reddy, Sean R Lynch, Sandra A Dassenko, and James D Cook

ABSTRACT The effect of reducing the phytate in soy-protein isolates on nonheme-iron absorption was examined in 32 human subjects. Iron absorption was measured by using an extrinsic radioiron label in liquid-formula meals containing hydrolyzed corn starch, corn oil, and either egg white or one of a series of soy-protein isolates with different phytate contents. Iron absorption increased four- to fivefold when phytic acid was reduced from its native amount of 4.9–8.4 to < 0.01 mg/g of isolate. Even relatively small quantities of residual phytate were strongly inhibitory and phytic acid had to be reduced to < 0.3 mg/g of isolate (corresponding to < 10 mg phytic acid/meal) before a meaningful increase in iron absorption was observed. However, even after removal of virtually all the phytic acid, iron absorption from the soy-protein meal was still only half that of the egg white control. It is concluded that phytic acid is a major inhibitory factor of iron absorption in soy-protein isolates but that other factors contribute to the poor bioavailability of iron from these products. *Am J Clin Nutr* 1992:56:000–000.

KEY WORDS Soy-protein isolate, phytate, iron absorption

Introduction

Soy protein is a major ingredient in infant formulas especially in the United States where soy formulas now account for about one-quarter of infant-formula sales (1). The use of soy protein is also increasing in extended meat products, baked goods, and dairy-type foods. Good protein quality, low cost, plentiful supply, and excellent functional properties make it an attractive raw material for the development of new manufactured foods (2). One potential drawback to the use of soy protein is that it has an inhibitory effect on iron absorption in humans (3–7). Full-fat soy flour, textured soy flour, and isolated soy protein all markedly reduce nonheme-iron absorption. The isolated protein has the greatest inhibitory effect (3).

The nature of the substances in soybean products that inhibit iron absorption is unclear. However, soy-protein products are known to contain appreciable quantities of phytate, which is an important inhibitor of iron absorption in wheat bran (8).

The present study was designed to define the role of phytate in modifying nonheme-iron absorption from soybean-protein isolates in humans. A series of soy-protein isolates with a 1000-fold variation in phytate content were prepared. Nonheme-iron absorption from liquid-formula meals containing these soy-protein isolates was then measured in human volunteers with a radioisotopic method.

Materials and methods

Preparation of soybean-protein isolates

Eleven different soybean-protein isolates were prepared from three different batches of soy flour (**Table 1**). Isolates I–IV were standard isolates containing much of their native amount of phytic acid (4.9–8.4 mg/g of isolate). Isolates V–VII were low-phytate isolates (0.2–1.0 mg phytic acid/g) in which the phytic acid had been reduced by continuous acid-salt washing and ultrafiltration. In isolate VIII the phytic acid was reduced to < 0.01 mg/g by enzyme treatment. Isolate IX (< 0.01 mg phytic acid/g) was both enzyme treated and ultrafiltered. Finally, in isolates X and XI, phytic acid was restored to approximately its original amount by adding back sodium phytate to the low phytate isolates V and VIII, respectively. Isolates I and V were prepared from the first batch of soy flour and were fed in study 1. Isolates II, VI, and X were made from the second batch of soy flour and were fed in study 2. The remaining isolates were made from the third batch of soy flour and were fed in studies 3 and 4.

All isolates (≈1–2 kg) were prepared from commercial defatted soybean flour that was first soaked for 1 h in deionized water (flour to water ratio 1:7.5, wt:wt) and then centrifuged in a continuous system at 12000 × *g* at 37°C to remove the fibrous material. For the native phytate isolates (I–IV), the resulting soybean milk was adjusted to pH 5.2 to precipitate the protein, which was recovered by centrifugation, washed with deionized water, neutralized with potassium hydroxide, sterilized by steam injection at 140 °C, and spray-dried. The washing step removes some phytic acid and is the reason why our native phytate isolates contain 4.9–8.4 mg phytic acid/g compared with 9.0–17.0 mg/g in commercial isolates. Those isolates in which the phytate was removed by acid-salt washing and ultrafiltration (V–VII) were prepared in the same way except that after precipitation the protein fraction was ultrafiltered in a two-step process at pH 5.2 and pH 7 in the presence of sodium chloride (9).

For isolates in which the phytate was removed by enzyme treatment (VII and IX), the soybean milk was treated at pH 5.2

[1] From Nestec Ltd, Nestlé Research Centre, Lausanne, Switzerland, and the University of Kansas Medical Center, Kansas City, KS 66103.

[2] Supported by NIH grant DK39246 and AID Cooperative Agreement DAN-515-A-00-7908-00.

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TABLE 1
Analytical data on soybean-protein isolates*

Soy-isolate fraction†	Percent crude protein	Phytic acid	Trypsin inhibitor	Iron	Calcium	Inorganic phosphorus
	%, N × 6.25	mg/g	TIU/mg N	mg/g	mg/g	mg/g
Native phytate						
I[a]	96.4	8.4 ± 0.4‡	5.3	0.145	0.278	0.11
II[b]	87.7	7.2 ± 0	5.3	0.175	0.563	0.21
III[c]	89.1	6.5 ± 0.7	8.8	0.130	0.616	0.50
IV[c]	90.1	4.9 ± 1.1	6.7	0.140	0.462	0.09
Acid-salt-reduced phytate						
V[a]	92.0	0.2 ± 0	4.5	0.145	0.070	0.08
VI[b]	92.6	1.0 ± 0.1	3.6	0.180	0.440	0.19
VII[c]	90.1	0.3 ± 0.01	3.5	0.146	0.077	0.05
Enzyme-reduced phytate						
VIII[c]	89.7	≤ 0.01 ± 0.01	21.7	0.135	0.820	0.68
IX[c] §	91.8	≤ 0.01 ± 0	14.5	0.155	0.289	0.28
Restored phytate						
X[b] ‖	91.1	9.9 ± 0.2	7.2	0.162	0.364	0.17
XI[c] ¶	90.8	3.7 ± 0.2	8.8	0.145	0.142	0.22

* All analytical data were obtained on the spray-dried products without further moisture removal.
† Isolates with the same superscript were prepared from the same batch of soy flour.
‡ $\bar{x}$ ± SE.
§ Enzyme reduced followed by ultrafiltration.
‖ Produced by adding sodium phytate to isolate VI.
¶ Produced by adding sodium phytate to isolate VIII.

with a phytase from *Aspergillus niger* (Alko Ltd, Helsinki, Finland) before precipitation of the protein. Isolate VII was then centrifuged, washed, neutralized, sterilized, and spray-dried as described above. For isolate IX the coagulum was subjected to an additional ultrafiltration treatment to remove the low-molecular-weight compounds. Isolates X and XI were made by adding sodium phytate back to the acid-salt–washed isolate V and the enzyme-treated isolate VIII, respectively, before the neutralization step.

Analytical methods

Iron and calcium were determined by atomic-absorption spectroscopy after dry ashing. Total nitrogen was determined by using an automatic nitrogen analyzer (Type NA 1500; Carlo-Erba, Milan, Italy). Trypsin inhibitors were measured according to the method of Kakade et al (10) and trypsin inhibitor units (TIU) were expressed per milligram nitrogen.

Phytic acid was measured by using a modification of the method of Makover (11) in which cerium replaced iron in the precipitation step. Phytic acid was calculated from the phosphorous content of the precipitate by using a factor of 3.55.

Inorganic phosphorous was extracted from 200 mg dried isolate with 10 mL 1 mol H_2SO_4/L. Phosphorous was measured immediately by using a microtitration plate assay based on the complex formation of malachite green with phosphomolybdate under acidic conditions (12).

Iron-absorption studies

Four iron-absorption studies were carried out in groups of 7–9 human subjects. In study 1 the volunteers were fed two test meals, each containing one of the experimental soy-protein isolates and a control meal containing egg white. Three test meals and a control meal were given in studies 2, 3, and 4. All test meals were fed as a semisynthetic liquid formula containing 67 g hydrolyzed corn starch (Fro-Dex, American Maize Products, Hammond, IN), 36 g corn oil (Nugget Brand, Stockton, CA), 12 mL vanilla extract (McCormick and Co, Baltimore, MD), 200 mL deionized distilled water, and 30 g protein (nitrogen × 6.25) derived from either a soy-protein isolate or egg white (Monarch Egg Corporation, Kansas City, MO). The calcium content of the soy-protein-isolate meals within each study was equilibrated by adding calcium chloride ($CaCl_2 \cdot 2H_2O$) to raise the calcium content to 44 mg/meal in study 1, 19.2 mg in study 2, and 27.4 mg in studies 3 and 4. The amount of calcium (96 mg/meal) in the egg white–control meal was not modified.

The test meals for studies 1–4 are described in Table 2. The test meals in study 1 included soy-protein isolate (I) containing its native phytic acid content and a low-phytate isolate produced by continuous acid-salt washing and ultrafiltration (V). Both isolates were prepared from the same soy flour. The test meals for the second study comprised a soy-protein isolate containing its native amount of phytic acid (II), a low-phytate isolate produced by continuous acid-salt washing and ultrafiltration (VI), and the same isolate to which phytic acid had been added back (X). Again, all isolates were prepared from the same soy flour. In the third study the test meals included a control soy-protein isolate containing its native amount of phytic acid (III), an isolate from which the phytate had been removed by enzyme digestion (VIII), and the same phytate-free isolate to which phytic acid had been added back (XI). For study 4 the meals were a control isolate containing its native phytic acid (IV), a low-phytate isolate produced by acid-salt washing and ultrafiltration (VII), and an

TABLE 2
Effect of phytate removal on iron absorption from soy isolates

Study, subjects, and mean age	Mean packed cell volume	Serum ferritin*	Meals†	Iron absorption*	Absorption ratio vs meal A	Absorption ratio vs meal D
	%	μg/L		% of dose		
1. (6 M, 2 F), 24 y	44	59 (49–71)	A Isolate I (native phytate)	1.50 (1.10–2.06)	—	0.24‡
			B Isolate V (A-S–reduced phytate)	3.15 (2.32–4.28)	2.10‡	0.50
			D Egg white control	6.34 (4.72–8.51)	—	—
2. (5 M, 4 F), 23 y	43	38 (29–50)	A Isolate II (native phytate)	0.92 (0.65–1.32)	—	0.16§
			B Isolate VI (A-S–reduced phytate)	1.91 (1.34–2.71)	2.07‡	0.33§
			C Isolate X (restored phytate)	1.08 (0.75–1.54)	1.17	0.19§
			D Egg white control	5.75 (3.96–8.33)	—	—
3. (7 M, 1 F), 23 y	45	68 (60–77)	A Isolate III (native phytate)	0.53 (0.41–0.68)	—	0.10§
			B Isolate VIII (E-reduced phytate)	2.50 (2.10–2.97)	4.75§	0.46‡
			C Isolate XI (restored phytate)	0.78 (0.52–1.15)	1.45	0.17§
			D Egg white control	5.48 (3.63–5.94)	—	—
4. (3 M, 4 F), 22 y	43	35 (28–45)	A Isolate IV (native phytate)	1.36 (0.94–1.98)	—	0.14§
			B Isolate VII (A-S–reduced phytate)	4.17 (3.01–5.76)	3.06§	0.43‖
			C Isolate IX (E-reduced phytate)	5.48 (4.16–7.21)	4.02‡	0.56
			D Egg white control	9.72 (7.56–12.51)	—	—

* Geometric $\bar{x}$ (±1 SE).
† A-S, acid-salt–reduced phytate; E, enzyme-reduced phytate.
‡ $P < 0.05$.
§ $P < 0.001$.
‖ $P < 0.01$.

isolate from which the phytate had been removed by enzyme treatment followed by ultrafiltration (IX). All isolates fed in studies 3 and 4 were produced from the same batch of soy flour. As indicated above, isolate IX differed from isolate VIII by having been subjected to an additional ultrafiltration step to remove low-molecular-weight compounds.

The volunteer subjects ranged in age from 20 to 31 y with a mean age of 23 y. There were 21 men and 11 women. They exhibited a wide range of iron status as reflected by serum ferritin concentrations between 11 and 138 μg/L. All were in good health and denied a history of disorders that are known to influence the gastrointestinal absorption of iron. Written, informed consent was obtained from each volunteer before beginning the investigation and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Double sequential radioiron labels were used to measure iron absorption from four separate meals consumed by each subject. The meals were administered between 0700 and 0900 after an overnight fast and only water was allowed for the subsequent 3 h. All meals were labeled with the extrinsic label technique (13) by adding 37 kBq $^{59}FeCl_3$ or 111 kBq $^{55}FeCl_3$ to a solution of $^{58}FeCl_3$ in 0.01 mol HCl/L containing a quantity of iron sufficient to adjust the total iron content of each meal to 5.7 mg in study 1, 6.4 mg in study 2, and 5.5 mg in studies 3 and 4.

On the day preceding the first test meal, 15 mL blood was drawn for the measurement of packed cell volume, serum ferritin (14), and background radioactivity. The first and second test meals were labeled with either ^{59}Fe or ^{55}Fe and administered on days 2 and 3 of the study. Blood (25 mL) was drawn on day 16 to measure incorporated red cell radioactivity. A similarly labeled second pair of meals was given on days 16 and 17 in studies 2, 3, and 4. Only a single meal was fed on day 17 in study 1. The final blood sample was drawn 2 wk after the last test meal to measure the increase in circulating red cell radioactivity. Radioiron measurements were made on duplicate 10-mL samples of whole blood by a modification of the method of Eakins and Brown (15). Percentage absorption was calculated on the basis of the blood volume estimated from height and weight (16, 17) and an assumed red cell incorporation for absorbed radioactivity of 80% (18).

Percentage absorption values were converted to logarithms before statistical analysis and the results reconverted to antilogarithms to recover the original units (19). Because each study contained several independent manipulations of the test meals, paired t tests were used to compare absorption from selected test meals within the same study by determining whether the mean log absorption ratios differed significantly from zero.

Results

The results of the iron-absorption studies are shown in Table 2. In study 1, subjects fed the liquid-formula meal containing the control soy isolate (I) with a native phytic acid content of 8.4 mg/g had a mean iron absorption of 1.5%, which increased to 3.15% ($P = 0.01$) when the phytic acid content of the soy isolate was reduced to 0.2 mg/g by acid-salt washing and ultrafiltration (isolate V). In study 2 there was a similar twofold increase in iron absorption on feeding another low phytate isolate produced by acid-salt washing and, in addition, absorption returned to approximately its original amount when the phytic acid was added back. In this study, decreasing the phytic acid

from 7.2 (isolate II) to 1.0 mg/g (isolate VI) increased iron absorption from 0.92% to 1.91% ($P < 0.02$). Adding back phytic acid to an amount of 9.9 mg/g reduced iron absorption to 1.08%, which was not significantly different from its original amount ($P > 0.2$).

A low phytate isolate produced by enzyme treatment was investigated in study 3. This isolate had a phytic acid content of ≤ 0.01 mg/g, compared with 0.2–1.0 mg/g in the low-phytate isolates produced by acid-salt washing and ultrafiltration (Table 1). In this study (Table 2), reducing the native phytic acid content of the control isolate (III) from 6.5 to ≤ 0.01 mg/g (isolate VIII) increased iron absorption almost fivefold from 0.53% to 2.50% ($P < 0.001$). Again, adding back phytic acid to 3.7 mg/g decreased iron absorption to 0.78%, which was not significantly different from its original amount ($P > 0.2$).

Study 4 compared directly a low-phytate isolate produced by acid-salt washing (isolate VII) with a similar isolate produced by enzyme treatment (isolate IX). Both isolates were ultrafiltered to remove the low-molecular-weight compounds. Mean iron absorption from the control isolate (VI) containing 4.9 mg phytic acid/g was 1.36%. Reducing phytic acid to 0.3 mg/g by acid-salt washing increased absorption to 4.17% ($P < 0.001$) whereas reducing phytic acid to ≤ 0.01 mg/g by enzyme treatment increased absorption to 5.48% ($P < 0.05$). In this study there was no significant difference in iron absorption between these two low-phytate isolates ($P > 0.05$). However, when all the absorption ratios of the low-phytate isolates relative to their respective controls were combined (Fig 1), it is seen that acid-salt washing to produce isolates with 0.2–1.0 mg phytic acid/g increased iron absorption 2.3-fold, whereas enzyme treatment to give isolates with ≤ 0.01 mg/g phytic acid produced a significantly greater 4.4-fold increase ($P < 0.01$).

The mean iron absorption from the egg white–control meal was 6.34%, 5.75%, 5.48%, and 9.78%, respectively, in studies 1–4. As absorption from the egg white–control meal was measured in all subjects, it is possible to compare the absorption from different meals between studies by comparing their absorption

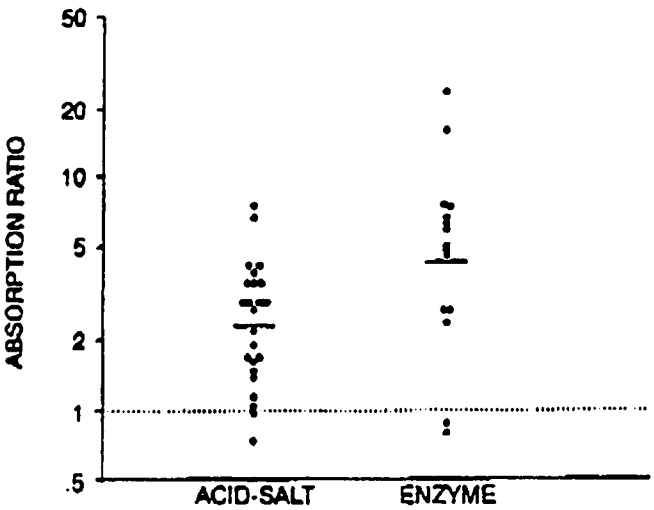

FIG 1. Iron-absorption ratios between reduced phytate soy-protein isolates and their corresponding control isolate containing its native phytic acid content. Acid-salt–reduced isolates contained 0.2–1.0 mg phytic acid/g and enzyme-reduced isolates contained ≤ 0.01 mg phytic acid/g. Short horizontal lines represent mean values.

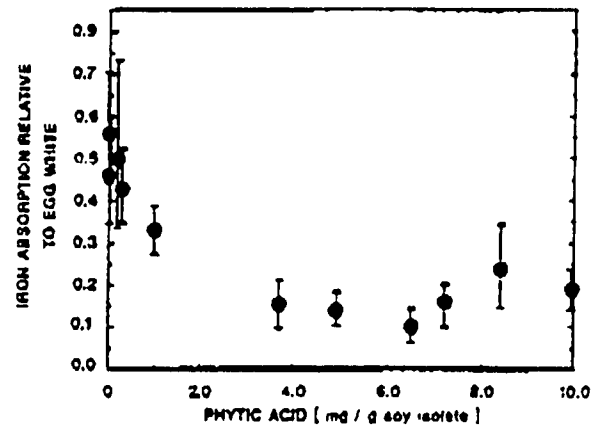

FIG 2. Relationship between phytate content (mg/g) of soy-protein isolate and mean iron absorption ± 1 SE of each soy-protein-isolate meal relative to its respective egg white control meal. (Data compiled from studies 1–4 as listed in Table 2). Approximate phytic acid content (mg) per meal can be calculated by multiplying the phytic acid content (mg/g) of the soy-protein isolates by 33.

relative to the egg white control. Thus, in Figure 2, iron absorption from meals containing the different soy-protein isolates relative to that from the egg white–control meal fed in the same subject (relative absorption = 1.0) is compared with the phytic acid content of the isolates. At phytic acid contents between 9.9 and 3.7 mg/g, iron absorption relative to the egg white control was low and varied randomly from 0.10 to 0.24. Only after phytic acid was reduced to ≤ 0.3 mg/g was there a substantial increase in iron absorption to 0.43–0.56 of the egg white control. Relative iron absorption can also be compared with the approximate phytic acid content of the meal. Because the hydrolyzed corn starch contained no measurable phytic acid, the soy-protein isolates were the only phytic acid–containing components of the meal. Each meal contained 30 g crude protein from the test isolate, and, because the protein contents of the isolates differed slightly (Table 1), is equivalent to ≈33 g isolate per meal. The phytic acid content per meal can be obtained by multiplying the phytic acid content of the isolate (mg/g) by 33. It can be seen that a substantial increase in iron absorption occurred only after phytic acid was reduced to < 10 mg/meal.

Discussion

The observation that consumption of wheat bran reduces iron absorption led Widdowson and McCance (20) to suspect that phytate may be an important inhibitor of nonheme food iron absorption. Subsequent investigations with bran have confirmed its inhibitory effect but other studies have yielded contradictory results as to the inhibitory nature of phytate specifically. In one study (21) the reduction of phytate in wheat bran was reported to have no effect on nonheme-iron absorption and monoferric phytate, which represents half the iron in wheat bran (22), was reported to be well absorbed. In other studies, the reduction of phytic acid in wheat bran did improve iron absorption (8) and adding phytic acid to wheat rolls inhibited iron absorption dose-dependently (23). The role of phytate in modifying nonheme-iron absorption from soy products was even more unclear as neither the removal of phytate from soy flour by acid washing (6) nor a twofold variation in the phytate content of soybeans

produced under different growing conditions (24) influenced nonheme-iron absorption.

The present findings, however, now strongly suggest that phytic acid is a major inhibitory factor in soy-protein isolates. Removal of phytic acid to ≤ 0.01 mg/g of isolate increased iron absorption four- to fivefold whereas adding back the phytic acid reduced iron absorption to its original low value. Our results also demonstrate that relatively small amounts of phytic acid can still strongly inhibit iron absorption and that the phytic acid concentration in isolates must be reduced to ≥ 1.0 mg/g and optimally to < 0.3 mg/100 g to ensure a meaningful increase in iron absorption. The latter figure corresponds to < 10 mg phytic acid in a meal containing ≈5 mg Fe. The necessity for these very low amounts of phytic acid could explain why earlier studies failed to demonstrate a beneficial effect of reducing phytic acid in soy products (8, 24). By modifying the growing conditions, Beard et al (24) reduced the phytic acid content of soybeans from 7.04 to 3.76 mg/g. They fed the cooked beans as a soup or purée in meals providing ~220 mg phytic acid in the high-phytate meal and 110 mg phytic acid in the low-phytate meal. They showed that reducing phytic acid by these amounts did not increase iron absorption relative to their reference meal. Our results would also suggest that decreasing the phytic acid content of a meal from 220 to 110 mg would have little effect on iron absorption but that by decreasing the phytic acid to < 10 mg/meal, iron absorption would be increased substantially.

Enzyme treatment was more effective at removing phytic acid than was acid-salt washing combined with ultrafiltration, giving isolates with ≤ 0.01 mg phytic acid/g compared with 0.2–1.0 mg/g. Acid washing with ultrafiltration, in addition to removing the phytic acid, removes a variety of low-molecular-weight compounds, which could also influence iron absorption. To investigate this possibility we subjected an enzyme-treated isolate to a further acid-salt washing with ultrafiltration. Our results would indicate that the ultrafiltration step did not further improve iron absorption. Absorption from the meal containing the enzyme-reduced phytate isolate VIII (Table 2) was 2.50% compared with 5.48% for the egg white–control meal. Absorption from soy isolate VIII relative to the egg white–control meal was thus 0.46. Absorption from the enzyme-reduced phytate isolate IX (Table 2) subjected to an additional ultrafiltration was 5.48% compared with 9.72% from the egg white–control meal in the same subjects. Absorption of soy isolate IX relative to the control meal was 0.56. The phytic acid content of both isolates was ≤ 0.01 mg/g.

Earlier studies comparing different protein sources incorporated into the same liquid-formula meals as administered in the present investigation demonstrated that soy-protein isolate was the most inhibitory of the protein sources tested. Iron absorption from a soy-protein-isolate meal with its native phytic acid content was 20% of the egg white–control meal (3) compared with 31% for wheat gluten, 108% for whey protein (25), 55% or 100% for casein (3, 25), 190% for bovine serum albumin (26), 300% for beef muscle (27), and 363% for the protein-free meal (26). In the present study, removal of virtually all the phytic acid from soy-protein isolates increased iron absorption from 10–24% to ≈55% of the absorption from the egg white–control meal, indicating that even after the removal of phytate, soy protein itself is still relatively inhibitory to iron absorption.

Earlier studies have shown that, at the same amount of ascorbic acid or in the formula, iron absorption from milk-based infant formulas is two to three times higher than that from soy formulas (28). Our results indicate that iron absorption from formulas made from phytate-free soy isolate would be similar to that from milk-based formula. Phytate-free soy-protein isolate, casein, and whey are still moderately inhibitory to iron absorption. The inhibitory nature of whey would appear to be due to its high calcium and phosphorous contents and not to its protein component (29). The inhibitory nature of casein and phytate-free soy isolate on the other hand is due probably to the binding of iron to insoluble peptides in the duodenum. The iron-binding peptides from casein are those that contain serine phosphate (29). The iron-binding peptides from soy could be those containing a high proportion of carboxylic acid groups.

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Assessment of dietary determinants of nonheme-iron absorption in humans and rats[1-3]

Manju B Reddy and James D Cook

ABSTRACT Prior investigations have shown that rats are less sensitive than humans to dietary factors that influence the absorption of nonheme iron. This investigation was undertaken to determine whether this disparity is due to differences in the methods used to measure absorption in the two species. By use of identical methodology and test meals, absorption studies were performed in rats and humans to compare the effect of known dietary enhancers (ascorbic acid and meat) and inhibitors (tea, bran, and soy protein) on nonheme-iron absorption. Meat and tea had a marked effect on absorption in humans but did not influence absorption in rats. Although the effect of ascorbic acid, soy protein, and bran on absorption was statistically significant in rats, the absorptive response was far less than it was in humans. Our studies indicate that rodents cannot be used to assess the quantitative importance of dietary factors in human iron nutrition. *Am J Clin Nutr* 1991;54:723–8.

KEY WORDS Nonheme-iron absorption, dietary bioavailability, species differences

Introduction

During the past two decades, application of the extrinsic-tag method for measuring nonheme-iron absorption has led to the identification of a large array of dietary factors affecting iron absorption in humans. Recent studies have attempted to define the quantitative importance of specific biochemical determinants such as ascorbic acid, phytate, polyphenols, and proteins, both singly and in combination (1–5). However, progress in this area has been slow because of the limited number of radioisotope studies that can be reasonably performed in human volunteers. Further refinement in our understanding of the role of the diet in human iron nutrition would be facilitated by the development of a reliable animal model.

Most studies of iron bioavailability in small laboratory animals have been performed in growing rats. Rodents have been particularly useful for assessing the bioavailability of iron compounds used in the food industry. However, when factors affecting the absorption of dietary iron are evaluated, there are disparities between studies in rats and humans (6, 7). It is not clear whether these reflect species variation or are simply related to differences in the methods used to measure iron absorption in the two species. In the present study, we measured nonheme-iron absorption in humans and rats by using identical methodology and test meals to assess the importance of inherent species differences in dietary influences on nonheme-iron absorption.

Methods

Human studies

The absorption of dietary nonheme iron was measured by extrinsic radioiron labeling in volunteer subjects aged 18–40 y. All subjects were in good health and had no history of disorders known to influence gastrointestinal absorption. None was anemic, as defined by a hemoglobin concentration > 120 g/L in women and > 130 g/L in men. Written informed consent was obtained from each volunteer before the investigation and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Absorption from two to four separate meals was determined in each subject by using ^{55}Fe and ^{59}Fe. The labeled meals were given between 0700 and 0900 after an overnight fast; water only was permitted for the next 3 h. The meals were labeled extrinsically by adding 37 kBq $^{59}FeCl_3$ or 111 kBq $^{55}FeCl_3$ (New England Nuclear, North Billerica, MA) to a solution containing 0.1 mg $FeCl_3$ in 1 mL 0.01 mol HCl/L. When absorption from four different meals was measured in each subject, the first pair of meals was fed on 2 successive days after blood was obtained for measuring serum ferritin (8) and background radioactivity. Blood was obtained 14 d later to measure incorporated red blood cell radioactivity. A second pair of labeled meals was fed on two successive mornings. A final blood sample was obtained 2 wk later to measure the increase in red-cell radioactivity. Blood radioactivity was measured in duplicate 10-mL samples of whole blood by using a modification of the method of Eakins and Brown (9). Percentage absorption was calculated from blood volume estimated from height and weight (10, 11). Red-cell incorporation of absorbed radioactivity was assumed to be 80% (12). Studies of the effect of ascorbic acid (13) and soy proteins on iron absorption in humans (14) were previously published.

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Animal studies

Absorption measurements were performed in male Sprague-Dawley rats (Sasco Inc, Omaha) weighing 125–150 g. The animals were housed in individual, suspended, stainless steel cages with wide mesh bottoms designed to prevent coprophagia. Alternating 12-h light and dark cycles were used throughout the study. The rats were placed on a diet containing 50 μg Fe/g diet (United Biochemical Corp, Cleveland) at least 7 d before the first test meal and were maintained on this diet for the remainder of the study. The diet contained corn starch (56%), hydrogenated vegetable oil (14%), vitamin-free casein (27%), a salt mixture (3%), and a vitamin mixture (1 kg/45.4 kg diet). The animals were allowed food and deionized water ad libitum. Guidelines established by the University of Kansas Medical Center for the care and use of animals were followed.

As in human studies, absorption was measured in each rat from two labeled meals fed on successive days. These consisted of a control meal and the same meal (test) containing one of the factors known to influence iron absorption. For training purposes the two meals were fed without a radioisotope label on two mornings during the preceding week to ensure that the meal was fully consumed within 30 min. The labeled meals were fed in the morning after a 16-h fast. The food was placed in a porcelain bowl fixed to the side of the cage to minimize spillage and was usually eaten within 15 min. All feedings were carefully supervised and any spilled food was collected on drop pads and returned to the feeding bowl. Meals were tagged extrinsically with either 18.5 kBq ^{59}Fe or 55.5 kBq ^{55}Fe by mixing 0.05 mL 0.01 mol HCl/L containing 1 μg Fe as $FeCl_3$ with the food (15). The variation in radioactivity between weighed portions of the labeled food was $< \pm 2\%$. Triplicate standards for whole-body counting were prepared from meals labeled with ^{59}Fe.

One week after administration of the meals, the ^{59}Fe radioactivity retained in each rat was measured by obtaining duplicate 1-min counts in a whole-body counter (16) together with counting standards prepared at the time of meal administration. The animals were then anesthetized with ethyl ether and exsanguinated through the abdominal aorta, and the liver was removed for duplicate measurements of nonheme iron (17).

The measurement of ^{55}Fe and ^{59}Fe in whole blood was made by a modification of the technique of Moore (18). Whole blood (0.1 mL) was mixed with an equal volume of 0.01 mol HCl/L and added to 0.5 mL soluene 350 (1:1, soluene:isopropyl alcohol; United Technologies, Packard, Downer's Grove, IL). After thorough mixing, the sample was incubated at 40 °C for 60 min. Hydrogen peroxide (0.5 mL) was added and the incubation continued for 15 min at room temperature and then 30 min at 40 °C. Fifteen milliliters of Instagel-XF:0.05 mol HCl/L (9:1, Packard) was added to the mixture and the sample was counted by liquid-scintillation counting. Standards were diluted and prepared identically except that 0.1 mL radioiron in HCl was mixed with an equal volume of unlabeled rat blood. Absorption from meals tagged with ^{55}Fe was determined from the ratio of ^{55}Fe to ^{59}Fe in circulating blood and the absorption of ^{59}Fe as measured by whole-body counting. This dual-radioisotope technique was validated by tagging the same meal simultaneously with ^{55}Fe and ^{59}Fe. In 26 animals the correlation between absorption of the two isotopes was 0.996 (Fig 1).

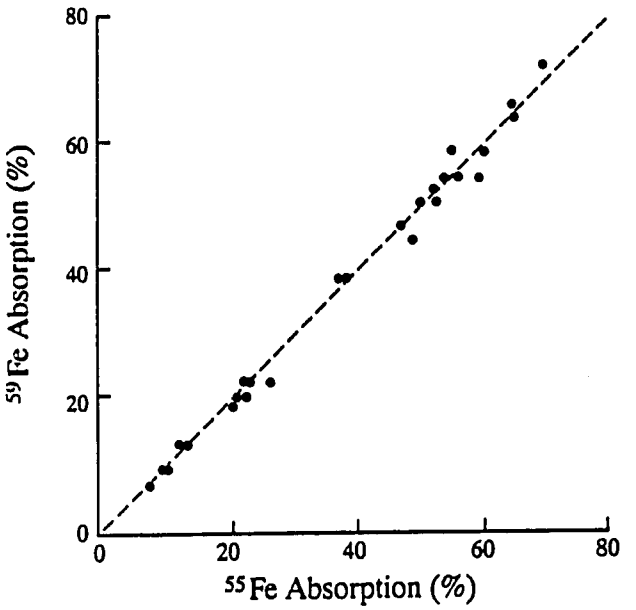

FIG 1. Percent absorption of ^{55}Fe and ^{59}Fe added to the same meal. The interrupted line is the line of identity.

Control and test meals

For each dietary factor the same pair of control and test meals was fed to humans and rats. For rats the test meals were homogenized before feeding and the quantity of food was reduced 80-fold as compared with clinical measurements.

When the effect of meat was assessed, the control meal consisted of a 75-g bun, 68 g French fries, and a 50-g milkshake. The test meal contained, in addition, 80 g ground beef. The control meal contained 1.9 mg Fe and 2600 kJ food energy whereas the test meal contained 4.1 mg Fe and 3400 kJ food energy. In all remaining studies the control meal contained 29.4 g protein as egg albumen, 68 g carbohydrate as dextrimaltose, and 35 g fat as corn oil (19). Ferric chloride was added to bring the iron content to 4.1 mg. When the effect of tea was studied, one cup of tea, single or triple strength, was fed with the test meal. One cup of single-strength tea was prepared by adding 1.75 g tea (Lipton Inc, Engelwood Cliff, NJ) to 200 mL boiling water, steeping for 4 min, and straining. Triple-strength tea was prepared similarly with 5.25 g tea. For animal studies the tea was freeze dried and mixed with the control meal. The effect of bran was examined by adding 10% bran (by weight) obtained from AACC, St Paul. The effect of soy protein was evaluated by substituting isolated soy protein (Nestlé, Vevey, Switzerland) isonitrogenously for the egg albumen in the control meal.

Additional rat studies were performed to determine whether the sensitivity of dietary factors was altered by lowering percentage absorption from the control meal. This was achieved by iron loading the rats either orally by placing them on a diet containing 500 μg Fe/g diet for 1 wk before the study or parenterally by giving two intraperitoneal injections of 5 mg Fe as iron dextran (Fisons Corp, Bedford, MA) during the week before the study.

Statistical analysis

Values for percentage absorption and the ratio of test to control-meal absorption were converted to logarithms for statistical

analysis. The results were reconverted as antilogarithms to recover the original units. Paired t tests were used to compare absorption from the control and test meals by determining whether the mean log absorption ratio differed from zero. Although percentage absorption is less skewed in rats, the same statistical approach was used to maintain methodological uniformity.

Results

Initial studies were performed to examine the effect of ascorbic acid and meat, the two main enhancers of nonheme-iron absorption. The addition of beef to a meatless meal increased mean absorption in seven men significantly from 1.96 to 3.90, an increase of 99% (**Table 1**). In contrast, absorption in rats increased from 45.3% without meat to 46.8% with meat, an insignificant 3% change (**Table 2, Fig 2**). In humans the addition of 100 mg ascorbic acid to the control meal increased absorption nearly fourfold, from 2.08% to 7.86%, whereas 5 mg ascorbic acid in rats increased absorption by only 23%, from 44.9% to 55.1% (Fig 2).

Most of the dietary inhibitors evaluated produced a significant decrease in iron absorption in rats but the inhibition was small as compared with humans (**Fig 3**, Tables 1 and 2). With tea a marked decrease from 7.45% to 1.23% was observed in humans whereas in rats absorption fell from 49.8% to only 45.7% ($P > 0.05$). When the amount of tea was increased, a more significant decrease from 62.6% to 44.7% was observed in rats and a further decrease was also observed in humans. Bran reduced absorption markedly in humans to 10% of basal, from 7.45 to 0.75%, but in rats the decrease was only 23%. The strongest inhibitory factor in rats was isolated soy protein, which reduced absorption from 69.7% to 45.4%. In humans the reduction was still more pronounced, from 2.49% to 0.46%.

One obvious difference between absorption in rats and humans in these studies was the basal absorption from the control meal. In humans this ranged between 2% and 8% whereas in rats absorption was invariably > 50%. To determine whether the limited sensitivity of the rat to dietary factors was related to their higher basal absorption, we reduced it to < 30% by pretreating the animals with oral or parenteral iron. When the effect of ascorbic acid was reexamined, an increase in absorption of 26% was observed, similar to that seen in normal rats (**Fig 4**). When liver nonheme iron was raised further with parenteral iron, the effect of ascorbic acid was even less apparent. Similarly, parenteral loading did not increase the effect of bran on iron absorption as compared with that observed in normal rats.

Discussion

Rats have been used extensively to evaluate the bioavailability of iron compounds used for food fortification. The usual approach is to induce severe iron deficiency in growing rats by dietary iron restriction and/or repeated phlebotomy and then to assess the rate of hemoglobin regeneration on various intakes of the fortification iron. By comparing the rate of response to that observed with a highly bioavailable form (ferrous sulfate), the relative biological value of fortification iron is calculated. In a recently published comparison of several methods for evaluating the bioavailability of fortification iron compounds, including solubility in acid, hemoglobin regeneration, isotopically measured absorption, and in vitro digestion, the closest correspondence with clinical measurements was obtained with the hemoglobin regeneration model as recommended by the Association of Official Analytical Chemists (20). Promising results were also obtained by in vitro digestion.

The hemoglobin-regeneration measurement in rats has several limitations when used to evaluate the bioavailability of dietary

TABLE 1
Nonheme-iron absorption in human subjects by dietary variable

Dietary variable	Age*	Serum ferritin†	Iron absorption†		Absorption ratio (test:control)†
			Control	Test	
	y	*μg/L*	%		
Meat (n = 7)	24 (19–37)	76 (65–88)	1.96 (1.61, 2.38)	3.90 (3.44, 4.43)	1.99‡ (1.73, 2.29)
Ascorbic acid (n = 13)	20 (18–23)	45 (38–54)	2.08 (0.59, 7.30)	7.86 (3.62, 17.1)	3.77‡ (2.01, 7.04)
Tea (n = 9)	28 (22–40)	47 (37–61)	7.45 (5.90, 9.41)	1.23 (0.79, 1.93)	0.17‡ (0.12, 0.23)
Tea, triple strength (n = 9)	28 (22–40)	47 (37–61)	7.45 (5.90, 9.41)	0.64 (0.45, 0.92)	0.09‡ (0.06, 0.12)
Bran (n = 9)	28 (22–40)	47 (37–61)	7.45 (5.90, 9.41)	0.75 (0.51, 1.10)	0.10‡ (0.08, 0.14)
Soy protein (n = 15)	26 (24–30)	53 (45–62)	2.49 (2.08, 2.98)	0.46 (0.35, 0.62)	0.19‡ (0.15, 0.24)

* $\bar{x}$ (range).
† Geometric $\bar{x}$ (± 1 SE).
‡ Ratio significantly different from 1, $P < 0.01$.

TABLE 2
Iron-absorption measurements in normal and iron-overloaded rats by dietary variable

Dietary variable	Iron conditioning	Weight	Liver iron*	Iron absorption†		Absorption ratio (test:control)†
				Control	Test	
		g	*μmol/g*	%		
Meat (n = 7)	None	213	1.65 ± 0.05	45.3 (43.7, 46.9)	46.8 (45.0, 48.6)	1.03 (0.98, 1.09)
Ascorbic acid (n = 8)	None	208	1.16 ± 0.11	44.9 (41.1, 48.9)	55.1 (53.6, 56.6)	1.23‡ (1.13, 1.33)
Tea (n = 8)	None	206	1.32 ± 0.11	49.8 (47.6, 52.2)	45.7 (43.9, 47.5)	0.92 (0.85, 0.98)
Tea, triple strength (n = 10)	None	209	1.13 ± 0.11	62.6 (60.7, 64.5)	44.7 (42.7, 46.9)	0.71§ (0.69, 0.74)
Bran (n = 10)	None	207	1.38 ± 0.11	61.4 (59.6, 63.2)	47.3 (45.9, 48.8)	0.77§ (0.75, 0.79)
Soy protein (n = 10)	None	222	1.11 ± 0.09	69.7 66.9, 72.6)	45.4 (43.6, 47.2)	0.65§ (0.61, 0.69)
Ascorbic acid (n = 10)	Oral excess	217	2.61 ± 0.29	27.2 (24.3, 30.4)	34.14 (32.3, 36.1)	1.26 (1.13, 1.40)
Ascorbic acid (n = 10)	Parenteral excess	216	6.45 ± 0.57	26.2 (23.8, 28.7)	26.8 (24.4, 29.4)	1.02 (0.93, 1.12)
Bran (n = 10)	Parenteral excess	214	5.59 ± 0.45	20.4 (18.6, 22.5)	20.7 (19.1, 22.6)	1.00 (0.97, 1.06)

* $\bar{x}$ ± SE.
† Geometric $\bar{x}$ (±1 SE).
‡§ Ratio significantly different from 1, ‡$P < 0.05$, §$P < 0.01$.

iron. Iron absorption from the diet is lower than from added fortification iron, resulting in a blunted hemoglobin response. Furthermore, dietary manipulations produce smaller differences in iron intake than can be achieved by adding fortification iron to the diet. Maximal iron absorption resulting from severe iron deficiency in rats may further minimize the influence of dietary factors on iron absorption. Finally, the use of hemoglobin-regeneration measurements to assess food iron availability requires modification of a rat diet that may not be relevant to human nutrition.

Measurements of food iron availability in iron-replete rats have been performed by using either metabolic-balance studies or radioisotope labeling. Isotope techniques have gained favor because of their convenience and simplicity although the two approaches gave similar results when performed under the same experimental conditions (21, 22). With isotope measurements the usual approach has been to feed radiolabeled test meals to fasting animals and measure the retention of radioiron by whole-body counting, fecal excretion, or incorporated red-cell radioactivity. In other respects, the technique for measuring food iron absorption in rats has varied widely among laboratories with regard to the type of diet examined (rat vs human), the amount of food given, age and iron status of the animals, and method of test-dose administration. Regardless of the specific technique, dietary factors known to influence iron absorption in humans have had much less effect in rats. For example, several workers demonstrated a profound inhibiting effect of phytate or bran in humans (23–26) whereas several studies in rats failed to demonstrate an inhibiting effect (22, 27–30). Soy protein or soy flour is known to reduce the absorption of nonheme iron in humans (14) whereas only slight differences (6, 31), if any (32), were demonstrated in rats. Similarly, tea was reported to have a less-inhibitory effect on nonheme-iron absorption in rats (33) than in humans (34). Except for a recent study with a water-soluble extract of beef (21), meat has not been shown to facilitate nonheme-iron absorption in rats (35). These findings have raised serious doubts about the validity of using rats to assess bioavailability of diets for humans.

In the present study we eliminated, insofar as possible, any methodological factors that might account for differences in the effect of dietary factors on absorption in rats and humans. The control and test meals were identical. Absorption ratios of control and test meals were based in both species on the ratio of ^{59}Fe and ^{55}Fe incorporated into circulating whole blood. The method of labeling the test meals and the fasting period preceding each test were also similar. Any disparity in the response to dietary enhancers and inhibitors can therefore be attributed to a true species difference.

The relative insensitivity of rats to factors that influence the absorption of nonheme iron in humans was demonstrated clearly in the present study (Figs 2 and 3). The basis of this difference, however, is unknown. Percentage absorption from the control meals was > 10-fold higher in rats although their sensitivity to dietary factors was not improved by reducing absorption by prior

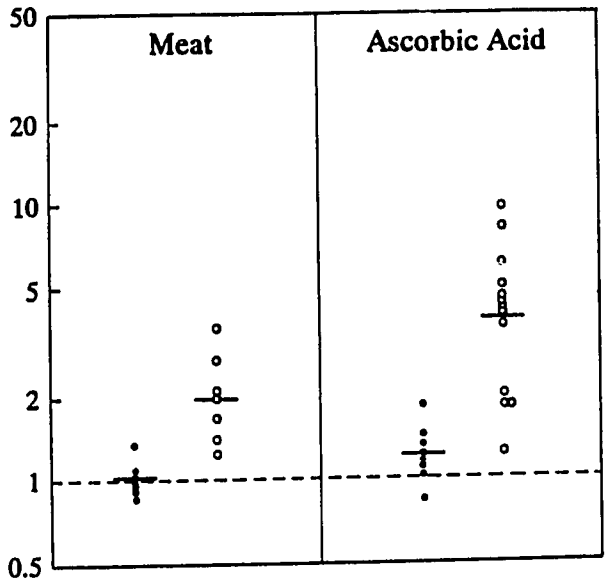

FIG 2. Effect of dietary enhancers on iron absorption in rats (●) and humans (O). The results are expressed as absorption ratios between the test and control meals.

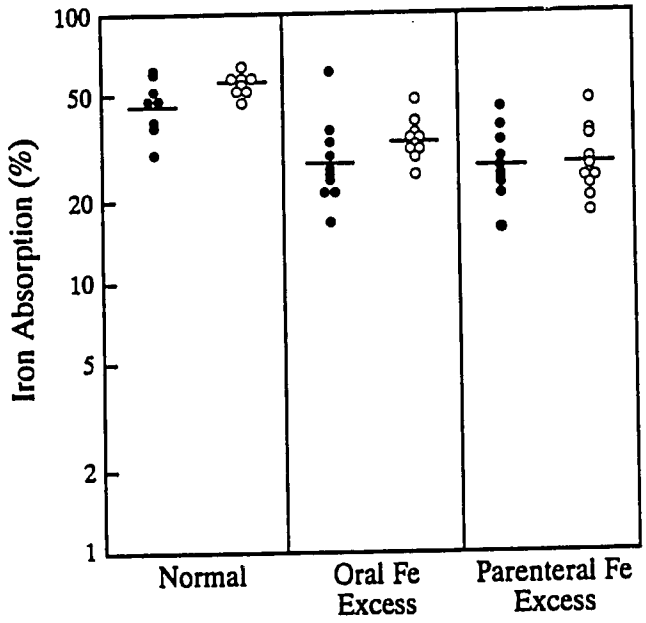

FIG 4. Effect of ascorbic acid on iron absorption in normal and iron-overloaded rats. Test and control meals were fed without (●) or with (O) ascorbic acid.

iron administration. This finding agrees with a previous report showing no influence of iron status on the effect of dietary factors in rats (6). The reduced sensitivity of rats to dietary factors may reflect differences in either the luminal environment of the small intestine or in the behavior of the intestinal mucosal cell. Several differences between rats and humans in mucosal iron transport have been demonstrated. Unlike humans, rats absorb heme iron poorly (36, 37), increase the concentration of mucosal transferrin with iron deficiency (38, 39), and do not absorb ferrous iron preferentially (40); rats also excrete a larger proportion of parenteral iron through their intestinal tract than do humans (40). Rats are also known to have a high content of ascorbic acid in their luminal secretion. Nevertheless, the reason for the reduced sensitivity of rats to dietary effects are unknown.

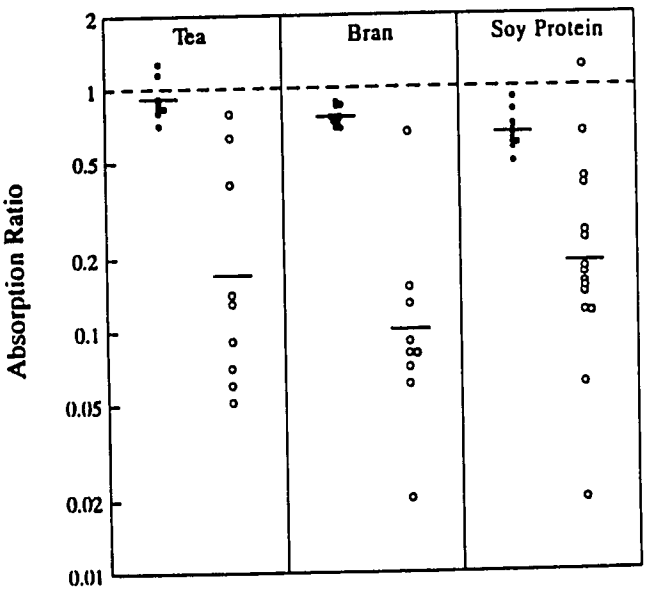

FIG 3. Effect of dietary inhibitors on iron absorption in rats (●) and humans (O). The results are expressed as absorption ratios between the test and control meals.

Our present findings indicate that nonheme-iron absorption data in rats can be extrapolated to humans only with caution. Not only was the influence of dietary enhancers and inhibitors much less, but certain dietary components such as meat or tea failed to influence absorption. It is apparent from our findings that the absence of an effect in rats cannot be taken as evidence against such an influence in humans. Although rats can still be used to screen dietary determinants, a negative result is of limited value. It is also clear that rat studies cannot be used to assess the quantitative importance of dietary factors in human iron nutrition. In vitro digestion methods may be a more reliable guide to factors affecting nonheme-iron absorption in humans (3, 41). Identification of the factor responsible for the diminished sensitivity of rats to dietary effects may result in the development of a more effective rodent model for assessing dietary iron availability in humans. ■

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Assessment of the role of nonheme-iron availability in iron balance[1-3]

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ABSTRACT To assess the nutritional relevance of absorption studies that use extrinsically labeled single meals, we developed a method for measuring nonheme-iron absorption from the diet and compared the results with absorption from single meals. When subjects consumed their usual diet, there was good agreement between dietary absorption (6.4%) and representative single meals fed in the laboratory (6.1%). Nonheme-iron availability, as estimated by a model that incorporated the effect of both enhancers and inhibitors, correlated significantly with absorption from single meals but not with dietary absorption. When the diet was modified to promote iron absorption maximally, dietary absorption increased only slightly (8.0%) and remained significantly lower than it was from single meals (13.5%). With an inhibitory diet, the decrease in absorption from single meals was similarly exaggerated. These results indicate that in the context of a varied Western diet, nonheme-iron bioavailability is less important than absorption studies with single meals would suggest. *Am J Clin Nutr* 1991;54:717–22.

KEY WORDS Nonheme-iron bioavailability, radioiron absorption, iron balance

Introduction

Iron balance in adult humans is determined by the rate of iron loss and the effectiveness of adaptive changes in the rate of its absorption from the diet (1). Dietary iron comprises a small, readily absorbed heme fraction and a large nonheme component, the absorption of which varies widely depending on body iron stores and meal composition. When iron requirements increase, body iron stores become depleted and the percentage absorption of bioavailable nonheme iron rises markedly. However, radioisotope studies of single meals have demonstrated that meal composition also has a major impact on determining how much of the nonheme iron is absorbed. Factors such as ascorbic acid markedly increase nonheme-iron absorption whereas inhibitory components such as polyphenols or phytates impair its assimilation to a similar extent (2, 3). Depending on the balance between enhancers and inhibitors, percentage absorption varies as much as 20-fold in the same individual. These findings have led to the assumption that the type of diet consumed by adults has a significant and independent influence on iron status that modifies the effect of iron requirements.

The importance commonly attributed to dietary bioavailability is based largely on these radioisotope measurements of iron absorption from individual meals. However, there is little direct evidence that iron status is influenced by dietary composition. In general, large population surveys have not demonstrated a clear relationship between iron status and the daily consumption of dietary factors such as ascorbic acid or tea that have been shown in single-meal studies to have a major effect on iron absorption. Attempts to modify the iron status of healthy individuals by enhancing dietary iron availability have not usually been successful. In one study no effect on iron stores was observed when 2 g ascorbic acid/d was added to the diet of normal American volunteers over a 2-y period (4). A more recent study indicated that ascorbic acid supplementation increases dietary iron absorption in iron-depleted women (5).

The importance of dietary nonheme-iron bioavailability as a determinant of iron status may have been exaggerated by studying absorption from isolated meals under laboratory conditions. The net effect of various enhancers or inhibitors of absorption may be less marked with a highly varied diet because no single factor may be contained in a sufficient number of meals to influence iron balance. To examine this possibility, we developed a method for measuring iron absorption from the diet to assess the nutritional relevance of results obtained with single-meal measurements.

Methods

Subjects

Iron-absorption measurements were performed in 45 volunteer subjects aged 21–40 y. They were divided into three groups to study absorption from diets designed to have low, average, and high bioavailability on the basis of single-meal studies. The volunteers were carefully screened before participating in the investigation to establish their willingness to maintain careful dietary records during the study and to modify their customary diet when this was required. A preliminary serum ferritin concentration was used to select a group of subjects with a broad range of iron status within each study group. All subjects were

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in good health and denied any history of disorders known to influence the gastrointestinal absorption of iron. None of the volunteers was anemic; hemoglobin concentrations were > 130 g/L in men and > 120 g/L in women. Written, informed consent was obtained from all subjects before the investigation. All experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Test meals

Four separate iron-absorption measurements were made in each subject by using two different radioiron labels sequentially. The first absorption test measured nonheme-iron absorption from the diet. This was performed by feeding an extrinsically tagged bread roll weighing 15 g and containing 3.7 kBq ^{55}Fe with 28 separate meals over a 2-wk period. Prior studies showed that extrinsic tagging of one food item is sufficient to label the nonheme iron component of the complete meal (6). The rolls were labeled extrinsically by mixing 0.1 mg iron as $^{55}FeCl_3$ with the dough before baking. Four separate lots of labeled rolls were prepared during the investigation and were stored at −20 °C until required. The subjects were instructed to eat the rolls slowly during the course of their meal. To avoid labeling meals containing only small amounts of iron, the subjects were instructed to eat the labeled bread rolls with two rather than three meals each day because preliminary dietary evaluation indicated that most of the volunteers consumed only two large meals each day. The subjects were required to keep a record of the food items consumed with the labeled rolls as well as all other foods consumed separately. These dietary records were carefully reviewed with the individual volunteers on four separate occasions during the 2-wk period of labeling.

A different diet was used in each of the three study groups. In the first group (SS group) absorption was measured from a self-selected diet. On the basis of a preliminary dietary evaluation, these subjects were chosen because their food preferences represented a wide spectrum of predicted dietary nonheme-iron bioavailability. They were instructed to consume their usual diet during the 2-wk labeling period. Analysis of their dietary records indicated that the average caloric content of the meals consumed with the labeled rolls was 3200 ± 1037 kJ ($\bar{x}$ ± SD) and the average iron content was 5.49 ± 2.44 mg. The foods consumed with the labeled meals represented an average of 76.1% of the caloric intake during the 14-d labeling period. The second group of subjects (EN group) was required to modify their diet in a manner that would maximally enhance the absorption of nonheme iron. All meals were required to contain ≥ 90 g of meat, poultry, or fish and sufficient fresh fruit, citrus juice, or fresh vegetables to provide ≥ 100 mg vitamin C. They were not allowed to drink tea or coffee with their meals, and eggs or foods with a high content of bran were not permitted.

In the last group (IN group) the diet was modified to inhibit the absorption of nonheme iron maximally. No meat products were allowed and no more than four of the 28 meals could contain a limited serving of fish or chicken. No fresh vegetables or fruits, citrus juices, or foods fortified with ascorbic acid were permitted. Dairy products were not restricted and the intake of whole eggs or egg yolks was encouraged. The subjects were requested to include generous servings of legumes, cereals, and foods rich in bran, such as whole-wheat bread and bran muffins. At least one cup of hot tea or coffee or a glass of iced tea was taken with each meal.

The second absorption test was performed to measure absorption from a single meal that was designed to represent the diet consumed during the period of dietary labeling. In the SS group all subjects were fed a different meal, each chosen to represent the individual's customary diet. In the remaining study groups the same meal was fed to all subjects. In the EN group this consisted of a meat pot pie, a bread roll, and 240 mL frozen orange juice. In the IN group the meal contained cheese pasta, whole-wheat bread, and either 150 mL iced tea or hot coffee. These typical meals were fed in the laboratory to fasting subjects on four successive mornings during the second week of dietary labeling. The meals were tagged extrinsically by distributing 1 mL 0.05 mol HCl/L containing 0.1 mg iron as 9 kBq $^{59}FeCl_3$ over the food items.

For the remaining two absorption measurements the same test meal was fed to subjects in all three study groups. Both tests were designed to characterize the absorptive behavior of each subject. The first was a standard meal containing 113 g ground beef, 53 g bun, 68 g French fries, and 148 g milk shake; the total iron content was 4.8 mg of which 3.4 mg was nonheme (7, 8). The meal was tagged extrinsically by pipetting $^{55}FeCl_3$ onto the bun as described previously. The second test meal was a reference dose of inorganic iron consisting of 3 mg iron as $^{59}FeSO_4 \cdot 7H_2O$ in 50 mL water (9, 10). Immediately before administration, 18.9 mg freshly prepared ascorbic acid was added, sufficient to give a 2:1 molar ratio of ascorbate to iron.

Absorption measurements

Except for dietary tagging, all test meals were administered between 0700 and 0900 after a 10-h fast. Thirty milliliters of blood was drawn on the day preceding the first test, for measurement of serum ferritin (11) and background blood radioactivity. The total dose of radioactivity in each test was 37 kBq ^{59}Fe or 111 kBq ^{55}Fe. On day 28 of the study, 14 d after the period of dietary labeling, blood was drawn for measuring incorporated red-cell radioactivity. The standard meal and reference dose were fed on the next two mornings and a final blood sample was drawn 2 wk later to measure the increase in red-cell radioactivity. The latter was measured in duplicate 10-mL blood samples by using a modification of the method of Eakins and Brown (12). Percentage absorption was calculated on the basis of blood volume estimated from height and weight (13, 14). Red-cell incorporation of absorbed radioactivity was assumed to be 80% in subjects with a serum ferritin > 15 μg/L and 100% in subjects with a serum ferritin ≤ 15 μg/L (15).

Iron-bioavailability estimate

A scoring system was developed to estimate nonheme-iron availability. The approach was similar to that used previously for predicting the enhancing effects of meat, fish, poultry, and ascorbic acid (16) but included the effect of absorptive inhibitors. A score of 5 was taken to represent a neutral meal with regard to bioavailability. Points were added for enhancers as follows: 30–120 g meat, fish, or poultry, 1 point; > 120 g meat, fish, or poultry, 2 points; 25–75 mg ascorbic acid, 1 point; > 75 mg ascorbic acid, 2 points. Points were subtracted for inhibitors as follows: 120–240 mL tea, 1 point; > 240 mL tea, 2 points; ≥ 180 mL coffee, 1 point; 30 g whole-wheat bread, bran muffin, or bran cereal, 1 point; > 40 g egg, 1 point. Scores for individual meals ranged from 1 to 10. For estimates of bioavailability of

TABLE 1
Age, iron status, and absorption data for subjects ingesting diets that were self-selected, enhancing, or inhibiting to iron absorption

	Self-selected	Enhancing	Inhibiting
Number of subjects (M/F)	15 (6/9)	15 (4/11)	15 (5/10)
Age (y)*	25 (21–30)	25 (21–34)	26 (21–40)
Serum ferritin (μg/L)*	34 (8–242)	48 (6–252)	37 (12–236)
Absorption†			
Dietary labeling	7.4 (5.6–9.9)	6.6 (5.4–8.0)	3.4 (2.6–4.6)
Typical meal	7.2 (5.3–9.7)	11.1 (8.7–14.3)	2.5 (1.8–3.4)
Standard meal	8.9 (7.0–11.4)	6.4 (5.4–7.5)	8.8 (6.7–11.5)
Reference dose	41.2 (33.5–50.6)	33.7 (27.5–41.3)	36.8 (30.9–43.8)
Dietary:typical	1.03 (0.87–1.23)	0.59 (0.52–0.67)	1.38 (1.26–1.52)
Corrected absorption (ferritin method)†			
Dietary labeling	6.4 (5.5–7.3)	8.0 (7.0–9.2)	3.2 (2.8–3.6)
Typical meal	6.1 (4.7–7.9)	13.5 (11.9–15.4)	2.3 (2.0–2.6)
Meal score†	5.4 (4.5–5.9)	7.0 (6.5–7.8)	3.8 (2.6–4.4)

* $\bar{x}$ (range).
† Geometric $\bar{x}$ (±1 SE).

the diet, each of the 28 meals was scored separately and the average was calculated.

Iron-status correction

Because of the marked influence of iron status on absorption, comparisons of dietary absorption in the three study groups required a technique for correcting individual absorption values to a common reference point. Three methods were examined. The first, used extensively in prior studies, was based on absorption of the reference dose (10). Dietary absorption was corrected to a mean reference value of 40% in each subject by multiplying by 40/R, where R is the reference-dose absorption. The second method was similar but used standard meal absorption and an assumed average value of 8%. The third method was based on serum ferritin concentrations, which bear a close inverse relationship to iron absorption (10, 17, 18). The serum ferritin value used in each subject was an average of five separate measurements obtained every third day during the 2-wk labeling period. On the basis of prior studies, we used a slope of −1.0 for the regression of log iron absorption on log serum ferritin. Dietary absorption in each subject was corrected to a value corresponding to a serum ferritin of 40 μg/L by using the following equation:

$$\text{Log } A_c = \text{Log } A_o + \text{Log } F_o - \text{Log } 40$$

where A_c is corrected dietary absorption, A_o is observed absorption, and F_o is observed serum ferritin.

Statistical analysis

Percentage-absorption values were converted to logarithms for statistical analysis and the results were retransformed to recover the original units (19). A paired *t* test was used to compare absorption from dietary, typical, and standard meals. This was accomplished by determining whether the mean log absorption ratios differed significantly from zero, which is equivalent to determining whether the mean ratio of percentage absorption differs from 1. Serum ferritin concentrations in the three study groups were compared by analysis of variance, and multiple-regression analysis was used to compare the influence of iron status and bioavailability on iron absorption. The *ABSTAT* program (Anderson Bell Corp, Parker, CO) was used for statistical analyses.

Results

The three study groups were similar with respect to age and range of iron status (**Table 1**). Mean dietary absorption in subjects consuming their usual diet was 7.4%. Mean absorption from an inhibiting diet was sharply lower (3.4%) but, unexpectedly, absorption from the enhancing diet was also slightly lower (6.6%). This finding was apparently due to higher iron stores in the EN group. Although serum ferritin concentrations did not differ significantly among the three groups when tested by analysis of variance ($P > 0.10$), the mean serum ferritin in the EN group was appreciably higher and absorption of both the standard meal and reference dose was lower than in the other two study groups. A valid comparison of dietary absorption in the three groups therefore required a correction for iron status.

A correction based on reference-dose absorption proved to be less suitable than corrections using either standard-meal absorption or serum ferritin. For example, correlation coefficients for dietary and reference absorption were far lower in the SS, EN, and IN groups (0.496, 0.287, and 0.587, respectively) than were the correlation coefficients for dietary absorption and either standard-meal absorption (0.846, 0.865, and 0.860, respectively) or serum ferritin (−0.877, −0.857, and −0.903, respectively). Furthermore, the variability in dietary absorption values within each group, which is due mainly to individual differences in iron status, was not reduced significantly with corrections based on reference dose. On the other hand, marked reductions occurred with corrections based on either standard meal or serum ferritin (**Fig 1**). The variance of log absorption in the SS group, for example, was reduced from 1.231 to 0.35 and 0.29 when corrected by standard meal and serum ferritin, respectively, reductions of 72% and 77%. Corrected mean absorption values in the SS, EN, and IN groups were 6.7%, 8.2%, and 3.1% based on the standard-meal correction, and almost identical values of 6.4%, 8.0%, and 3.2% were obtained by using the serum ferritin correction (Table 1). We selected the latter method because serum

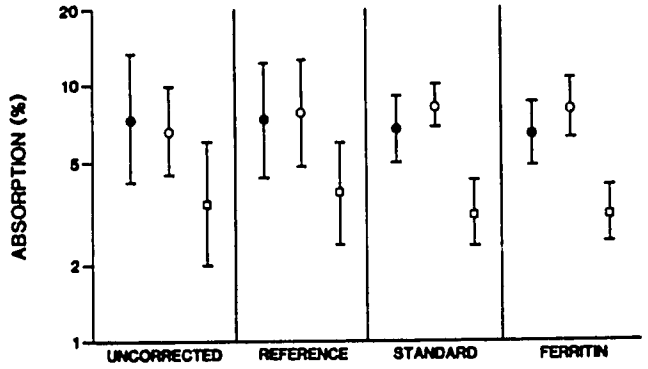

FIG 1. Dietary iron absorption in groups consuming a self-selected (●), enhancing (○), or inhibiting (□) diet. Results are shown for uncorrected values and values corrected by reference-dose absorption, standard-meal absorption, or serum ferritin. The vertical bars represent ±2 SE.

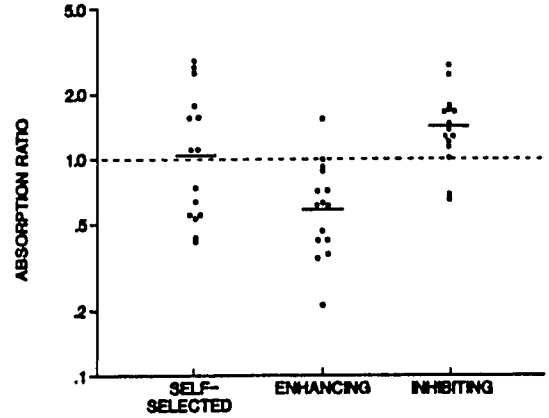

FIG 2. Ratio of iron absorption from dietary and typical meals in subjects consuming a self-selected, enhancing, or inhibiting diet. The horizontal interrupted line depicts a ratio of 1.

ferritin is a more widely used index of iron status. Corrected dietary absorption in the EN group was higher than in the SS group (8.0% vs 6.4%) but this difference was not statistically significant ($P > 0.10$). On the other hand, the difference in mean absorption of 3.2% between the SS and IN groups (6.4% vs 3.2%) was significant ($P < 0.01$).

Dietary absorption values were highly correlated with single (typical) meal values in the three study groups ($r = 0.824$, 0.863, and 0.950 in the SS, EN, and IN groups, respectively). In the SS group excellent agreement was also observed in mean values for dietary (7.4%) and typical (7.2%) absorption (Table 1). The mean absorption ratio for the two meals was 1.03 (0.87–1.23, ±1 SE). In the other study groups, however, there was a significant disparity between mean dietary and typical meal absorption (Fig 2). In the EN group, absorption from the typical meal averaged 13.5% as compared with only 8.0% from the diet; the difference was highly significant as reflected by the mean ratio of 1.41 ($t = 4.0$, $P < 0.001$). In the IN group a similar disparity of 38% was observed but in the reverse direction; dietary absorption averaged 3.4% as compared with 2.5% from the typical meal ($t = 3.31$, $P < 0.01$).

Estimates of iron bioavailability of the diets ingested during the labeling period differed markedly in the three study groups (Table 1). The meal scores averaged 5.4 in the SS group, 7.7 in the EN group, and 3.8 in the IN group and there was virtually no overlap among the three groups (Fig 3). The bioavailability scores corresponded well to measurements of dietary absorption. For example, dietary absorption was 25% higher in the EN group than in the SS group and the meal score was also 30% higher. In the IN group the decreases in dietary absorption and meal scores were comparable, 50% and 46%, respectively. The meal score and typical meal-absorption values were highly correlated in the SS group ($r = 0.641$, $P < 0.01$) but this relationship could not be examined in other groups because the same meal was fed to all subjects. In the total group of 45 subjects, a highly significant correlation was observed between meal scores and dietary absorption ($r = 0.681$, $P < 0.001$) but this was not significant when examined in each group separately. This finding presumably reflects the relatively narrow range in bioavailability scores within each group, even in the SS group of subjects who were allowed to select their diet without restriction.

To compare the relative importance of iron status and bioavailability as determinants of dietary absorption, multiple-regression analysis was performed by using dietary absorption as the dependent variable. There was a highly significant relationship with serum ferritin ($t = 7.399$, $P < 0.0001$) but not with meal score ($t = 1.90$, $P = 0.081$). Similarly, when dietary absorption was corrected to a constant concentration of iron reserves by using the ferritin method, the correlation between absorption and bioavailability score increased from 0.103 to 0.464 but remained insignificant ($P = 0.082$). These results indicate that iron status rather than dietary bioavailability is the major factor determining the percentage absorption of dietary nonheme iron.

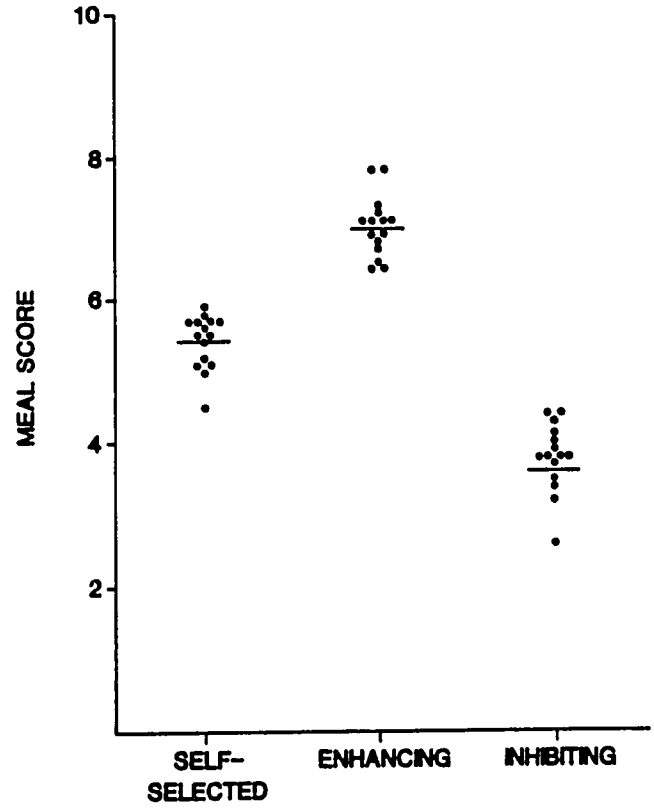

FIG 3. Meal scores of bioavailability in groups consuming a self-selected, enhancing, or inhibiting diet.

Discussion

There is abundant evidence that the biochemical composition of a meal influences nonheme-iron absorption. Hallberg (3) reported that percentage absorption from complex meals ranged from 2.2% to 45.0% depending on the content of meat, ascorbic acid, organic acids, bran, and other determinants. The striking influence of meal composition on nonheme-iron absorption from individual meals was also observed in the present study. With a meal designed to maximize iron absorption by including both ascorbic acid and meat, corrected values averaged 13.5%, whereas with an inhibitory meal absorption was only 2.3%. This 5.9-fold difference implies that dietary bioavailability should be a major determinant of iron status in otherwise healthy individuals. However, when absorption was measured from the total diet, the difference between an inhibiting and enhancing diet was much smaller. Mean absorption from the EN and IN diets was 8.0% and 3.2%, a difference of only 2.5-fold.

Several possible explanations for the apparent difference in the effect that bioavailability has on the absorption of iron from single meals and from the diet were considered. In the EN and IN groups the single meal fed in the laboratory was selected only by bioavailability and may have differed in other important respects from the meals consumed with the labeled rolls. For example, the iron content of the single meal was fixed whereas the iron content of the meals consumed with the labeled rolls was not controlled and varied substantially because of differences in the caloric intake of our male and female subjects. However, meal iron content was shown to have only a small influence on percentage iron absorption (1, 20). Subjects who were fed in the laboratory had been fasting for 8–10 h whereas a shorter and more variable time elapsed between prior food intake and meals tagged to measure dietary absorption. As compared with fasting subjects, residual food in the stomach or small intestine may have dampened the effect of inhibitors and enhancers. Another possibility is that our subjects were not fully compliant with the imposed dietary modifications even though they were carefully screened and were considered highly motivated when interviewed during the study. A more plausible explanation is that the influence of dietary inhibitors and enhancers is diluted in a normal diet by meals that have no overall effect on nonheme-iron absorption.

Additional evidence indicating that there is a limited range of iron bioavailability in a highly varied diet was provided by absorption data in subjects who were allowed to consume their own diets. We expected to find a wide range for both meal scores and dietary iron absorption because subjects were chosen because they were believed to consume diets that differed maximally in their content of factors known to affect nonheme-iron bioavailability. Some subjects consumed meat, fish, or poultry with most meals whereas others consumed a vegetarian-type diet. Representative meals confirmed these differing preferences with bioavailability scores ranging from four to nine. Moreover, the correlation between meal score and single-meal absorption was highly significant. On the other hand, when meal scores were averaged for the 28 meals tagged during the 2-wk period of self-selected dietary consumption, the range was relatively narrow (4.5–5.9). A correlation coefficient of −0.877 was observed between dietary absorption and serum ferritin in these subjects, indicating that most of the variation in dietary absorption was due to differences in iron status. Multiple-regression analysis indicated that bioavailability may have accounted for some of the variability ($P = 0.082$). Note that the high correlation between dietary absorption and serum ferritin reflects the wide range in iron status of our subjects. Although it is not possible to draw any firm conclusions from this limited study, the observation does suggest that the wide range of foods available to most Americans and their preference for dietary variability makes it unlikely that a quantitatively significant imbalance between powerful enhancers and inhibitors of iron absorption would be present in a sufficient number of meals to make bioavailability an important factor in determining iron status.

The method for estimating bioavailability used in this study represents the first attempt to incorporate the effects of both enhancers and inhibitors on nonheme-iron absorption. The scoring system was able to predict absorption from typical meals in the SS group, and a significant correlation was also observed between bioavailability scores and dietary absorption in the composite group of 45 subjects. Absorption data indicated that in the context of the American diet, inhibitors are more important than enhancers. In using a model to predict dietary bioavailability, an important technical problem is whether to base estimates directly on specific food items or on actual biochemical measurements such as polyphenol or phytate content. The latter approach is obviously more desirable but requires more information than is presently available on the biochemical composition of different foods, the magnitude of the effect of different determinants, and their relative importance when contained in the same meal.

An important finding in this study is that the representative American diets consumed by our volunteer subjects were nearly optimal with respect to food-iron availability. Mean dietary absorption in the SS group was only slightly lower (16%) than that from a standard hamburger meal containing a generous portion of meat and from a diet designed to enhance iron-absorption maximally. The diets selected by subjects in the SS group are typical of those eaten by many Americans but clearly do not represent all segments of the population. It is impractical to use isotopic methods to examine all types of diets, but the bioavailability scores used in this study did correlate with iron absorption and could provide more information on this point if based on accurate meal records collected over a sufficient period of time.

One of the major difficulties in evaluating food-iron absorption is the exquisite sensitivity of the absorptive process to iron status. The influence of iron stores was reduced in prior studies by relating food-iron absorption in each subject to a reference dose of inorganic iron. In the present investigation we found that this corrected absorption was more variable when based on reference-dose absorption than when based on either standard-meal absorption or serum ferritin, although the corrected means were similar with all three methods. Absorption of inorganic iron is apparently more variable than the absorption of food iron or is perhaps more influenced by recent food intake in the occasional subject who is not compliant with fasting. Corrections based on standard-meal absorption and serum ferritin were comparable in accuracy and precision but serum ferritin has certain advantages. The assay is widely available, can be accurately standardized (21), and eliminates the need for an independent radioisotope measure of absorption. Absorption values were corrected to a serum ferritin value of 40 μg/L in the present study because this value was close to the overall average in our volunteers. However, absorption can be predicted at any iron status. Because

of a significant day-to-day variation in the serum ferritin concentrations, we believe it is important to base the corrections of absorption values on several serum ferritin determinations obtained during the course of a study.

One question that arises when the ferritin method is used is whether the regression slope varies with different diets or meals. We found no evidence to indicate this. The regression slope of dietary absorption against serum ferritin was −0.88 in the composite group. When values were calculated in the three study groups separately, the differences were not statistically significant despite wide variation in absorption. In addition, a moderate difference in slope has only a small effect on the corrected absorption value. For example, if a regression slope of −0.8 rather than −1.0 is used to correct absorption of dietary iron in the SS group, a mean absorption value of 6.6% rather than 6.4% is obtained. A slope of 1 is convenient when predicting the correspondence between iron absorption and iron stores. For example, if an individual subject in the SS group with a serum ferritin value of 40 μg/L began eating an inhibiting diet with 50% less absorption, the serum ferritin would have to fall to 20 μg/L to recover iron balance by increasing absorption of nonheme iron.

The present observations have important practical considerations for concerns about including foods known to markedly affect nonheme-iron bioavailability in the American diet. For example, the partial substitution of alternative protein sources that reduce nonheme-iron bioavailability for meat is unlikely to have a major effect on overall iron nutrition unless the quantity of heme iron consumed is reduced. Conversely, the common practice of ingesting large quantities of vitamin C would not be expected to lead to excessive nonheme-iron absorption in normal individuals. Although factors affecting nonheme-iron bioavailability may have limited influence, the dietary content of heme is obviously important because the fraction is avidly absorbed. A correlation between iron status and meat intake was demonstrated in epidemiologic studies (22, 23), and meat intake was shown to correlate with iron status in college students (24). It is also inappropriate to extrapolate our findings to developing countries, where the diet is usually less varied and contains many inhibitors (25). The more monotonous the diet, the more likely that bioavailability is important. Differences in dietary composition undoubtedly contribute to geographic variations in the prevalence of iron deficiency in developing countries.

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Screening Strategies for Nutritional Iron Deficiency

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The usual approach to combating nutritional iron deficiency is to provide additional iron to the population at risk. An alternative approach is to screen the population first and to give iron only to those who are identified as iron-deficient by laboratory measurements. One advantage of this approach is that iron is not given to individuals who do not require it. This is not a concern with short-term interventions such as iron supplementation in pregnancy or cereal fortification in infancy, but there are reasons to avoid giving iron indefinitely to iron-replete individuals. Idiopathic hemochromatosis is now estimated to affect about one in 300 individuals of European descent (1,2), and there is little doubt that the progression of this disease is accelerated by increasing iron intake (3). Another concern with untargeted iron fortification or supplementation is that genetically normal individuals with higher iron reserves may be at greater risk of developing cancer (4,5). The risk of accelerated the progress of hemochromatosis and increasing the rate of malignancy would be eliminated by limiting iron administration to those with a proven deficiency.

The main disadvantages of screening programs are cost and inconvenience. Personnel are needed to obtain the blood sample, perform the laboratory measurement, and provide counseling. Many laboratory measurements cannot be performed immediately, necessitating a return visit. Laboratory costs vary widely with different screening strategies, but there is some expense with all laboratory measurements. Despite the cost, screening programs to identify iron deficiency can be justified in certain segments of the population with a high prevalence of iron deficiency, especially when the liabilities of iron deficiency in the target group are significant. Compliance and therapeutic efficacy will also improve with individual detection and counseling.

GROUPS AT RISK OF IRON DEFICIENCY

Four segments of the population at high risk of developing iron deficiency might benefit from a screening program. These are (a) rapidly growing individuals, (b) pregnant women, (c) persons undergoing endurance training, and (4) vegetarians.

Rapid Body Growth

Iron deficiency is common when rapid body growth exceeds the available dietary iron supply. This can occur at any time during the first two decades of life, but infants and preschool children are especially vulnerable. Teenagers are also susceptible to iron deficiency because of menarche and the adolescent growth spurt. Screening to detect iron deficiency is not commonly performed after infancy, but identification of iron deficiency in preschool children may be particularly rewarding because of the associated defects in cognitive performance and learning ability.

Pregnancy

Screening programs have been used extensively to identify iron deficiency during pregnancy, but the benefit of routine hematological assessment is still widely debated. Many obstetricians believe that anemia in pregnancy is usually dilutional, resulting from a disproportionate increase in circulating plasma volume. Nevertheless, there is now firm evidence for an association between anemia in pregnancy and prematurity (6,7). For screening to be effective in preventing this complication, iron deficiency must be identified early enough in gestation to permit a hematological response to iron before the third trimester.

Endurance Training

Individuals engaged in endurance training are at increased risk of developing iron deficiency (8). In one Canadian report, 82% of female and 29% of male distance runners had serum ferritin levels in the iron-deficient range (9). Various factors have been proposed to account for this, including poor dietary habits, iron loss due to red cell destruction, gastrointestinal blood loss due to gut ischemia, and hemodilution from an expanded plasma volume. While most studies have identified an increased frequency of iron deficiency, overt anemia is relatively uncommon. Because there is evidence that iron deficiency, even without anemia, may impair athletic performance, there is mounting interest in screening programs to identify iron deficiency in high-school or endurance athletes.

Vegetarianism

The relationship between iron status and either the amount or type of dietary iron is an unresolved question in human iron nutrition. While dietary iron intake is commonly assessed by nutritional counseling, there is no firm evidence that individuals at risk of iron deficiency can be identified by dietary history. The most important component of the diet with respect to iron status is the intake of meat, not only because the heme iron it contains is well absorbed but because it also promotes the

absorption of non-heme iron. In a recent study of healthy adults in a meat-eating population, a correlation was demonstrated between serum ferritin levels and meat consumption (10). While there may be some benefit in screening vegetarians for iron deficiency, there is still no firm evidence that this group is at significant risk of iron deficiency. Many dietary components such as ascorbic acid, phytate, bran, calcium, tea, and coffee have been shown to influence non-heme iron absorption, but none of these have consistently been identified in epidemiologic studies as determinants of iron status.

Other Causes of Iron Deficiency

There are many other causes of iron deficiency that are not included in this listing, either because they are not primarily nutritional in nature or because they do not identify groups that are particularly suitable for screening programs. Some examples include frequent blood donations, menorrhagia, hookworm and other parasitic infestations, prolonged lactation, high parity, and abject poverty.

LABORATORY STRATEGIES FOR IDENTIFYING IRON DEFICIENCY

There is a large array of laboratory methods to identify iron deficiency and assess its magnitude (11). The present discussion is limited to methods that are suitable for screening purposes. Techniques that are simple and inexpensive and that require a finger prick rather than venous sampling are obviously preferred. More elaborate methods are often justified because of the greater information they provide. Sensitivity and specificity are particularly important when only one or two measurements are used. As isolated measurements, plasma transport variables (such as the serum iron, total iron-binding capacity, or transferrin saturation) and red cell indices (such as the mean corpuscular volume or red cell distribution width) are either too costly, cumbersome, or not specific enough for iron deficiency to be used for screening. Four laboratory measurements, used either singly or in combination, are suitable

TABLE 1. *Laboratory screening for iron deficiency*

Single measures
Hemoglobin
Serum ferritin
Erythrocyte protoporphyrin
Serum transferrin receptor
Dual measures
Serum ferritin + hemoglobin
Erythrocyte protoporphyrin + hemoglobin
Serum receptor + hemoglobin
Serum ferritin + serum receptor

for screening purposes: hemoglobin or hematocrit, serum ferritin, erythrocyte protoporphyrin, and serum transferrin receptor (Table 1).

INDIVIDUAL MEASUREMENTS

Hemoglobin Concentration

Many screening programs are based on either the hemoglobin concentration or the hematocrit as the only laboratory measure. Hemoglobin determination is one of the most convenient screening methods and has been further simplified by the introduction of small portable instruments and self-filling disposable pipets. The detection of anemia is more useful when the prevalence of iron deficiency is high, as in pregnancy or infancy. The major limitation of hemoglobin measurement is low specificity. For example, the hemoglobin concentration alone does not distinguish between iron deficiency anemia and anemia due to chronic infection. The sensitivity of the hemoglobin measurement is also limited by the marked overlap in values between normal and iron-deficient populations (12,13). The usefulness of hemoglobin measurements is greatly enhanced by including a second, more specific index of iron deficiency.

Serum Ferritin

The serum ferritin is a sensitive laboratory index of iron status (14,15), but it has certain limitations as a screening method. It is a costly procedure and some laboratories require several days to obtain a result. A serum ferritin below 12 μg/l is highly specific for iron deficiency but gives no information about its magnitude. The serum ferritin is of lesser value when iron deficiency is common, as in infants and pregnant women.

Erythrocyte Protoporphyrin

Earlier techniques for measuring erythrocyte protoporphyrin employed cumbersome extraction methods that restricted its use for screening purposes. The introduction of the hematofluorometer was a major advance because it gives an immediate result and requires little or no technical experience (16). A battery-operated model is of particular value for screening programs in the field. Raised erythrocyte protoporphyrin levels also occur in lead poisoning, which may limit the value of the test as a single measure of iron deficiency in children. A raised protoporphyrin level identifies a more advanced stage of iron deficiency, and because the level increases only slowly after the onset of iron deficiency, it is less useful for identifying iron deficiency during pregnancy.

Serum Transferrin Receptor

The newest iron measure is the serum transferrin receptor, which is destined to play an important role in the assessment of iron status. The measurement is an outgrowth of intensive efforts during the past decade to define the mechanisms by which cells acquire iron from their environment. The protein plays a key role in receptor-mediated endocytosis, the process by which transferrin iron is delivered to the cytosol. When a cell perceives a need for additional iron, there is an up-regulation of transferrin receptor synthesis which allows the cell to compete more effectively for circulating transferrin iron. Reports from several laboratories (17–19) have shown that small amounts of transferrin receptor can be detected in the serum with sensitive immunological assays. The serum receptor increases with enhanced red cell production, but iron deficiency is the only disorder in which there is increased serum receptor combined with a low level of red cell production. The circulating receptor is analogous to the serum ferritin in that minute quantities of a circulating protein reflect the total body mass of the protein. The serum ferritin reflects body iron stores, whereas the serum receptor measures tissue iron need. The serum receptor is measured by the same enzyme-linked immunoabsorbent assay (ELISA) system as the serum ferritin and requires only a few microliters of plasma or serum. The major obstacle in measuring serum transferrin receptor is that commercial assay kits are not yet available. However, techniques for isolating the protein from human placenta are well defined, and laboratories experienced with immunoassay should have no difficulty in establishing the assay.

Measurements of serum transferrin receptor offer certain advantages in a screening program. Unlike the serum ferritin, which only identifies iron deficiency, the serum transferrin receptor measures its severity. Certain disorders such as sickle cell anemia, thalassemia, or malaria may be confused with iron deficiency, but these hematologic disorders affect only certain populations and can usually be identified in other ways. Further studies are needed to determine the influence of folate deficiency on serum transferrin receptor concentrations, but increased values are likely. One important advantage of the serum receptor level is that it becomes raised shortly after the onset of tissue iron lack, much earlier than red cell indices or erythrocyte protoporphyrin (20). The measurement is therefore of particular value in identifying iron deficiency during pregnancy (21). Another important feature of serum transferrin receptor is that, unlike many other iron measurements, the level remains normal in patients with the anemia of chronic inflammation of infection (22) and therefore assists in identifying iron deficiency in populations where chronic infection is common.

MULTIPLE INDICES

Most of the liabilities associated with iron deficiency have been linked to the associated anemia, and consequently the hemoglobin remains a key screening mea-

surement. However, because of its low sensitivity and specificity, its usefulness is greatly enhanced by coupling it with a more specific index of iron status. A very useful combination of measurements is the *hemoglobin and serum ferritin* (Table 1). If both measurements are normal, iron deficiency is excluded; if both are low, iron deficiency anemia is unequivocally identified. If the serum ferritin is low but the hemoglobin is normal, the individual is at risk of iron deficiency, while if the hemoglobin is low but the serum ferritin is normal, further hematological assessment is required to identify the cause of anemia.

The main drawback of using both the hemoglobin and serum ferritin for screening is the cost of the serum ferritin assay and the delay in obtaining a result. If cost is a constraint or if an immediate result is needed, the *hemoglobin and erythrocyte protoporphyrin* are a useful combination. Results can be obtained shortly after taking the blood sample, and battery-powered instruments are available for field use. Iron deficiency without anemia can also be identified reliably with the *hemoglobin and serum receptor*. If both are normal, tissue iron deficiency can be excluded, whereas a raised serum receptor and a normal hemoglobin detects milder iron deficiency. Further experience with this combination in screening programs is needed.

Another important combination is the *serum ferritin and serum receptor*. The advantage of these measurements is that they portray the entire spectrum of iron status ranging from normal to severe iron deficiency. They can be performed efficiently in the laboratory because the methodology is identical. The serum ferritin measures residual iron stores, whereas the serum receptor measures the deficit in tissue iron. In a recent study in which a wide spectrum of iron status was induced by repeated phlebotomies, body iron could be accurately assessed from the serum ferritin and transferrin receptor, either used separately or expressed as a ratio (Fig. 1) (20).

The optimal combination of laboratory measurements depends on the target population. When the prevalence of iron deficiency is high, the hemoglobin and erythrocyte protoporphyrin are useful but the hemoglobin and transferrin receptor may be an even better combination. Because of the rapid change in transferrin receptor with the onset of iron deficiency, the latter combination is of particular value for

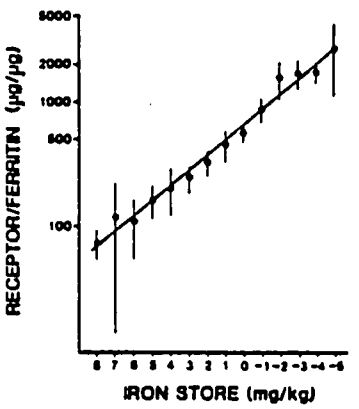

FIG. 1. Relationship between iron stores and serum receptor/ferritin ratio during phlebotomy (20). The vertical bars represent ± 2 SEM.

assessing iron status during pregnancy. With milder degrees of iron deficiency, the hemoglobin and serum ferritin are a useful combination although they do not distinguish between storage iron depletion and tissue iron deficiency without anemia. The latter can be detected by using the transferrin receptor and serum ferritin in tandem. Fortunately, there are several laboratory options for identifying iron deficiency in screening programs.

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DISCUSSION

Dr. Finch: The studies of plasma transferrin receptors have established this measurement as valuable in the detection of iron deficiency. Each of a number of measures of iron deficiency has its own peculiarities. Transferrin saturation indicates the supply of iron to the tissues: red cell protoporphyrin is sensitive to the iron supply to the individual erythroid cell: serum ferritin reflects the state of the iron stores and also responds as an acute phase protein in inflammation; measurement of transferrin receptors reflects the adequacy of iron supply to body cells at large—it does not appear to be affected by inflammation but is influenced by changes in the rate of erythropoiesis. A combination of serum ferritin and transferrin receptor measurements would permit the evaluation of iron status even in the face of inflammation or increased erythropoiesis.

Dr. Bothwell: Will this new tool make it any easier to diagnose iron deficiency in places such as Africa where there are often complicating chronic inflammatory states?

Dr. Cook: Our information on transferrin receptor levels when iron deficiency and infection coexist is based on clinical studies. Although we do not have direct information on this, I suspect that a rise in receptors occurs in proportion to the degree to which iron deficiency is contributing to the anemia. However, it is difficult to assess the relative importance of inflammation and iron deficiency when these disorders coexist in an anemic individual. Our clinical observations suggest that the serum receptor will increase in the presence of inflammation if iron deficiency is the more important cause of the anemia.

Dr. Hershko: This new test has the major advantage of being sensitive at the lower end of the scale. Ferritin is excellent for iron overload, but once you get values of 12 ng/ml or less you really don't know how severe the iron deficiency is.

Dr. Hallberg: In screening for iron deficiency in developing countries, I think that hemoglobin should always be included as a measure of the severity of anemia.

I should like to mention some of our findings in relation to sustained endurance training. We have shown that under these conditions there is increased intravascular hemolysis with reduced serum haptoglobin, increased uptake of the hemoglobin–haptoglobin complex in the heptocytes, and a reduced uptake of dying red cells in the reticuloendothelial system, the latter being probably responsible for the reduction in serum ferritin. The elite swimmers and runners we have examined showed no sign of iron deficiency or iron-deficient erythropoiesis, but they had lower serum ferritin and less (but never zero) iron in their bone marrow smears. The classical indices of iron status may therefore be misleading due to the change in iron metabolism in such individuals.

Dr. Hercberg: Serum ferritin is very difficult to interpret during inflammation. Red cell ferritin seems more stable in this context. Do you have any experience in measuring this variable?

Dr. Cook: We have some experience with red cell ferritin measurements. The major problem in my view is the additional processing time required. I am not convinced that the information gained can justify the additional time and expense.

Dr. Viteri: What happens to transferrin receptors when you give a single large dose of iron to an iron-deficient individual? On the one hand, this would act to reduce the increased numbers of receptors which are present because of the iron deficiency; on the other hand, the receptors should be increased because of increased erythroid activity. Have you tested this?

Dr. Cook: In subjects in whom iron deficiency anemia was induced by repeated phlebot-

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omy, we did not observe a further increase in transferrin receptors following oral iron replacement. At the same time, the transferrin receptor level does not return promptly to normal, changing more slowly than the serum ferritin level. The slower decline in receptors may reflect increased erythropoiesis.

Dr. Viteri: This is an important issue. One of the problems of ferritin estimations in trying to estimate the increase in iron reserves in populations supplemented with iron is the fact that ferritin rises in response to iron intake. Can you comment on the level of daily supplementation at which ferritin loses its value in the estimation of iron stores?

Dr. Cook: I believe that serum ferritin is a valid measure of iron reserves in the face of iron administration. If serum ferritin rises, it is because iron is actually being deposited in the iron stores.

Dr. Cooper: All proliferating cells have transferrin receptors, and Rose Johnstone has measured these on microvesicles in plasma. She feels these reflect the status of the receptors on the cells of the body. Is your receptor specific for erythroid cells or does the level increase in patients with abscesses, cancers, etc.?

Dr. Cook: We have searched hard for exosomes in the serum of normal individuals and cannot find them. We have also used specific peptide antibodies against intact and soluble receptors and again cannot find evidence of intact receptor in human serum. However, we have not searched in all clinical conditions for the presence of exosomes, and we have obtained evidence of exosomal production by K562 erythroleukemia cells. We have also measured serum receptor levels in a wide variety of hematological malignancies and, except in the case of chronic lymphatic leukemia, serum receptor usually remains normal.

Dr. Fomon: Could Dr. Finch comment on a remark he made to me about the need for non-erythroid indices of iron deficiency?

Dr. Finch: Most of the measurements we use to detect iron deficiency relate to red cell production—that is, the hemoglobin concentration, the red cell protoporphyrin, the mean corpuscular volume. This seems appropriate since the iron requirements of the erythron are so high and it is so sensitive to iron lack. Iron deficiency of the erythron may not be associated with iron deficiency in other tissues. The only measurement directly reflecting iron supply to other tissues is the plasma transferrin saturation, the important aspect being the amount of diferric transferrin. Even this does not tell us the relationships between transferrin iron content, tissue requirement, and the capacity of the tissue to extract iron. When we discuss possible effects of iron deficiency on brain function, we are guided only by the mean iron supply (as evaluated by the plasma iron and transferrin saturation) and by the average total transferrin receptor value, which is dominated by the erythroid receptors, whereas the brain has its own transferrin system. Since individual tissues differ in their vulnerability to iron deficiency, it would seem that we need more direct measurements of tissue iron status to relate to our studies of the effects of iron deficiency.

Dr. Cook: I believe that the rise in serum transferrin receptor in iron deficiency is greater than can be explained by the increased density of transferrin receptors on erythroid precursors. I suspect that the serum receptor increase is derived in part from non-erythroid tissues and reflects tissue iron supply in general, though this may be difficult to prove.

Dr. Anderson: A possible approach might be along the lines that are being followed in using platelet serotonin content and serotonin receptor measurement as an index of central nervous system serotonin. Attempts have been made to use platelet serotonin as an indication of what is happening in serotoninergic neurons centrally. I wonder whether there are iron receptors on platelets. The extent to which they take up iron might reflect the central nervous system concentration.

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Dr. Brabin: Is it possible that there could be soluble substances in the blood—antigens or antibodies—that could block the transferrin receptor on the plasma membrane. We know, for example, that in malaria endemic areas there are high levels of autoantibodies such as anti-DNA and anti-connective tissue antibodies, and these are present sometimes in 100% of the population at a very early age. It is conceivable that if such a blocking antibody were present it could explain the high prevalence if iron deficiency in malarial zones.

Dr. Cook: I know of at least one report suggesting that an autoantibody directed against the binding site for the receptor impairs iron uptake and results in anemia. However, I suspect this is a rare phenomenon, since it will require a highly specific antibody to interrupt the binding of transferrin by its receptor.

Dr. Fomon: Dr. Siimes, is the serum transferrin receptor likely to help in evaluating small preterm infants?

Dr. Siimes: I assume there are no data on infants yet, but it would be most interesting to see how this assay behaves during the time when ferritin is increased while simultaneously there is evidence of iron deficiency, as for example may occur in a 3-month-old preterm infant. The mobilization of iron stores accumulated in fetal life seems to be more difficult in such infants; a preterm infant may often develop iron deficiency with decreasing iron stores, while still having increased erythropoiesis. It would be of great interest to see how the transferrin receptor behaves in this situation and whether it detects the iron deficiency while ferritin is still raised.

Dr. Walter: The age at which we most commonly see iron deficiency—infancy—is a difficult one with regard to the widespread prevalence of viral infections. We have examined the influence of a mild predictable model of viral infection—measles vaccination—on measures of iron status. In 30 infants who were given measles immunization, we measured various indices of iron status on days 0, 4, and 9 after the injection. At 9 days there were significant changes in hemoglobin, MCV, serum iron, and serum ferritin, but no change in the mean value for transferrin receptors. We are hoping that transferrin receptor measurement will prove useful in infancy, when mild viral infections which interfere with the other iron indices are so prevalent and difficult to diagnose.

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IRON DEFICIENCY AND THE MEASUREMENT OF IRON STATUS

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INTRODUCTION

It is highly unlikely that life in any form can exist without iron. Its role in mammalian metabolism includes a variety of diverse functions such as reversible oxygen binding to the haem containing proteins haemoglobin and myoglobin which are involved in oxygen transport and storage, the stepwise release of energy by the haem-containing proteins of the mitochondrial electron transport apparatus, the controlled interactions with molecular oxygen by haem iron proteins, iron sulphur proteins, and non-haem iron-containing oxygenases, and the conversion of ribose to deoxyribose nucleic acids by the iron-containing ribonucleotide reductase which is required for the propagation of genetic information. Given the critical dependence of body tissues on iron, elaborate mechanisms have evolved for its efficient absorption, transport, cellular uptake, storage and conservation. While the biochemical liabilities of deficiency are evident, the efficacy of body iron conservation and iron's ability to generate reactive species should caution against supplying excess iron to those with adequate iron reserves.

The continuing growth in our knowledge about the importance of iron in human nutrition has been paralleled by an increasing variety and sophistication of laboratory methods to assess iron status. The emphasis in the present review is on methods suitable

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for evaluating human iron nutrition. Before discussing these methods, the functional consequences of a deficiency or excess in body iron will be briefly reviewed.

CONSEQUENCES OF IRON DEFICIENCY OR EXCESS

Most of the nutritional literature concerning iron is devoted to the liabilities of a deficiency whereas the clinical literature is focused on the effects of iron overload. Because of our ability to manipulate the iron status of a population through programmes of iron fortification or supplementation, the effects of either a lack or surplus of body iron are important from a nutritional standpoint.

IRON DEFICIENCY

Because the major portion of body iron is contained in circulating red cells, anaemia has long been considered the major liability of iron deficiency. However, nutritional anaemia severe enough to produce symptoms such as weakness, shortness of breath, and dizziness is relatively uncommon, even in developing countries. Anaemia is a useful index of the severity of iron deficiency but the significant liabilities of iron deficiency are more related to a deficiency in tissue iron. The major consequences of iron lack include defects in cognitive function, reduced work capacity and premature delivery.

Impairment in psychomotor development and cognitive function are one of the most important deficits associated with iron deficiency (Lozoff, 1988; Dallman, 1989). Following the original description of behavioural abnormalities in iron-deficient children (Oski & Honig, 1978) interest has focused on either infants between 9 and 24 months of age or school children between 9 and 12 years of age. Studies in Costa Rica (Lozoff, 1989) and Chile (Walter, 1989) have shown that iron-deficient infants score lower on mental and motor measurements of infant development as compared with iron-replete children, and despite correction of the deficiency deficits in motor and cognitive function may persist. Similar abnormalities have been described in iron-deficient school children who have poor school achievement scores as compared with non-anaemic children, a deficit that is not fully corrected by iron replacement (Pollitt *et al.* 1989). These abnormalities appear to be largely due to a diminished attention span. Some have suggested that the cognitive disorder may be due to the poor socio-economic background which is commonly associated with iron deficiency, but a recent prospective randomized study appears to exclude this possibility (Walter, 1989).

There is convincing evidence that iron deficiency is associated with a deficit in work performance (Dallman, 1982; Cook & Lynch, 1986). The most important liability is a limitation in endurance work rather than brisk aerobic activity. When work output can be measured accurately, as for example in tea pickers or latex tappers, studies have shown that iron deficiency even without anaemia reduces work productivity. These findings have important economic implications, especially in developing countries. In industrialized countries, attention has focused recently on defects in exercise tolerance resulting from iron deficiency. When adults with iron deficiency are subjected to brief strenuous exercise, lactic acidosis and a greater degree of tachycardia develops than in iron-replete controls (Charlton *et al.* 1977; Gardner *et al.* 1977). The extent to which iron deficiency limits the performance of the elite athlete is currently of much interest.

The consequences of anaemia during pregnancy have been debated for many years. Some investigators believe that moderate anaemia is largely a physiological phenomenon due primarily to haemodilution from an expanded plasma volume. However, at least two large

epidemiological studies have recently shown that even a modest decrease in circulating haemoglobin during pregnancy is associated with a significant risk to both the mother and fetus. In one large study, low birth weight, prematurity and perinatal mortality were significantly increased in pregnant women with a haemoglobin concentration < 104 g/l at 24 weeks gestation (Murphy *et al.* 1986). A retrospective study in the US identified an association between a low haemoglobin concentration and an increased frequency of low birth weight and perinatal mortality (Garn *et al.* 1981).

IRON EXCESS

In contrast with the relatively subtle defects associated with iron deficiency, an excess in body iron has more serious consequences and can be lethal. Because of the ease with which additional iron can be provided to iron-replete individuals through iron-fortified foods or iron supplements and the limited ability to excrete the mineral, the consequences of iron excess are as relevant nutritionally as the liabilities of iron deficiency.

Individuals with hereditary haemochromatosis, a disorder characterized by a life-long inability to regulate the absorption of dietary iron from the gastrointestinal tract, are at greatest risk of iron overload (Bothwell *et al.* 1979, 1989). Certain haematological disorders such as thalassaemia are also associated with iron overload, but these patients are usually detected readily by their physical or laboratory abnormalities. Individuals with haemochromatosis, however, usually go unrecognized for several decades before clinical manifestations develop. Excess iron in the liver leads to fibrosis and cirrhosis, in the skin to excess pigmentation, in the pancreas to diabetes, in the joints to arthritis, and in the heart to cardiac failure and fatal arrhythmias. This disorder has been recognized for nearly a century but its genetic basis has only recently been defined. It is an autosomal recessive disease with the abnormal gene lying close to the histocompatibility locus antigen (HLA) complex on chromosome 6. About 1 out of 10 individuals of European descent carries one haemochromatosis gene (heterozygote) and 1 in every 300 to 400 individuals is homozygous (Edwards *et al.* 1988). The large number of heterozygotes in the population do not appear to be at significant risk of developing iron overload, but homozygous individuals certainly are. Excessive iron intake does not induce haemochromatosis, but it obviously hastens the development of clinical manifestations (Bezwoda *et al.* 1981). The best approach to controlling the disorder is earlier laboratory recognition because, if detected before irreversible tissue damage has occurred, the iron excess can be fully removed by vigorous phlebotomy. Haemochromatosis is one of the most common genetic abnormalities in humans and should not be neglected in discussions of iron deficiency and methods to alleviate it. Iron overload is of particular concern with iron fortification programmes which provide large amounts of iron to segments of the population that do not require it. Emphasis should be placed on more effective screening methods to identify affected individuals before irreparable damage has occurred.

Of perhaps even greater concern is recent epidemiological evidence indicating that individuals with higher levels of body iron may be at increased risk of developing cancer. Humans have an exceptional ability to regulate the amount of iron absorbed from their diet and to maintain body iron within narrow limits (Bothwell *et al.* 1979). It has long been assumed that moderate increases in dietary iron intake do not increase body iron stores significantly in genetically normal individuals. In a recent study, ten year follow-up examinations were performed in 14,000 adults participating in a National Health Survey (Stevens *et al.* 1988). In 242 men who subsequently developed cancer, serum iron and transferrin saturation were significantly higher and the iron-binding capacity significantly

lower at the time of the original survey than in those who remained free of malignancy. The highest association between iron status and malignancy occurred with cancer of the colon and lung. Large prospective studies employing sensitive and specific indices of iron status are needed before iron can be regarded as a carcinogen in otherwise normal subjects. In the interim, aggressive fortification or supplementation programmes which supply large amounts of iron to normal subjects must be viewed with some reservation.

LABORATORY ASSESSMENT OF IRON STATUS

It is useful to review measurements of iron status in relation to the specific iron compartments they reflect: storage, transport, and erythroid iron (Cook, 1982; Cook & Skikne, 1989). These three main iron compartments are also affected sequentially with increasing deficits in body iron. A deficiency in storage iron occurs first, followed by deficits in the iron transport and erythroid compartments. Numerous laboratory methods are available for assessing these changes. The key measurements and the diagnostic ranges are listed in Table 1.

STORAGE IRON

Iron stores have no physiological function other than to serve as a buffer against increasing iron demands such as occur during pregnancy or with acute blood loss. Storage depletion represents an increased risk of developing iron deficiency but by itself is not associated with any known liabilities. The most accurate method of measuring the size of the storage iron compartment, which is contained primarily in the reticuloendothelial cells of the liver, spleen, and bone marrow, is by quantitative phlebotomy. Normal subjects are regularly phlebotomized until frank anaemia develops; iron stores are then calculated as the difference between the amount of iron removed by phlebotomy and the induced deficit in circulating haemoglobin iron. For clinical purposes, iron stores can also be assessed qualitatively by examining the stainable iron on an aspirated bone marrow sample. Both of these approaches have now been largely supplanted by serum ferritin measurements, one of the most important determinations of iron status.

Ferritin is the storage protein for iron and has been extensively characterized from both a physiological and biochemical standpoint. Comprehensive reviews of our knowledge about this protein have been published (Jacobs, 1985; Worwood, 1986, 1990). Apoferritin, the iron-free protein, has a molecular weight of 460 000 and consists of 24 protein subunits surrounding a hollow core. Iron is deposited within this core as insoluble ferric hydroxide phosphate. There are large quantities of ferritin in iron storage tissues such as the liver and spleen, but only minute quantities are present in human serum, normally between 12 and 300 μg/l. Circulating ferritin is essentially free of iron and does not, therefore, contribute to internal iron transport. The importance of the serum ferritin is that it provides a precise quantitative measure of the total iron in the storage compartment.

A variety of sensitive immunologic techniques have been developed to measure serum ferritin and there are several commercial kits now available. Early methods requiring radioactive labelling of either the antigen or antibody have now been replaced by enzyme-linked immunosorbent assays (ELISA) because of their greater simplicity and longer reagent shelf life. Commercial laboratories have developed large automated systems which can perform over 1 million assays annually. Despite the variety of commercial kits that are marketed for serum ferritin determination, there appears to be good agreement in results obtained with different assays. This is due, in large part, to the development of a WHO reference standard for ferritin which can be obtained from the National Bureau of Health

Table 1. *Laboratory measurements of iron status*

	Diagnostic range	
	Iron deficiency	Iron excess
Iron stores		
Serum ferritin (μg/l)	< 12	> 300
Iron transport		
Serum iron (μg/l)	< 60♂	> 180♂
TIBC (μg/l)	> 400♀	< 2500
Transferrin saturation (%)	< 16	> 60
Red cell parameters		
Haemoglobin (g/l)	< 130♂, 120♀	—
MCV (femtolitre)	< 80	—
Erythrocyte protoporphyrin (μg/l RBC)	> 700	—
RDW (%)	> 16	—
Tissue iron needs		
Serum transferrin receptor (mg/l)	> 8·5	—

Abbreviations: TIBC, total iron-binding capacity; MCV, mean corpuscular volume; RBC, red blood cells; RDW, red cell distribution width.

Standards in the UK (International Committee for Standardization in Haematology, 1984). Work on developing a reference method for serum ferritin is nearing completion (Worwood *et al.* 1991).

Factors influencing the serum ferritin concentration have been studied extensively (Finch *et al.* 1986). Phlebotomy studies have demonstrated that 1 μg/l serum ferritin corresponds to 8–10 mg of storage iron in an average sized adult. There are marked effects of age and sex on serum ferritin which reflect the known physiological variations in iron status. Serum ferritin is relatively high in newborns but falls rapidly to the iron-deficient range during the first few months of life as iron stores are mobilized for the expanding red cell mass. The concentration increases slowly throughout childhood until late adolescence when values in males increase to roughly 3-times those in women. The serum ferritin remains lower in women during their childbearing years, but following menopause, a period of more favourable iron balance, the serum ferritin rises through the fifth and sixth decades, eventually approaching values in males. When using serum ferritin to gauge the nutritional iron status of a population, the importance of establishing reference values specific for age and sex has been emphasized (Vicente *et al.* 1990).

Serum ferritin measures iron reserves and is therefore a better index of iron sufficiency than iron deficiency. The serum ferritin is especially useful for monitoring long-term changes in the iron-replete segment of a population. For example, serum ferritin values were recently shown to correlate with meat intake in healthy individuals, presumably reflecting the higher assimilation of dietary haem iron (Leggett *et al.* 1990). The serum ferritin will play a key role in defining the relationship between iron stores and cancer risk because it is only quantitative index of storage iron that is suitable for epidemiological purposes. The serum ferritin is of some value in screening for idiopathic haemochromatosis, but the concentration is not invariably increased in these patients.

Once iron stores are depleted as defined by a fall in serum ferritin below 12 μg/l, the measurement gives no indication of the severity of the iron deficiency. Serum ferritin is therefore a less reliable indicator of iron status in populations with a high prevalence of iron

deficiency anaemia. For example, the serum ferritin by itself is of limited value in assessing iron status during infancy or pregnancy because mean values are often close to the iron-deficient range. The measurement is useful in these populations for gauging the efficacy of iron interventions such as fortified infant cereals or iron supplements in pregnancy. Because of the inability of the serum ferritin to portray iron status once stores are fully exhausted, it is most helpful when coupled with measurements which reflect more advanced degrees of iron deficiency. Another drawback with serum ferritin is that certain disorders such as chronic inflammation, malignancy, or liver disease are associated with a disproportionate rise in values relative to iron stores. These changes complicate the clinical use of serum ferritin measurements for the assessment of the anaemic patient. This disorders usually occur too infrequently in the population to diminish the value of serum ferritin in nutritional surveys but their effects should not be ignored. For example, heavy ethanol use was associated with higher serum ferritin values in a recent study even in the absence of liver disease (Leggett *et al.* 1990). Serum ferritin values are also less reliable in populations with a high prevalence of infection such as those often encountered in developing countries.

IRON TRANSPORT

Once iron stores are fully depleted, any further decline in body iron is accompanied by a reduction in the concentration of plasma iron, one of the earliest measurements of iron status (Bothwell *et al.* 1979). Plasma iron is measured colorimetrically after acidification and precipitation of plasma proteins and is included in the automated chemistry profile performed in larger hospital laboratories. The plasma iron is usually measured in tandem with transferrin, its specific plasma transport protein. Transferrin is often determined in the laboratory as the total iron-binding capacity (TIBC) which is the amount of added iron that can be specifically bound by plasma. Transferrin can also be determined immunologically. Because the plasma iron and TIBC move in a reciprocal fashion in iron deficiency and iron overload, the most informative expression of plasma transport is the plasma iron expressed as a percentage of the TIBC, referred to as the transferrin saturation. The main limitation of the transferrin saturation relates to the wide diurnal variations in plasma iron concentration. Concentrations in healthy subjects may vary by as much as 100% during a 24-h interval. This variation is not diminished significantly by sampling at a uniform time each day because roughly one-third of the subjects cycle in the reverse direction. Another technical problem that plagued earlier manual methods was iron contamination, but this difficulty has been largely eliminated by using disposable plastic ware and automated chemistry systems.

A reduction in transferrin saturation below 16% is a reliable index of an undersupply of iron to the developing red cell (Bothwell *et al.* 1979). Since iron-deficient erythropoiesis also occurs in disorders other than iron deficiency such as acute and chronic inflammation or malignant disease, the specificity of a reduced transferrin saturation is limited. Because the TIBC increases in iron deficiency but falls with inflammation, it provides some additional discriminating evidence although it is usually within the normal range when iron deficiency and chronic inflammation co-exist. Transferrin saturation values are actually more useful in screening for iron overload than for iron deficiency. An elevated transferrin saturation above 55% is now regarded as the most reliable laboratory screen for idiopathic haemochromatosis (Bassett *et al.* 1988; Edwards *et al.* 1988), more so than the serum ferritin. The transferrin saturation is also valuable in populations with a high prevalence of thalassaemia. Both iron deficiency and thalassaemia produce microcytic hypochromic anaemia, but the transferrin saturation is invariably elevated in thalassaemia major due to

the excessive dietary iron absorption whereas the transferrin saturation is reduced in iron deficiency. Despite the high variability and low specificity of the transferrin saturation, the ease of the determination with automated chemistry systems and the long experience with its interpretation ensure its continued use in population studies to identify both iron deficiency and iron excess.

RED CELL PARAMETERS

Since the largest proportion of body iron is contained in blood, laboratory measurements to detect evidence of reduced haemoglobin formation in circulating red cells are important in the detection of overt iron deficiency. Distinction of the various causes of this impaired haemoglobinization in the anaemic patient is discussed extensively in the haematological literature. The three major causes are iron deficiency, thalassaemia, and chronic infection or inflammation, but in nutritional surveys one can usually assume that iron deficiency is the cause of impaired haemoglobin formation. Changes in circulating red cells provide useful information about iron status in nutritional surveys even in the absence of anaemia.

The introduction of electronic counters for examining the number and size of circulating red cells has greatly enhanced the reliability of red cell indices and changed their order of sensitivity as compared to older manual or microscopic methods. A reduction in the size of circulating red cells, termed the mean corpuscular volume (MCV), is a reliable index of reduced haemoglobin synthesis, values below 80 femtolitres indicating iron-deficient erythropoiesis. As with many measurements of iron status, an abnormally low MCV is more useful in identifying iron deficiency than a normal value is in excluding it. The major limitation of the MCV, in common with other haematologic parameters, is the time required after the onset of iron deficiency for the level to become abnormal. Because the life span of circulating red cells is greater than three months, several weeks must elapse before a sufficient number of microcytic cells have been released to influence the MCV.

A new and closely related electronic parameter of red cell morphology is the red cell distribution width (RDW) (Bessman & Feinstein, 1979; McClure *et al.* 1985). Microcytic cells in iron deficiency are smaller but vary significantly in the extent of the size reduction. This results in a wider frequency distribution of circulating red cell size that can be measured electronically with newer counting systems. The same widening does not occur in patients with thalassaemia minor, providing useful discrimination in populations with a high prevalence of this inherited disorder. Early reports also suggested that the RDW could distinguish iron deficiency from chronic infection, but subsequent reports have shown significant overlap in RDW values between these disorders (Baynes *et al.* 1986; Flynn *et al.* 1986). Experience with the RDW is still early, but the major advantage of this parameter appears to be that the changes occur earlier following the onset of iron deficiency than other haematologic indices such as the MCV.

A reduction in iron supply to the developing red cell results in an excess of free protoporphyrin within the red cell which would otherwise combine with iron to form haem (Bothwell *et al.* 1979; Schifman & Rivers, 1987; Labbe & Rettmer, 1989; Jensen *et al.* 1990). Measurements of this surplus protoporphyrin in circulating blood have proved to be a sensitive measure of iron-deficient erythropoiesis. Interest in red cell protoporphyrin measurements increased greatly with the development of the haematofluorometer, a specialized instrument designed to measure the reflected fluorescence from zinc protoporphyrin on a thin film of blood. A drop of blood is simply placed on a glass slide, inserted into the instrument and the result displayed electronically. Reagents and disposable equipment costs are negligible and reliable measurements can be obtained with a minimum

of laboratory training. Battery operated versions of the haematofluorometer are useful for field studies of nutritional status. There is still no consensus about the optimal method of expressing the results or the preferred technique for standardization.

An elevation in erythrocyte protoporphyrin has about the same diagnostic significance as a decrease in MCV. Like the latter measurement, the erythrocyte protoporphyrin does not become elevated until several weeks after the onset of iron deficiency and returns to normal only slowly following its repair. Because it detects a diminished iron supply to the developing red cell from any cause, it does not distinguish between iron deficiency and the anaemia of chronic disease. An important limitation of the erythrocyte protoporphyrin when assessing iron status, especially in children, is that the concentration is also elevated in lead poisoning (Piomelli *et al.* 1973). In a given individual, iron deficiency can be distinguished from lead poisoning by history, blood lead or ancillary iron measurements or a therapeutic iron trial, but this distinction is more difficult in epidemiological studies. Erythrocyte protoporphyrin is useful for assessing iron status in populations at increased risk of iron deficiency such as infants, pregnant women, and blood donors, and may be more useful in these populations than haemoglobin determinations (Jensen *et al.* 1990).

Haemoglobin or haematocrit determinations have been used longer and more widely as a laboratory index of iron status than any other iron parameter. Any assessment of iron status must include the haemoglobin concentration because it defines a more advanced stage of iron lack and it is the only laboratory assay that provides a quantitative measure of the severity of iron deficiency once anaemia has developed. Haemoglobin and haematocrit determinations are basically interchangeable with respect to assessment of iron status, the choice depending on the available instrumentation. The recent development of reliable battery operated instruments has facilitated the use of these measurements which are the simplest and least expensive assays available for detecting iron deficiency.

The major limitation of haemoglobin determinations is that they lack both sensitivity and specificity. The sensitivity of the haemoglobin as a measure of anaemia is severely limited by the wide overlap in the frequency distribution of haemoglobin values between normal and iron-deficient populations. When iron deficiency was defined as a significant rise in haemoglobin following oral iron, roughly 20% of anaemic women, as defined by a single cutoff level of haemoglobin, were misclassified as normal and nearly one-third of normal women were wrongly considered anaemic (Garby *et al.* 1969). The specificity of haemoglobin measurements as an index of iron deficiency anaemia is also very low because many factors other than iron lack limit red cell production. Anaemia due to chronic infection, protein calorie malnutrition, or certain haemoglobinopathies produce anaemia that may be wrongly attributed to iron lack in nutritional surveys. Recent works suggest that the anaemia of chronic disease may encompass a broader spectrum of diseases than the infectious and inflammatory disorders traditionally believed to produced it (Cash & Sears, 1989). It has been estimated that perhaps 50% of the global prevalence of anaemia is due to iron deficiency (DeMaeyer & Adiels-Tegman, 1985). Fortunately, these limitations of isolated haemoglobin determinations can be readily circumvented by combining the measurement with more specific indices of iron status. Hopefully few, if any, modern epidemiological surveys will rely on haemoglobin determinations as the sole index of iron status.

TISSUE IRON NEED

One of the major recent advances in iron metabolism is knowledge of the process by which cells acquire iron. It has long been known that iron is transported in the body by transferrin, but the process of iron uptake by the cell has been defined only recently

(Seligman, 1983; Huebers & Finch, 1987; Irie & Tavassoli, 1987; Ward, 1987). It has been shown that circulating diferric transferrin binds to a specific receptor on the cell surface followed by invagination of the ligand–receptor complex in an endocytic vesicle. The subsequent fall in pH within this vesicle reduces the affinity of transferrin for iron which is then released and transported into the cytosol. The apotransferrin remains bound to its receptor until it returns to physiological pH at the cell surface where it is released to participate in another cycle of iron transport. One of the key features of this process is that when a cell senses a need for iron, the synthesis of transferrin receptor is upregulated, allowing it to compete more effectively for circulating transferrin iron. The density of transferrin receptors reflects the tissue needs for iron and is consequently highest in rapidly dividing cells, in red cell precursors engaged in haemoglobin synthesis. and in the placenta which requires a continuous supply of iron for fetal growth. Human transferrin receptor has been cloned and fully characterized biochemically (Omary & Trowbridge, 1981; McClelland *et al.* 1984). It is a transmembrane glycoprotein containing two identical subunits, each of 95000 kDa mass and linked by 2 disulphide bridges. Each unit contains 760 amino acids: 61 in the N-terminal cytoplasmic domain, 28 in the intermembrane portion, and 671 in the large extracellular domain.

Despite the importance of receptor-mediated endocytosis in the process of cellular iron procurement, the transferrin receptor played no role in assessing iron status until it was shown that soluble transferrin receptor can be detected and quantified in human serum using sensitive immunologic assays (Kohgo *et al.* 1986; Flowers *et al.* 1989; Huebers *et al.* 1990). Measurements of serum receptor are analogous to serum ferritin in that minute quantities of the proteins in the circulation provide a reliable index of the total body content. The serum transferrin receptor concentration has been shown to correlate closely with the total number of erythroid precursors. In the assessment of haematologic disorders, the serum transferrin receptor therefore provides a convenient measure of total erythropoiesis that could be obtained previously only by ferrokinetic measurements or less quantitatively by bone marrow examination. Despite the high concentration of transferrin receptor in rapidly proliferating tissues, the serum receptor level remains normal in most haematologic malignancies (Klemow *et al.* 1990). Only about 50% of the circulating receptor appears to be derived from the erythroid marrow, based on measurements in patients with aplastic anaemia or marrow ablation performed in preparation for a bone marrow transplant (Flowers *et al.* 1989). Recent biochemical studies have shown that the circulating receptor is a monomeric fragment of intact receptor with a cleavage point in the extracellular domain just beyond the cell surface (Shih *et al.* 1990). Because the serum contains a large excess of transferrin relative to circulating receptor the receptor is bound to transferrin in the circulation.

The value of serum receptor measurements stems from the fact that receptor synthesis is upregulated in iron-deprived tissues. Using an ELISA developed with monoclonal antibodies, a mean level of $5{\cdot}6 \pm 1{\cdot}2$ mg/l in 82 normal male and female volunteers was sharply increased to $18{\cdot}0 \pm 11{\cdot}4$ mg/l in patients with iron deficiency anaemia (Flowers *et al.* 1989). When phlebotomies were performed in 14 normal volunteers to provide a wide spectrum of iron status, the serum receptor levels remained normal during the period of storage depletion (Skikne *et al.* 1990*b*). When the serum ferritin fell below 12 μg/l, the serum receptor began to rise and continued to increase throughout the remainder of the bleeding programme. As an index of iron status, two important advantages of the serum receptor were identified. First, the concentration increased earlier than traditional haematologic indices such as the MCV or erythrocyte protoporphyrin. Second, there was a close inverse relationship between serum receptor and the induced deficit in functional iron. This study demonstrates that the entire spectrum of iron status can be evaluated by

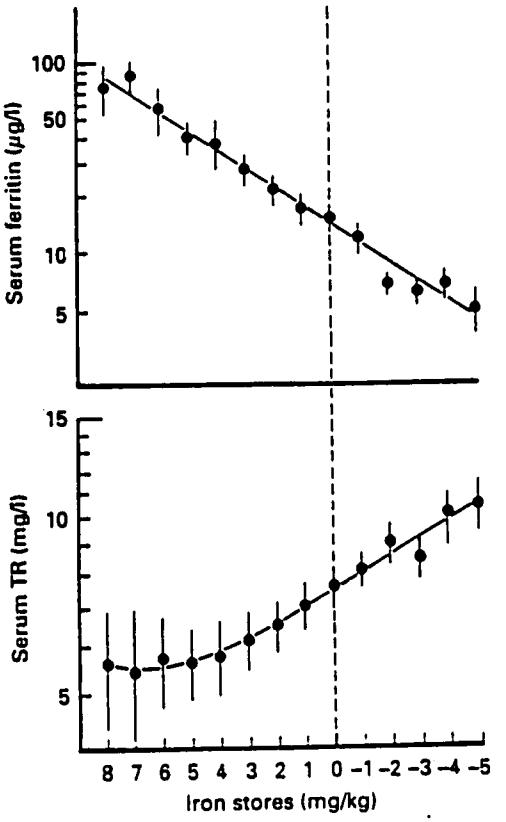

Fig. 1. Changes in body iron during repeated phlebotomy of healthy subjects (Skikne *et al.* 1990*b*). Serum ferritin measurements are shown in the upper panel and serum transferrin receptor (TR) measurements in the lower panel (mean ± 1 SE). Negative values for iron stores represent the induced deficit in tissue iron or the amount of iron that has to be returned before iron stores can develop. The interrupted vertical line represents the point of storage iron depletion.

only two measurements: serum ferritin as a measure of iron stores and serum receptor as a measure of tissue iron deficiency (Fig. 1).

One important application of serum receptor measurements will be in the assessment of iron status during pregnancy. Haemoglobin measurements are notoriously unreliable during pregnancy because of the marked shifts in plasma volume and red cell mass that occur during gestation. Changes in haematologic indices such as MCV or erythrocyte protoporphyrin occur too slowly to detect iron deficiency that develops during pregnancy. Contrary to a recent report that the serum receptor rises during pregnancy irrespective of iron status (Kohgo *et al.* 1988), we found that significant increases occur only in women with iron deficiency as defined by other laboratory parameters (Carriaga *et al.* 1991). Because of the elevation in transferrin receptor in early iron deficiency, this measurement may prove to be the most reliable index of an impairment in tissue iron supply during gestation.

An important question relating to serum transferrin receptor measurements is whether they will distinguish true iron deficiency from the anaemia of chronic disease. We have recently completed studies in patients with liver disease and inflammation, both acute and chronic. In contrast with serum ferritin concentrations which are falsely elevated in these disorders, we found that the transferrin receptor remains normal in the majority (Skikne

et al. 1990*a*). This may have major implications when assessing the iron status of populations with a high prevalence of infection.

SELECTION OF AN OPTIMAL LABORATORY APPROACH

Given the large array of laboratory techniques to assess iron status, it is important to select the optimal method or combination of methods for a particular purpose. There are two main applications from a nutritional standpoint: screening for iron deficiency in high risk groups and assessing iron status in the population as a whole

SCREENING FOR IRON DEFICIENCY

In segments of the population with a high prevalence of iron deficiency, it may be more cost-effective to identify iron lack on an individual basis. The main populations in which screening may be effective are infants, preschool children, and pregnant women. Certain other factors such as regular blood donation, a low meat intake, and endurance training also increase the risk of iron deficiency and may warrant additional screening efforts. One important advantage of screening for iron deficiency is that iron supplements are given only to those in need of additional iron. Because of economic constraints, screening programmes usually rely on only one or perhaps two laboratory measurements to identify iron deficiency. If only a single laboratory measurement is used, the choice depends on the prevalence of iron deficiency in the targeted population. If the prevalence is low, an early index of iron lack such as the serum ferritin is needed, whereas if anaemia is highly prevalent, the haemoglobin concentration is most informative. If two methods are used in tandem, the serum ferritin and haemoglobin concentration are useful because they monitor a broad spectrum of iron status and distinguish iron deficiency from other causes of anaemia. If both measurements are normal iron deficiency may be confidently excluded, whereas if both values are abnormal, iron deficiency is identified unequivocally. A low ferritin and normal haemoglobin concentration indicate storage iron depletion whereas a low haemoglobin and normal serum ferritin warrant further haematologic evaluation. Our preliminary experience suggests that the serum ferritin and serum receptor may provide the most comprehensive assessment of iron status in screening programmes.

ASSESSMENT OF THE IRON STATUS OF A POPULATION

Iron status measurements are commonly included in surveys to assess the nutritional status of a population. Population surveys are also important for measuring the impact of iron supplementation or fortification and for detecting long-term trends in the iron status of the population. In recent years, there has been a tendency to include an increasing number of iron parameters in surveys, thus providing a comprehensive assessment of a sufficiency or deficiency in iron. In general, the greater the number of laboratory measurements, the more informative the nutritional survey will be. However, the serum ferritin, serum transferrin receptor, and haemoglobin concentration should monitor the entire spectrum of iron status in the population and have the advantage that venous sampling can be avoided.

When a battery of iron parameters is measured the statistical treatment of the data may be as important as the particular selection of laboratory indices. The traditional approach has been to examine each laboratory measurement independently of the other using single cut-off levels to define normality. This approach results in multiple and often conflicting

estimates of prevalence. A more effective method is to use various combinations of measurements to enhance the specificity of prevalence estimates or to define varying stages of iron lack. In one report, varying combinations of serum ferritin, transferrin saturation, MCV, erythrocyte protoporphyrin, and haemoglobin were used to defined storage iron depletion, iron deficiency without anaemia, and more advanced iron deficiency anaemia (Pilch & Senti, 1984). This approach has the advantage over the use of isolated criteria, but does not detect changes in the iron-replete segment of the population.

In a recent study we attempted to define body iron levels in each surveyed individual (Cook *et al.* 1986). In iron-replete subjects, iron stores were estimated quantitatively from the serum ferritin value. In individuals with iron deficiency anaemia, the deficit in circulating haemoglobin was used to measure the degree of functional iron deficiency. Quantitation of body iron between these extremes is more difficult. One approach has been to use empirical mathematical formulae based on the degree of abnormality in transferrin saturation, serum ferritin, and erythrocyte protoporphyrin. Incorporation of the serum transferrin receptor measurement should permit further refinements in this approach.

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THE EFFECT OF ENHANCED ERYTHROPOIESIS ON IRON ABSORPTION

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SUMMARY

To examine the influence of erythropoiesis on iron absorption, radioiron absorption tests were performed in normal subjects before and following a course of recombinant erythropoietin. The absorption of heme and nonheme iron from a standard meal was measured in 9 subjects and the absorption of a therapeutic dose of ferrous sulfate given with or without food was determined in an additional 11 subjects. The subcutaneous administration of 100 U recombinant erythropoietin/kg body weight given on 10 successive days over a two week period induced a brisk increase in erythropoiesis and a sharp fall in iron stores. With the standard meal, there was a modest increase in heme iron absorption from 47.0 to 58.6% ($p<0.05$) and a dramatic 5-fold rise in nonheme iron absorption from 5.9 to 31.8% ($p<0.001$). The absorption of 50 mg iron as ferrous sulfate increased from 2.0 to 17.9% when given with food ($p<0.001$) and from 7.0 to 24.6% when given with water ($p<0.001$). To assess the effect of erythropoiesis independently of the induced changes in iron status, the absorption data were adjusted to a common serum ferritin level. The relative increase in iron absorption was still significant for both dietary nonheme iron (ratio 2.51, $p<0.02$) and ferrous sulfate given with food (ratio 2.99, $p<0.01$). It is concluded that the striking enhancement of iron absorption following regular erythropoietin administration in normal subjects is due to the combined effect of diminished iron stores and augmented erythropoiesis.

Running head: Erythropoiesis and iron absorption

Abbreviations: rHEPO - recombinant human erythropoietin; TIBC - total iron binding capacity

INTRODUCTION

The amount of iron in body stores is the main factor controlling the absorption of iron from the gastrointestinal tract. Even a small change in iron stores as reflected by serum ferritin values is accompanied by a reciprocal alteration in iron absorption.[1-4] The rate of erythropoiesis is also believed to influence iron absorption but its quantitative importance is less clearly defined. Evidence for an effect of erythropoiesis on iron absorption is based primarily on animal studies in which erythropoiesis can be altered more dramatically than in humans.[5-7] Clinical studies have shown that the ineffective erythropoiesis in patients with thalassemia major or sideroblastic anemia is a potent stimulus to iron absorption.[8-10] However, when enhanced erythropoiesis is effective, as in patients with hereditary spherocytosis or glucose-6-phosphate dehydrogenase, only a modest increase in inorganic iron absorption has been demonstrated and these disorders are not usually associated with a significant increase in body iron.[11] Despite the diminished erythropoiesis in patients with chronic renal failure, no obvious abnormalities in iron absorption have been demonstrated.[12,13]

The recent availability of rHEPO has afforded an opportunity to examine the relationship between erythropoiesis and iron absorption in normal subjects. In the present study, dual radioisotopic measurements of iron absorption were performed before and following regular rHEPO administration to normal subjects.

MATERIALS AND METHODS

Iron absorption measurements were performed in 7 men and 13 women ranging in age from 22 to 43 years. The mean weight of the subjects was 77.7 kg (range 53.2 to 101 kg). All subjects were in good health and denied a history of hematologic or gastrointestinal disorders known to influence iron absorption. Apart from one subject who had mild iron deficiency anemia, the participants were hematologically normal and all had residual iron stores as defined by a serum ferritin level >12 µg/L. All subjects gave written, informed consent before participating in the study which was carried out in accordance with the procedures of the Human Subjects Committee at the University of Kansas Medical Center.

The effect of rHEPO on food iron absorption was evaluated in 9 subjects and on the absorption of a therapeutic dose of ferrous sulfate in the remaining 11 subjects. By employing ^{55}Fe and ^{59}Fe simultaneously, four separate absorption measurements were obtained in each subject, two before and two following rHEPO administration. All test meals were administered between 0700 and 0900 following an overnight fast and no further food or liquid was allowed for 4 hrs following ingestion of the test meal. A standard hamburger meal that has been used in several prior studies from this laboratory[14,15] was fed to measure the absorption of food iron. This meal consisted of ground beef (113 g), a bun (53 g), french fries (68 g) and a vanilla milkshake (150 ml). The meal contained 820 kCal and 4.8 mg iron of which 1.4 mg was in the form of heme iron. The heme and

nonheme iron compartments were labelled separately using extrinsic radioiron tags of ^{55}Fe and ^{59}Fe as follows. The nonheme iron compartment was labelled by adding 0.1 mg iron and 3.7 kBq radioactivity as $^{59}FeCl_3$ in 0.5 ml 0.01N HCl to the bun. The heme iron compartment was similarly tagged by dispensing 111 kBq ^{55}Fe-labelled hemoglobin in 0.5 ml onto the hamburger patty. The radiolabelled hemoglobin was obtained by administering 74 MBq $^{55}FeCl_3$ intravenously to an iron deficient pathogen-free rabbit and bleeding the animal one week later.

The absorption of a therapeutic dose of ferrous sulfate given either alone or with food was measured in a second group of 11 subjects. On the first morning, a solution containing 50 mg iron as ferrous sulfate and 37 kBq of ^{59}Fe was given followed by 150 ml of water. On the following morning the same dose of ferrous sulfate tagged with 111 kBq ^{55}Fe was taken with a standard meal. This meal was the same as that used in the first group of subjects except that the hamburger patty was omitted. The meal contained 600 kCal and 2.4 mg nonheme iron.

In both study groups, iron absorption was measured 14 days after administering the test meals using a combination of whole body counting and incorporated red cell activity. Absorption of the ^{59}Fe was measured in a shadow-shield whole body counter designed specifically for radioiron absorption measurements.[16] After obtaining whole body counts, 30 ml of blood was drawn for radioactivity measurements and baseline hematologic and iron parameters preceding rHEPO administration. Duplicate measurements of ^{59}Fe and

^{55}Fe activity in 10 ml samples of whole blood were performed using a modification of the method of Eakins and Brown.[17] Absorption of the ^{55}Fe label was calculated from the retained ^{59}Fe whole body activity and the ratio of $^{59}Fe/^{55}Fe$ in circulating red blood cells. Sufficient counts were obtained on the digested blood samples to reduce the net counting rate error for each isotope to ±2% or less in subjects absorbing over >1% of the test dose.

On the following day, the subjects were given rHEPO (Ortho Pharmaceutical Corp., Biotech Division, and R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ) in a dose of 100 U/kg body weight subcutaneously on five successive days each week for a total of 10 doses. On the day following the final injection, blood was drawn for measurement of baseline ^{59}Fe and ^{55}Fe activity and hematologic parameters. After obtaining a baseline ^{59}Fe whole body count, two further iron absorption tests were performed in each subject using the same procedures as used prior to erythropoietin administration. Fourteen days following the second test dose, whole body counts were obtained to measure the rise in ^{59}Fe radioactivity and a final blood sample was obtained to measure the absorption of ^{55}Fe from the ratio of the further rise in ^{55}Fe and ^{59}Fe whole blood radioactivity.

Measurements of hemoglobin, hematocrit, and red blood cell count were performed with an automated counter (Coulter STKR; Coulter Electronics, Inc., Hialeah, FL). Reticulocyte counts were measured by a standard chamber method. Serum iron and TIBC were

measured by manual methods recommended by the Iron Panel of the International Committee for Standardization in Hematology.[18,19] Serum ferritin and serum transferrin receptor were determined by enzyme-linked immunosorbent assays established with monoclonal antibodies.[20,21]

Because of the marked influence of iron status on iron absorption, a method was required to adjust for the effect of differences in storage iron induced by erythropoietin administration. The correction method was based on the serum ferritin level which bears a close inverse relationship to iron absorption.[22] Extensive studies of food iron absorption in normal subjects in this laboratory have shown that the regression of log nonheme iron absorption on log serum ferritin has a slope of -1. The percentage absorption of dietary nonheme iron in each subject was corrected to a value corresponding to a serum ferritin of 40 μg/L using the following equation:

$$\text{Log}A_c = \text{Log}A_o + \text{Log}F_o - \text{Log}40$$

where $\text{Log}A_c$ is corrected absorption, $\text{Log}A_o$ is observed absorption, and $\text{Log}F_o$ is observed serum ferritin. This correction was applied to measurements of nonheme iron absorption and of ferrous sulfate given with food but not to measurements of heme iron absorption or ferrous sulfate in the absence of food.

For statistical analysis, percentage absorption values were converted to logarithms and the results retransformed to recover the original units. A paired-t test was used to examine the effect of erythropoiesis on iron absorption by determining whether mean log absorption ratios differed significantly from 0.

RESULTS

The administration of rHEPO produced significant changes in the majority of hematologic indices (Table 1). The increases in hematocrit and red cell count but not hemoglobin concentration were statistically significant. The enhanced erythropoiesis was documented by a 2-fold increase in reticulocyte count and a 3-fold rise in serum transferrin receptor. The fall in serum iron and transferrin saturation, but not the rise in TIBC, were highly significant. There was a dramatic fall in serum ferritin from 42.5 µg/L to 13.0 µg/L, reflecting a 3-fold decline in storage iron. Following rHEPO, the serum ferritin level fell within the iron deficient range (<12 µg/L) in 9 of the 20 subjects.

rHEPO resulted in a significant increase in the absorption of both heme and nonheme iron (Table 2). There was only a modest 25% increase in heme iron absorption from 47.0 to 58.6% ($p<0.05$). In contrast, there was a dramatic 500% rise in nonheme iron absorption from 5.9% to 31.8% giving a mean absorption ratio of 5.39 (±1SEM, 3.58-8.12).

A similar dramatic effect of erythropoietin administration was seen on the absorption of ferrous sulfate (Table 3). When taken without food, mean absorption increased from 7.0 to 24.6%, giving a mean absorption ratio of 3.53 (1.43-8.70)($p<0.001$). An even more dramatic rise in absorption occurred when ferrous sulfate was given with food. Mean absorption increased from 2.0 to 17.9%, giving a mean absorption ratio of 8.82 (7.04-11.06)($p<0.001$).

The marked changes in iron absorption following rHEPO were due in part to alterations in body iron stores as reflected by serum ferritin values. Nevertheless, after correcting the absorption data to a serum ferritin of 40 µg/L, the increase in iron absorption was still significant. Using the corrected values, the pre-treatment nonheme iron absorption of 6.0% increased to 13.1% following rHEPO; the mean absorption ratio of 2.17 (1.60-2.95) was statistically significant ($p<0.02$). A similar increase in absorption was observed for ferrous sulfate given with food; mean absorption increased from 2.5 to 7.6% giving an absorption ratio of 2.99 (2.29-3.90)($p=0.001$). Thus, after correcting for differences in iron status, the increase in nonheme iron absorption induced by enhanced erythropoiesis was still significant and remarkably similar whether or not the meal contained added therapeutic iron (Figure 1).

DISCUSSION

The major obstacle in assessing the effect of erythropoiesis on iron absorption independently of iron status is that significant changes in erythropoiesis are invariably accompanied by alterations in plasma iron turnover and laboratory indices of iron status.[11] Thus, experimental manipulations in both animals and humans such as phlebotomy, hypertransfusion, and transport from sea level to high altitude or return, are all accompanied by changes in both the level of erythropoiesis and internal measurements of iron status. This was also true in the present study where a highly specific erythropoietic stimulus produced an increase in erythropoiesis as well as a marked change in body iron distribution. The serum ferritin, which is a reliable measure of body iron reserves, fell approximately 3-fold. To examine the effect of erythropoiesis per se on iron absorption, it was therefore necessary to develop a method for eliminating the effect of differences in iron status on iron absorption.

The method used for correcting for iron status differences was derived by examining the relationship between serum ferritin and food iron absorption in a large number of absorption studies. The validity of the method was established by comparison with corrections based on a second absorption test using either a standard meal or reference dose of inorganic iron.[22] The latter has been used for many years to eliminate the effect of differences in iron status when studying dietary variables influencing food iron absorption. Because the relationship between heme iron absorption and serum ferritin or

the absorption of ferrous sulfate taken with water and serum ferritin has not been adequately defined, the correction for iron status was applied only to nonheme iron absorption and ferrous sulfate taken with food.

One possible concern in using the serum ferritin correction is whether the alteration induced by rHEPO reflects a true difference in body iron stores. One possible source of error is that the formula used to adjust iron absorption for differences in iron status was based on serum ferritin measurements obtained at a steady state, whereas the serum ferritin values in the current study were obtained during a phase of decreasing iron stores. However, quantitative estimates of the shift in body iron suggest that it does reflect true changes in iron stores. In the composite group of volunteer subjects, the baseline mean serum ferritin of 42.5 µg/L corresponds to iron stores of approximately 500 mg iron using a logarithmic conversion.[23] The mean serum ferritin of 13 µg/L following rHEPO indicates that iron stores were essentially depleted. The decline in storage iron can be accounted for, in part, by the increase in circulating hemoglobin iron, the average increase of 8 g/L hemoglobin corresponding to 120 mg of iron. We assume that the balance of iron mobilized from the storage compartment was located in marrow erythroid precursors. It is estimated that an average of 6.5% of the iron in the erythron compartment is contained in the marrow, about two-thirds in the nucleated red cell compartment and one-third in marrow reticulocytes.[24] The serum transferrin receptor, which is a reliable index of the size of the erythroid marrow,[25] was increased roughly 3-fold in our subjects following rHEPO administration. The contribution of iron deficient erythropoiesis to the raised

transferrin receptor level is unknown but the 2-fold rise in the reticulocyte count indicates that the rise in serum receptor is due mainly to enhanced erythropoiesis. Assuming that 6.5%, or approximately 125 mg, of erythron iron is contained in the marrow in a 70 kg man, a 3-fold rise in the size of this compartment would correspond to an increase of about 370 mg iron. Thus, the total increase in erythron iron of 490 mg agrees with the calculated loss from iron stores.

The 400-500 mg iron required for the increase in erythron iron following a course of rHEPO corresponds to a daily iron requirement of nearly 35 mg, an amount that is far greater than can be absorbed from the diet. It is estimated that the basal iron requirement of 0.9 mg/day for an adult man is balanced by an absorption of 0.6 mg nonheme dietary iron and 0.3 mg of dietary heme iron.[26] Using these basal estimates, rHEPO administration produced only a modest increase in heme absorption from 0.3 to 0.4 mg and an increase in nonheme iron absorption from 0.6 to 3.0 mg daily. Thus, under the effect of erythropoietin administration, a normal diet can furnish only 2-3 mg of additional iron daily or about 10% of the iron required for the enhanced erythropoiesis induced by rHEPO. A much larger proportion of the iron demand can be met if ferrous sulfate is given. When taken separately from food, 25% of ferrous sulfate was absorbed or 12.5 mg (Table 3) and nearly 10 mg when taken with a meal. Thus, 2-3 ferrous sulfate tablets daily taken separately from a meal or 3-4 tablets taken with food could meet the increased iron needs following rHEPO administration. Because iron absorption decreases

at higher serum ferritin levels, it is unlikely that even this amount of iron could fully prevent a decline in serum ferritin with regular rHEPO administration.

Despite marked differences in the form and amount of iron administered, the effect of rHEPO on iron absorption was remarkably similar. With nonheme dietary iron, a 5.39 increase was observed of which 2.51 was due to enhanced erythropoiesis per se. With ferrous sulfate taken with food, an 8.82 increase occurred of which 2.99 was due to enhanced erythropoiesis. It is of interest that the effect of erythropoietin independent of changes in iron status was similar despite an appreciable difference in the amount of administered iron.

The manner in which enhanced erythropoiesis stimulates iron absorption is unknown. It has been postulated that iron absorption is influenced by the rate of tissue iron uptake and by the size of a labile iron pool in various body tissues.[27] This hypothesis predicts that iron absorption will be enhanced if there is an increased outflow of iron from plasma, the latter is determined primarily by the mass of transferrin receptors contained in the erythroid compartment of the bone marrow. In support of this hypothesis, exchange transfusions with high reticulocyte blood in rats produced a significant increase in absorption without altering plasma iron concentration or body iron stores.[28] In the present study, rHEPO induced both a decline in the labile iron compartment and an increase in tissue iron uptake as evidenced by an increase in reticulocyte count, hematocrit, and serum transferrin receptor. Thus, both factors suggested by the Cavill hypothesis could

have produced enhanced absorption. It should be noted, however, that there is no evidence that basal erythropoiesis influences iron absorption in normal individuals. It was recently shown that in the absence of iron deficiency, tissue receptor mass, as reflected by serum transferrin receptor levels, had no discernable relationship with iron absorption.[4]

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Table 1
Laboratory measurements before and following the administration of erythropoietin in normal subjects (Mean ±SD).

Measurements	Pre	Post	P Value
			0:28
Hemoglobin (g/dl)	142±13	150±16	NS*
Hematocrit (%)	41.7±3.4	45.5±4.7	<0.05
Red Cell Count (10^{12}/L)	4.58±0.42	5.01±0.50	<0.05
Reticulocytes (%)	2.6±1.4	5.7±1.8	<0.001
Serum transferrin receptor (mg/L)	5.4±0.8	14.8±2.4	<0.001
Serum Iron (µg/dl)	72±16	38±13	<0.001
TIBC (µg/dl)	378±74	409±97	NS*
Transferrin Saturation (%)	20±7	10±5	<0.01
Serum ferritin† (µg/L)	42.5 (21.9-82.3)	13.0 (6.3-26.9)	<0.001

* Not significant
† Geometric mean±1SD

Table 2
The absorption of heme and nonheme iron before (pre) and after (post) erythropoietin administration

Age/Sex	Hemoglobin		Serum ferritin		Heme Iron Absorption		Nonheme Iron Absorption			
							Original		Adjusted*	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	(g/L)		(µg/L)		(% of dose)		(% of dose)			
32M	154	172	83	24	26.1	51.7	1.5	29.5	3.1	17.7
35M	166	184	72	20	65.0	42.4	11.7	57.2	21.0	28.6
29F	136	145	53	19	24.6	39.7	0.6	16.9	0.7	8.0
30F	142	143	48	13	48.5	46.6	6.5	25.6	7.8	8.3
29F	126	137	36	8	60.7	65.6	9.7	54.5	8.7	13.6
27F	139	152	35	8	51.6	59.3	1.3	22.0	1.2	5.5
33F	134	134	26	19	40.0	67.5	25.7	37.6	16.7	17.9
30F	140	141	16	4	94.1	100.0	20.3	71.0	8.1	17.8
37F	118	119	10	5	46.8	77.4	20.3	14.1	20.3	14.1
MEAN*	139	147	35	11	47.0	58.6	5.9	31.8	6.0	13.1
-SEM			28	9	40.7	53.1	3.7	26.3	4.0	11.0
+SEM			44	14	54.1	64.8	9.4	38.4	9.1	15.5

* Absorption adjusted to a serum ferritin of 40 µg/l

Table 3

Absorption of 50 mg ferrous sulfate given with and without food before (pre) and after (post) erythropoietin administration

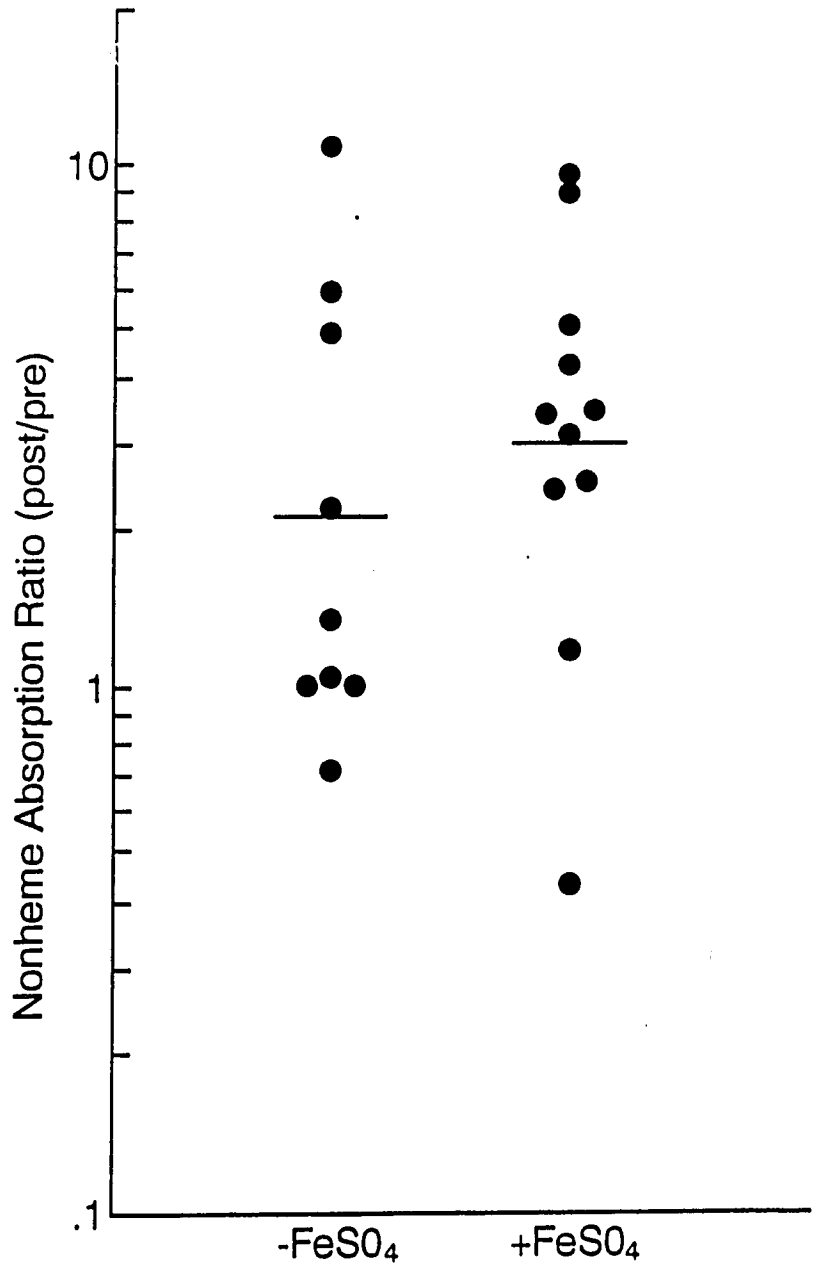
Age/Sex	Hemoglobin		Serum Ferritin		Absorption Without Food		Absorption With Food			
							Original		Adjusted*	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
	(g/L)		(µg/L)		(% of dose)		(% of dose)			
43M	163	176	151	65	1.9	35.2	1.3	30.0	5.1	48.8
36M	157	170	126	39	2.2	24.5	1.8	19.0	5.6	18.5
28F	130	151	76	23	12.0	14.7	1.3	13.8	2.5	7.9
38F	130	139	54	23	6.3	17.2	1.7	19.4	2.3	11.1
23M	153	169	54	16	13.3	23.8	2.3	25.4	3.0	10.2
22F	151	152	50	9	3.9	10.7	4.3	8.9	5.4	2.2
35F	138	147	40	8	14.3	20.6	1.5	14.6	1.5	3.6
37M	149	154	34	13	9.2	31.1	5.0	15.5	4.2	5.0
28M	141	157	27	11	16.0	46.1	2.1	30.9	1.4	5.8
24F	133	141	27	6	3.8	38.6	0.9	21.1	0.6	5.3
26F	138	145	22	5	14.7	31.0	3.5	18.2	1.9	4.5
MEAN*	144	155	54	15	7.0	24.6	2.0	17.9	2.5	7.6
-SEM			41	12	5.5	21.5	1.7	16.3	2.1	3.2
+SEM			60	19	8.8	28.1	2.4	19.8	3.1	17.6

* Absorption adjusted to a serum ferritin of 40 µg/L

LEGENDS

Figure 1. Nonheme iron absorption ratio from the standard meal (-$FeSO_4$) and from a meal taken with 50 mg ferrous sulfate (+$FeSO_4$) after correction for the serum ferritin (see text).

Figure 1



APPENDIX B

APPENDIX B-1

SELECTED PAPERS WITH POLICY IMPLICATIONS

FROM

ENDING HIDDEN HUNGER
(A Policy Conference on Micronutrient Malnutrition)

Montréal, Québec, Canada

October 10-12, 1991

Co-Sponsors:

WHO • UNICEF • WORLD BANK • CANADIAN INTERNATIONAL DEVELOPMENT AGENCY •
U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT • FAO • UNDP

SECRETARIAT:
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PREFACE

The International Conference on "Ending Hidden Hunger" (A Policy Conference on Micronutrient Malnutrition) was convened in Montréal, Québec, Canada from October 10-12, 1991. This meeting was co-sponsored by the World Health Organization, UNICEF, The World Bank, the Canadian International Development Agency, U.S. Agency for International Development, the Food and Agriculture Organization of the United Nations, and United Nations Development Programme. The Conference also had the active support of the International Council for Control of Iodine Deficiency Disorders, the International Vitamin A Consultative Group, and the International Nutritional Anemia Consultative Group.

This was the first meeting on a global scale to be called to pursue the goals of the World Summit for Children, which was held in New York in September 1990. Participants discussed and exchanged experiences on national plans to address micronutrient malnutrition and to accelerate progress towards the goals set out in the World Declaration on the Survival, Protection and Development of Children and Plan of Action for its implementation.

The goals endorsed by the Summit were the virtual elimination by the end of the century of the disorders resulting from deficiencies of iodine and vitamin A, and reduction of iron deficiency anemia in women by one-third. The Plan of Action adopted at the Summit committed governments to address these issues in their national development plans.

The Conference was attended by approximately 300 people from 60 countries.

This document contains selected papers from the Conference on "Ending Hidden Hunger" which have policy implications. This publication will be disseminated to a broad spectrum of interested countries and organizations.

LIFTING THE TRAGIC BURDEN OF MICRONUTRIENT DEFICIENCY

Dr. Hiroshi Nakajima
Director-General
World Health Organization

Ministers, distinguished colleagues, ladies and gentlemen,

We have gathered, some from close at hand, others from the far reaches of the earth, with a common purpose -- to end once and for all the "hidden hunger" of micronutrient malnutrition.

In warmly welcoming you, I am deeply aware of the historical potential of this conference. It should substantially reinforce the already rising global momentum of national and international action, commitment and resources so that, by the year 2000, two of the greatest scourges of mankind -- iodine deficiency disorders and vitamin A deficiency -- can be eliminated and the massive worldwide problem of iron deficiency greatly reduced.

During the course of this conference, speakers will draw your attention to the tragedy of the enormous magnitude of global micronutrient malnutrition -- about 200 million of our fellow humans brain-damaged, blind or anaemic for want of small amounts of iodine, vitamin A or iron in their daily diet. Then you will hear of the growing confidence and global commitment as we see technology, national development, expanding awareness and resolution combining to successfully and permanently eliminate these deficiencies.

Let me remind you of what our goals are, and briefly put them in perspective. Over the past 20 years, but especially during the past decade, many countries have become more aware of their iodine deficiency disorder problems (goitre and cretinism), vitamin A deficiency blindness and nutritional anaemia. Many countries have developed their own reduction or elimination targets for these nutritional deficiencies, as part of intensified primary health care for achieving health for all by the year 2000.

In the regions of the world where these deficiencies are severest, affecting many millions, particularly in South-East Asia, Africa, the Western Pacific and Latin America, Member States have already individually and collectively resolved, by WHO regional committee resolutions, to accelerate action and/or eliminate iodine deficiency, vitamin A deficiency and iron deficiency anaemia.

From a global perspective, in 1984 and 1986, before we believed that the global elimination of iodine deficiency disorders and vitamin A deficiency were realistic goals, resolutions were adopted by the World Health Assembly urging Member States to give high priority to their prevention and control. Then, in 1990, at the Forty-third World Health Assembly, delegates from country after country stood up and urged WHO to aim at eliminating iodine deficiency disorders as a public health problem by the year 2000. This goal was indeed unanimously adopted by resolution WHA 43.2.

Among the many achievements of the 1990 World Summit for Children was endorsement, and indeed reinforcement, of these micronutrient goals. Specifically these goals are:

(i) the virtual elimination of iodine deficiency disorders;

(ii) the virtual elimination of vitamin A deficiency and its consequences, including blindness; and

(iii) reduction by one-third of 1990 levels of iron deficiency anaemia in women.

The challenge of reaching these goals is clearly enormous, but it can be achieved.

Of all the three micronutrients, success is most likely for iodine deficiency disorders. Iodine deficiency is recognized as the world's leading cause of preventable mental retardation. Globally, over 1,000 million people live in significantly iodine-deficient environments, and it is estimated that over 200 million have goitre, while 20 million are brain damaged.

Yet I wish to emphasize the great progress achieved and the growing global momentum of commitment and action that has resulted in successful national control programmes.

At least 18 countries, among them Australia, Canada, Costa Rico and Switzerland, have successfully eliminated iodine deficiency disorders, while 46, among them Ecuador, Nepal and Thailand, have well-established programmes showing evidence of success. However, others still need to strengthen, develop and accelerate national control programmes. With proven cost-effective interventions, iodized salt as well-established long-term sustainable method, and oral or injected iodized oil as a short-term stop-gap measure in selected, severely affected populations, our task is to work together to support the establishment of effective national control programmes. Already much of the necessary commitment, infrastructure and resolve are there.

I have already mentioned the individual targets of Member States, the regional resolve demonstrated at the WHO regional committees, the World Health Assembly and the World Summit for Children.

With the ever-growing development of national control programmes, supported by regional working groups and the remarkable global collaborative network of the International Council for Control of Iodine Deficiency Disorders, represented here today by Dr. Hetzel and Dr. Ramalingaswami, the essential infrastructure for global elimination is already in place. Now required are the resources to reinforce the national programmes which will drive this global support system. If these resources are forthcoming, I am certain we shall see the virtual elimination of iodine deficiency disorders by the year 2000.

Vitamin A deficiency is the most common cause of preventable childhood blindness. It is estimated that because of this at least 13 million pre-school children have damaged eyes and that well over 40 million pre-school children are physiologically vitamin A deficient. Every year, some 500,000 new pre-school children develop active corneal disease, and it is known that a large proportion of these children die as a result. At any one moment, some 3 million children under the age of 10 years are blind from vitamin A deficiency.

Over and above this problem of blinding malnutrition, there is persuasive new evidence, accumulating from several completed and current intervention trials, suggesting that in deprived areas where vitamin A deficiency is a problem of public health importance, improving vitamin A status may substantially reduce mortality in older infants and young pre-school children.

I am keenly aware of the crucial importance of these findings and the debate on their significance. In May this year, in collaboration with USAID and the United States National Institutes of Health, WHO convened in Geneva a unique technical consultation of principal investigators to review the scientific evidence. I know that Dr. Al Sommer will cogently apprise you of where we now stand on this issue.

From a global perspective, vitamin A deficiency appears to be a serious public health problem in at least 37 countries, and possibly in some areas of a number of other countries. Many of you are well aware of this problem and have already developed national vitamin A programmes.

I would emphasize, as I have in connection with iodine deficiency, that the aim must be to develop long-term, sustainable strategies to ensure that all people are able to consume an adequate dietary intake of vitamin A.

During the Conference we shall hear how this can be, and has been, achieved through an appropriate balance of dietary, public health, agricultural and developmental strategies.

Of all the micronutrient deficiencies, iron deficiency is by far the most widespread. It is estimated that some 1,500 million people are anaemic from all causes, 700-800 million of these being affected by iron deficiency anaemia. Women of childbearing age, particularly pregnant women, and young children are worst affected. Even this is a conservative estimate, since iron deficiency anaemia is the visible end-stage of a relatively long process of deterioration of iron stores. Many more people than those manifesting anaemia are in fact suffering from deficiency, with its adverse affect on health and stamina.

You will hear from Dr. Viteri of the consequences of iron deficiency, which includes maternal and fetal morbidity and mortality, low birth weight, growth retardation, impaired cognitive, motor and mental development in infants and children, and decreased work capacity in adults.

As with iodine and vitamin A deficiencies, much work in the area of communication needs to be done to bring about the necessary recognition, including by those affected, of the importance of iron deficiency. We have learned from many a past failure that attempting simply to provide iron supplements to vulnerable populations does not succeed. Strategies need to aim at an adequate dietary intake and absorption of iron, the fortification of foods where possible, and iron supplementation of selected groups where necessary, supported by public health measures to control hookworm, malaria and parasitic infestation. All these require a sufficiently developed primary health care system, a reliable delivery system, public education and motivation, and the monitoring and evaluation of both process and impact.

This Conference gives us a precious opportunity to gather our collective strength, to visualize our goals, to comprehend the urgent action that we must undertake as individuals, as Member States, as international organizations, and as scientific and other bodies. It also allows us to generate the enthusiasm, commitment, resources and support that are essential, if we, as a global movement, are to lift or lighten the tragic burden of iodine deficiency, vitamin A deficiency and iron deficiency from the face of the earth.

I welcome you all and wish you a successful, inspirational and productive meeting.

THE END OF "HIDDEN HUNGER" IS IN SIGHT

James P. Grant
Executive Director
UNICEF

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It is a pleasure and a privilege to be here today at this potentially historic milestone event in the long history of humankind's struggle against hunger -- in this case, the "hidden hunger" of micronutrient malnutrition. If knowledge is power -- as the wise saying goes -- this is certainly a most powerful gathering. We have here the world's foremost scientists and experts in the field to whom we owe so much for their years of dedication and often lonely pioneering work. If national decision makers are key to action, in this room we have an impressive array of government ministers and senior officials (many from countries where the problems we are addressing are most severe), high-level representatives from international agencies and public health leaders, all of whom are in a position to translate knowledge into policy consensus...common strategies...new resources and, most importantly, accelerated global and national action. If social mobilization and health education is another key, we also have in this room an impressive array of health educators, social mobilizers, and communication experts. Your agreed common goal is another key. The World Summit for Children, the greatest gathering of power holders ever, has instructed us to eliminate vitamin A, iodine and iron as major deficiencies.

Given the centrality of nutrition to virtually all aspects of human and economic development, a successful global offensive against micronutrient malnutrition will give a decisive push to human progress in the 1990s and, particularly, to meeting the broad range of goals adopted by the World Summit for Children, held just a year ago. The task awaiting us is a formidable one -- to convert the potential in this room into an historic breakthrough -- but I know you will all agree it can and must be done.

<u>An enabling international environment</u>

Our deliberations here this week are greatly facilitated by a new international climate in which truly global cooperation is possible for the first time in more than half a century: the Cold War has ended; defense expenditures are being reduced; democracy is expanding throughout the world.

In addition, we are powered by the extraordinary mandate for concerted action that all of us -- each in our specific spheres of activity -- received from the World Summit for Children following years of consultation and agreement among experts and international agencies. The success of worldwide efforts to reach universal child immunization (UCI 1990), certified and celebrated earlier this week at United Nations headquarters, provides us with a momentum of confidence, mobilization and communication on which our micronutrient offensive can build. In part -- only in part, but an important part -- it provides us also with one of the key frameworks, a set of strategic allies anxious for additional challenge, a vehicle for some strategic components of that offensive.

In fact, this is a gathering of many of the same individuals and organizations who made these recent breakthroughs possible. It was, to a great extent, the success of the immunization effort many of you pioneered and all supported that led to the setting of a broader range of new goals by the World Health Assembly, the UNICEF Executive Board and other bodies, which the world's political leaders then embraced at the World Summit for Children. The gratification of having made a difference -- of accomplishing what many thought impossible -- should now inform and energize our discussions here in Montréal.

To date, the World Declaration on the Survival, Protection and Development of Children and its accompanying Plan of Action has been signed by 123 heads of state or government, and initialed by senior representatives of another 27 countries. No other document in history bears the signature of so many world leaders and their commitment for specific action within a specific time period. It marks the point at which the world officially woke up to the fact that child death and child malnutrition on today's scale are no longer inevitable and are therefore no longer acceptable. Political will -- long the missing link between what can be done and what will be done -- is engaged, and it is now up to us -- up to governments, UN agencies, NGOs, industry and others in the private sector, communities and individuals everywhere, for which we in this room are the representatives -- to see to it that world leaders are helped to keep their remarkable promise made to children a year ago.

Malnutrition: a key focus of the World Summit

The presidents, prime ministers and monarchs who attended the World Summit gave prominence to the problem of malnutrition. In the 10-point programme they adopted, they state:

> "We will work for optimal growth and development in childhood, through measures to eradicate hunger, malnutrition and famine, and thus to relieve millions of children of tragic sufferings in a world that has the means to feed all its citizens."

In a section dedicated specifically to food and nutrition, their Plan of Action states:

> "With the right policies, appropriate institutional arrangements and political priority, the world is now in a position to feed all the world's children and to overcome the worst forms of malnutrition, i.e., drastically to reduce diseases that contribute to malnutrition, to halve protein-energy malnutrition, virtually to eliminate vitamin A deficiency and iodine deficiency disorders and to reduce nutritional anaemia significantly."

These goals -- endorsements, really, of targets adopted prior to the World Summit by the governing bodies of WHO and UNICEF -- are, in effect, both our "marching orders" and our political opportunity. This is why we are here, to strategize on ways to "keeping the promise" in implementing the Summit goals related to iodine, vitamin A and iron deficiencies, in the framework of efforts to cut malnutrition in half by the end of the decade.

The fight against malnutrition lies at the very core of human development, with intimate linkages to the entire array of goals for the 1990s. Because of their exceedingly high impact and low cost, efforts to eliminate micronutrient deficiencies can constitute the cutting edge of the attack on malnutrition, and -- as with UCI -- provide the encouragement that comes from knowing that what once seemed unattainable can, in fact, be accomplished.

Micronutrients are really "super-micronutrients"

As will be made abundantly clear here this week, and as USAID's Richard Bissell correctly insisted months ago, the term "micronutrients" hardly does justice to the significance of these essential dietary constituents. Named that way because of the minute amounts of them we require each day and the difference they make to our well-being, they are really "super-micronutrients", or "super-nutrients", if one takes into account their great importance for the economic and social development of a country, as well as for the welfare and quality of life of its people. Deficiencies in iodine, vitamin A and iron constitute three out of the four nutritional problems of most public health significance today. Dr. Nakajima has already explained the magnitude of this problem facing fully twenty per cent of the world's population -- concentrated primarily in the developing countries. And what makes this situation all the more unacceptable is that this massive loss of life and waste of human potential is entirely preventable...at a genuinely "micro" cost.

Prevention of "micro" deficiencies will result in major macro benefits. It will raise the average intelligence quotient among deficient populations, reduce infant mortality and boost work productivity. Just a few weeks ago, the Leeds Castle International Conference on the Prevention of Disability underscored the advances that could be made against physical and mental disabilities through provision of this triad of essential nutrients.

An eminently "do-able" proposition

What makes our effort against micronutrient malnutrition so eminently "do-able" is the fact that nothing new has to be invented: the tools we need are already in the workshop -- effective and low-cost remedies which have existed for many years. Where they are applied, the results are truly extraordinary. Two vitamin A capsules, costing four US cents, can stave off deficiencies in an individual for a year. The iron pills needed for the recommended three to four month treatment period cost a quarter of a US dollar. Iodization of salt works out to about five US cents per person per year. Of course, there

are other costs associated with training and distribution, and establishing local production capabilities, but to the extent that the fight against micronutrient deficiencies is integrated into other programmes, these costs can be kept quite low. It would be difficult to find a health intervention more cost-effective than the nutrition education, the dietary fortification and supplementation remedies now available for the global effort against hidden hunger.

The way forward

While the conditions prevailing in each country will, of course, determine the concrete strategies to be adopted for each micronutrient (and a situation analysis in each country is the first requirement), the way forward thereafter is clear; the solution lies in the proper combination of dietary diversification, food fortification, supplementation, and public health strategies that address the factors preventing adequate absorption or utilization of micronutrients. However, long-term sustainable approaches to prevention and control will need to be accompanied by short-term measures to assure achievement of our year 2000 goals.

Interventions can address multiple deficiencies at the same time. Public education campaigns can promote dietary changes to correct iodine, vitamin A and iron deficiencies; salt can be fortified with both iodine and iron; clean water programmes can include an iodization component. Some existing channels -- can and should be used to deliver, where needed, all three micronutrients. To be successful, our offensive against hidden hunger will need to be conducted, in most cases, through primary health care systems and agricultural extension services, and through schools, commercial networks, the communications media...strengthening their capabilities in the process.

The need for public education and mobilization

Micronutrient deficiency does not produce hunger as we know it: it gnaws at the core of health, but not in the belly. Most of its consequences are not readily perceived; like the iceberg, its bulk lies beneath the surface. Even its most apparent effects -- such as blindness and cretinism -- seem to most people to be unrelated to diet. That is why we call it "hidden hunger" and why such an extraordinary effort must be made -- through every available channel -- to drag it into the open, make it visible as an issue at the political level, and empower families with the prevention knowledge they need.

Communication is our most powerful tool. Take the case of the remote Andean mountain regions of Ecuador, where a million people were believed to be iodine deficient in the mid-1980s. Remarkable progress has been made there -- in a short time -- in getting the local population to switch over to iodized salt and, where appropriate, be injected with iodized oil. This was accomplished through a sophisticated -- but low-cost -- social marketing strategy relying on a flexible combination of radio broadcasting of nutrition messages (in Spanish and Quechua), health education by schoolteachers and intensive training of village leaders and health workers in the most change-resistant communities. Over 80 per cent of those formerly at risk are no longer so, and complete coverage is on the horizon.

The time for bold action is now

What is now required to win our war against hidden hunger is a global mobilization of the type and magnitude that has made the immunization programme such a success story. Much greater cooperation and acceleration of governmental, private sector, NGO and community efforts is now needed. Aggressive policy action and regulatory enforcement on the part of governments is necessary; greater initiative and cooperative on the part of the food industry will be critical; and all the time-tested techniques of public education and social mobilization will need to be employed to facilitate the transition from supply-oriented to demand-based programmes.

I am pleased that there is a new major private sector initiative that will give our offensive against "hidden hunger" a real boost. The pharmaceutical giant, Hoffman-LaRoche, has informed WHO and UNICEF that it will be providing, free of charge, sufficient vitamin A to dose 115 million children in 37 countries over the next three years. This generous donation is aimed, specifically, at giving impetus to a newly-launched WHO/UNICEF initiative linking vitamin A supplementation with measles immunization.

This "piggy-backing" approach points us in the direction we need to be headed: integration and coordination of programmes for resource savings and maximum efficiency. Where relevant and feasible, countries should consider using existing programmes of high coverage, such as the EPI, for reducing and eliminating micronutrient deficiencies -- along with fortification and other dietary strategies.

Most governments now preparing their 10-year Programmes of Action to implement the goals of the World Summit for Children will want to include detailed plans for eliminating hidden hunger. Upon returning to their countries, I am sure that many health ministers and other government officials attending this conference will want to ensure inclusion of such plans in these national programmes, due by the end of this year.

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Many industrialized countries are now reviewing their development assistance budgets to ensure that they are supportive of the Summit goals, and I am confident that the donor community will act decisively to see to it that programmes to address micronutrient malnutrition will be adequately funded. And it is not only money that is needed: your scientists who are doing such promising research in the micronutrient field can share their expertise with their developing country counterparts. Producer countries can supply nutrients needed for our accelerated effort.

This conference could not have taken place at a more opportune moment -- just when human development and aid plans are being drafted for the decade ahead. But this also means we have no time to lose. Most often there is a lag of many months and even years between the holding of a major international policy conference and the implementation of the agenda it adopts; in the present case, given the "window of opportunity" we now have, this is a luxury we simply cannot afford. We must get things rolling on an entirely new qualitative level, starting right now.

Many countries may wish to accelerate plans to achieve the micronutrient goals well before the year 2000 -- indeed, some have already set themselves such targets. Bolivia, for example, now seeks to achieve the goal for iodine deficiencies by the end of 1993. Earlier attainment has political attraction; it will provide encouragement by example, permit action to enter a "maintenance" phase, and enable priority attention to be given to Summit goals yet unreached.

The Grand Alliance for Children which has gathered new partners and unprecedented strength in recent years can be the foundation and springboard for a powerful Global Alliance to End Hidden Hunger. This week the leadership of this alliance has assembled in Canada, most appropriately, inasmuch as Canada, together with the United States, has taken a leadership role in this "super-nutrient" effort. The end of hidden hunger is in sight in this decade of the 1990s. We know what must be done and how to do it, and our presidents and prime ministers have committed their support. I am confident that, together, we can meet the great challenge posed by micronutrient malnutrition, leaving this conference with common strategies and strengthened resolve to succeed in this great undertaking to eliminate this hidden enemy.

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CHALLENGES AND OPPORTUNITIES

Dr. V. Ramalingaswami
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One Vitamin and Two Minerals

This is the story of one vitamin and two minerals, an unfinished story of a needless human tragedy being enacted on the developing world stage. The vitamin, vitamin A, and one of two minerals, iodine, have to be taken in microgram quantities and the other mineral, iron, in milligram quantities in daily diets for human well being. In such minute quantities, they are essential as constituents of vital enzymes and proteins for the normal processes of growth, development, maintenance and resistance to infection. In their absence, individuals (and families) suffer serious consequences expressed as increased mortality, morbidity and disability rates; communities and nations suffer losses in human potential, the social and economic costs of which no country can afford. The disturbances produced by lack of these essential nutrients are not confined to single organs as was formerly believed, but affect multiple organ systems. Thus, iodine deficiency does not result in just a goitre, nor iron deficiency in just nutritional anaemia, nor vitamin A deficiency in just xerophthalmia. These three micronutrients and their physiological roles were known for a considerable period of time and were prominent in the early history of nutritional science. Their deficiencies had long since been brought under control in the industrialized world, while they continue to persist in large parts of the developing world as vestiges of pre-transitional health scenario constituting the unfinished agenda of health development of the Third World. Technology is not the limiting factor in addressing micronutrient deficiencies.

Recent studies reveal adverse consequences of milder deficiency states and more fundamental roles for these micronutrients in growth, development, and immunity than was formerly believed, thus seeking to underscore the urgency of prevention and control of micronutrient malnutrition.

To call them "Micronutrients" may be in conformity with the minute quantities needed by the human body, but it is certainly not in consensus with the nature and extent of damage being brought about by their deficiencies in individuals and societies on such a vast scale today. The are rightly called "Supernutrients" by Mr. Grant, and their deficiencies constitute today a major constraint on future human development. The young, the poor, and the female in some cultures, are the worst affected, but micronutrient malnutrition is a pervasive phenomenon and its consequences are felt at all stages of the human life cycle, from the moment the fertilized ovum starts upon its perilous journey in the darkness of the mother's womb. Pre-conceptual nutrition (nutrition in anticipation of pregnancy) is a key concept in public health nutrition today.

The Panorama of Micronutrient Malnutrition

The numbers of humans affected by micronutrient malnutrition are truly staggering and are a blot on society. To narrate them in extenso would be like playing on the piano in fortissimo for far too long.

Iron:

Briefly, one billion persons are affected by iron deficiency; this deficiency is particularly common in women in the reproductive age group and in young growing pre-school children in tropical and sub-tropical zones. Iron deficiency also impacts seriously on school children and working adult males. If uncorrected, it leads to anaemia of increased severity, reduced work capacity, diminished learning ability, increased susceptibility to infection and greater risk of death of women in pregnancy and child birth[1]. Roughly 20% of all maternal deaths in West Africa and India, when blood transfusion was not available, are believed to be directly attributable to anaemia[2]. The elimination of this single micronutrient deficiency can do more than any other single programme to achieve the goals of development, because all age groups and both sexes are affected[3]. Provision of iron supplementation during pregnancy, lactation and early childhood has been demonstrated to be effective under controlled and supervised conditions, but under routine health care conditions, the effectiveness of control programmes against iron deficiency anaemia had been most unimpressive. Iron deficiency is the most neglected and the most widespread of all nutritional deficiencies constituting a real brake on human development. Its poignancy is underlined by the fact that the therapeutic effect of iron on anaemia was demonstrated by the English physician Thomas Sydenham more than two hundred years ago. Recent studies have blown up the myth that the necessity for repeated daily administration of iron tablets and problems with patient compliance were major bottlenecks in the iron deficiency control programme. While these factors are no doubt important, the studies clearly show the importance of supply and logistic problems in not permitting the steady flow of iron supplements to mothers. Here lies an extraordinary opportunity for a determined attack in iron deficiency, not fully reflected in the rather conservative Summit goal of reducing iron deficiency by one-third by the year 2000. I would urge this conference to go beyond the Summit goal for iron.

The addition of folic acid to the iron supplement in anticipation of pregnancy can now be firmly recommended for the prevention of neural tube defects in women with an affected pregnancy. The powerful role of folic acid in this respect, and also in improving anaemia in some populations, led to the suggestion that staple foods be fortified with folic acid in such areas. Nutrition is thus becoming increasingly an anticipatory science. Mass chemotherapy of school children with endemic hookworm infestation has led to improved growth and cognitive abilities of children, presumably by improving their iron nutrition.

Iodine:

The total population at risk of iodine deficiency is estimated to be in excess of one billion; 200 million have goitres and 20 million suffer varying degrees of mental deficiency related to iodine deficiency[4]. Iodine deficiency is the leading preventable cause of intellectual and neurological impairment in the world today. Iodine supplementation prevents this widespread brain damage manifested in the spectrum of hearing, speech, locomotor, and learning disorders and mental deficiency culminating in the extreme form of cretinism. In severely endemic areas, as many as 10-15% of the general population may be cretins. Iodine deficiency is entirely preventable in all its manifestations. The elimination of this ancient scourge of mankind, a unique example of a place disease, may be comparable to (Dr. Maberly of Australia thinks it may conceivably exceed) the impact of the global eradication of smallpox[5]. Iodination of salt has been shown repeatedly to be a low cost, highly effective means of preventing iodine deficiency and that was how the problem was eliminated in most parts of the industrialized world. There is some recent evidence to suggest that iodine deficiency is a significant problem in the countries of Europe, Eastern and Western. Iodized oil by mouth or by injection can be used as an interim measure in endemias where provision of iodized slat may not be promptly feasible, as in remote, isolated communities while an iodized salt system is being developed and established[4]. To date, in excess of 60 million doses of iodized oil were given either by injection or by mouth over the past 15 years in Asia, Africa and Latin America. The cost of iodized salt works out at 5-10 cents per head per year and of iodized oil at 20 cents per head per year. Iodine deficiency should be corrected at the earliest possible moment in life, preferably before conception; if not, early in pregnancy; if not, early in infancy. Despite its simplicity, low cost, and extreme effectiveness and despite governments generally encouraging control against IDD, and some tangible progress made in recent years, control programmes against IDD progress had been generally uneven, and large populations remain unprotected, especially in Africa and Asia.

Vitamin A:

About 40 million children in the world under the age of 5 are physiologically deficient in vitamin A. Of these, about 400,000 die of severe vitamin A deficiency each year and more than 250,000 become partially or completely blind from lack of vitamin A[6]. The role of vitamin A in preventing nutritional blindness is well documented. In addition, evidence accumulating in recent years show that vitamin A supplementation of children in communities with vitamin A deficiency as a public health problem exerts a measurable positive impact on child mortality[7]. This effect has also been most recently demonstrated in infants in the second 6 months of their lives[8]. Variations in the extent of response in different studies are to be expected, considering the variations in the severity of vitamin A deficiency to start with in different areas and the contribution of other ecological factors should not be exaggerated. The question of extent of benefit to be derived in very young infants from vitamin A supplementation in the first six months of life and the optimal dosages thereof may not have been resolved as yet, but "meanwhile," as an editorial in the Lancet says: "Children have a right to the best means of improving their

vitamin A status."[9]. There can be no excuse for inaction. Surely it is the prerogative of governments to decide upon a national policy for the prevention and correction of vitamin A deficiency based on their informed judgment, the severity of the problem, and the infrastructural facilities available.

The World Summit for Children

Against this panorama of micronutrient malnutrition, the historic and unprecedented gathering of world leaders in the World Summit for Children last year issued a World Declaration on the Survival, Protection and Development of Children and a Plan of Action for the 1990s.[10]. The plan included seven major goals and twenty-six supporting/sectoral goals, of which three relate to micronutrient malnutrition. The actions leading to the fulfillment of all the goals need to be taken in an interlinked, inter-disciplinary, inter-sectoral and synergistic manner so as to subserve the over-arching goals of reducing infant and pre-school child mortality rates by a third and of maternal mortality rates by one-half by the end of the 1990s. Although the three micronutrient goals seemingly constitute only a fraction of all the goals, they are the cutting edge; they are amenable to fulfillment in the shortest possible time; their cost benefit ratio are highly favorable; and along with other nutritional goals, they provide nutritional science and technology with a unique opportunity to promote nutritional well-being as fundamental to sustainable human development on a scale so far not witnessed. Indeed, the Summit goals have become a Mission for the entire U.N. family, for the aid agencies, for governments, and non-governmental organizations. They beckon the United Nations to turn increasingly from the issues of War and Peace to those of human development, representing a watershed signalled by the Human Development Report of the UNDP.[11] I venture to think that the forthcoming International Conference on Nutrition in Rome next year, also an effort by the U.N. family, will serve to accelerate the achievement of the Summit's nutrition goals. The challenge, of course, will be to get the development agencies and the U.N. system with all its agencies to orchestrate their efforts at the national level in reinforcing the priority of micronutrient malnutrition and in coordinating national plans and programmes to combat it. Much will depend upon how each of the relevant technical assistance and development agencies will contribute to the systematic implementation of strategies, with the elimination of micronutrient deficiencies as the central focus[12]. A forum of technical and funding agencies to harmonize policies had been suggested. Donor fatigue and waning confidence in development assistance to the extent that they exist need to be resolutely overcome.

The Prime Minister of Canada, Co-chairman of the Summit declared: "Every child will become an adult who inherits lifelong responsibilities towards humanity and towards the Earth....Nations can now apply efforts and resources towards achieving impressive goals for children and can do so successfully because the world in which we live is now a little safer."[13].

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Lessons of the Past

Technology and Society:

As we enter a new phase of technology application in respect to micronutrient malnutrition, an understanding of the reasons for our failure to utilize optimally some of the commonplace contributions of science and technology would be useful. The problems essentially seem to lie at the interface between technology and society. Political commitment, resource mobilization, and sound programme management, including logistical support, information support for policy formulation and goal setting, monitoring, and public understanding of programme purposes emerge as key elements for success. Many a programme has foundered from lack of dynamic national information systems to feed essential information on a continuing basis to detect trends and shape policy. National averages make little sense unless they are disaggregated in terms of groups at greatest risk, their geographical distribution, income levels, and social stratification. The information that a policymaker needs does not flow automatically from technology. Questions of costs, effectiveness, benefits, and risks in relation to gains; performance of the technology under the environmental, socio-economic, and cultural milieu of the recipient population, the identification of intended beneficiaries, their needs and their social and economic circumstances, all these enter into the agenda for policy formulation and are not often examined in depth ab initio. Surveillance is crucial to guide policy and for programme correction. Surveillance should provide not only a feedback, but also a feed forward. We may recollect how a feedback and feed forward system galvanized the workers in the smallpox eradication campaign. The micronutrient initiative must take into account the politics of policy formulation, the functioning of bureaucracy, the orientation of the providers of health care and the socio-cultural and behavioral characteristics of communities which the initiative serves. One of the lessons learnt from successful national development programmes is the role of social communication. Bridges must be built between those that have the science and technology, those that deliver the services, and those that have the power to make the political and financial decisions.

Technology and the Delivery System

Technology cannot be divested from the delivery system, nor can it be substituted for it. Since Alma Ata, developing countries have been making efforts to establish functional primary health care systems with varying degrees of success. In principle, when the micronutrient initiative is embedded in a functional primary care system, its sustainability becomes assured. The initiative itself adds strength to the health care system through its linkages with other elements in the system. In practice, the record of the micronutrient initiative closely parallels that of the health care system and establish parallel programmes for the micronutrient initiative or any other, but to strengthen the system and make the micronutrient initiative an integral part of the ongoing service programmes.

Linkage with EPI

EPI has now reached coverage levels of 80% or more of all the children under one year of age throughout the developing countries. It is poised to achieve 90% coverage levels by the end of this decade. Over 500 million contacts take place each year between health service agents and the people under this programme. Immunization services are now available to children throughout the developing world. They are provided by the routine health services of countries, not by exogenous agencies. In many places, mothers are beginning to demand these services for the protection of their children, a critical turning point from a supply-oriented to a demand-based health service. EPI offers a valuable window of opportunity for the delivery of services to prevent micronutrient malnutrition. Such a linkage has more than logistic value. Immunization breaks the dangerous partnership between malnutrition and infection in the first, most dangerous year of life. When immunization and nutrition join forces with clean water and sanitation, we have a quartet of complete technologies ushering in a renewed era of primary prevention in public health. Far from being a mono-culture disturbing the peace and balance of health care, as part of Primary Health Care, EPI has great potential for creating real assets for comprehensive services. This is the challenge of the 1990s. Good health services facilitate immunization, immunization services, in turn, could strengthen the health infrastructure, facilitate decentralization, improve management capabilities, including the setting up of surveillance systems. The micronutrient initiative cannot but benefit from these assets, but the detailed modalities need to be worked out. WHO and UNICEF are taking new steps to establish viable linkages between EPI and micronutrient programmes at the operational level. Supplementation of mothers and young infants with vitamin A, iodine, and iron would be greatly facilitated by these linkages.

The availability of effective technologies often leads to vertical programming of the delivery process in the first instance. But as they evolve, they lateralize and attempt connections. A strong movement is now needed to make the strengths of the vertical programmes subserve broader, cohesive actions, and at the same time enhance autonomy and self-determination on the part of communities in their own health development. I visualize the 1990s as a period of transition from focused goals to interlinked goals with out loss of focus, but with equity as the driving force.

Leadership at appropriate ministerial levels is a prerequisite, also at other levels including consumer groups and public health activists to obtain political commitment and to raise resources from donors and industry. There is a need to cultivate leadership in health at various levels to initiate and foster a process of change. The improvement of services, their outreach and humanization entail change. Not enough is being done to cultivate leadership in health, to foster the vision, the values and skills of individuals who are in a position to mobilize others. The challenge lies in getting people to take greater responsibility for their own health, to participate collectively in health activities and to change the perception and value system of health providers.

A Pluralistic and Inclusive Approach

The second generation of technology application would require a pluralistic, participatory and inclusive approach in which new and multiple partnerships are fostered, new linkages are established and health care providers, health administrators, policy and decision makers and community representatives function together along with researchers to establish priorities, measure programme results and couple knowledge to action. The common area of intersection of all these elements is where genuine human development takes place.

Communities

No discussion of technology/society interface will be complete without considering the role of communities. "Community is not too grandiose an idea when shorn of its utopian connotation. Community is where there is shared perception of the value of individual lives and social commitment to protect them equitably."[14] In the pluralism of the new era, communities occupy a central place. Development takes place within the economic, political, social, cultural, and moral context of communities.

Role of Research

Developing countries in general do not make effective use of research for guiding policy and action. The concept of Essential National Health Research (ENHR) was put forward by the Commission on Health Research for Development and the Task Force on Health Research for Development, the successor body to the Commission, is now engaged in operationalizing the concept at country level.[15] ENHR is conceived as an integrated strategy to promote health and development on the basis of equity and social justice. It holds the key to the achievement of the goals of the Summit. The technical discussions on Essential Health Research at the World Health Assembly last year are leading to follow up actions by the Advisory Committee on Health Research of the WHO. The New Paradigm being propounded by Dr. Nakajima, Director General of the World Health Organization, injects a new economic vocabulary into the health section and advocates health as central to human development, as a political issue, and pleads for better use of science and technology for human development.[16] Any micronutrient initiative as well as other initiatives to be undertaken for achieving the goals of the Summit should be responsive to these developments.

FUTURE STRATEGIES

Sustainable Actions

The most fundamental strategy to achieve the Summit goals should be aimed at long-term approaches to prevention and control, but in many cases if the goals are established in time, long-term sustainable actions need to be combined with short-term measures for immediate impact. Each country should establish a policy appropriate to its own situation.

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Strategies to overcome micronutrient malnutrition should be pursued in the larger context of primary health care and overall development strategies. Close association with measures for infection control such as immunization and measures for improvement of maternal and child health and family planning is imperative. The use of common targets, common entry points, and common agents of service delivery enhances efficiency, reinforces primary care and reduces costs. Building institutional capacity for management of micronutrient programmes is of strategic importance.

In general, deficiencies of micronutrients may be addressed by appropriate combinations of the following strategies:

Diversification of diets as a long-term measure

Diversification of diets so as to provide the required quantities of the three essential nutrients on a daily continuing basis is a long-term measure which should be the ultimate goal of the micronutrient initiative. This strategy is particularly relevant to vitamin A and iron. Promotion of breastfeeding and improving maternal nutrition are of critical importance. Education is a vital component of this strategy so as to bring about desired change in feeding behavior. Improved production, availability and access of nutrient rich foods at affordable prices is another component. In the education programmes one must go beyond information supply, beyond awareness building. Awareness must be converted into initiative and action on the part of the target groups. The power of the message, its appropriateness and the medium used to communicate it may help to drive awareness to action. But as AIDS education by UNICEF in East Africa has shown, a second generation of activities is needed in this area to bring about behavioral change. Inter-active, face-to-face discussion, with a face that is familiar and trusting, greatly facilitates this process. It must be remembered that every man and woman is a secret teacher, as Ivan Illich said once.

There is a tendency to down-play the importance of long-term approaches on the grounds that they take time, that the results are uncertain, and that induction of behavioral change is a difficult process, not to speak of the other doubts raised regarding increasing the access of the poor to productive assets for raising nutrient-rich crops in their own homes or their small land holdings. It must be emphasized that there is a great scope for home production in urban and rural areas; that the dietary approach should be seen as a component of community based strategies for Household Food Security. The activities include education, agriculture, and horticulture extension, home gardens, school gardens coupled with nutrition education of school children.[17]

Food fortification

Food fortification as a basic strategy for overcoming micronutrient malnutrition is especially relevant to iodine, but also for iron and vitamin A using vehicles such as salt, sugar, flour, MSG, etc. Fortification has worked well with industrialized countries. Historically speaking, dietary improvement and food fortification were the main strategies that led to the virtual elimination of micronutrient malnutrition in today's industrialized

countries. Country-specific and country-wide foods are used for fortification. The chief problem with fortification is not lack of technical solutions, but the means of implementation in developing countries. The legal requirement of iodized salt has not eliminated iodine deficiency diseases in most developing countries because the regulations are inadequately enforced. It is imperative that national capabilities be developed in respect of writing fortification legislation, setting up regulatory structures, motivating consumers, carrying out surveillance and improving compliance. Double fortification of salt with iron and iodine offers an exciting opportunity.

Supplementation

Supplementation, utilizing oral or injectable routes is immediately effective and is a useful short-term measure, while longer term systems are being developed. It could pave the way through careful policy design for long-term, sustainable measures which address basic causes. Delivery channels include the EPI systems, other primary health care outlets, school systems and extension services. Oral iodized oil capsules may be taken once in every two years; oral vitamin A supplements may be administered once in every six months, and oral iron tablets will need to be taken daily. Supplementation with high dose liquid vitamin A in oil by capsule, spoon or dispenser has great potential for rapid impact. The extent of overlap of target beneficiaries is not known with precision. But areas of special emphasis in the programme should be kept in mind: for iron deficiency, special attention must be given to pregnant women; for iodine deficiency, to women before and during pregnancy and to infants; for vitamin A deficiency, to mothers and children below 6 years of age.

Operational targets

Operational targets need to be set and observed for fulfillment of the goals, such as, for example, that by the year 1995, all countries should have established a national coordinating mechanism, a monitoring and control unit, a dietary diversification programme, a salt iodisation programme where appropriate, and systematic monitoring of indicators related to attainment of goals for each micronutrient. The monitoring of process indicators should include coverage, level of fortification at various levels of distribution including the retail end in all parts of the country and sensitive assays on valid samples of target population for adequacy of supply of each micronutrient. By the year 2000, all countries with significant micronutrient malnutrition should have fully operational programmes of long term, short term, or combinational nature as decided by each country.

Research of the nature of Essential National Health Research dealing with policy and management issues will be needed as a constant companion to the operational programmes. Research should also facilitate the rapid emergence of feasible new technologies such a slow release preparations of iron, double fortification of salt with iron and iodine, and transdermal and subcutaneous dosing systems.

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WORKSHOPS REPORT

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Our conference "Ending Hidden Hunger" has drawn over 300 policy leaders, scientists, and representatives of international agencies from about 55 countries. We are a multi-sectoral group coming from diverse fields as nutrition, health, agriculture, education, information, planning, and industry - both public and private. Through 15 conference presentations, informal conversations in the exhibition hall and over coffee and meals, and 10 working group sessions this morning, we have learned of recent scientific advances and shared rich and diverse experiences.

This summary report is necessarily tentative. Working group chairpersons met in two planning sessions and the 10 rapporteurs synthesized their respective working group discussions over a one hour session at noon today, thus forfeiting both our lunch and post-lunch nap! A comprehensive and balanced summary, therefore, is compromised by brevity of exchange and synthesis. "Time is our enemy!"[1] I calculated that our 300 plus viewpoints compressed into a 20-minute summary would leave an average of four seconds each. Thus, if any four seconds of this summary touches upon any of your viewpoints, please consider that your views have been diligently represented!

My theme for this summary is "three Ps" - **Promise, Policy, and Progress.** These three Ps are linked together - fulfilling the promise through policy actions for making progress.

The Promise

This conference has been a milestone event, a follow-up action to fulfill the promise made by Heads of State at the World Summit for Children in 1990. It has been an unprecedented event, both scientifically and politically. Never before has the Director-General of WHO and the Executive-Director of UNICEF jointly issued conference invitations directly to Heads of State, and most of us here were dispatched to the conference in response to these summit invitations. The highest-level of political support should unleash our energies and strengthen our resolve.

The goal of the conference is "putting castles in the sky...now put foundations under them."[2] We are here to launch a universal effort against the menace of the three micronutrient deficiencies (iron, iodine, and vitamin A) that together constitute "hidden hunger." These problems may be "micro" or invisible to the senses, but they are **"macro-problems."** The number of people affected is vast - about 1 billion each. And their consequences, as we were reminded, are not simply health and nutritional, but also cognitive development, work performance, and economic productivity.[3] At stake is the very path of socioeconomic development.

The Policy-Action Challenge

The policy-action challenge was posed to us at the opening.[4] It took 170 years from the invention of Jenner's vaccine to the eradication of smallpox. We are already 50 years into the scientific discovery of essential vitamins and minerals for human health. How much longer will it take us to eliminate or control these micronutrient deficiencies?

The scientific basis for policy action is well established and already at hand. Three key nutrients and three basic approaches to problem-solving - diet diversification and quality improvement, fortification of food and other vehicles, and direct and targeted supplementation. These interventions have been demonstrated to be feasible, effective, and affordable.

Translating knowledge into effective action will require the establishment of systems for linking policy action to human need.[5] The successful experience in Indonesia identified four basic components to these systems.[6] These are the four "wares:" people (human-ware), tools (techno-ware), delivery systems (systems-ware), and policy (policy-ware).

People: In terms of people, the most important are the target groups, customarily identified as children, mothers, and families. Participants stressed a focus on the most vulnerable, the disadvantaged, including refugees and displaced persons. The concept of target groups should also be broadened to include women not only as mothers but during "preconception," especially during adolescence when young girls are building up their nutritional reserves for their adult roles in economic production and family reproduction.[7]

People also involves the policy actors. Hidden hunger needs visible advocates. Most of our conference participants are high-level policy leaders representing governments around the world. We identified four additional groups that must be brought into the policy process, to enhance participation and to strengthen the basis of policy-support.

Industry - especially the local food production, processing and distribution business in developing countries;

Non-governmental agencies - especially women's groups who are concerned about, and can be mobilized around, this need;

Scientific and professional groups - for re-orienting their education in support of enhanced priority attention for eliminating hidden hunger; and

Field workers - in all sectors that reach vulnerable families, such as school teachers, agricultural extension workers, and primary health care workers.

Tools: The three basic tools of diet, fortification, and supplementation were extensively reviewed. Other supporting tools were suggested by participants. "Stop the leakage" of micronutrients was one such proposal.[8] Interventions that control gastrointestinal parasitic infestations and prevent the catabolic effects of malaria are examples of actions that can enhance the impact of diet, fortification, and supplementation. Other complementary interventions include the promotion of breastfeeding, clean drinking water, adequate sanitation, and improved hygiene.

Still other tools include approaches important to micronutrient absorption and effective utilization, for example folate and vitamin C supplementation to combat iron deficiency anemia. Finally, working groups noted the urgent need for low-cost, simplified diagnostic and monitoring field measurement techniques, essential for the guidance of successful interventions. One example for effective consumer awareness and regulatory control of salt fortification were rapid and inexpensive chemical markers for the detection of iodine in commercial salt, as employed in India.[9]

Delivery Systems: No one argued for entirely vertical delivery systems, and all pushed for avoidance of isolation.[10] Our common themes were integration, multi-sectoral coordination, and sustainability. The key is to reach all vulnerable families and communities. Pragmatism of what works predominated in the recommendations - home gardens, primary schools, agricultural extension services, primary health care including maternal-child health, and piggy-backing onto expanded programmes in immunization. Noteworthy were the exciting initiatives already underway in many countries. These need to be built upon, adding fresh new elements and new structures to plug gaps and to capture novel opportunities. "Bridges must be built and walls must be destroyed across sectors."[11]

The systems for attacking micronutrient deficiencies should be permanent, for these problems never entirely disappear and regression or slippage is an ever-present danger. One debate on sustainability is the relative priority to be accorded to longer-term dietary improvements in comparison to shorter-term supplementation strategies. These, of course, are not mutually exclusive, and the debate can never be fully resolved as theoretical issues on a global basis. The challenge is country-specific, shaped to the unique circumstances and decided by responsible parties in diverse situations around the world - all striving for harmony and synergy in action.

Policy: The proposed term "policy-ware" could be even more appropriately called "policy-care," for if policy leaders cared about micronutrient deficiency problems, policy actions would be effective. Policy action against micronutrient deficiencies are part of overall socioeconomic development goals of social equity and justice, especially the attack against mass poverty. Targeted interventions against "hidden hunger," however, must be linked to "visible hunger," for public support can be mobilized around such a linkage.[12] This dichotomy need not be

divisive, for addressing micronutrient deficiencies can be an "entry point" to the longer-term objective of good food and a healthy diet for all. These are basic human entitlements and fundamental human rights.

Micronutrient deficiencies are not simply "hidden hunger" because of the absence biologically of hunger pangs. Hidden hunger is silent also because of the lack of public awareness and the insensitivity of policy leaders.[13] Successful policy action, therefore, will critically depend upon translating the silence of hidden hunger into outspoken voices, of moving the problem from the closet into the open view of government and the general public. "Popularization" of the problems and their remedies through communications is critical. Examples of social mobilization include marketing concepts such as "super-nutrients" or "smart foods."

Achieving Progress

Harnessing these "wares" for achieving progress depends upon successful action in diverse national and community settings.[14] The problems vary enormously between and within countries. In many settings, the nature and magnitude of the problems are simply unknown. Also under-explored is the value or handicap of traditional diets that have evolved from centuries of human settlements in specific ecologic settings.

The key, therefore, is action in every country against each of the major micronutrient deficiencies. The triple A loop - assessment, analysis, and action - describes the components of what must be done in specific communities and nations. For each micronutrient, each country and, in turn, each community must:

Assessment - determine the significance of the problems;
- prioritize hidden hunger amongst competing demands;

Analysis - examine its own unique record and history;
- consider how science and other experiences are relevant to its unique situation; and

Action - design, test, expand, and monitor interventions shaped to its circumstances; and
- build local capacity for sustainability.

Food dietary improvement strategy and supplementation activities are complementary. Emphasis on one or the other shall take into account the sustainability of these programs and the socio-economic condition of the country or region.

These country- and community-specific actions from the bottom-up need to be complemented by actions from the top-down, for the solution to micronutrient deficiencies cannot be solved entirely from either direction alone.

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Our conference presentations and the working group discussions brought forth many examples of the "triple A loop." The goiter control programme in Ecuador is one example of targeted assessment, analysis, and action.[15] Another is the innovative communications strategy employed to incorporate nutrient-rich gourds into the Thai diet.[16] The very strong intersectoral coordination and training of field workers in the micronutrient programme of Tanzania is another example.[17] Assessments in Eastern European countries undergoing economic reform into free-market economies are needed for bringing the agriculture and health sector into a "marriage" to orchestrate a comprehensive "nutritional symphony" in rapidly changing societies.[18]

The Next Steps

Large conferences come and go. In the end, the central challenge facing the participants is: **What will we each do after the conference?** What we do will determine whether we fulfill the promise through policy actions for making progress. The follow-up actions of three participant groups have been identified.

Policy-Makers: Participants concurred on the need for urgent action without delay upon their return home. While time may be our enemy at this brief conference, time can also be a friend affording the opportunity for follow-up action in our home countries. Because the policy-makers attending this conference have been dispatched here by Heads of State, they can form **National Spearhead Teams**, acting as the central focus for launching accelerated national activities. The conference presentations and discussions have "sharpened" the points of these spearhead teams, and the "cutting edge" of these teams will be National Programmes of Action.

National Programmes of Action will incorporate at least four key elements. Firstly, participation should be broadened and support should be strengthened through inclusive strategies of bringing other interested groups into micronutrient initiatives, such as the food industry, non-governmental agencies, and women's groups. Secondly, national goals should be determined including process indicators, targets, and timetables for completion. Thirdly, sustainable interventions should be launched shaped to the unique circumstances of diverse countries. And finally, systematic methods of assessment and monitoring should be put into place to guide and accelerate action. These national activities may be fostered through workshops and other promotive activities at the national level.

National action plans should be strengthened through **regional cooperation.**[19] In many regions, such as Central America and South America, there is already broad recognition of a common problem that calls for joint approaches to common solutions for the mutual benefit of all regional partners.

The Scientific Community deserve our applause for bringing us this far.[20] This conference marks the transition of science moving from the sidelines into the (bigger and trickier) international playing field. The scientific questions are shifting from simply "what" are the problems to, more importantly, "how" do we solve them?[21] Science in support of action will require development of complementary disciplines, especially the policy, management, social, and behavioral sciences. When science moves into mass action, there is the ever-present danger of "scientific Balkanization," in which the rigor of scientific discipline can be confused with the decisions and actions needed even under circumstances of incomplete information. This tension is highlighted by the adage: "the best can be an enemy of the good."

International Agencies are needed to support these national and scientific initiatives. Conference participants noted the need for donor coordination, non-duplication, and demand-driven response to the diverse needs of countries. Access to international information, technology, and training was requested. Everyone called for more resources: "send money!"[22] Needed in external assistance will be approximately US $1 billion annually to match the US $2 billion that will have to be mobilized annually from domestic resources within developing countries.[23] Four-fifths of these estimates relate to the elimination of iron deficiency anaemia. International agencies are also requested to secure Technical Teams to assist each country or region, as well as to "keeping the promise" for immediate action by the country government.

Conclusion

One participant noted at the closing that "We have been sensitized to the problem. We have also been illuminated of what is possible."[24] Can we harness the power of the participants to form a global compact to eliminate hidden hunger? Can we channel our diverse talents and energies - amongst policy leaders, scientists, and international agency representatives - into solidarity to attack one of the major problems of contemporary humankind? Together, let us move forward to redeem that **Promise** through effective **Policy Actions** for achieving **Progress** in **each and every one of our countries** around the world.

Thank you.

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3. Dr. Hiroshi Nakajima, Director-General, World Health Organization, Geneva.

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6. Dr. Soekirman, Deputy for Social and Cultural Affairs, National Development Planning Agency, Government of Indonesia.

7. Dr. Mary Anne Anderson, Deputy Chief, Health Services, United States Agency for International Development, Washington, DC.

8. Dr. Michael C. Lathem, Director, Program on International Nutrition, Cornell Food and Nutrition Policy Program, Cornell University.

9. Dr. Vinodini Reddy, Director, National Institute of Nutrition, Indian Council of Medical Research, India.

10. Mr. J.M. Ali, Permanent Secretary, Ministry of Planning, Zanzibar, Tanzania.

11. Dr. V. Ramalingaswami, Chairman, Task Force on Health Research for Development, Geneva.

12. Mrs. Alberta T. Quartey, Chairperson, Ghana National Commission on Children, Ghana.

13. Professor Darwin Karyadi, Director, Nutrition Research and Development, Ministry of Health, Government of Indonesia.

14. Dr. Shirma K. Gyoh, Director General, Federal Ministry of Health, Government of Nigeria.

15. Dr. M.A. Rivadeneira, Director, Andean Sub-regional Programme for Control of Iodine Deficiency Disorders, Ecuador.

16. Dr. Suttilak Smitasiri, Head, Division of Communications, Institute of Nutrition, Mahidol University, Thailand.

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17. Dr. Festo Kavishe, Director, Tanzania Food and Nutrition Centre, Tanzania.

18. Professor Stan Berger, Institute of Human Nutrition, Warsaw Agricultural University, Poland.

19. Ing. Enrique Garcia Galiano, Universidad Nacional de Mexico Av., Mexico.

20. Dr. Alfred Sommer, Dean, School of Hygiene and Public Health, Johns Hopkins University, USA; Dr. Fernando E. Viteri, Professor, Department of Nutritional Science, University of California, Berkeley, USA; and Dr. Basil Hetzel, International Council for the Control of Iodine Deficiency Disorders, Adelaide Australia.

21. Mr. Alan Berg, The World Bank, Washington, DC.

22. Dr. Oscar Daza Marquez, Under-Secretary of Industry and Tourism, Ministerio de Industria, Comercio Y Turismo, Bolivia.

23. Ms. Marjory Dam, World Health Organization, Washington, DC.

24. His Excellency, Mr. M. Mokammel Haque, Secretary, Ministry of Health and Family Planning, Government of Bangladesh.

APPENDIX B-2

COORDINATED STRATEGIES FOR CONTROLLING MICRONUTRIENT MALNUTRITION: A TECHNICAL WORKSHOP

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ABSTRACT A workshop was convened in November 1991 to explore the technical feasibility of coordinated strategies for controlling malnutrition due to iodine, iron, vitamin A, and other micronutrients. While recognizing many unanswered questions, workshop participants concluded that coordinated strategies for controlling micronutrient malnutrition are technically feasible and should be given consideration in planning control efforts. Opportunities for possible coordination exist in the area of **information collection.** Coordinated surveys with simultaneous collection of samples for biochemical assessment of multiple micronutrients are feasible. Dietary assessment can also be used for vitamin A, iron, and other micronutrients as well as for additional dietary factors such as energy and protein. Furthermore, since target groups of concern (e.g., infants, children at school entry, and/or women of childbearing age) overlap, they may prove a useful focus for examination of multiple micronutrients. Multiple **fortification** is also possible using vehicles such as salt (fortified with iron and iodine), processed rice and sugar (fortified with vitamin A and iron), and citrus drinks and cookies for schoolchildren. **Supplementation** efforts can be integrated with existing delivery systems such as maternal and child health or primary health care programs. **Food-based strategies** have also proven effective. The most impressive examples have been community-based and have included a strong nutrition and health education component designed to change food consumption patterns, improve food preservation and preparation practices, and link income generating activities with food production activities. Workshop participants agreed, however, that the success of a coordinated control effort rests not only on the technical feasibility, but also on the presence of a strong political commitment and supportive infrastructure. Close and consistent communication is essential between policymakers, technical experts, industry representatives, and health workers at all levels - international, national, and local - to assure commonality of purpose throughout design and implementation phases of micronutrient malnutrition control efforts.

INDEXING KEY WORDS:

- micronutrient malnutrition

BACKGROUND

Twenty percent of the worlds' population, concentrated primarily in the developing world, are at risk of micronutrient malnutrition. Deficiencies in iodine, iron, and vitamin A comprise three nutritional problems of greatest public health importance because of their high prevalence, related morbidity and mortality, and preventability. About 16-18 percent of the world's population (or approximately 1 billion people) live in **iodine** deficient areas, placing them at risk for permanent brain damage, mental retardation, reproductive failure, decreased child survival, goiter, and socioeconomic stagnation. **Iron** deficiency is the most prevalent micronutrient deficiency of public health importance in the world, and the most common cause of nutritional anemia in children and women of reproductive age. The major

consequences of iron deficiency anemia include impaired child development and reduced work capacity and economic outputs. In developing regions, 51 percent of children 0-4 years old and 59 percent of women of child-bearing age (15-49 years) are estimated to have anemia. Worldwide, approximately 1 billion people are anemic due to iron deficiency. **Vitamin A** deficiency, the most preventable cause of irreversible blindness, affects nearly 14 million children clinically and 50 million more subclinically in developing nations. Approximately 190 million children are at risk of vitamin A deficiency. At least 350,000 new cases of irreversible blindness each year are caused by vitamin A deficiency and more than 60 percent of these children die within one year of diagnosis. In addition, children with subclinical cases of vitamin A deficiency are at increased risk of dying from infections.

Prevention and control of micronutrient malnutrition would have a substantial impact on worldwide socioeconomic development through increased survival, learning potential, productivity, self-reliance, and quality of life. Effective control strategies exist for each micronutrient deficiency but the opportunity for coordinating those strategies has not yet been fully explored. A variety of factors make such exploration imperative:

- At-risk population groups for several micronutrient deficiencies overlap frequently;
- Surveillance and assessment mechanisms used to monitor control of one micronutrient may be adaptable for others; and
- Intervention delivery systems, technical skills, education, and facilities for control of one micronutrient may be the same as or compatible with those for other micronutrients.

Taking advantage of these factors through a variety of coordinated control strategies would avoid significant duplication of effort and costs, thereby improving the effectiveness and efficiency of programs for controlling micronutrient malnutrition.

The importance of and opportunities for coordinated control have received increasing international support during the past few years. During the 1990 World Summit for Children sponsored by UNICEF, seventy Heads of State endorsed the World Declaration and Plan of Action on the Survival, Protection and Development of Children calling for elimination of vitamin A deficiency and iodine deficiency disorders and significant reduction in iron deficiency anemia. Country representatives attending this meeting committed themselves to develop national plans for coordinated control of micronutrient malnutrition. Shortly thereafter, the World Health Organization (WHO) and UNICEF cosponsored a follow-up meeting in Montreal to establish a policy framework for international action in eliminating micronutrient deficiencies. This meeting, held in October 1991, resulted in the identification of major policy assumptions that should underly national and international plans.

With these two policy conferences as a backdrop, the challenge remained to identify the specific scientific and technical opportunities for and constraints to implementing effective micronutrient malnutrition control programs. To meet this challenge, a workshop was convened in November 1991 titled, "Coordinated Strategies for Controlling Micronutrient Malnutrition: A Technical Workshop." The objectives of this workshop were:

- To identify coordinated strategies for controlling micronutrient malnutrition due to iodine, vitamin A, iron, and other micronutrients; and
- To identify priorities for applied research to strengthen the scientific and technical base for micronutrient deficiency control.

The workshop was sponsored by the International Life Sciences Institute (ILSI), a public, nonprofit foundation dedicated to research and information exchange on nutrition, food safety, environmental hazards, and other health-related topics. Collaborating agencies included the Centers for Disease Control (CDC) and the Program Against Micronutrient Malnutrition (PAMM). PAMM is designed to assist governments to achieve sustained elimination of micronutrient malnutrition through training and technical support tailored to country-specific needs and existing capacity. A Steering Committee of technical experts was formed to design the workshop agenda.

Participants at the workshop were drawn from multiple agencies and organizations from the public and private sectors (e.g., government, industry and academia) as well as a wide range of disciplines and technical expertise, including nutritionists, nutritional epidemiologists, nutritional biochemists, nutritional anthropologists, health care workers, agricultural experts, economists, and food technologists. The workshop began with brief presentations on possible coordinated strategies and on selected country experiences in controlling micronutrient malnutrition from India, Thailand, Indonesia and Philippines. These presentations were followed by three concurrent work group sessions on:

Information Collection and Dissemination
Fortification and Supplementation
Food-based Approaches.

The work groups were charged with defining the current "best" approaches in their area. This included discussing the possibilities for and constraints to coordinated approaches among the three major micronutrient deficiencies, and identifying gaps in knowledge and priorities for future research. Participants were assigned to workshops based on their areas of expertise. The workshop concluded with presentations by the work group leaders summarizing their deliberations, and a final panel discussion to further explore potential for coordinated approaches and recommendations for research.

This summary is intended to present the major approaches generally acknowledged by workshop participants to be the most successful for controlling each micronutrient, to identify options for coordinating these approaches, and to suggest priorities for further research. The first section of the summary presents the conclusions of each of the three work group sessions, followed by a section on major implementation issues.

SUMMARY OF WORK GROUP SESSIONS

Information Collection and Dissemination

Scope and Rationale

The Information Collection and Dissemination work group identified three valuable roles for information in relation to controlling micronutrient malnutrition. First, information can be used for advocacy to increase awareness of the problem at a national or international level and to garner political and financial support for control efforts from key decision-makers. Second, information can be useful in identifying high risk populations and designing effective targeted programs to control micronutrient malnutrition. And lastly, information

is essential for monitoring and evaluating program impact so that progress toward eliminating the problem can be measured and further control efforts planned.

As the name of the work group suggests, information can only be useful if it is both collected properly and communicated effectively to the appropriate individuals. The importance of dissemination cannot be underestimated, for only if decision-makers have access to useful and relevant data can they make informed decisions.

Opportunities and Constraints

Advocacy: The proper acquisition and use of information for advocacy is one of the most important aspects of information management. In designing an advocacy effort, it is critical to define what decisions the effort should influence and who will be making those decisions.

Advocacy efforts at national and international levels can be strengthened by using the following types of information:

- The prevalence and severity of the malnutrition problem. To the extent possible, this information should be compatible worldwide, using common definitions and criteria, so that comparisons can be made among populations, countries and regions. Information collection efforts should be designed to produce a compelling argument for the need to address the micronutrient problem.
- The significance or impact of the micronutrient deficiency. This would include information on the physiologic, psychosocial, and economic consequences of the deficiency on individuals, populations, and entire countries, and on the benefits of alleviating the deficiency.
- The cost-effectiveness of feasible solutions for addressing the problem. Information pertaining to these solutions were discussed by the other two work groups.

Much of this information currently exists but needs to be shared more widely with individuals who can best use it, including government officials and decision-makers, community leaders, health professionals (e.g., general practitioners), and students in training programs (e.g., in medical, nursing, nutrition, and agriculture programs). The concepts of social marketing can be applied to increasing micronutrient consumption as they have been by the Expanded Programme on Immunization (EPI) during the past decade. Industry can be useful in this area to provide technical expertise, reach at risk populations, and advocate for increased awareness of the problem by political officials.

Planning: The following table summarizes the work group's discussion of the relative merits of various clinical and biochemical indicators that can be used to define baseline prevalence and to characterize the nature and extent of the problem in a population. The second column lists the methodologies that are currently available and adaptable for field-oriented operations. The third column lists those methodologies that are either currently available but too expensive for widespread field application, or need further research and development to overcome technical and operational constraints.

TABLE 1: INDICATORS FOR SURVEILLANCE OF MICRONUTRIENTS

Micronutrients	*Indicators Currently Available*	*Indicators Available In 2-5 Years*
Vitamin A	Night blindness by history Bitot's spots Serum retinol Conjunctiva impression cytology Relative dose response Modified relative dose response	Dark adaptation measurement Blood spot retinol Retinol by fluorescence (capillary tube sample)
Iodine	Goiter by palpation Blood spot thyroid stimulating hormone (TSH) Urinary iodine Thyroid size by ultrasound	
Iron	Hematocrit Hemoglobin Erythrocyte protoporphryn Serum ferritin	Blood spot ferritin Blood spot transferrin receptor

Several opportunities exist for coordinated assessment of micronutrients and should be pursued. Coordinated surveys can be conducted with simultaneous collection of blood samples for biochemical assessment of iron, vitamin A and iodine deficiencies. Dietary assessment can also be used for vitamin A, iron, and other micronutrients as well as for additional dietary factors such as energy and protein. It should be recognized, however, that dietary assessment may not always produce a precise definition of the problem.

Concentration of effort on one targeted age group (e.g., infants or children at school entry) may also be practical. Care must be taken to assure that assessment efforts focused on one group do not ignore the importance of intervention strategies focused on different populations (e.g., women, particularly those of childbearing age).

Use of any of these coordinated strategies should be planned in collaboration with local community health workers to assure that the strategies meet local needs and constraints. As much as possible, these health workers or other volunteers from the local area should be trained to conduct or assist in the assessments, rather than relying solely on outside expertise. This training would not only provide willing and able hands for baseline surveys but would also build the area's capacity to conduct routine monitoring and intervention programs independently in the future.

A major constraint on implementing coordinated assessment is the lack of uniform guidelines to assure standardized application of appropriate methodologies. Such guidelines could address common methods for establishing baseline prevalence, conducting surveys, analyzing data, and using those analyses to plan effective programs. They would not only reduce the need for direct technical assistance to countries wishing to embark on large-scale

assessment efforts but would also facilitate communication among countries, donor agencies, industry, and academia.

A coordinated data base, such as the one being initiated by WHO, would also be useful. WHO's effort should be supported and strengthened to include baseline data on the prevalence of micronutrient deficiencies in all countries and to provide information on appropriate indicators to measure success of control efforts. Use of these indicators worldwide would allow consistent and valid monitoring of international progress toward the Year 2000 goal of eliminating micronutrient malnutrition. The data base could be retained in a "clearinghouse" for use by all interested individuals.

Evaluation: Many of the indicators listed in the section on Planning are also relevant for program evaluation. In addition, process measures are needed to determine progress toward achieving program goals. Such measures should include:

- Knowledge, attitudes and beliefs of health workers about micronutrient deficiency and ways to control it. Such information could be used to determine the association between knowledge of the deficiency and its prevalence.
- Individual/consumer behaviors associated with micronutrient intake.

National surveillance systems should be strengthened to enable continuous program monitoring and evaluation. However, design of control programs need not wait until these systems are fully operational.

Country Experiences

India: A nationwide survey in 1974 showed that, of the 9 million blind individuals in the country, 2 percent were due to vitamin A deficiency. In a more recent survey, vitamin A deficiency contributed to only .04 percent of the total blindness. This may not reveal the true picture as only survivors are examined in such surveys, but the decreasing trend was reinforced by a nutrition survey among pre-school children. However, there is still considerable regional variation.

Iron deficiency anemia is most prevalent among women of childbearing age. A recent survey revealed that more than 60 percent of pregnant women and pre-school children are anemic. Cereals form the major sources of iron in the Indian diet; isotopic studies have shown that iron absorption from this native diet is only 3-5 percent. Other factors that aggravate anemia in some areas include hookworm disease and malaria.

A recent survey in 14 districts of different states showed that the prevalence of goiter ranged from 6-65 percent. More alarming is the prevalence of endemic cretinism in all the states. Other forms of iodine deficiency disorders like dwarfism, mental retardation and deaf-mutism are also common in endemic areas.

Thailand: In 1973, 37 percent of adult males and females suffered from iron deficiency anemia. In 1974-75, the prevalence among pregnant women was about 30-31 percent in Bangkok and 41-70 percent in the provincial rural area. Recent data available from antenatal clinics at the provincial hospital indicate that the prevalence has declined among this population to about 20 percent.

A 1988 survey showed a 43 percent prevalence rate of iodine deficiency in 65 districts. Within one year of implementing a control program, the goiter rate in one high prevalence province dropped to 17 percent.

Indonesia: A National Xerophthalmia Survey conducted in 23 of Indonesia's 27 provinces in 1978 identified 15 provinces having prevalence higher than the criteria classification for public health significance. Limited repeat surveys in 5 provinces in 1983-1986 showed that the prevalence of xerophthalmia declined significantly from 1.16 to 0.67 percent. A recent 1991 survey in 4 provinces of eastern Indonesia showed that xerophthalmia is a public health problem in one of the four provinces and vitamin A deficiency (as measured by blood serum vitamin levels) is a problem in all four provinces.

In the past, goiter was found in almost every major island of Indonesia, with a prevalence as high as 92.5 percent in certain endemic areas. After implementing a goiter prevention program in 1976, a national goiter survey of primary school children in 26 provinces in 1980-82 revealed that 68.3 percent of 666 subdistricts were severely endemic and 10 percent of the villages had endemic cretinism. Parallel surveys conducted in 1987 and 1990 showed significant decreases in the prevalence of both total goiter rate and visible goiter rate (from 37.2 to 27.7 percent and from 9.2 to 6.8 percent, respectively).

Current prevalence of anemia among pregnant women ranges from 39.7 to 63.4 percent, with the national average about 52 percent. The prevalence of anemia among low income female workers is 30-40 percent; among adult non-lactating and non-pregnant women who usually visit community health centers and Posyandu (integrated health service posts) the prevalence is 20-45 percent. The 1991 eastern islands prevalence study in 4 provinces in 1991 revealed that the average prevalence rates among pregnant women and pre-school children are 50.1 percent and 49.2 percent, respectively.

Philippines: National Nutrition Surveys and smaller studies since 1978 have shown that the proportion of specific population groups with deficient plasma vitamin A levels ranges from 2.3 to 3.4 percent. Children 7-12 years old and pregnant women have the highest rates of vitamin A deficiency. Prevalence of anemia is as high as 70 percent among infants and is also quite high among school children 7-12, pre-school children, pregnant and nursing women, and the elderly. Overall, the prevalence of anemia in the country is about 37 percent. In 1982, prevalence of xerophthalmia and Bitot's spots was 3.6 percent; by 1987 this rate was reduced greatly but in certain areas still hovers around 4 percent.

For goiter, the same pattern holds true; for the country as a whole, the prevalence is only about 3.5 percent but in selected areas, especially mountainous regions, prevalence rates as high as 66 percent can be found among children 7 years of age and older. A rapid assessment procedure has been developed for goiter prevalence, involving interviews with school children to determine if they have any family members with enlarged goiters. This procedure provides a rough picture of endemic areas that can be used for program planning.

Knowledge Gaps

Several gaps in knowledge were identified by the work group that can serve as the basis for future research priorities. These knowledge gaps include the following:

- The physiological interactions of micronutrients (e.g., how they support and interfere with each other). Where iron deficiency is common, vitamin A also seems to be more prevalent. Conversely, treatment of vitamin A deficiency often results in changes or improvement in biochemical indicators for iron. The implications of this relationship need to be defined precisely for monitoring and assessment purposes. Although similar

relationships between iodine deficiency and vitamin A have already been explored (e.g., in Senegal), further studies are needed.
- More sensitive assessment tests that are feasible for use in the field.
- The performance (e.g., sensitivity) of the indicators listed above for different target groups.
- The age or gender groups which would most feasibly serve as optimal target groups for multiple micronutrient assessment and surveillance.
- Guidelines for conducting coordinated assessments of multiple micronutrients (e.g., sample size, data management and analysis).
- Effects of various dietary interventions on dietary intake.

Fortification and Supplementation

Scope and Rationale

Fortification and supplementation have proven to be effective means of increasing micronutrient intake to acceptable levels. Fortification involves adding micronutrients to selected foods during the production process, while supplementation requires injection or oral intake of micronutrients in medicinal form (e.g., tablets or capsules). The Fortification and Supplementation work group discussed issues related to iodine, iron, and vitamin A specifically as well as issues related to multiple micronutrients.

Opportunities and Constraints

Iodine: Iodine fortification with salt has met with great success and was felt by the work group to warrant expansion to all developing countries. Dosages depend on per capita consumption of salt and anticipated storage losses, but range from 20 to 100 ppm (parts per million). Toxicity issues are negligible and cost considerations are fairly small. Potassium iodate is the preferred additive because it is more stable. Storage and handling issues should be addressed carefully since the volatile iodine is easily lost. Concerns about safety are addressed in the recent report of the Joint FAO/WHO Expert Committee on Food Additives.

Supplementation was seen as an important short-term adjunct to an effective salt fortification program, with the latter clearly addressing the long-term problem more effectively in most countries. The major forms of iodine supplementation are iodized oil, either injectable or oral, and iodized water. The effectiveness and safety of iodized oil programs has been amply demonstrated; from preliminary data it appears that an oral oil capsule with a dose of 480 milligrams of iodine will give protection for about one year. Injection can increase this protection to about three years. The major problems with injection and oral supplementation programs have been with logistics and difficulties in initiating an injection program. There are theoretical toxicity concerns for iodine supplementation of pregnant women but these are minor in areas with high incidence of iodine deficiency.

Iron: Fortification of foods with iron is much more complex than fortification of salt with iodine because of variations in dietary absorption and dietary patterns in different countries

which influence iron availability and absorption. In addition, the required dosage of iron is at least 50 times more than the required dosage of iodine, causing taste and color changes in fortified foods. Iron fortification of salt has proven to be feasible, effective, and free of adverse effects.

The vehicles used for iron include wheat flour, cereals, sugar, and salt. Some specialty foods such as citrus drinks for children have been tested with good results. However, these may not be appropriate for distribution on the open market because of the variability in the amount consumed. In controlled situations such as schools, these products can be helpful.

Iron EDTA is a promising fortificant because it is absorbed much better, smaller amounts can be used, and color and taste are more acceptable. Generally, use of iron EDTA in countries with high prevalence of nutritional anemia seems desirable. Although EDTA is an approved food additive, iron EDTA is more expensive than other iron fortificants and has not yet been approved by any nation or by the Joint Expert Committee on Food Additives (JECFA). A positive recommendation on iron EDTA from a single country would encourage other countries to use it also.

Supplementation with iron is again more complex because of the amount and dosage schedule, and because of absorption and toxicity issues. Supplementation is usually by tablet and there are a number of types. Absorption depends on the presence of food in the stomach and on the type of food present; thus, there is great variability on individual and population levels. Iron excess is a possibility but is felt to be a limited problem in countries with high anemia rates. Side effects are significant, are increased with increasing doses, and are common in the major target group - pregnant women. Side effects are minimized if the dose is taken on a full stomach, but this limits absorption as well. Taking the dose at night on an empty stomach may alleviate this problem. A new product, the gastric delivery system (GDS), may help with both the side effect and absorption problems. Further testing on this product should be pursued.

Vitamin A: Vehicles used for vitamin A fortification include sugar, rice, and some specialty foods. Vitamin A is lost with exposure to heat when air (oxygen) is also present but is relatively stable when heat and air are not both present. In addition, vitamin A fortification is complicated by the color and slight taste imparted to the food. There is some concern about toxicity, particularly in pregnant women.

The work group was intrigued with the concept of rice fortification using broken grains and reprocessing techniques. This process may enable pre-mix development where issues of color and stability are controlled and allow double fortification with iron. The rice is being produced but needs extensive testing to assure quality control, consumer acceptability, and stability of fortificant.

Supplementation with oral dispensing vitamin A capsules has been tested in research projects in many countries. Doses have been reviewed at length and standard guidelines have been published by WHO and IVACG. Cost is minimal but some issues of toxicity remain, particularly for pregnant women.

Coordinated Strategies: Examples of multiple fortification opportunities identified by the work group are depicted in Table 2:

TABLE 2: POSSIBLE COORDINATED FORTIFICATION STRATEGIES

Food	*Iodine*	*Iron*	*Vitamin A*	*Comments*
Salt	X	X		Technology is now available to produce a stable formulation, but it still needs considerable testing in terms of stability and absorption using different dietary patterns. Double fortification may require a change from iodate to iodide, posing logistical difficulties in countries with salt iodation programs underway.
Rice		X	X	
Sugar		X	X	
Specialty foods, citrus drinks for schoolchildren, soft drinks, cookies, nutricubes	X	X	X	Fortification is risky because these food items are consumed in irregular patterns, making intake monitoring difficult. The work group cautioned against fortifying a wide range of products for similar reasons.

Supplementation efforts should be integrated with existing delivery systems wherever possible. Systems such as maternal and child health (MCH) programs, primary health care programs, and EPI should be explored. For example, if supplementation were linked with EPI, pregnant women could receive an iron tablet and iodized oil with their tetanus toxoid immunization and children could receive vitamin A supplementation during their routine vaccination regimen.

When planning coordinated strategies, care should be taken to address a variety of potential obstacles and constraints by:

- Involving all relevant sectors (government, industry and consumer groups) in planning and implementation. Industry is a key player in the control of micronutrient malnutrition and should be supported in its free market, profit-oriented approach. Industry's expertise in market development and price determination should be tapped.
- Communicating with consumers to determine perceived needs and educating them to demand a better product and accept a slightly higher price for that product.
- Building an adequate infrastructure, including trained manpower, market penetration, and program management. Countries should identify administrative structures to suit their needs and unique situations.
- Recognizing and taking advantage of opportunities for fortifying new products to increase market penetration.
- Identifying mechanisms (e.g., subsidies) to reduce costs to consumers. Such costs are marginal for iodine fortification, for example, but become more significant for iron.
- Monitoring and evaluating process and outcome variables. A poorly monitored program is liable to fail or be ineffective. Programs should be envisioned as long-lasting, with

evaluation as an essential component to identify progress, problems, and needs. Monitoring should encompass process measures such as the quality of the food and the level of the fortificants at different stages in the production and delivery system, and status indicators as discussed by the Information work group.

Country Experiences

India: The Indian Government has recently liberalized the production of iodized salt to include the private sector as well as the public sector. It has also committed itself to iodize all edible salt in the country by 1992. A subsidy is provided to manufacturers to offset the cost of potassium iodate. Goiter control cells have been established in all states to monitor progress, using a simple kit to detect the iodine in the fortified salt.

The National Anemia Control Programme has been in operation for the last two decades. Under this program pregnant and lactating women are given tablets containing 60 milligrams of iron and 500 micrograms of folic acid, while children receive one-half that dose. Each beneficiary is given one tablet per day for 100 days each year through primary health centers and MCH clinics. Implementation has been very slow, with a recent evaluation showing poor coverage in all states due to inadequate supplies, lack of awareness among health staff, and poor compliance of women. Steps are being taken to address these problems. Initially all nutrition interventions were started as isolated, vertical programs, but subsequently they were integrated into other health care services including food and nutrient supplementation, immunization, treatment of minor illness, nutrition and health education.

Fortified salt, developed by the National Institute of Nutrition (NIN) in Hyderabad, can provide 10-15 milligrams of iron per day, with an absorption rate of about 3-6 percent. In Calcutta, where hookworm infestation is common, the prevalence of anemia was reduced from 90 to 50-60 percent after introducing iron-fortified salt. Attempts to produce iron-fortified salt on commercial scale have been quite successful.

The technology for double fortification of salt with iodine and iron has been successfully developed by NIN. Laboratory studies have shown satisfactory results with respect to stability and bioavailability of both micronutrients and large-scale community trials are now underway.

A vitamin A distribution program is now in operation in all states in the country, covering about 30 million children between 1 and 5 years of age. Each child is given one spoonful of vitamin A syrup containing 200,000 units every six months. The program is implemented by auxiliary nurse midwives through primary health centers. In areas where the program has been implemented well, the prevalence of xerophthalmia has decreased significantly. Reasons for poor coverage in other areas included inadequate supplies of vitamin A, lack of coordination between various health professionals, adoption of a clinic approach instead of house-to-house visits, and lack of community awareness. Efforts are now being made to improve logistics of distribution and to strengthen training and education components.

Thailand: Supplementation of iodine is accomplished through 50 ppm (parts per million) of iodine in salt and 2 drops of iodine solution to 10 liters of water in school and household reservoirs. Kits are used to test iodine levels in water. Six drops of iodine solution can also be added to 750 milliliters of fish sauce in a bottle the size of a whisky bottle. This allows preparation to be done at the village level in a highly available and accessible container. In

addition, iodine capsules of 200 milligrams are distributed every 6-12 months in endemic areas.

Thailand advocates the provision of one ferrous sulfate tablet and one multivitamin tablet daily to all pregnant women. Health officials feel that compliance is improved if only one tablet is required.

In 1976, Thailand established guidelines for vitamin A fortification of sweetened condensed milk after observing that blindness due to vitamin A deficiency occurred primarily among pre-school children. Guidelines also exist for the promotion of vitamin A rich foods at different ages, e.g., breast feeding for infants, supplementary feeding with rice and bananas after four months of age, chicken liver and egg yolks at five months, ivy gourd at six months, etc.

Indonesia: To attain the goal that no cretin will be born in the Year 2000, a national Iodine Deficiency Disorders committee was established by Ministry of Health Decree in 1990. The national program consists of three strategies: iodinated oil injection as a short term measure in severely endemic areas; iodized salt for human consumption, as a permanent long-term strategy; and iodinated water as an appropriate technology alternative to be integrated with safe drinking water in high risk areas.

Indonesia's intervention program for controlling nutritional anemia includes iron supplementation to pregnant women through Posyandu and community health centers; nutrition education to increase the consumption of iron-rich foods; and fortification of foods with iron. The fifth in a series of five-year development plans states that by the end of 1994, the prevalence of nutritional anemia among pregnant women should be reduced from 50 to 40 percent. About 80 percent of all pregnant women are to be covered by iron tablet distribution. For the last three years, approximately 50 percent of all pregnant women have been given 90-100 tablets during their pregnancy, but the real number of pregnant women taking iron tablets every day remains unknown. The government plans to conduct a pilot project to establish an anemia information system and to promote iron sources for pregnant women.

Xerophthalmia has been reduced significantly in some areas due to a combination of several strategies: distribution of vitamin A capsules; nutrition education through Posyandu; fortification with vitamin A of MSG; and public health intervention through high immunization coverage, better sanitation and improved health services. It is difficult to prove which strategy has had the highest impact.

Philippines: The Philippine micronutrient strategy is part of an overall food and nutrition program. In areas endemic for goiter, iodized oil capsules are mandated for children up to 14 years of age and women of childbearing age. In non-endemic areas, capsules are given only to persons with goiter. Pregnant women receive 120 milligrams of iron or two tablets of ferrous sulfate daily, starting from the twentieth week of pregnancy. Coverage is about 20-40 percent of all pregnant women because the program reaches only those who attend prenatal clinics at health centers. Two tablets of iron are also given to nursing women and anemic infants and children. High-dose capsules of vitamin A are provided, targeted to children with severe diarrhea, those suffering from moderate or severe protein energy malnutrition, and those with lower respiratory infections.

Knowledge Gaps

The work group felt that research was needed in the following areas:

- To determine the effects of iron fortification, use of new products, color and stability of fortified foods, and absorption with different vehicles under different dietary conditions. Current products have been subject to some of this analysis but new products will need full review to assure safety.
- To determine the optimal number of iron tablets for pregnant women and the length of time that dosage is needed.
- To develop a supplement containing both iodine and vitamin A.
- To fortify sugar with sodium EDTA and iron EDTA. EDTA is not absorbed well on its own, but enhances the absorption of dietary iron. In countries where EDTA consumption levels are fairly low, iron EDTA could well be approved.
- To determine the recommended dosage regimen for vitamin A supplements, i.e., whether smaller, more frequent doses have a greater impact than larger, longer lasting doses.
- To clarify the appropriate number of doses of vitamin A for children with measles. Current recommendations by WHO and UNICEF promote one dose, while recent trials are being conducted on two doses.
- To show the measurable benefits of fortification by conducting small pilot projects in one or two countries, funded by several agencies and industry.
- To measure the synergistic effects of integrating micronutrient programs with other public health programs in terms of disease trends and costs, and/or the impact of water and other development programs on the problem of micronutrient malnutrition.

The group also felt that efforts by WHO and others to establish an information bank should be supported and expanded. Information on country experience, particularly as they relate to fortification and supplementation, should be collected and disseminated.

Food-based Approaches

Scope and Rationale

Food-based approaches for the control of micronutrient malnutrition are designed to increase local production and consumption of foods that are high in specific micronutrients. The three micronutrients discussed during the workshop vary in their suitability for food-based approaches, with vitamin A (found in yellow/orange fruits and vegetables, dark green leafy vegetables, liver of poultry, fish liver oils, whole milk and butter, and eggs) being the most suitable and iodine (found in fish, seaweed, and plants from soil rich in iodine) being the least suitable. The suitability of iron for food-based strategies is moderate, since foods with the best bioavailability of iron are generally expensive (e.g., meat products) while more commonly available foods tend to have lower bioavailability (e.g., legumes).

Several key advantages of food-based strategies, as identified by the work group, are presented in Table 3:

TABLE 3: ADVANTAGES OF FOOD-BASED APPROACHES

Food-based Approaches Are Valuable Because They:
• Can address multiple micronutrient problems; • Have a community-based emphasis, promoting general community and human development; • Are feasible in most countries but, under some circumstances, may need augmentation with other interventions such as fortification or supplementation to meet all micronutrient needs; • Result in potentially sustainable long-term changes by promoting positive behavioral changes in consumption patterns, infant and child feeding practices, and preparation practices; and • Are cost-effective over time with benefits to individuals, communities and countries in nutrition, health and economic status.

Food-based strategies are usually implemented when food sources are available but are underutilized, or when food sources are unavailable or limited but can be augmented through horticultural approaches or transported from other areas. They may need to be implemented in concert with supplementation or fortification programs, particularly for iron and iodine.

Opportunities and Constraints

Food Sources Available But Underutilized: The major strategy for food-based approaches when food sources are available but underutilized is nutrition and health education. Such education is most effective if it is based on strategies designed to:

- Change food consumption patterns. Education strategies should promote increased consumption of locally available foods rich in micronutrients, such as eggs, papayas or other yellow fruits and vegetables, dark green leaves, as well as animal products when these are affordable.
- Change food preservation and preparation practices. Food preservation can help to bridge seasonality factors and guarantee a supply of micronutrient rich foods all year long. Proper preparation would increase the bioavailability and dietary appeal of some foods, particularly to those in greatest need (i.e., women and young children). For example, mothers can be educated to dry certain fruits and vegetables (e.g., mangoes or green leaves), preserve, and grind them into a powder, then add the powder to gruel for infants and toddlers.
- Link income generating activities with food production activities (which often also generate income) where economics is a primary constraint to food availability. Care should be taken not to promote the production of income at the expense of proper nutrition.

Food-based approaches should be community-based to identify local micronutrient problems and potential local food sources. Lists of locally available, micronutrient-rich foods that can either be grown or bought would be extremely valuable. It was suggested by the work group that such lists be prepared by each country as part of intervention planning, with assistance from FAO. The lists should recognize the fact that some foods must be processed before their micronutrients become available (e.g., sorghum and maize).

The work group felt that nutrition and health education to increase proper food intake would be most effective if it were:

- Effectively communicated as a feasible approach to all levels (national, regional and local) in order to develop supportive constituencies;
- Appropriately developed and tailored to bring about behavioral changes among consumers in how to select, process, prepare and preserve foods so that micronutrient consumption is optimal;
- Directed at policy officials to advocate for improved conceptual structures and better technical and logistic support; and
- Integrated with other ongoing programs. Nutrition education messages should not be disseminated separately but should be coordinated with other health education, agricultural, and social messages being conveyed at the community level. This may increase the effectiveness of the nutrition messages and enhance their sustainability.

Based on formative research, a methodology exists for tailoring education messages appropriately to meet the needs of unique target audiences. A range of rapid assessment procedures should be utilized, including both qualitative and quantitative techniques. Active community participation should be solicited for these assessments, preferably through selection of small purposive time-limited samples rather than large groups over lengthy time periods. A variety of media should be used (e.g., press, radio, billboards) to deliver the messages, and the public sector should mobilize the community by educating local community health workers to develop and disseminate compatible messages.

Food Sources Unavailable: Food-based strategies when food sources are unavailable or limited must address the issues of food production and transportation. These issues need to be considered both for urban and rural settings. For example, use of food coupons and promotion of kitchen gardens are two possible strategies for urban settings. Small backyard gardens might be a more appropriate strategy for rural settings. Long-term sustainability of home gardens should take into account water availability, land availability and cost.

All too often, micronutrient-rich foods are produced in a location distant from the site in need of those micronutrients; hence, these foods must be transported to and marketed in the area of need. Analysis of existing infrastructures is critical to assure distribution of these foods to the local level without incurring additional unnecessary expense. Transported foods should be packaged hygienically to preserve their quality and appeal. Strategies for improving food availability must be coupled with nutrition education as described above to assure consumption of the new foods.

The work group recognized the importance of agricultural policies that are supportive of dietary diversification to meet micronutrient needs, but felt that immediate impacts are more likely to occur through horticultural and animal production at the community and household levels. In most instances, such efforts will likely involve women or women's groups; hence,

consideration must be given to the multiple demands on women's time and energy. Strategies to overcome constraints to food availability may involve not only provision of resources such as seeds and fertilizer, but also relief from time and energy consuming burdens such as hauling water. Community development programs that reduce these burdens on women should be emphasized. In implementing interventions to increased food production, support is also needed from agricultural extension workers who may need training in how best to work with women since they are more accustomed to dealing with male farmers.

The work group the following factors that should be considered when designing food-based approaches. The extent to which they represent opportunities or constraints will vary considerably from country to country, depending on the sociopolitical environment in each country and the effectiveness of program planning efforts.

- The time required to change behavior may not be prolonged if strategies are appropriately tailored to local needs and cultures.
- Distribution channels may need to be improved to assure that the food reaches the vulnerable populations (e.g., young children).
- Communication needs to take into account educational status, particularly literacy, among women and to encourage their active participation in interventions to improve diet diversification.
- The status and role of women as decision-makers in attaining and utilizing food may need to be strengthened.
- Because food-based approaches involve greatly diverse situations, close cooperation is needed between local and national levels during program development to adapt approaches to suit circumstances at the community level.
- Agricultural policies may need to be focused to support dietary diversification.
- Political commitment may need to be developed at all geographic and political levels.
- Technology that is appropriate for the community level may need to be designed.

<u>Country Experiences</u>

India: A pilot project was undertaken recently to evaluate the feasibility of food-based strategies. Thirty villages in three different agroclimactic regions were selected. A baseline survey was conducted to obtain information on horticultural practices, food habits, and available vegetables and fruits with high bioavailability of micronutrients. Based on this survey, an intervention program was launched combining nutrition education and horticultural interventions. Home gardening was promoted to increase the availability of vegetables and fruits at the household level, emphasizing perennial varieties of green, leafy vegetables (e.g., Indian spinach - a creeper that requires little water, grows all year, and is rich in beta-carotene, iron and calcium).

Thailand: After a concentrated effort to reduce chicken mortality through promotion of increased hygiene, vaccination, and improved minimal care, the death rate of chickens dropped from 60-70 percent to 10-15 percent. This decrease resulted in an increased supply of poultry, chicken livers, and eggs. Thailand also emphasizes production of nutritious foods (primarily rice, legumes, and sesame), gardening, use of organic fertilizer, correction of sterilized soils, and village fisheries.

Philippines: Nutrition education concentrates on breastfeeding promotion and increased intake of green leafy and yellow vegetables, increasing intake of foods rich in vitamin C, using iodized salt in food preparation, and increasing intake of foods containing goitrogens (e.g., cassava). A bio-intensive gardening program was initiated as a pilot but has been adopted nationwide. It is carried out by the Ministry of Agriculture with oversight by the Ministry of Public Health, and involves significant training and education of local villagers to produce their own micronutrient foods.

Knowledge Gaps

The work group felt that research priorities should be linked to implementation issues, focusing on applied and operational questions such as:

- What is the basic infrastructure required to implement food-based strategies?
- Where such a basic infrastructure does not now exist to implement food-based strategies, how can it best be developed?
- How can existing infrastructures be better utilized to focus on alleviation of micronutrient deficiencies through food-based strategies?
- Where appropriate, how can existing infrastructures for food-based strategies be augmented by supplementation and fortification strategies?

Other areas for further research and development include the following:

- Development of conceptual models, particularly for maternal and infant feeding practices, to define micronutrient requirements for vulnerable groups and ways to meet those requirements under different scenarios. These models can be accompanied by guidelines for use by national level planners in adapting the models to meet local situations. Models should include information on locally available foods rich in micronutrients, their bioavailability, and ways to preserve those nutrients.
- Design of methodologies to increase policymakers' understanding of the cost-effectiveness of a food-based strategy, particularly in regards to the small quantities of micronutrient-rich foods that are actually needed and the small plots of land needed to grow adequate supplies of vegetables rich in micronutrients (e.g., for vitamin A household-level production).
- Simplification of educational messages and approaches into a limited number of easy steps with specific actions, so that they can be tailored easily to a variety of populations.
- Specification of technical and logistic support required for agricultural programs designed to increase micronutrient content of foods.
- Documentation of food-based strategies (e.g., horticultural production in backyard gardens) that have increased micronutrient intake.
- Analysis of experiences in which successful food-based strategies (including those initiated by nongovernmental organizations) spread horizontally from village to village or vertically from small projects to national programs. Understanding of this unique process would have significant implications for the control of many malnutrition problems. Micronutrient deficiencies that most frequently occur in pockets may be best addressed by local programs that are allowed spread.

IMPLEMENTATION OF COORDINATED STRATEGIES

As the above summary indicates, coordinated strategies for controlling micronutrient malnutrition are technically feasible. In the area of **information collection**, coordinated surveys with simultaneous collection of samples for biochemical assessment of multiple micronutrients are feasible. Dietary assessment can also be used for vitamin A, iron, and other micronutrients as well as for additional dietary factors such as energy and protein. Furthermore, since target groups of concern (e.g., infants, children at school entry, and/or women of childbearing age) overlap, they may prove a useful focus for examination of multiple micronutrients.

Multiple **fortification** is also possible using vehicles such as salt (fortified with iron and iodine), processed rice and sugar (fortified with vitamin A and iron), and citrus drinks and cookies for schoolchildren. **Supplementation** efforts can be integrated with existing delivery systems such as maternal and child health programs, primary health care programs, and EPI.

Food-based strategies have also proven effective. The most impressive examples have been community-based, recognizing local micronutrient problems and potential local food sources. They have also included a strong nutrition and health education component designed to change food consumption patterns, to improve food preservation and preparation practices, and to link income generating activities with food production activities.

After much discussion of the strengths and weaknesses of coordinated control strategies, workshop participants concluded that pilot projects are needed to test some of these strategies. While recognizing the many unanswered questions, the group felt that sufficient technical knowledge exists to support a major concerted international control effort. It was generally agreed, however, that technical expertise alone is not sufficient to assure success. A well-designed, coordinated control strategy cannot be effectively implemented without a comparably strong political commitment and a supportive infrastructure. It was acknowledged that independent expert groups and industries advocating for increased effort to address a particular micronutrient (be it iron, iodine, vitamin A, etc.) must work together closely to implement coordinated strategies. Likewise, policymakers and health workers at all levels - international, national, and local - must communicate clearly and frequently to assure commonality of purpose. Only through concerted effort in both the technical and the political arenas can coordinated control of micronutrient malnutrition be achieved. Three specific suggestions for improving coordination among policymakers, technical experts, and health professionals are summarized below.

National Coordinating Bodies: In light of the wide range of strategies suggested and discussed during the workshop, the participants felt that strong political support is needed at the national level in order to facilitate technical progress in micronutrient control. Establishment of some type of national coordinating body was suggested for each country interested in initiating or strengthening its program against micronutrient malnutrition. This body should involve relevant parties in all stages of program planning, from inception through evaluation, so that they are able to advocate for improved micronutrient status. The group should include representation from technical experts in public health, social science, food technology, and communications and should represent all interested sectors (e.g., industry, ministries, and consumers). It might even include a representative from the

national planning commission. It is important that such committees not be viewed as vertical structures, separate and apart from other related health, social, and economic efforts. To the extent possible, ministries of interior, agriculture, education, and public health should be involved, along with universities and the private sector.

The committee's efforts would be most successful if they were an integral part of general public health and socioeconomic development and were linked to a well-developed national plan of implementation. The organizational structure of this policy-setting body would vary from country to country, depending on existing structures and philosophies.

One possible structure would consist of a national nutrition committee with subcommittees for special micronutrients. The Philippines has such a structure organized by the Ministry of Public Health. Individuals at other levels are involved, including the governor and officials at the provincial, district and community levels. Indonesia has a similar structure with a committee on nutrition improvement under the coordination of the Ministry of Social Welfare and subcommittees on iodine deficiency disorders and family nutrition. Micronutrient intervention is part of an overall Family Nutrition Improvement Program, involving several government sectors with complementary roles and active community participation. Plans have been developed in the context of the country's overall national development strategy which emphasizes three goals: equitable distribution of development and its benefits, accelerated economic growth, and political stability. Thailand also plans its food and nutrition programs in the context of broader national economic and social development plans. Preparation and implementation of these plans has increased awareness at all levels of the important link between nutrition and socioeconomic status.

Communication: The workshop participants also recognized the need for close communication between all relevant and interested parties involved in micronutrient deficiency control. This includes policymakers, government officials and staff, the medical profession, industry, and consumers. Effective networks are needed to assure that consistent messages are being conveyed at all levels.

Communication strategies should be consistent with national communications plans, clearly identify target audiences, be tailored to those audiences, and be based on communication methodologies and tactics that will reach those target audiences effectively. National and local journalists should be utilized fully since they have expertise in this field and have access to a significant proportion of the target audience. Face-to-face interactions between health workers and consumers should reinforce more broad-based media approaches.

Future Technical Exchange: The participants also felt a strong need for continued interchange on new technical developments toward the elimination of micronutrient deficiencies. They suggested that a mechanism be established to assure continuous sharing of new technical knowledge (e.g., through a task force, additional international conferences, newsletters, manuals). Future conferences to address specific technical issues or areas could be sponsored by interested agencies or organizations. For example, FAO is exploring the possibility of coordinating a technical conference on food-based approaches. The participants also supported the need for additional training and technical assistance of health professionals, to enable them to analyze problems in their own countries and develop appropriate control strategies.

APPENDIX B-3

EVALUATION OF A GASTRIC DELIVERY SYSTEM FOR IRON SUPPLEMENTATION IN PREGNANCY

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Running head: Iron Supplementation in Pregnancy

ABSTRACT

The present investigation was undertaken to assess the efficacy of oral iron supplementation during pregnancy using a gastric delivery system (GDS). Three hundred and seventy-six pregnant women between 16-35 years of age and 14-22 weeks gestation were selected if mild anemia was present (hemoglobin concentration 80-110 g/L), The participants were randomized to one of three study groups given either no iron, two $FeSO_4$ tablets (100 mg iron) daily, or one GDS capsule (50 mg iron) daily. Blood was obtained initially and after 6 and 12 weeks for measurement of red cell and iron parameters including serum transferrin receptor. There was a significant and comparable improvement in hematologic and iron status measurements in the two groups of women given iron where as iron deficiency evolved in women given no iron supplement. We conclude that by eliminating gastrointestinal side effects and reducing the administration frequency of an iron supplement to once daily, GDS offers significant advantages for iron supplementation of pregnant women.

Key Words: Iron deficiency; Pregnancy; Iron supplementation

INTRODUCTION

It is presently estimated that over a billion people in the world are anemic (1) and that the major cause of this anemia is a deficiency in the supply of dietary iron relative to the iron needs of a population. The population segment most vulnerable to this nutritional iron deficiency is pregnant women (2). The global prevalence of nutritional anemia during pregnancy exceeds 50% and although this figure is much higher in Third World countries, no region or society is exempt. Recent studies have highlighted the significant consequences of iron deficiency anemia during pregnancy. Mild anemia is associated with an increase in the rate of premature delivery and low birth weight (3-5) whereas more severe anemia is estimated to account for up to 20% of maternal deaths during parturition. In light of the dismal success in reducing the global prevalence of anemia in this highly vulnerable segment of the population, there is a need to develop novel methods to improve the iron status of pregnant women.

For the past several decades, the provision of oral iron supplements to pregnant women has been the primary method to decrease the prevalence of anemia. A commonly cited reason for the ineffectiveness of iron supplementation in developing countries is the reluctance of women to adhere to a regimen that is often associated with gastrointestinal side effects. One way of circumventing this problem is to supply iron in a slow release form while retaining it in the stomach proximal to the iron absorbing region of the small intestine, a formulation referred to as a gastric delivery system (GDS). Recent studies indicate that this preparation not only eliminates the

side effects of oral iron but also results in a more efficient assimilation of the nutrient (6). The present investigation was undertaken to evaluate the hematological efficacy of GDS in a field setting.

MATERIALS AND METHODS

The primary objective of this trial was to compare the efficacy of the officially recommended program for iron supplementation of pregnant women in Jamaica with a regimen employing GDS. Women were recruited from eight maternal and child-health (MCH) centers in Kingston, St. Andrews, and Spanish Town, Jamaica, West Indies. All pregnant women who agreed to participate in the investigation were assigned randomly to one of three study groups, a no iron group given one placebo tablet daily, an $FeSO_4$ group given the recommended program for iron supplementation in Jamaica consisting of two $FeSO_4$ tablets or 100 mg elemental iron daily, and one GDS capsule or 50 mg elemental iron daily. All participants were given 400 mg folic acid daily. This study was approved by the Ethics Committee at the University of the West Indies and conducted by personnel of the Caribbean Food and Nutrition Institute.

The selection criteria for participation in the investigation were a maternal age 16-35 years, gestational age 14-22 weeks, and hemoglobin concentration between 80-110 g/L as determined by the Hemocue system (DeCentech Inc., St. Paul, MN). The randomization procedure was as follows. Using a random number table, cards

designating the assignment to one of the three study groups were placed in sealed envelopes and distributed to one of the eight maternal and child-health (MCH) centers. In women who fulfilled the entry criteria and gave written, informed consent to participate in the trial, an additional 5 ml of blood was drawn into EDTA for further hematological and biochemical laboratory measurements. The envelope was then opened to determine the assigned study group. Women were given 16-18 days supply of tablets and were requested to return at two week intervals for the duration of the 12 week trial. It should be noted that this was not a double blind study because the placebo, $FeSO_4$ tablets, and GDS capsules differed in their appearance. However, the women were not told which of these preparations contained iron. All women were given a small travel allowance to defray the cost of returning to the prenatal clinic for their biweekly visit.

At each return visit during the 12 week trial, a questionnaire was administered to assess the number of supplements taken and recorded any associated side effects. The women were requested to return any unused tablets which were then counted to provide an independent measure of adherence to the supplementation program. Additional blood samples were obtained at the 6 and 12 week follow-up visit to monitor the hematological response. If the hemoglobin concentration at the 6 week follow-up visit fell below 70 g/L, the participant was removed from the study, given full therapeutic doses of oral iron, and referred to a physician for follow-up medical care.

At the conclusion of the 12 week trial, all participants were given sufficient iron tablets to continue iron supplementation until parturition.

Blood samples were processed on the same day that they were obtained for measurement of zinc protoporphyrin using an Aviv 202 hematofluorometer (Aviv Biomedical Inc., Lakewood, NJ) and for electronic measurements of hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), red cell distribution width (RDW), and white blood count (WBC) using a model Coulter S Plus IV Counter (Coulter Electronics Inc., Hialeah, FL). Plasma was distributed in small iron-free plastic vials and stored at -20°C for later measurement of serum iron (7), total iron binding capacity (TIBC) (8), and enzyme-linked immunosorbent assays established with monoclonal antibodies for measurement of serum ferritin (9) and serum transferrin receptor (10) as described previously. The immunoassays were deferred until the conclusion of the trial to permit measurements on the baseline, 6 week, and 12 week samples in each individual to be performed in the same assay plate.

All samples were screened for a hemoglobinopathy using established methods (11). All participants found to have sickle cell disease or thalassemia were later rejected. These included all patients with SS, SC (2 subjects), α-chain or β-chain variants (3 subjects), hemoglobin Lepore (1 subject), and β-thalassemia (6 subjects). Of 363 women who were evaluated at the initial clinic visit, 305 (84%) had a normal (AA) hemoglobin phenotype, 47 (12.9%) had sickle cell trait (AS), and 11 (3.0%) had

hemoglobin C trait (AC). Subsequent analysis of hematological and iron measurements failed to reveal significant differences in women with sickle cell trait or hemoglobin C trait.

The hematological and biochemical laboratory data were compared at each study point by one-way analysis of variance after converting serum ferritin measurements to logarithms (12). Differences between specific study groups were tested by Scheffe's post-hoc test. The differences in the various laboratory measurements within each subject between the baseline and 6 week sample and between the baseline and 12 week sample were likewise assessed by the analysis of variance.

RESULTS

The combined total of women initially registered in the trial was 376. Their mean age was 23 years and was virtually identical in the three study groups (Table 1). In the baseline sample, 31.9% of the women were below 20 years of age, and 10.4% were 30 years or older. Sixty-nine percent were not employed outside of the home and of the remainder, 9.7% were employed in domestic work, 8.1% in factory work, 5.9% as street vendors, and 7.3% in miscellaneous jobs. Fifty-nine percent of the women completed primary education and 42% of the sample completed secondary education. The mean body weight at the time of entry to the study was 61.2 kg and was similar in the three study groups as was the average parity. The mean gestational age was the

approximate mid-point of the second trimester and was five weeks later in the iron supplemented groups than in the group receiving no iron. None of the sample characteristics listed in Table 1 were significant when analyzed by analysis of variance.

There was a progressive reduction in all three study groups throughout the 12 week trial in the number of women who returned for their biweekly clinic visit. Twenty-eight women (7.5%) did not return for the initial two week follow-up and the initial participants were reduced to 85.5% at the midpoint of the investigation. Two-hundred and seventy-five women (73.1%) completed the trial with a relatively even distribution of the drop-outs among the three study groups ranging from 23% in the no iron group to 30% in the GDS group. These differences were not statistically significant when tested by chi-square ($p>0.10$). In order to permit comparison of the laboratory results within each subject at the initial, 6 week, and 12 week follow-up visits, a further 27 women were excluded because of incomplete laboratory information on one of these occasions. The final sample, for which all hematological and biochemical data were available, was comprised of 86 women in the no iron group, 79 in the $FeSO_4$ group, and 83 in the GDS group.

Adherence to the supplementation program exceeded 90% in all study groups during the 12 week trial as determined by tablet counts and questionnaires. The adherence rate did not differ significantly among the three study groups. These results, together

with the reported side effects that might be attributed to iron supplementation will be reported subsequently.

There were significant differences for certain laboratory measurements among the three study groups at the time of initial registration (Table 2). The hemoglobin concentration was significantly higher in the no iron group than in the GDS group ($p<0.001$) and the difference in hemoglobin concentration between the no iron and $FeSO_4$ group also approached statistical significance ($0.05<p<0.10$). Several of the other iron parameters also indicated that the iron status of the no iron group was slightly better than the GDS but not the $FeSO_4$ group. Thus, the MCV and transferrin saturation were both significantly higher and the TIBC and serum transferrin receptor significantly lower in the no iron group as compared with the GDS group. Importantly, none of the hematological or biochemical laboratory measurements differed between the two groups given iron supplements.

When examined at the 6 week midpoint of the trial, the mean hemoglobin concentration had fallen significantly below that of the $FeSO_4$ group but did not differ from the GDS group (Table 3). The RDW remained lower in the no iron group suggesting that iron supplementation had resulted in a greater variability in circulating red cell diameter in those women receiving some form of iron. The lower geometric serum ferritin in the no iron group of 8.3 µg/L was significant as compared to the groups receiving iron (16.5 and 13.6 µg/L) and highly significant differences were also

observed for the serum iron and transferrin saturation. Both the TIBC and serum transferrin receptor were significantly higher in women receiving no iron than in the $FeSO_4$ but not the GDS group. For none of the laboratory parameters was the difference between the two iron groups significant at the 6 week follow-up.

A comparison of the laboratory data at the conclusion of the three month trial showed a further evolution of iron deficiency in women given folate only (Table 4). The hemoglobin concentration in the no iron group was nearly 10 g/L lower than in the iron-supplemented groups reflecting a further fall as compared to the 6 week follow-up (Figure 1). A similar magnitude of difference was observed with the majority of the remaining laboratory measurements. In women receiving no iron, the FEP, TIBC, and transferrin receptor were significantly higher and the serum ferritin, serum iron, and transferrin saturation lower than in women given iron supplements. The reciprocal change in serum transferrin receptor over time in the same three study groups was similar to that observed with hemoglobin measurements (Figure 2). The only laboratory parameter showing a difference between the two groups receiving an iron supplement was the serum iron ($p=0.05$) and transferrin saturation ($p=0.02$), both of which were higher in the women given $FeSO_4$. These were not fasting blood samples and the women were not instructed to defer their iron supplement on the day of their clinic visit. Because of the slower iron release with GDS, no significant rise in serum iron occurs following oral administration whereas a prompt elevation occurs following $FeSO_4$ (Skikne et al., unpublished observations).

Because the iron status of the three study groups differed somewhat at the entry point of the women to this trial, an analysis was also performed to compare the change in laboratory values within each subject at the 6 and 12 week follow-up (Table 5). By reducing the effect of intersubject variation in laboratory measurements, the contrast between the study groups was more apparent. At the 6 week follow-up, the mean changes in the $FeSO_4$ and GDS groups for all laboratory parameters was similar whereas changes in the no iron group were in the reverse direction. The contrast for the iron parameters was especially notable. The serum ferritin fell by 20% in the no iron group whereas it increased by 22 and 17% in the $FeSO_4$ and GDS arms respectively (F=34.9). The change in the serum transferrin receptor was also highly significant increasing by an average of 1.5 mg/L in the no iron group as compared to modest declines in the iron supplemented groups.

The mean differences in laboratory parameters between the baseline and 12 week follow-up sample were greater among the study groups than at 6 weeks. The only F value that did not attain statistical significance at the 0.1% level was the RDW. In no instance did the mean changes differ for the two groups receiving an iron supplement. The mean change in hemoglobin concentration was slightly greater in the GDS group than in the $FeSO_4$ group perhaps because of the somewhat lower hemoglobin concentration in the GDS group at the time of the entry into the trial (Figure 3). The mean serum ferritin level had fallen by 36% in women receiving no iron as compared to increases of 75% in the $FeSO_4$ group and 47% in the GDS group. The mean

change in serum transferrin receptor demonstrated the further evolution of iron deficient erythropoiesis in women receiving no iron as compared with little or no change in the women receiving an iron supplement (Figure 4).

DISCUSSION

The measures presently available for reducing the prevalence of iron deficiency anemia during pregnancy are limited both in number and effectiveness. Improving the diet with respect to either the total iron content or, more importantly, the bioavailability of food iron should improve the iron status of women entering pregnancy. However, the duration of gestation is too short and the benefit of modifying the diet too limited to have a meaningful impact on the evolution of iron deficiency anemia during pregnancy. The provision of a highly fortified food supplement to pregnant women offers more promise but the inhibiting effect of food on iron absorption remains an important constraint when attempting to modifying iron balance in a limited time period. These considerations have led to a focus in the past several decades on the provision of a medicinal iron supplement to pregnant women as the only reasonable intervention measure. Even in the US where the prevalence of gestational iron deficiency is lower than in most regions of the world, it is recommended that women consume a supplement containing at least 30 mg elemental iron daily separate from meals during

the second and third trimesters to diminish the risk of developing iron deficiency anemia (13).

The efficacy of iron supplementation in pregnancy has been repeatedly demonstrated in controlled trials both in highly industrialized and lesser developed regions of the world. At the same time, it is widely believed that when undertaken as a public health measure, iron supplementation has had little impact on anemia prevalence in pregnant women. Of various reasons that have been cited to explain the marginal effectiveness of iron supplementation at the population level, limited coverage of national primary health care systems is considered important in most Third World countries (14). Problems in the procurement and distribution of iron tablets restrict the supply to pregnant women and even when tablets are available at MCH centers, primary care workers often fail to instruct or encourage women to take their supplement regularly.

The extent to which the major gastrointestinal side effects of medicinal iron such as nausea, vomiting, and epigastric pain, account for the diminished effectiveness of iron supplementation in pregnancy is unclear. However, these side effects are a well known obstacle in conducting field trials in which the motivation of those administering the supplements is high. When probiems in the procurement and distribution of iron tablets are eliminated, it is highly likely that constraints imposed by the side effects of oral iron will again emerge as the major difficulty with iron supplementation.

The GDS employed in the present trial has the major advantage that upper gastrointestinal side effects are eliminated (6). Although delayed release forms of iron have been provided by pharmaceutical companies for several decades, their ineffectiveness is well recognized by medical practitioners because the release of iron in the intestinal tract is delayed until the tablet is beyond the maximal absorbing area in the proximal small bowel. With the GDS, $FeSO_4$ is incorporated into a hydrocolloid matrix that becomes buoyant on exposure to gastric secretions and is thereby retained for prolonged periods in the acidic environment of the stomach where iron solubility is favored. Upper gastrointestinal symptoms are eliminated because the iron is released slowly over a period of hours. Of equal importance is the fact that iron absorption in this form may be 2- to 4-fold higher than absorption of $FeSO_4$ when given with food, presumably because the GDS retains iron in the stomach until the bulk of the meal and its absorption inhibitors has emptied. The present trial was undertaken in part because of this absorptive advantage. We reasoned that because of the higher absorption of iron in GDS, a single capsule daily containing 50 mg elemental iron would be equivalent to a standard $FeSO_4$ tablet taken twice daily.

In the present study, a progressive deterioration in iron status occurred in women who received no iron supplements as compared to those that were given either $FeSO_4$ or GDS. Using a wide variety of hematological and iron related parameters, no obvious differences in the response to $FeSO_4$ and GDS were demonstrated. The hematological response was not identical in the two groups when examined at the 6 week midpoint

of the study as compared to the conclusion of the three month trial (Figure 1) but this difference may reflect the lower hemoglobin levels in the GDS group at the beginning of the trial. Indeed, when tested as the change in hemoglobin levels before and following iron supplementation, the response in the two groups receiving iron was indistinguishable (Figure 3). Except for a slightly higher serum iron and transferrin saturation in the $FeSO_4$ group at the 6 week point of the trial, apparently due to the more rapid release of $FeSO_4$ to the circulation, all other laboratory measurements in the two groups receiving iron were indistinguishable.

One of the curious aspects of the present investigation is that despite randomization, the hemoglobin concentration in women receiving no iron was significantly higher than in the iron supplemented groups at the time of the initial registration. The nature of this difference remains unexplained. It is probably related to the fact that the women receiving no iron were registered on the average of 5 weeks earlier during gestation. Because iron status usually deteriorates during the second trimester, the later gestational period in the iron supplemented groups presumably accounts for this difference. Because the major focus in this study was a comparison between two different programs of iron supplementation, the interpretation of the findings is not influenced.

It remains to be established that GDS can significantly improve the adherence to an iron supplementation program but there are several reasons to believe that it will. A

single GDS tablet daily is as effective as two $FeSO_4$ tablets and compliance is likely to be improved by reducing the administration frequency. Another major advantage is that the troublesome upper gastrointestinal side effects are eliminated with GDS but persist in a substantial portion of women taking a conventional $FeSO_4$ tablet. The present investigation was not designed optimally to assess compliance because it was not possible to obtain the placebo, $FeSO_4$ tablets, and GDS in an indistinguishable form. Even if this had been possible, differences would have existed in the frequency of tablet administration in the two iron supplemented groups. Our evaluation of compliance and side effects do indicate an advantage of the GDS tablets (Simmons et al., unpublished observations) but there is a need for further field studies performed in a double blind fashion.

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Table 1. Characteristics of study groups at baseline

	No Iron	$FeSO_4$	GDS
Sample Size			
Baseline	121	123	132
6 weeks (% of baseline)	102 (83)	105 (85)	115 (87)
12 weeks (% of baseline)	93 (77)	89 (72)	93 (70)
Age (years)	23.3±5.0*	23.4±4.4	23.0±4.9
Weight (kg)	61.1±11.7	62.3±13.4	60.4±8.7
Parity	1.10±1.24	1.20±1.37	1.27±1.41
Gestational age (days)	135±17	140±22	141±18

Mean ±1SD

Table 2. Laboratory Parameters at Baseline

Laboratory Measurement	No Iron (N)	$FeSO_4$ (F)	GDS (G)	F value	Intergroup Comparisons (p values) N:F	N:G	G:F
N	86	79	83				
Hemoglobin (g/l)*	105.0±2.0	101.4±2.2	98.9±2.4	7.8**	0.073	<0.001	ns‡
MCV (fl)	88.1±1.6	87.0±1.6	85.1±1.9	3.4§	ns	0.036	ns
RDW (%)	14.0±0.3	14.0±0.3	14.5±0.4	2.2	ns	ns	ns
FEP (µmol/L RBC)	64.5±5.3	68.2±8.0	75.7±9.3	2.2	ns	ns	ns
Serum Ferritin (µg/L)†	13.9 (11.2-17.2)	10.7 (8.4-13.5)	9.4 (7.4-11.9)	3.2§	ns	0.051	ns
Serum iron (µg/100 ml)	83.1±7.1	77.5±8.6	69.6±7.6	3.0§	ns	0.051	ns
TIBC (µg/100 ml)	416±15	435±17	453±17	5.3¶	ns	0.006	ns
Saturation (%)	20.8±2.1	19.0±2.4	16.3±2.1	4.2§	ns	0.017	ns
Transferrin receptor (mg/L)	7.24±0.54	7.81±0.83	8.63±0.83	3.6§	ns	0.028	ns

* ±2SE
† Geometric mean (±2SE)
‡ Not significant (p>0.10)
§ p<0.05
¶ p<0.01
** p<0.001

Table 3. Laboratory Parameters at 6 week follow-up

Laboratory Measurement	No Iron (N)	$FeSO_4$ (F)	GDS (G)	F value	Intergroup Comparisons (p values) N:F	N:G	G:F
N	86	79	83				
Hemoglobin (g/l)*	100.9±2.3	105.0±2.3	102.3±2.3	3.2§	0.042	ns‡	ns
MCV (fl)	87.1±1.5	89.2±1.6	87.2±1.7	2.1	ns	ns	ns
RDW (%)	13.8±0.38	15.1±0.8	15.4±0.8	6.8¶	0.027	0.003	ns
FEP (µmol/L RBC)	68.9±6.5	62.7±6.1	69.4±8.9	1.0	ns	ns	ns
Serum Ferritin (µg/L)†	8.3 (6.8-10.1)	16.5 (13.8-19.8)	13.6 (11.5-16.0)	15.7**	<0.001	<0.001	ns
Serum iron (µg/100 ml)	66.0±6.1	94.9±9.1	91.6±7.0	12.6**	<0.001	<0.001	ns
TIBC (µg/100 ml)	471±13	433±13	453±16	7.4**	<0.001	ns	ns
Saturation (%)	14.4±1.5	22.4±2.2	21.0±2.8	15.9**	<0.001	<0.001	ns
Transferrin receptor (mg/L)	8.75±0.69	7.45±0.68	8.40±0.67	3.8§	0.03	ns	ns

* ±2SE
† Geometric mean (±2SE)
‡ Not significant ($p>0.10$)
§ $p<0.05$
¶ $p<0.01$
** $p<0.001$

Table 4. Laboratory Parameters at 12 week follow-up

Laboratory Measurement	No Iron (N)	$FeSO_4$ (F)	GDS (G)	F value	Intergroup Comparisons (p values) N:F	N:G	G:F
N	86	79	83				
Hemoglobin (g/l)*	99.3±2.6	108.1±2.6	108.3±2.5	16.5¶	<0.001	<0.001	ns‡
MCV (fl)	84.6±1.7	88.9±1.6	87.1±1.7	7.0§	0.002	ns	ns
RDW (%)	14.2±3.9	14.6±5.3	15.0±5.9	2.3	ns	ns	ns
FEP (µmol/L RBC)	91.4±9.3	64.5±5.4	74.7±7.2	13.0¶	<0.001	0.007	ns
Serum Ferritin (µg/L)†	8.8 (7.4-10.3)	18.8 (15.3-23.0)	13.9 (11.4-17.0)	17.2¶	<0.001	0.002	ns
Serum iron (µg/100 ml)	64.7±10.4	100.2±10.2	83.8±7.6	14.2¶	<0.001	0.016	0.053
TIBC (µg/100 ml)	493±17	437±16	459±15	12.7¶	<0.001	0.01	ns
Saturation (%)	14.1±2.7	23.6±2.6	18.9±1.9	15.9¶	<0.001	0.017	0.022
Transferrin receptor (mg/L)	10.44±0.91	7.30±0.78	8.31±0.64	16.7¶	<0.001	<0.001	ns

* ±2SE
† Geometric mean (±2SE)
‡ Not significant (p>0.10)
§ p<0.01
¶ p<0.001

Table 5. Differences in laboratory measurements at 6 week and 12 week follow-up

	Difference between 6 week and baseline				Difference between 12 week and baseline			
	No Iron	$FeSO_4$	GDS	F value	No Iron	$FeSO_4$	GDS	F value
Hemoglobin (g/l)*	-4.2±1.8	3.5±2.4	4.0±2.1	18.7¶	-5.7±2.4	6.6±2.8	9.4±2.8	37.8¶
MCV (fl)	-1.0±0.8	2.2±0.7	2.2±1.1	18.9¶	-3.6±1.1	1.9±0.8	2.2±1.2	33.8¶
RDW (%)	-0.2±0.3	0.9±0.6	1.0±0.6	7.1§	0.2±0.4	0.5±0.4	0.5±0.5	0.4‡
FEP (µmol/L RBC)	4.2±4.4	-6.0±5.8	-4.3±10.4	2.3‡	26.9±7.2	-4.0±6.0	-0.2±7.8	23.0¶
Serum Ferritin (µg/L)†	0.80 (0.73-0.87)	1.22 (1.12-1.32)	1.17 (1.08-1.26)	34.9¶	0.64 (0.51-0.80)	1.75 (1.41-2.16)	1.47 (1.17-1.86)	23.5¶
Serum iron (µg/100 ml)	-16.5±6.9	17.1±10.6	21.9±6.0	18.0¶	-18.4±12.2	22.8±12.4	14.2±7.8	15.5¶
TIBC (µg/100 ml)	54.9±13.8	-2.0±14.8	-0.7±14.8	21.2¶	77.7±17.8	2.7±15.9	6.0±17.1	25.3¶
Saturation (%)	-6.3±1.7	3.4±2.6	4.7±2.8	25.8¶	-6.7±3.0	4.7±3.2	2.5±2.0	19.4¶
Transferrin receptor (mg/L)	1.50±0.49	-0.27±0.50	-0.23±0.50	37.8¶	3.27±0.73	-0.49±0.68	-0.30±0.68	36.8¶

* Mean difference ±2SE
† Ratio ±2SE
‡ Not significant
§ $p<0.001$
¶ $p<0.0001$

LEGENDS

Figure 1. Mean hemoglobin concentration at baseline, 6 weeks, and 12 week follow-up in groups receiving no iron (O), 100 mg iron as $FeSO_4$ daily (●), or 50 mg iron as GDS daily (□). The vertical bars represent ±2SE.

Figure 2. Mean serum transferrin receptor concentration at baseline, 6 weeks, and 12 week follow-up in groups receiving no iron (O), 100 mg iron as $FeSO_4$ (●), or 50 mg iron as GDS daily (□). The vertical bars represent ±2SE.

Figure 3. Change in hemoglobin concentration within each subject between baseline and 12 week follow-up in groups receiving no iron, 100 mg iron as $FeSO_4$, or 50 mg iron as GDS daily.

Figure 4. Change in serum transferrin receptor concentration within each subject between baseline and 12 week follow-up in groups receiving no iron, 100 mg iron as $FeSO_4$, or 50 mg iron as GDS daily.

Figure 1

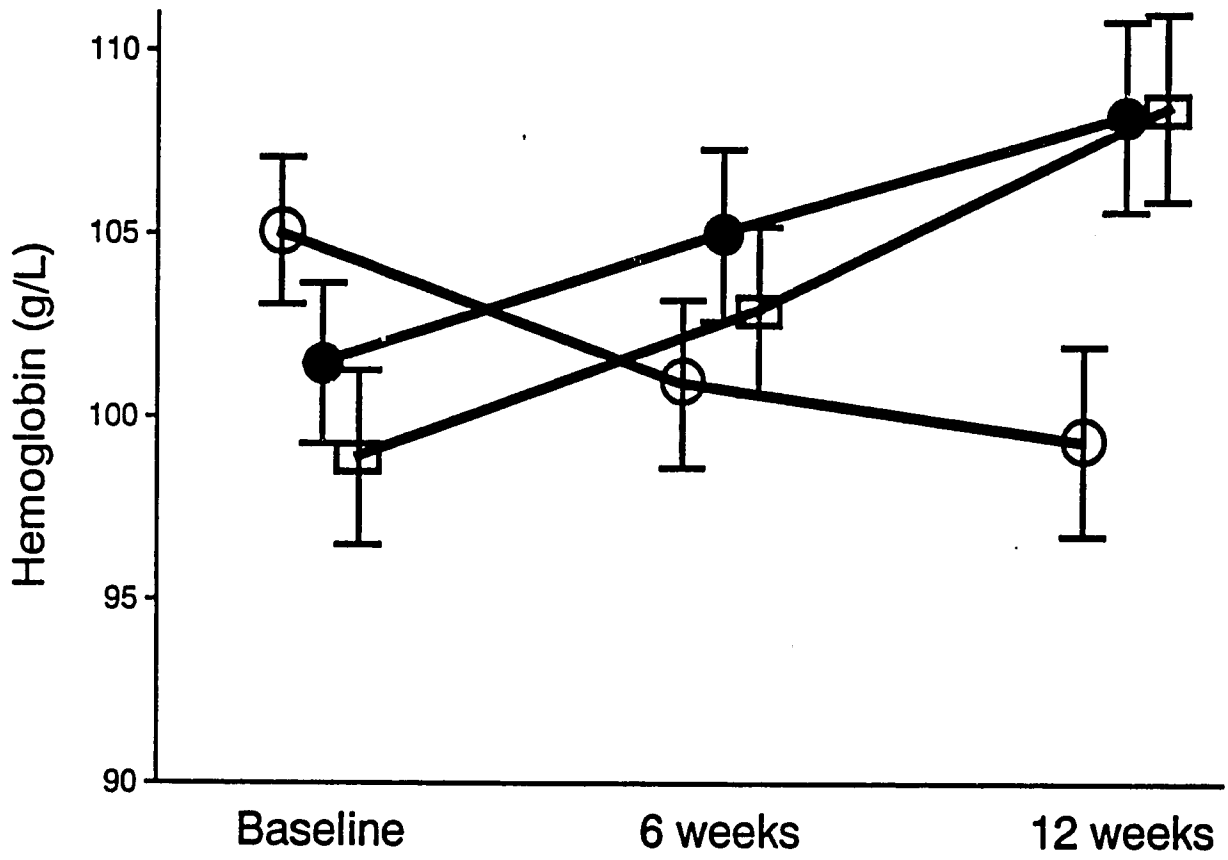


Figure 2

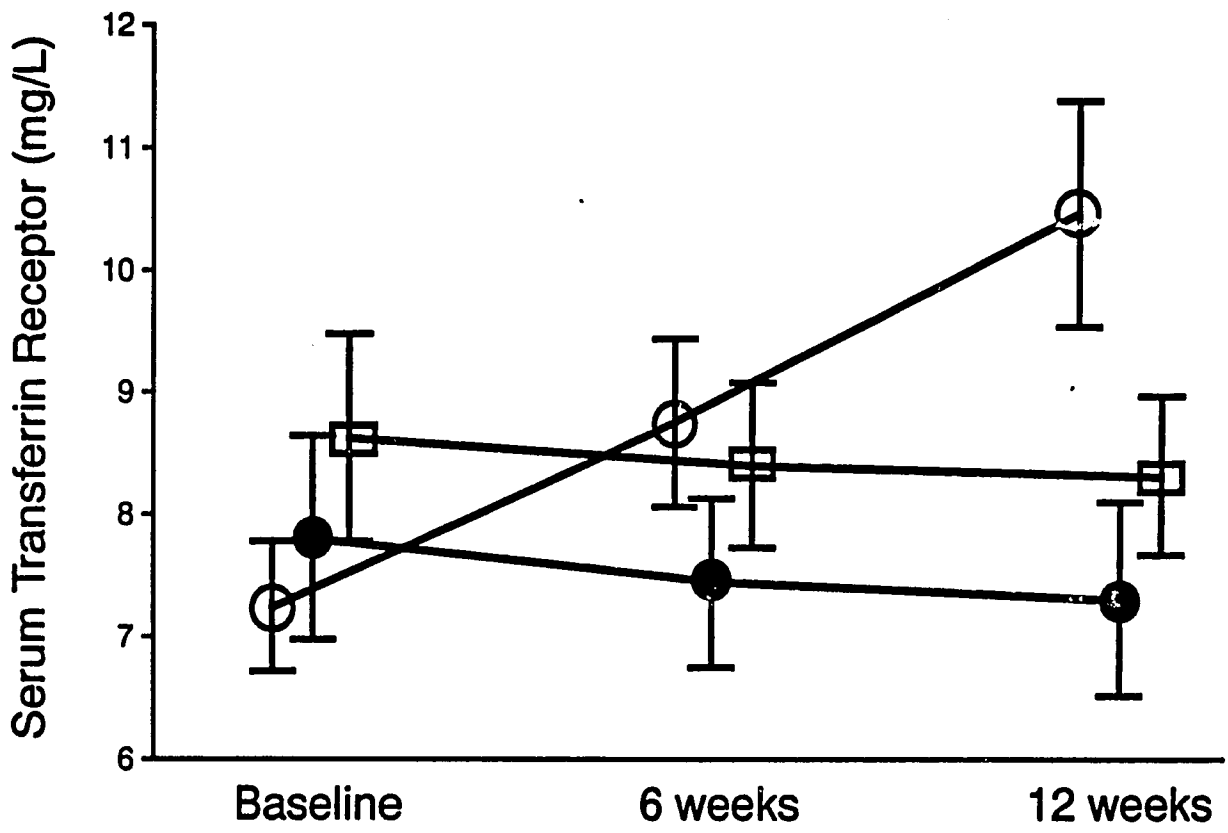

Serum Transferrin Receptor (mg/L)
12
11
10
9
8
7
6
Baseline
6 weeks
12 weeks

Figure 3

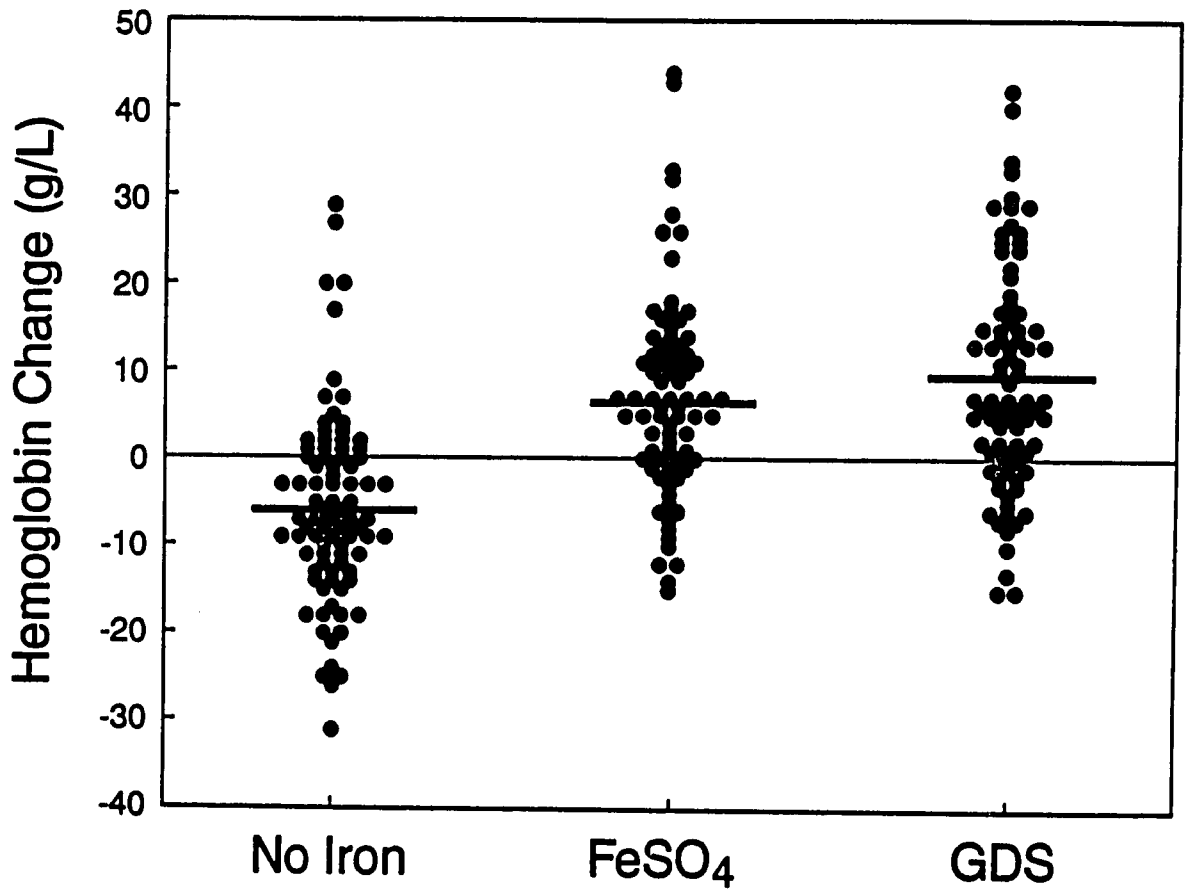
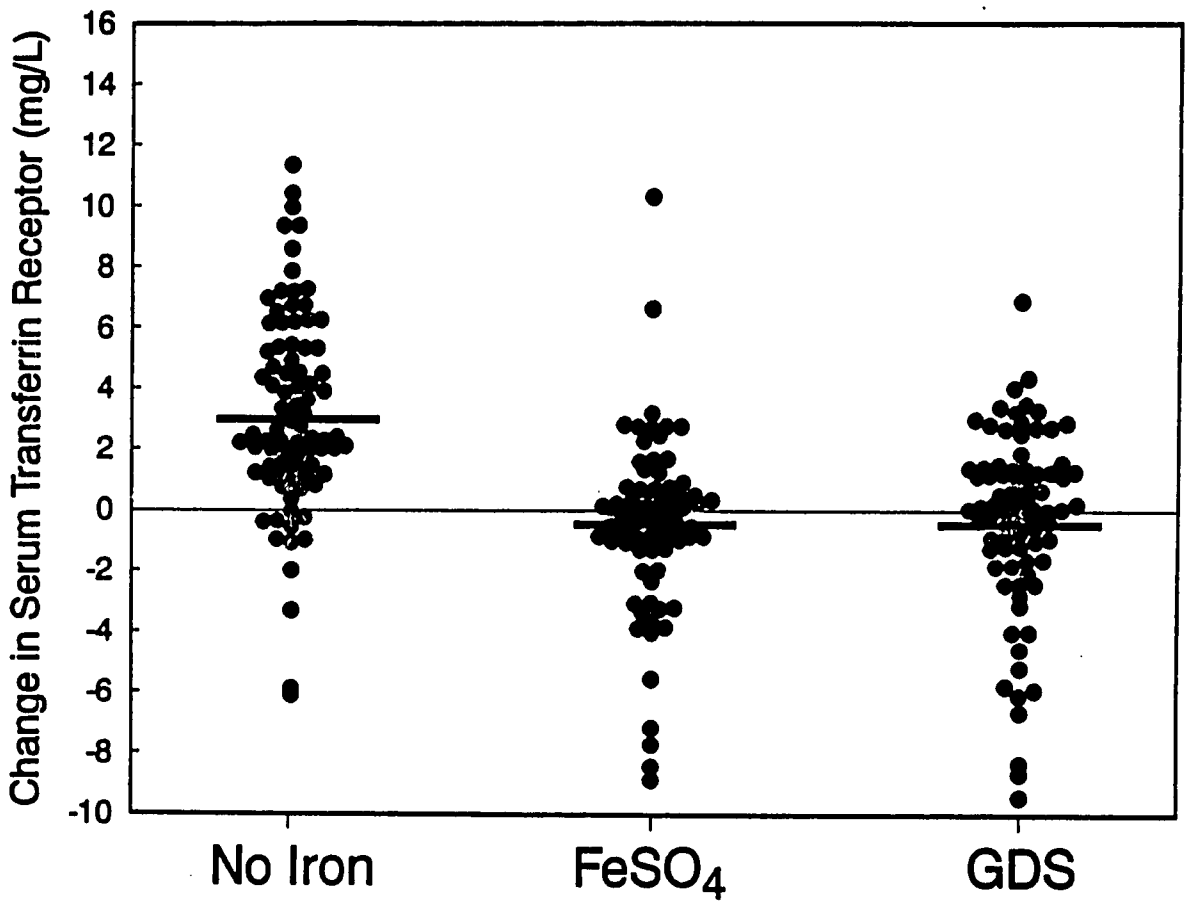

Hemoglobin Change (g/L)
50
40
30
20
10
0
-10
-20
-30
-40
No Iron
FeSO4
GDS

Figure 4


Change in Serum Transferrin Receptor (mg/L)
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
No Iron
FeSO4
GDS

APPENDIX B-4

DRAFT

A PROJECT PROPOSAL

A PARTNERSHIP FOR IMPROVING IRON NUTRITION IN GRENADA

THE FORTIFICATION OF WHEAT FLOUR (Using The Nutribusiness Model)

Collaborative Project With

THE GOVERNMENT OF GRENADA

the

CARIBBEAN AGRO INDUSTRIES Ltd.

and

THE CARIBBEAN FOOD AND NUTRITION INSTITUTE

Caribbean Food and Nutrition Institute
Kingston 7, Jamaica

Pan American Health Organization
Pan American Sanitary Bureau, Regional
Office of the World Health Organization

1991

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EXECUTIVE SUMMARY

Previous studies have shown that anaemia is a public health problem in Grenada. An islandwide anaemia survey of that country was conducted in 1985-86. The results of the study showed that anaemia and iron deficiency were serious problems throughout the entire population. The prevalence of deficient plasma ferritin levels indicating no iron reserves was as high as 62% in some population groups.

Iron supplements or iron fortified foods have been found to be cost-effective in raising haemoglobin levels of iron deficient populations resulting in increased productivity.

Studies have shown that wheat flour and wheat products are the principal contributors to energy and protein among most Caribbean populations. In the case of Grenada, wheat flour is the staple food for the population and would thus be a logical choice as a vehicle for iron fortification.

In Grenada, wheat is imported and is milled locally at a central location. Baking flour which is used in the baking trade, and for home use, is fortified with iron and B-complex vitamins. Thirty-seven milligrams of iron per kilogram of baking flour is used. The counter flour which is sold for home use and mainly in rural areas in Grenada is not fortified with iron or B-complex vitamins. The ratio of sales of baking flour to counter flour is 55:45. This means that 45% of the wheat flour (counter), which is the main source of wheat flour for home consumption, is not fortified with iron or B-complex vitamins.

The purpose of this study will be to fortify all wheat flour in Grenada with a suitable type and level of iron, evaluate the impact of the fortification programme and institute an anaemia monitoring system. This will mean adding 44mg of iron per kilogram of counter flour. The type of iron added will be ferrous sulphate.

The project will use the nutribusiness strategy. This strategy promotes indigenous private sector enterprises in food processing, thus enabling these enterprises to produce higher quality foods. It is a partnership among private enterprise and governmental organizations. Nutribusiness links nutrition and the private sector to address well-defined nutritional needs of the population.

The project will last for three years and will be evaluated by sensitive hematological parameters. The objective of the study is to significantly reduce iron deficiency in the entire Grenadian population. The evaluation will be based on analyses of blood samples and questionnaire responses obtained from randomly chosen households and from antenatals attending clinics in depressed areas. Government statistics relating to health and productivity will also be used to determine trends.

This proposal seeks funding of US$56,500 to start the study.

I. INTRODUCTION

Existing data indicate that anaemia exists in all countries of the English-speaking Caribbean. It is most common in pregnant and lactating women, and in pre-school children, with country prevalences (using WHO criteria) between 27-100% and 11% and 76%, respectively.

Severe anaemia, with a haemoglobin concentration in the blood below 8 g/dl, is found in about 6% of pregnant and lactating women and 11% of pre-school children in some of the countries.

There are three major causes of anaemia in the Caribbean:

1. Poor nutrition;
2. Specific parasitic infestations; and
3. Haemoglobinopathies.

All three causes are important from a public health point of view, and all three may interact.

The major (dietary) nutritional cause of anaemia is iron deficiency, probably due to a low absorption in the intestine and an inadequate dietary intake. A dietary deficiency of folate is sometimes also responsible. Changes in food habits of some sections of the population are associated in some instances with a deficiency of vitamin B_{12}. This appears to be currently a minor problem in the English-speaking Caribbean, but it could increase.

Hookworm and whipworm (trichuris) are the two main parasites associated with anaemia. Infestations of hookworm are isolated in certain areas of the population. The major haemoglobinopathy causing anaemia is sickle-cell disease [1-11].

In 1972, a nutrition survey was conducted in the village of La Poterie in Grenada. Anaemia was found to be a problem in all ages studied in the survey. Thirty-eight percent of pre-school age children, 65% of school-age children, 22% of adult males, and 49% percent of non-pregnant, non-lactating females were found to be anaemic by the World Health Organization (WHO) standards. Low serum iron and folates were found in the pre-school age children and adult females [12].

Another study conducted in 1976 indicated that 51% of ante-natals were anaemic, according to suggested WHO haemoglobin standards. Eighteen point five percent of pre-school age children had haemoglobin levels below 10.5% g/dl [13]. The WHO recommended haemoglobin standard for pre-school age children is 11.0 g/dl [4].

Most recent data obtained from health clinics showed that of the 400 women assessed prenatally, 234 (58.5%) had haemoglobin levels below 11 g/dl in 1983, and in 1984, of the 479 women tested, 353 (73.7%) had low haemoglobin levels, i.e. below 11 g/dl [15].

The Government of Grenada is concerned about the poor performance of its students on the CXC Examination. Questions have been raised about the possibility

of iron deficiency contributing to the poor school performance of Grenadian school children.

In 1985, two females died of post-partum haemorrhage, which could be caused from anaemia. Because of the fear that anaemia could be a major public health problem in Grenada, the Government of Grenada requested that the Caribbean Food and Nutrition Institute (CFNI) study the problem of anaemia in Grenada and develop a programme for its control.

As a first step, a population survey of iron status was planned. The variables to be studied were demographic, social, dietary, parasitic and haematological. The blood tests were haemoglobin, hematocrit, sickling, and serum ferritin. Qualitative counts of parasites in stool samples were done by CAREC. The dietary data concentrated on frequency of consumption on iron-rich food, enhancers and inhibitors on iron absorption, and possible vehicles for iron fortification.

The sample was statistically representative, with sub-samples of pregnant and lactating women. The study was conducted in November 1985.

In summary, the findings were that anaemia was widely prevalent in all ages and gender groups ranging from a low of 14% in young men to a high of 74% in pregnant women. The next most vulnerable groups were children aged 0-5 years (with a peak of 12-24 months) and lactating women. Serum ferritin results demonstrated an almost universal lack of iron stores, pointing to iron deficiency as a significant aetiological factor. SS haemoglobinopathy was found to be present in

0.5% of the sample, and 15% had an AS genotype. Hookworm was found in 6% of the stool samples. Although other parasites were found, the load was not considered to be sufficient to be a cause of the anaemia demonstrated [16].

The qualitative pattern of the diet was satisfactory, enhancers of iron absorption were consumed often and inhibitors rarely. However, quantitative data on the diet were not collected.

In June 1986, a workshop on the Grenada Anaemia Survey was convened to discuss the results of the survey and to develop a strategy for the control of anaemia in Grenada [17].

The participants at the workshop were divided into five working groups and were requested to work out a simple, workable strategy. The groups recommended specific procedures to improve supply management, supplementation programmes, haemoglobin screening, education, and fortification. It was recommended that all flour be fortified and that the effectiveness of such a programme be evaluated by the initial blood samples and the subsequent monitoring of blood samples every six months, up to two years. [7]

After the workshop, considerable work was done on the control of anaemia in pregnant women and pre-school age children. In pre-school children regular administration of iron is difficult. In pregnant women supplementation maintains the iron status but does not correct the initial deficiency. However, to reach the entire

population other approaches must be considered. The fortification of wheat flour in Grenada should reach this objective.

Wheat flour has been fortified with iron in numerous countries around the world. Some countries have had these fortification programmes for as long as forty years. However, the fortification of wheat flour with iron has never been evaluated on a national scale.

The nutribusiness concept could be the vehicle to ensure a constructive and lasting programme. Nutribusiness links nutrition and the private sector to address well-defined nutritional needs of populations. Operationally, nutribusiness provides quality foods and food products at affordable prices through private-sector approaches. It uses environmentally regenerative production, storage, and distribution techniques and avoids pollution and inappropriate or excessive use of energy and other natural resources. Nutribusiness thus ensures optimal nutritional quality, safety, and acceptability of foods provided through commercial channels and in keeping with local cultural and environmental imperatives. The nutribusiness model addresses "well defined nutritional needs and populations". When nutritional needs are specified and target populations carefully defined, it is possible to examine how needs can be met (Annex 1).

The programme will be implemented by Caribbean Agro Industries Ltd. which is the flour mill in Grenada. Guidance and suggested levels of fortification will be provided by the Government of Grenada. The project will be evaluated by Caribbean

Agro Industries Ltd, The Government of Grenada and the Caribbean Food and Nutrition Institute (Annex 2).

Once such a programme is implemented and evaluated, it is believed that the prevalence of iron deficiency anaemia in Grenada should be reduced. It is also believed that the methodology and experience gained in Grenada would be applicable and could be used in other Caribbean countries and, possibly, in other parts of the world. A description of Grenada can be seen in Annex 3.

HOPEFUL MAJOR BREAKTHROUGHS

The following initiatives and evaluations will be attempted for the first time in this project:

First Time

1) A Government, a private sector company and an international agency will collaborate on an iron nutrition project through the Nutribusiness Approach.

First Time

2) The fortification of wheat flour with iron will be evaluated on a national scale.

First Time

3) The plasma transferrin receptor will be used to evaluate a programme on a national scale (see section VI).

First Time

4) A cost/benefit analysis will be conducted after fortifying wheat flour with iron.

II. FOOD FORTIFICATION

The fortification of foods - the addition of selected nutrients to foods in order to improve their nutritive value - is a commonly used strategy for improving or maintaining the nutritional status of a population by increasing the daily intake of specific nutrients. The technology for adding nutrients to foods is well developed and has been used in many countries for many years. Primary examples are the addition of iodine to salt, vitamin A to fats and sugar, and vitamin C to juices. The most widely used food fortification technology is for the addition of B-vitamins and iron to cereal foods such as flour, bread, meals, and pasta. The fortification of wheat flour with vitamins and iron is presently being used in more than 20 developed and developing countries. Also, in at least 12 countries that do not produce their own flour, the imported flour is required to be fortified with vitamins and minerals. The fortification of foods has been shown to be a simple, effective and relatively inexpensive way of introducing nutrients, such as bioavailable iron, into the daily diet.

The technology for the fortification of cereal food, such as wheat flour with iron, involves two major components:

1. The installation and operation of the equipment for adding the nutrient;
    a. in reasonable amounts
    b. on a fairly regular basis
2. Consumption by those in the lower economic levels, the ones most in need of the nutrients. Since cereal foods are the primary staple food for so many

of the world's population, especially in developing countries, they are the most frequently selected vehicles for fortification.

II.1 Wheat Flour Fortification in the English-speaking Caribbean

Wheat flour and wheat products are the principal foodstuffs consumed in the Caribbean, except Guyana. As can be seen in Figure 1, this wheat and wheat flour is imported from West Germany, the United States, Canada and Puerto Rico. Some of the pre-ground wheat flour imported into the Caribbean is fortified with vitamins and iron.

Most wheat flour imported into the Caribbean and ground locally is fortified with vitamins and iron except in Suriname. These wheat products are fortified at the following levels:

1.	Thiamine	-	4.4	-	5.5	mg/kg
2.	Riboflavin	-	2.6	-	3.3	mg/kg
3.	Niacin	-	35.0	-	46.0	mg/kg
4.	Iron			-	28.0 36.0	mg/kg
5.	Calcium	-	1.1	-	1.4	mg/kg

Fortification with calcium is optional and is usually not done.

The iron added in both pre-ground as well as locally ground wheat is in the form of either ferrous sulphate or reduced iron. Ferrous sulphate has a higher bioavailability than does reduced iron of a large particle size. The reduced iron size used is 44 mu in size. Studies have shown that this large particle size reduced iron is

absorbed to a limited size. Since wheat provides 17 and 24% of available energy and 18 to 30% of protein for those Caribbean countries from which information is available, this produce needs special mention in relation to fortification with iron [18].

II.2 Present Fortification Programme in Grenada

In the case of Grenada, wheat flour is the staple food of the population. The amount of wheat flour produced and available on a per capita basis can be seen in Table 1. This is the logical choice as a vehicle for an iron fortification programme.

In Grenada, presently 99% of wheat flour used is milled locally at the central location. Both baking flour and counter flour are produced. Baking flour, which is used in the baking trade and some home use, is fortified with iron, thiamine, riboflavin and niacin. The iron is added at 37.0 mg/kg of baking flour and is in the form of ferrous sulphate. Forty-five percent of the flour is counter flour. This means that 45% of the wheat flour (counter flour) which is the main source of wheat flour for home consumption and probably the flour purchased by the lower socio-economic group, is not fortified with iron or B-complex vitamins.

Both baking flour and counter flour are sold in 100 lb. bags to bakeries and supermarkets. The supermarkets usually divide both wheat flours in 2, 8, 10 or 15 lb. bags. A two-pound bag of baking flour costs EC$1.66, and a two-pound bag of counter flour sells for EC$1.56. Baking flour is also sold in five-pound labelled packets.

II.3 Change in Iron Fortification Programme in Grenada

As part of an iron fortification programme of wheat flour in Grenada, specifications will be established and plans developed with the Grenadian Caribbean Agro

Industries Limited (The flour mill), to ensure that the form of iron added to all wheat flour produced in the country be ferrous sulphate.

The wheat flour component of the fortification programme can be viewed both as:

1. An upgrade of the nutritional value: the change in iron form used in a food already part of an intervention; and
2. As a new nutrition intervention involving the fortification of counter flour with ferrous sulphate or electrolytic reduced iron and B-vitamins.

This intervention will make iron fortified flour available to people who have not had access to fortified flour.

About 55% of the wheat flour used in the country is baking flour and is produced at the Caribbean Agro Industries Limited in St. George's, Grenada. The type of flour currently produced is used in commercial bakeries to produce breads of all types and is available for some home use. The flour is fortified with B-vitamins and iron by use of a specially prepared premix which is added to the flour using typical food fortification technology. This premix contains enzymes and oxidation materials in addition to the vitamins and iron. These ingredients are not usually included in standard premixes. The form of iron used in the premix is ferrous sulphate and is added at the level of 37 mg/kg of baking flour.

Although precise costs are not available at this time, preliminary calculations suggest a slight increase in cost for adding the premix to the counter flour. The producer has agreed to absorb the increase in the cost as part of his effort for improving the nutritional value of the flour.

The remaining 45% of the flour used in Grenada is "counter flour". This flour traditionally is used by the majority of the population for general home use and probably used by the lower socio-economic group, since it is less expensive than the baking flour. Available information indicates that this flour is not fortified with any nutrients, including iron, so that the general population which consumes counter flour have not been recipients of any added iron in their daily diet through the use of counter flour. The counter flour should be fortified with B-vitamins and iron. Ferrous sulphate could be considered as the iron source of choice. A premix for this use will be much less complicated, ingredient-wise, than that used for the bread flour. It is expected that this premix also will undergo a long holding time in the mill before use, but no problems are anticipated with the use of ferrous sulphate (which is the standard for comparison) which is available from producers of premises. The cost to fortify wheat flour with B-vitamins and iron (ferrous sulphate) to current U.S. standards is about US$0.90 per tonne. The iron level for this fortification would be 44 mg/kg of flour.

In review, the iron source contained in the premix, used to fortify the wheat flour produced in the country, should be ferrous sulphate. The required iron level in all wheat flours should be determined regardless of iron source used.

A programme for the iron fortification of counter flour will involve the addition of iron such as ferrous sulphate in the mill at the time of production. The iron will be blended into a premix for ease and accuracy of addition to the meal. A level of 44 mg per kilogram of counter flour is suggested. Ferrous sulphate will be used.

The project will be self-sustaining in that Caribbean Agro Industries Ltd. has agreed to continue the fortification of wheat flour with B-complex vitamins and iron after the initial project has been completed.

III. OBJECTIVES OF THE STUDY

The general aim of the project is to significantly reduce iron deficiency in the entire Grenadian population through wheat flour fortification. To this end we will need to have all appropriate monitoring devices in place so that the benefits of the improved fortification can be observed. Such benefits may relate not only to health effects but also to worker productivity and academic achievement. Measures in the latter areas will of necessity be crude but their importance is such that they cannot be overlooked. Additionally, it will be necessary to look more closely at the high risk group: antenatals living in depressed areas.

Specific Objectives

1. To evaluate the short term impact of the change in fortification on iron status, general health and productivity of the entire population by:
   a) A survey of households which are a representative of the entire population.
      1) Hematology
      2) Food Consumption patterns
      3) Socio-economic parameters
   b) Analysis of Government statistics.
      1) Health Parameters
      2) Productivity
      3) Educational achievement

2. To evaluate the impact of the change in fortification on the detailed hematology of pregnant women attending clinics in depressed areas.
   1) Hematology
   2) Socio economic parameters
3. To improve the country wide surveillance system for monitoring anaemia and its effects. This system will have the following features:
   a) It will identify appropriate indices of productivity that are routinely part of Government statistics.
   b) It will identify appropriate health indicators that are or should be a part of Government statistics.
   c) It will ensure that appropriate observations are taken in the hospitals and clinics and transmitted to the health authorities.
   d) It will include a sub-system for the quality control of wheat flour through random sampling in food retail outlets activities and bakeries.

IV. METHODOLOGY

IV.1 Impact of Iron Fortification

The evaluation of the impact of the change of iron fortification of wheat flour derives from several sources. The first is the improved iron status of a random sample of households and after the institution of the change in fortification. The second relates to the measurement of maternal mortality and morbidity. The third

relates to productivity and academic attainment data collected by the establishment of an improved surveillance system. These changes will be evaluated for three years and some of the parameters will be continually measured and evaluated after the initial three year period of the project.

Household Survey

The population of Grenada is approximately 97,495 persons. A 1.02 percent sample will be 1,000 persons. If we assume approximately four persons per family, 250 families will be chosen for the study. However, to allow for families moving during the study 300 families will be chosen from a random sample of households chosen by enumeration districts (ED's) from the entire population. A single well-structured pre-tested questionnaire will be administered to the same households at the start of the project. The questionnaire will be administered by trained interviewers. It will focus on age, sex and employment status of household members and purchasing and consumption patterns.

The questionnaire will contain questions regarding the length of time flour is held at home before consumption. This would be important to ascertain whether the fortified flour is used within the designated shelf life. Qualitative food consumption data will be collected on each of the households. This will be done by asking questions on which food items are eaten rarely, once or twice weekly, three to seven times per week, or more than once per day. This should include some measurement of meat, fish, poultry, legumes, coffee, tea and citrus fruits and or other vitamin C rich foods. A larder survey may be conducted on a sub-sample of households.

A finger prick blood sample will be taken and the haemoglobin, plasma ferritin, and the transferrin receptor levels would be determined from each family member except pre-school age children under six months of age.

The same questionnaire including information on food consumption and finger prick blood samples will be collected at the eighteenth and thirty-six month on the same households.

Pregnant Women

The Government of Grenada has recently completed a study identifying Economically Depressed Communities in Grenada. A sample of 200 pregnant women that are attending antenatal clinics will be chosen from these depressed communities (See Annex 4). Only those pregnant women who enter the clinics at 16-20 weeks of pregnancy will be studied. Any woman with sickle cell disease will be excluded from the study. A questionnaire will be administered which will include name, age, and gestational period.

A blood sample will be taken and a hemoglobin, plasma ferritin and plasma transferrin receptor level determined. The blood samples will be taken at the beginning of the study and will be repeated at 18 and 36 months on ante-natals attending the same ante-natal clinics.

Other Parameters

The hospital system consists of main multidisciplinary hospital (General Hospital) in the capital (240 beds), a smaller general hospital in a rural area (40 beds) and a general hospital in Carriacou (32 beds).

Information will be collected on a yearly basis on mortality due to anaemia. Also in each of the three hospitals the number of patients admitted with a diagnosis

of anaemia will be recorded. The cost of treating these anaemic patients particularly patients admitted who are pregnant, will be recorded. This will include the cost of maintaining the bed, blood transfusions, etc. This information will be collected on a yearly basis.

Information will also be collected on a yearly basis to include passes in the CXC examination, low weight per age of pre-school age children, infant mortality rate, banana production, cocoa production, nutmeg production and gross national product.

V. ANALYSIS

V.1 Laboratory

The blood samples will be analysed at the Clinical Laboratory in Grenada and the Iron Nutrition Monitoring Laboratory at CFNI, Jamaica. The Department of Haematology, Kansas Medical Centre, Kansas City, U.S.A., will serve as a reference laboratory. A finger prick sample of blood will be collected from each person at the start. The sample will be taken in a plastic disposable blood collection device by using a disposable spring loader lancet. Twenty micro-litres of blood will be taken by Hemocue micro-cuvette and the micro-cuvette put into the Hemocue Photometer. The haemoglobin will be determined by the Hemocue method [20]. The whole blood sample will be kept cold and sent to the Central Hospital Laboratory, where it will be spun at 2,000 r.p.m. for 10 minutes. The plasma will be removed and frozen. The frozen plasma samples will be hand-carried to CFNI, Jamaica.

The plasma ferritin and the transferrin receptor level samples will be determined by the ELISA [21-22].

V.2 Statistical Analysis

1. Simple graphical analyses of trends in various Government statistics for productivity, educational attainment and health. These will include analysis of retrospective data where possible.
2. Simple graphical analysis of trends in various haematological measures as applied to the various sub population groups:

   0-5 years, 6-4 (M), 6-14 (F), 15-44 (M),

   45 and over (M), 45 and over (F), antenatals.

   This will be supplemented by ANOVAs to compare measures before and after the change in fortification.
3. An appropriate technique for statistical analysis of quality control data (flour) is yet to be worked out.
4. Data on flour consumption will be related to haematological parameters using correlational techniques.

VI. HEMATOLOGY

Estimating the severity of mild iron deficiency assumes greater importance in population studies. Earlier surveys relied on single cut-off levels of laboratory parameters to distinguish normal from iron-deficient segments of a population. In the evaluation of the Health and Nutrition Examination Survey (HANES II) in the United States, combinations of measurements were used to define prevalence of impaired

iron status or iron deficiency anaemia. (23) Another recent approach is to use algorithms to estimate body iron quantitatively in each sampled individual. (24) Because of the close correspondence between the serum ferritin and body iron stores, this calculation is reliable in otherwise healthy individuals if residual iron stores are present. At the other extreme in patients with overt anaemia, the deficit in body iron can be estimated quantitatively from the decrease in circulating haemoglobin concentration. Between these limits, estimating mild deficits in functional iron is more difficult. In previous report, combinations of the serum ferritin, transferrin saturation, and erythrocyte protoporphyrin were used to estimate body iron between the occurrence of storage depletion and the development of anaemia (24).

A recent study has shown that the serum reception level is a reliable index of early tissue iron deficiency. Of all the measurements examined, the serum receptor was the most reliable quantitative guide to a deficiency in the functional iron compartment. Therefore, the iron status of a population could be fully assessed by using serum ferritin as a measure of iron status, serum receptor as a measure of mild tissue iron deficiency, and haemoglobin concentration as a measure of advanced iron deficiency (25).

This could be a major methodological breakthrough. This will be the first time that the receptor level has been used on a national scale. Hopefully such a sensitive indicator as the receptor level could be shown to reduce the time necessary to evaluate an iron nutrition project.

The haemoglobin, ferritin and receptor levels can be estimated on a finger prick sample of blood which will greatly facilitate the conduct of the study. Also all these measurements will be conducted in Grenada and Jamaica and will be the first time that these two sensitive hematological indicators will be used in developing countries.

VII. SURVEILLANCE

A surveillance system will be designed and left in place after the fortification of counter flour has been evaluated. The present surveillance system consists of estimating the haemoglobin level on pregnant women. This system will continue. From 1985-1988 hemoglobin levels were collected on pre-school age children but was discontinued because of a lack of hemoglobinometers. Data will continually be collected on maternal mortality and morbidity, low birth weight infants, weight per age on infants and pre-school age children, etc. Quality control will be conducted on both counter and baking flour by Caribbean Agro Industries and the Produce Chemist Laboratory. (See
Section X. Quality Assurance).

VIII. ECONOMIC JUSTIFICATION OF FORTIFICATION

The end results in food fortification programmes are the improvement in iron status of the target population, increased productivity and reduced adverse consequences arising from poor iron status. Food fortification is justified when these positive attributes could be traced to the programme.

Approximately 10% of iron contained in wheat flour is absorbed. Thus 10% of the known level of iron added per unit wheat flour consumed is expected to enhance the iron status which in turn would enhance work output. The literature suggests worker output of between one and two percent is associated with a one percent

increase in the Hb status. Among populations with severe iron deficiency anaemia, supplementation has resulted in increases in Hb status of 25 percent and over (26). Under such conditions, worker productivity could increase 25-50%.

A series of economically unquantified adverse consequences are associated with poor iron status. These include maternal mortality and morbidity, poor growth and development, impaired immune system, reduced learning capacity and lassitude.

At the rate of 44 mg per kg of iron added to the wheat flour and an average per capita consumption of 0.15 kg flour per person per day, 0.66 mg or iron is expected to be absorbed. This represents 66% of the iron retention requirement of adult males and post-menopausal females. Since approximately 55% of the flour consumed is fortified at 37 mg/kg with iron whose bio-availability is suspect, an additional contribution of less than 33% is anticipated from the change. This additional contribution from a single fortified food source could have a significant impact on the haemoglobin status and thus on worker productivity. The cost of the supplementation estimated at $0.90 per tonne is negligible while the benefits could be great. Levin reports studies in which the benefit cost ratios of iron fortification were in the order of 5-8 in Indonesia, 30-47 in Kenya and 49-79 in Mexico (26). The fortification of wheat flour in Grenada is intuitively justifiable. This study will test the economic justification and other benefits of the proposed changes.

IX. MONITORING

The importance of establishing the efficiency of an iron fortification programme cannot be over-emphasized. It is seldom possible to measure the impact of a fortification programme once it is implemented, and this is particularly true with respect to iron fortification. In contrast to fortification with nutrients such as niacin, thiamine, or riboflavin, where the objective is to prevent the occurrence of isolated deficiencies, it is feasible to alter body iron stores of an entire population through dietary manipulation.

The customary approach to monitoring efficiency is to conduct a pilot study, but there are several drawbacks to this. During a preliminary trial, the supply of a fortified food is usually ensured whereas, this is not necessarily the case when implemented on a national scale. Another important limitation of preliminary iron trial is that because of the inhibiting effect of the diet on iron absorption, changes in iron status in a population produced by iron fortification occur very slowly, often only after several years. Pilot studies, therefore, delay the implementation of a fortification programme and are very costly because of the long duration of the trial.

It is therefore planned to start the study on a national scale and carefully monitor the population by using very sensitive haematological indices.

X. QUALITY ASSURANCE

During stage one and throughout an iron fortification programme, quality assurance activities must be carried out to verify the iron content of the fortified

flours. Sampling and testing for iron content and sorting out effect should be carried out routinely at the mills during the production of the products. The in-plant quality testing should be the responsibility of and carried out by the companies, since they have the laboratory facilities and good technical personnel with which to do this. This will be conducted by Caribbean Agro Industries Ltd.

Samples of products should be drawn periodically from the market shelf by designated government representatives and analyzed for iron content. This is needed to verify both the presence of added iron and in the proper amount. These samples will be analyzed at the Produce Chemist Laboratory, St. George's, Grenada. This level of quality assurance activity is necessary to ensure that there is a given level of iron intake each day based on expected consumption. A successful intervention can be accomplished only when the iron is present in the food on a continuing basis in the correct amount. Anything less than this could lead to unsatisfactory results due to lack of proper control rather than a failure of the intervention itself.

XI. SAFETY OF FORTIFICATION

The possible adverse effect of adding large amounts of iron to the diet in certain segments of a population has been discussed from time to time and deserves comment. In reviewing studies in which possible adverse effects of iron have been reported, it is important to define the route of iron administration. When large doses

of Imferon (iron dextran) were given by deep subcutaneous injection to new born infants, an increased frequency or fatal septicemia was noted, but it was not clear, whether this was due to iron or due to the intramuscular injection. There is some published evidence that the frequency of malaria may increase when full doses of therapeutic oral iron are administered to a susceptible population.

On the other hand, it must be stressed that no adverse effects have yet been demonstrated when conventional levels of iron fortification have been implemented in a population. Homozygotes for the iron loading gene, i.e. **idiopathic hemochromatosis**, have been demonstrated to occur in 1:300-500 American whites but this genetic disorder has never been clearly identified in a black individual. Countries such as Sweden are continuing to fortify the diet at a level that provides approximately 40% of dietary iron intake without demonstrable adverse effects. Therefore, there seems to be little basis for concerns for a fortification programme that will double the intake of dietary iron even in iron replete individuals. Nevertheless, because of the lack of information on the maximal consumption of wheat flour by older children and adults, it is desirable to establish consumption patterns of this food vehicle throughout the population.

XII. TRAINING

Preliminary discussions have taken place for training members of the Grenadian Food and Nutrition Council. This training will probably consist of the Systems

Analyst, U.W.I., Mona, Jamaica, in developing a training course for the computer analyst and members of the council attending training courses in the U.S.A. on Training for Project Managers. The CFNI Public Health Nutritionist will train all Public Health Nurses on the use of the Hemocue for determining the haemoglobin level and the latest techniques for taking blood samples.

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APPENDIX B-5

ADHERENCE TO IRON SUPPLEMENTATION DURING PREGNANCY: DETERMINANTS AND EFFECT ON HEMATOLOGIC VARIABLES.

A research proposal.

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September 1991

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I. BACKGROUND

Iron deficiency anemia among pregnant women has for a long time been recognized as a major public health problem. This is particularly true for developing countries where anemia makes a large contribution to pregnancy complications and maternal mortality (ACC/SCN 1991, WHO 1979, Fleming et al 1986).

It is quite clear from a number of studies that supplementation of elemental iron during pregnancy is a possible means to prevent a fall in hemoglobin levels and decrease the prevalence of anemia (Simmons 1990, Charoenlarp et al 1988, Fleming et al 1986) So far, most efforts to solve the anemia problem has focused on identifying appropriate dose, iron compound and formulation to ensure a positive change in the blood parameters. However, when attempts have been made to apply these clinical findings in public health programmes results have been less successful. Apart from logistic problems with distribution of the drug, a major reason for this is suggested to be non-compliance, or preferably non-adherence, to the iron regimen on part of the pregnant woman (WHO 1990).

Although adherence is a prerequisite for a successful iron supplementation programme limited research has been done on the determinants and the magnitude of the impact the constraining factors have. Therefore, this study will focus on the adherence aspect of iron supplementation, and in particular on impact of side-effects. However, measurements of hematologic outcome will also be included to assess if a variation in adherence is of importance for anemia prevalence.

II. CONCEPTUAL MODEL

A. Theoretical basis

The importance of using theoretical models in studying health behavior, like adherence to prescribed medication has been stressed (Christensen 1978, Leventhal 1985). Application of a theoretical model assists the researcher in understanding how predictive variables of adherence may interact and alter their respective impact on adherence. Use of models further facilitate comparisons to be made between studies in different settings.

Although there have been attempts to combine the numerous models that describe health related behavior (Cumming et al 1980) still the most frequently used model, in particular in studies of adherence, is the "Health Belief Model" (HBM). The model is a psychosocial model and as such it is limited to accounting for as much of the variance in the individuals' behavior as can be explained by their attitudes and beliefs (Janz and Becker 1984). It was originally developed by Rosenstock 1966 and further modified by Becker and Maiman 1975, Becker et al 1979, and Rosenstock et al 1988). The basic variables remain the same and the theory argues that whether a person will comply to

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recommended health behavior depends on the individual's: 1) perceived susceptibility of the disease or condition, 2) the perceived severity of the condition if contracted, 3) perceived benefits of the treatment recommended and 4) perceived difficulties or barriers to the recommended behavior.

The conceptual model (fig 1.) for this study has incorporated these attitude and belief variables of the HBM mentioned above but also included two other variables, self-efficacy and social support. Self-efficacy is the individual's assessment of her ability to follow the recommended behavour, in this case take the iron supplement. Although these variables fit conceptually in the HBM they have not generally been elaborated (Janz and Becker 1984). However, more recent studies of health related behavior tend to discuss and include these aspects more in depth (Becker 1985, Kelly et al 1991 and Rosenstock et al 1988)

The patient-doctor/provider interaction is another aspect discussed in health behavior studies (DiNicola and DiMatteo 1984, Hall et al 1988, Hulka 1979). In the present study, only one health center is to be included and the provider of the iron supplement will be the same for all subjects. Thus little variation in the interaction is expected, in particular since essential parts of the interaction, the information component, will be standardized. Because of this, only one aspect of the interaction, client's understanding of the regimen, will be included in the study.

B. Focus of study:

The focus of the intervention part of the study is the impact of side-effects on adherence. As a means to create a variation in the prevalence of side-effects, but also for evaluative purposes a new type of iron preparation will be compared with conventional iron supplements. The new iron supplement contain ordinary ferrous sulphate but combined in a matrix of hydrocolloid which in contact with gastric juices dissolve and form a mix which will float on top of the stomach content. The matrix is slowly disintegrated delivering the iron compound over an extended period of time to the upper part of the intestine. The gastric delivery system (GDS) has the advantages of giving less side-effects at the same time as it has a better absorption than both the conventional iron preparations and the so called slow-release types (Cook et al 1990, Simmons 1990).

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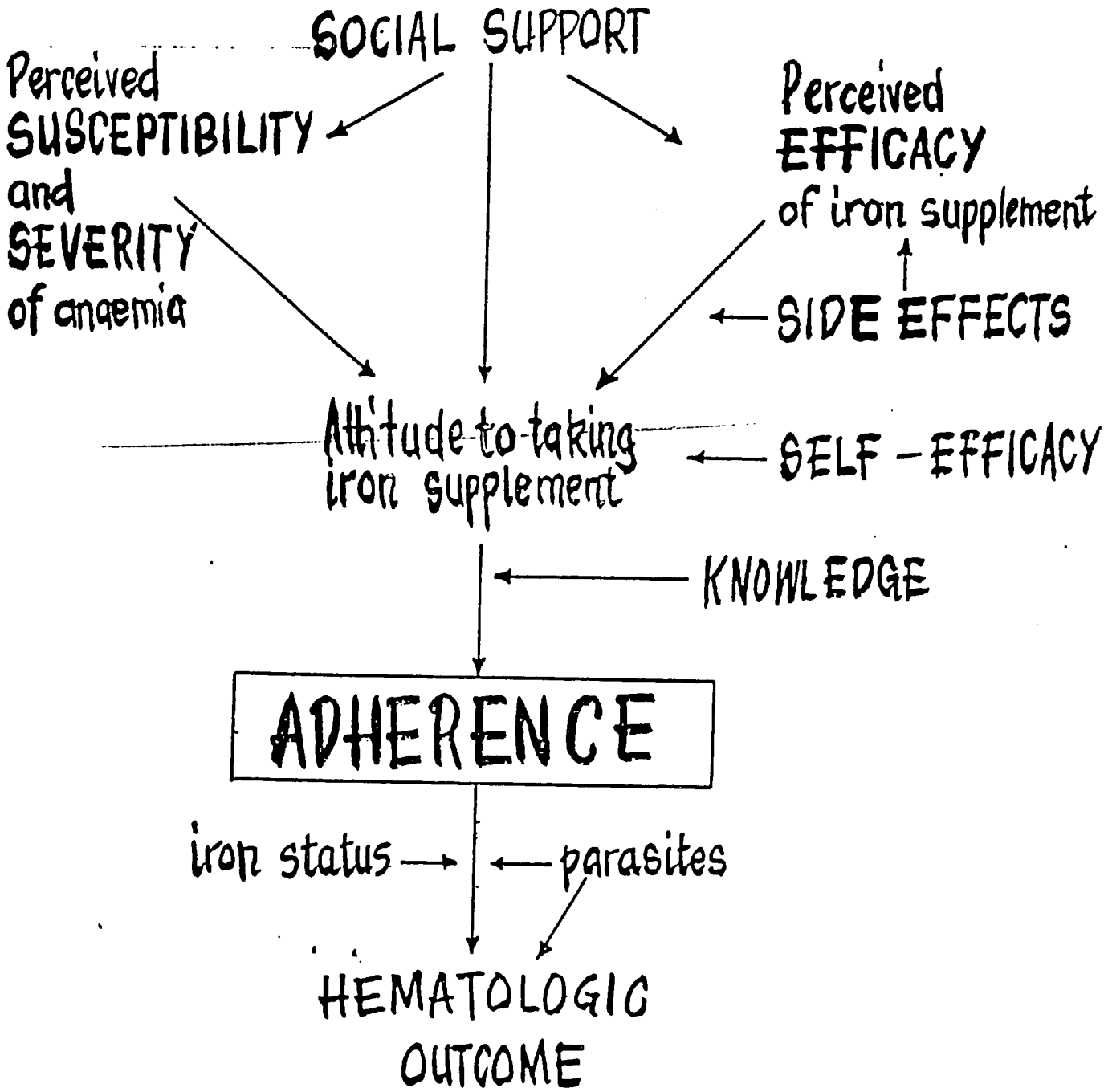
Figure 1 Conceptual framework



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III. OBJECTIVES

The overall objectives for the study are:

- Study patterns of adherence to iron supplementation and factors that determine it, focusing on importance of side-effects.

- Make a cost-effectiveness analysis of the usefulness of GDS iron supplement as a means to increase adherence and improve hematologic outcome.

IV. PREVIOUS STUDIES

Limited reliable information exists on levels of adherence to iron supplementation. This is probably partly due to difficulties in measuring adherence. The most applied techniques have been, pill count, recall by user, and biologic marker. All these techniques have their shortcomings. Pill count method may have an effect on adherence, pills may be kept in other containers, and pills may be dumped before counting episode. Recall by user have a tendency to be an overestimate of adherence and biologic marker is a costly method which also pose the problem of individual variation in the biologic measures even under conditions of perfect adherence. Neither does spot check measures represent long-term adherence (Becker and Rosenstock 1984, Cramer et al 1990, Feinstein 1990, Rudd et al 1989).

A new technique is now available consisting of a small microprocessor imbedded in an ordinary pill bottle cap which will record each time the pill bottle is opened (Cramer et al 1989, Rudd et al 1990). Use of this equipment may overcome many of the previous measurement problems (except cost!), and it is now possible to monitor adherence in a better way.

One of the major reasson suggested for poor adherence is the gastro-intestinal side-effects of the treatment (Afifi et al 1966, and DeMayer 1989). The prevalence of side-effects depends on dose, type of iron compound, dosage scheme, if it is taken with meal or not, and it also has a geographical variation (Bonnar et al 1969, Charoenlarp et al 1988, Cook et al 1990, Hallberg et al 1966, Hallberg and Sölvell 1966, Sjöstedt et al 1977). However, if present, side-effects will decrease if supplementation is continued and after about a week they will be significant reduced (Charoenlarp et al 1988, Cook et al 1990). suggesting that if side-effects have an impact on adherence it is of most importance in early stages of supplementation.

Limited information on side-effects' impact on adherence can be extracted from literature. In an iron supplementation trial in northeastern Thailand where the side-effects were high the subjects were persuaded by field personnel to continue taking the supplements (Charoenlarp et al 1988) and the high adherence observed may thus not be indicative of what would have happened outside the trial. In the same study, an unsupervised group receiving 240 mg elemental iron was compared to a supervised one.

Poor results in the unsupervised group could possibly be due to side-effects and decreased adherence as suggested by the authors (Charoenlarp et al 1988). In a recent iron supplementation trial in Jamaica where the side-effect prevalence, (depending on symptom) varied between 4-27 percent (Simmons 1990) adherence to supplement is also likely to have been affected by close supervision of field personnel. Furthermore, the measurements of adherence was reported as being unreliable (Simmons 1990).

Two studies have reported a relation between level of adherence and hemoglobin level at term of pregnancy. In a supplementation trial in Jerusalem self-reported level of adherence was divided into three groups; "good" "not so good" and "poor compliance". Although there was no significant difference in hemoglobin and hematocrit level in third trimester between the three groups there was a slight trend of better hematologic values for the "good" group followed by the "not so good" (Gofin et al 1989).

Another study in England, the subjects were divided into two groups depending on a hemoglobin level above or under 12 g/dl at term of pregnancy. Throughout pregnancy a number of stool samples had been taken and tested for iron as a measure of adherence to supplementation. The low hemoglobin level group had significantly lower number of positive tests, 25 percent compared to 86 percent in the high hemoglobin level group (Bonnar et al 1966).

Even though there are no conclusive results on the negative impact of side-effect on adherence, efforts have been made to develop iron preparations with less frequent side-effects. The so called "slow-release" preparations where the iron compound is released gradually on its passage through the intestine tried to meet this demand. Apart from being very expensive the "slow-release" preparations have had a reputation of poor absorption (Cook et al 1990).

The new gastric delivery system (GDS) preparation where the iron compound is released during an extended time period in the stomach does not have the problem of low absorption. On the contrary it has shown a 3-4-fold higher absorption compared to conventional iron preparations (Cook et al 1990). Since the ingredients of the formulation are not expensive it may be that the cost of GDS capsules could be competitive with conventional iron tablets (Simmons 1990).

Women's health beliefs may also affect adherence. A number of specific beliefs have been reported to have a negative impact. These include form of preparation of a medication (tablets, liquid, injections) where one of these can be regarded as more effective in general, or not compatible with the pregnant condition. Frequently it is mentioned that color of the iron supplement may play an important role. In case anemia is perceived as a weakness of the blood it may be favorable to have red iron tablet. Taste of the iron supplement may also play a role (WHO 1990, Galloway 1990).

A common worry among pregnant women is that they will have bic babies which will cause difficult deliveries. If the iron tablets is believed to make the fetus big and strong which has beer reported it is likely to have a negative effect on use of the supplement (WHO 1990).

V. RELEVANCE OF STUDY

In the overall efforts to reduce the high maternal mortality in developing countries one of the recognized strategies is to prevent iron deficiency anemia. It is established that iron supplementation during pregnancy has a positive effect on hemoglobin level, - if the supplement is taken as prescribed.

Although logistic problems of distribution of the iron supplement have sometimes been recognized as the major problem for adherence (ACC/SCN 1991) it is not necessarily the case that the supplement would be taken as prescribed if available. To ensure, once defaulting distribution systems are working, that the iron supplement provided will be effective it is essential to get a better understanding on adherence and its determinants.

Due to difficulties in measuring adherence limited reliable information is available on adherence level itself or on the magnitude of the impact of the factors that determine it. This is also true for the possible negative influence which side-effects may have.

As cultural factors play an important role in adherence it may be limitations for the extent to which generalizations can be made, even so it is still felt as important to investigate if reducing side-effects can be a possible strategy to improve adherence in a more limited setting. With the now available measurement technique it is possible to get valid information on adherence and in the case side-effects are a problem, the recently developed GDS formulation may be provide some remedy. A cost-effectiveness analysis will provide basis for decisions to substitute conventional iron preparations for GDS ones.

Regarding the study component of health beliefs and attitudes the application of a theoretical base in the conceptual framework will provide a more comprehensive picture of the determinants of adherence where the variables will have an explanatory capacity. Many studies do lack that quality and merely produce lists of descriptive variables that statistically do or don't correlate with adherence.

One could argue that even if the variables have an explanatory capacity the information is not useful unless it can be included in programme activities to improve adherence. Although there are no studies on adherence to iron supplementation that have used a theoretical base which can show this usefulness, the HBM has successfully been used for identifying intervention points for inducing behavioral change in other contexts (Becker et al 1979, Jones et al 1988).

VI. MAGNITUDE OF THE PROBLEM AND STUDY AREA

A. Nutritional anemia during pregnancy in Tanzania

Anemia during pregnancy is a major health problem in Tanzania. It is one of the most common reason for antenatal hospital admission in Dar es Salaam region and it is also a leading factors for maternal mortality (Kavishe 1982). A study at Muhimbili Medical Centre showed that in early 1988, 26 of 71 (37%) maternal deaths could be attributed to anemia (Kaisi 1988). A large stûdy of anemia during pregnancy was conducted in 1977. A total of 1317 women were evaluated when they came for their first antenatal clinic visit. The mean hemoglobin level was 9.3 gm% and none of the women had a hemoglobin level above the recommended cut-off of 11 gm% (Mwanukuzi E and Nhonoli AM 1972).

The major causes of anemia during pregnancy are malaria and iron deficiency. However there is limited information on their relative contribution to anemia. It is depending on malaria prevalence and thus on region and season. A recent study in Zanzibar (TFNC 1991) showed a prevalence of 41.2 of anemia in women using WHO cut-off of <12 g/dl for non-pregnant and <11g/dl for pregnant women. The same study demonstrated that 29.2 of the women were iron deficient as defined by a free erythrocyte protoporphyrin level above 70 microgram/dl of red blood cells. Anemia and iron deficiency showed a statistical significant correlation while malaria (prevalence 8.1 %) did not in this particular study. Although there is a need of more comprehensive information on anemia in Tanzania it is beyond all doubts that it is a major problem that need to be tended to.

B. Study site.

The study is proposed for Ilula villages in Iringa region about 45 km from Iringa town. The villages lie on the main road Dar es Salaam to the Zambian boarder. The census for the villages in 1978 was 5657. Health care is provided by the units, one Roman Catholic dispensary and a Lutheran dispensary and this study will have the Lutheran dispensary as a basis. From previous studies in the area it is reported that antenatal coverage is high but with a big variation in gestational age at register (mean 23, range 9-35 weeks) and number of visits to the clinic (mean 6.3 and range 0-12) (Möller et al 1989).

Ilula villages are comparable with other rural villages in Tanzania although they probably are somewhat more developed due to the closeness of the road. Still there is not electricity.

Although no quantitative information on anemia is available from Ilula anemia is assessed by the medical assistant at the MCH to be very common (Ballart 1991). In the previous study in the area anemia was noted as the most common pregnancy complication (Kavishe et al 1987).

VII. STUDY DESIGN

A. Introduction

The study is divided into three phases. The first phase include an assessment of the coverage of the antenatal clinic in Ilula base-line information on hemoglobin distribution near term o pregnancy and a sociologic study on beliefs of health and illnes during pregnancy as part of preparation of draft questionnaires.

The second phase is a pretest of the developed questionnaire a well as a test of the intervention components and the methodolog for their assessment, in particular the measurement of adherence. The last phase is the actual study where all the proposed factor affecting adherence will be measured as well as adherence itsel and hematologic outcome.

B. Phase I. October - November 1991

1. specific objectives

The specific objectives for phase 1 are:

- To assess coverage of pregnant women by the antenatal clinic in Ilula.
- To assess hemoglobin distribution near term of pregnancy in an unsupplemented population
- To describe common health beliefs related to pregnancy.
- To develop draft questionnaires.

2. Coverage of pregnant women by the MCH clinic.

In the villages to be included in the main study all household where a child has been born the last two months will be visited. The household to be visited will be identified by ten-cell leaders who knows the household composition and whether a child has been born in his ten-cell the last three months. At the visits information on mother's attendance at MCH clinic, such as number of visits, gestational age at registration and reason for participation or non-participation will be collected. The aim is to find out how large proportion of the pregnancies in the area are covered by the MCH clinic. It is expected that approximately 100 mother-newborn pairs will be interviewed.

A subgroup of this study population will be used for the study of health beliefs related to pregnancy.

3. Base-line information on hemoglobin distribution near term of pregnancy in an unsupplemented group.

According to present information there has not been any iron supplement available in Ilula health center for a long time. Before starting the distribution of iron supplement a base-line assessment of hemoglobin levels near term of pregnancy is going to be conducted. The possibility of using this information as a quasi control group in comparisons of hematologic outcome of iron

supplementation is going to be investigated. In order to do so background data on the subjects like socio-economic status, reproductive history, recalled morbidity and present malaria will be collected. Hemoglobin will be assessed by finger-prick and use of hemocue hemoglobinometer. Blood samples will be spotted on filter paper for later assessment of ferritin and erythroprotoporhyrin (see also phase III hematology).

Collection of information for two months is expected to provide a sample of approximately 100.

4. Description of common health belief during pregnancy and development of questionnaires.

(i) Objectives and study population

The study population for this section will be a subgroup of the subjects for the coverage study which are mothers with new born babies. Interviews will also be made with health personnel as well as traditional birth attendants.

The objectives of the study are

a) to describe general health beliefs and practices common during pregnancy.

b) identify a local concept of anemia which overlaps with modern medicine's definition.

This concept will be used in developing a cultural acceptable message related to the benefits of iron supplementation and in the intervention phase of the study for assessment of the perceived susceptibility and severity to anemia as well as other occasion when referring to anemia.

c) to create a list of illnesses and symptoms common during pregnancy.

For the main study, the most frequent side-effects of iron supplement will be included in the list which will act as an instrument to measure side-effects. The list will also be used for assessing symptoms of anemia which could trigger adherence to supplement.

d) investigate if there is a concept of preventive care which include medication of some sort.

If existing, parallels could be drawn with this concept in explanations of the function of iron supplement.

e) develop draft questionnaires

(ii) Methods

The specific methods for, in a structured way, collect

information of qualitative nature (like health beliefs) have been described by Bernard 1988 and Weller & Romney 1988 among others. The sequence or rather circular way of applying them has been proposed by Winch 1991.

The methods to be applied are a) identification of domain of interest, b) free listing c) charts d) pile sorts e) paired comparisons, and indicator questions, which are common methods applied in social science.

A brief description of these methods;

Identification of a domain is defining the area of interest in which the questions should focus on. For this research it is ilnesses or problems during pregnancy. Free listing is developement of a list of the most common problems and chart is a way to structure the cause, treatment and symptoms of each of the illnesses. Through pile sorting it is possible to find out which illnesses are related to each other and paired comparisons can be used as a tool for ranking how important or serious different illnesses are perceived to be. Indicator questions or statements are commonly used in assessment of attitudes.

A base-line questionnaire for assessment of household characteristics, including household composition, socio-economic status, and major means of living will be produced as well as one for the pregnant woman's reproductive history and obstetric information. A crucial variable in the study is gestational age. A previous study in the area showed that using last menstrual period gave a valid assessment of duration of pregnancy (Möller 1989). Even so fundus height will also be measured as a complementary method.

C. Phase II. Pilot test of questionnaires and outcome measures. December 1991-February 1992.

1. Specific objectives.

The specific objectives of phase 2 are;

- Pre-test of questionnaires.
- Pre-test of methodologies and procedures for assessing adherence and hematologic outcome.

2. Methodology.

Phase 2 involves a testing of all variables to be measured in the main study. For a period of three months data will be collected according to the schedule set for the main study. This involves a pretest of the questionnaires developed in phase 1 for the assessment of a) household characteristics; b) variables on health beliefs and attitudes in conceptual model and c) test of methods for assessing adherence and hematologic outcome.

Apart from testing the actual assessment instrument for the various variables this phase is a practical preparation for management of data collection in the main study.

3. Measures of adherence and hematologic outcome

(i) Hematology

Because of difficulties in handling venous blood samples under the existing field condition the hematologic measurements selected have been limited to those which can be done with capillary blood and do not need to be centrifuged or stored in a freezer. Capillary blood will be collected by finger prick and all samples will be taken by the same person. The measurements are: hemoglobin, free erythrocyte protoporphyrin (FEP), and sickle cell test.

Hemoglobin level will be assessed by Hemocue system which use disposable microcuvettes. The technique has been compared with standard laboratory hemoglobin methods and good reliability and accuracy has been found (Bridges et al 1987). The method is particular convenient for field conditions as it does not require any sample or reagent preparation and can be run by use of a battery.

FEP will be assessed by a micro-flourometric method (Orfanos et al 1977). The method needs a small amount of blood 2-3 drops which can be spotted on a filter paper and stored protected from sunlight for analysis later in the laboratory.

Sickle cell will assessed by microscopy.

At Kansas university a method is under development for assessment of ferritin in whole blood spotted on a filter paper. Samples will be collected in this study for the possibility of later assessment of ferritin.

Blood slides for assessment of malaria will be prepared.

(ii) Adherence

This study intend to use a new equipment for measuring adherence to medical treatment "Medication Event Monitor System" MEMS (Cramer et al 1989). It is a small microprocessor imbedded in a pill bottle cap. The microprocessor register each time and date the pill bottle is opened. Information about 1000 medication events can be stored for later retrieval by use of the computer software developed together with the microprocessor. This equipment allow detailed information about adherence to be collected without the knowledge from the pregnant women which decrease the risk of altering the adherence behavior due to the study intervention. The assumption is that every time the pill bottle is opened the tablet is actually taken.

The microprocessor method of measuring adherence will be used during the first month of supplementation where there is a need

of accurate information on a daily basis. As the possibility exist that the woman will open the pill bottle regularly and through away the tablet this method will be validated by testing for iron in stool samples (Afifi et al 1966).

For the follow-up of adherence assessment (week 4, 8 and 12 weeks) the method using stool samples will be used.

D. Phase III. Intervention study, March 1992- March 1993.

1. Specific objectives

The specific objectives for the intervention part of the study are the following;

- To assess level of initial adherence to iron supplements.
- Study patterns of adherence and its determinants.
- Test if use of gastric delivery system (GDS) iron preparation with reported lower frequency of side-effects has an improved effect on adherence compared to conventional preparations.
- Compare cost-effectiveness of conventional and GDS iron preparation on adherence.
- Compare effectiveness in improving hematological parameters related to anemia (in particular hemoglobin) between conventional and GDS iron preparations.
- Compare cost-effectiveness of conventional and GDS iron preparations on hemoglobin level.
- Test the explanatory capacity of the components in the health belief model on variation in adherence.

2. Study population and sample size

Of all women registering for MCH clinic at Ilula dispensary women with a gestational age less than 27 weeks will be offered to participate in the study and their consent to do so will be sought. Based on the health center's routine women with hemoglobin less than 8.5 g/dl will be excluded from the study and referred to Iringa hospital. Women with, or developing pregnancy complications such as: requiring blood transfusion; emergency obstetric intervention; eclampsia; or chronic diseases will also be excluded from the study.

The following calculations were made to estimate the number of women it may be possible to enroll per month. Based on information from the health center approximately 50 children are born at the clinic per month. If 80 percent of these have participated in MCH clinic before delivery there will be 40 children per month. Of these, assume 75 percent have participated before week 27 in pregnancy which lead to 30 pregnant women per month who can be offered to participate. If 80 percent accept to participate about 25 women per month can be entered in the study.

The proposed sample size is 250 women which will require a data collection of 13 months. A sample size of this magnitude will enable a difference in adherence proportion between the two iron

preparation of 20 percent to be detected and a difference in hemoglobin level of 0.35 g/dl to be identified. A difference of less than 0.5 g/dl is probably of little biologic importance (INACG 1984).

3. Treatment groups

Initially all women that agree to participate in the study will be treated for hookworm infestation with a single dose of Albendazole 400 mg.

The women will randomly be assigned to receive either conventional or GDS iron preparation. The conventional ferrous sulphate preparation will provide a dose of 120 mg elemental iron as recommended by WHO 1989. The GDS ferrous sulphate has shown to have a 3-4-fold higher absorption rate than ferrous sulphate elixir (Cook et al 1990), and a dose of 50 mg elemental iron in form of GDS capsules has proven, in an anemic (8-11 g/dl of hemoglobin) population, to give the same or better hematologic response than 100 mg elemental iron of conventional type (Simmons 1990, ACC/SCN 1991). The elemental iron dose for GDS preparation will thus be 50 mg per day.

The dose of conventional ferrous sulphate can be taken once a day or as two administrations of 60 mg each. To reduce the frequency of side-effects it is generally recommended to take the dose in two administrations which will be applied in this study. The GDS iron supplement is to be taken once a day with a meal which in this study will be the evening meal.

Since the positive effects of iron supplement is established in an anemic population it is not ethical to include a placebo or control group without iron in the present study.

However, designing a study without a placebo group create some problems. One of these is difficulties in evaluating the side-effects as there are a number of placebo side-effects. Neither is it possible to assess the absolute effect of iron supplementation.

However if, which is likely, there will be a non-compliant group in the study it may to certain extent be possible to use this group as a quasi-experimental control group. In interpretation of the results considerations have of course to be made to the fact that the group is a self selected one and not randomly assigned. Still the use of this group as a control group may be acceptable, in particular if it is possible to statistically show that it, regarding essential variables, is comparable with the rest of the study population. Suggestions for when it may be appropriate to use non-adherer as a control group is outlined in the analysis section. Furthermore, the base-line information of distribution of hemoglobin near term of pregnancy may be used as another quasi-control group.

4. Data collection frame

For each of the recruited subjects data will be collected at registration (week 0 of supplementation) and at week 2, 4, 8, and 12 of supplementation. The information that will be collected at these occasions are:

Registration:
- base-line information
- illnesses
- determinants of adherence
- hematology (incl malaria)

Week 2 of supplementation:
- illness and side-effects by 2 weeks recall
- adherence by microprocessor

Week 4 of supplementation:
- illness and side-effects by 2-weeks recall
- adherence by microprocessor and stool sample
- determinants of adherence, follow-up

Week 8 of supplementation:
- illness and side-effects by 4-weeks recall
- adherence by stool sample

Week 12 of supplementation:
- illness and side-effects by 4-week recall
- adherence by stool sample
- hematology (incl malaria)
- determinants of adherence, follow-up

Provision of iron supplements will be made until end of pregnancy.

5. Assessment of cost-effectiveness of iron supplementation.

To calculate cost the following information will be collected:

- cost of the two different iron supplement per pregnancy.
- cost for health personnel
- cost for transport
- cost for facilities

The first cost, the iron supplement, will differ between the two types of formulation. The cost difference will be used for calculation of marginal cost of using the GDS supplement instead of the conventional preparation.

The cost for the next three ingredients in the supplementation programme will not differ between the iron supplements. For later calculations of total programme cost the assumption will be that a basic health delivery system already exists and that the iron supplement will be one of several drugs delivered to the center. The cost factors for the mentioned items will thus be calculated

as shares of the total budget for the health centers cost for the mentioned ingredients. The share which will be attributed to the iron supplementation will be based on an assessment of how large proportion of the health center's time is used for information about, and distribution of iron supplement.

The effectiveness part of the calculation will have two variables. The first is a measure of the process of the supplementation programme, an output measure, which in this case will be number of adherers. The second variable is an outcome measure, per definition a biologic variable, in this case it will be hematologic outcome.

VIII. ANALYSIS

A. Hypothesis testing:

The intervention part of the study is the use of GDS iron preparation. A number of hypotheses regarding characteristics of the GDS supplement will be tested whether they are true or not. The analyses will be done using EPI-INFO and SPSS statistical packages which include statistical methods such as, t-tests, analysis of variance and multiple regrression analysis.

Hypothesis 1. GDS iron preparation produce less side-effects compared to conventional iron preparation.

Hypothesis 2. Use of GDS iron preparation will lead to better adherence to regimen.

Hypothesis 3. Use of GDS iron preparation will maintain or improve hematologic status better than conventional iron supplement.

Hypothesis 4. Side-effects of iron supplementation have a negative effect on adherence.

Hypothesis 5. A better adherence will result in better hematologic outcome.

Hypothesis 6. GDS iron supplement is more cost-effective in terms of adherence than conventional iron supplement.

Hypothesis 7. GDS iron supplement is more cost-effective in terms of hematologic outcome.

B. Analysis of the sociologic part.

The health belief model:
The components in the health belief model will be assessed on their respective importance for the variation observed in adherence by pathway analysis.

The health beliefs contribution to adherence patterns will be investigated by assessing the overall explanatory capacity of the model.

The general questions will be compiled into a descriptive section.

IX. ETHICAL CONSIDERATIONS

The subjects in the proposed research project are women in Ilula villages that, at start of study, within the last three months delivered a baby (phase 1; antenatal coverage study) and pregnant women attending antenatal clinic at the health center in Ilula (phase I; hemoglobin base-line study, phase II; pilot test of methodology and phase III; intervention study)

The subjects for the antenatal coverage study will be identified by approaching the community leaders, in particular ten-cell-leaders, who know each household and its composition within his/her administrative unit. Initial contact will be at their respective house. Subject for the other part studies will be approached when the visit antenatal the clinic.

At the first encounter with the subject candidate, before a request is made about participation in the study, they will be informed about the research project, its aims and what a participation would involve on their part. Since the population to large extent is illiterate written consent is not feasible and oral consent has to be taken. The subjects will be informed that if they choice to participate and later change their mind they can withdraw any time from the study without any penalties or consequences for their continuous participation and use of the clinic's service.

If the ongoing research for some reasons has to terminate and debriefing can't be done within the project's time frame the village leaders will be asked to inform the subjects.

The intervention part of the study is the test of two iron supplement which are randomly assigned to the women. Both preparations have the same active compound, ferrous sulphate. The dose of elemental iron is in the conventional formulation 120 mg as recommended by WHO. The GDS iron preparation has a 3-4-fold higher absorption rates (Cook et al 1991) and have resulted in the same or better hematologic response, in an anemic population, when the dose has been 50 mg of elemental iron (Simmons 1990). A dose of 50 mg of elemental iron will be used for the GDS iron preparation. Due to ethical reason this study will not have any placebo or control group.

In the intervention part of the study the data collection include taking two finger prick blood samples. Finger prick sample is already before the initiation of the study routinely taken in the clinic at registration but an additional sample is needed after 12 weeks of iron supplementation. Finger prick blood samples as such does not involve any risks for the patient. However, HIV virus is prevalent in Tanzania and strict precautions has to be taken to avoid transmission of the virus. The steps that will be taken are the following; only one person will take and handle all the blood samples in the study. This person will, even if previously trained, receive further training in safety measures regarding handling blood samples possibly contaminated with HIV virus. Training will be done by a medical doctor at the

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laboratory department of TFNC who has previously trained persons in blood sampling techniques. Protective gears for the person taking the blood samples will be made available as well as equipment for handling contaminated supplies and for its destruction. Finger prick samples will be taken by use of disposable supplies only.

The data collection and interviews will be carried out by field workers living in the area who are familiar with local customs and fluent in kiswahili.

The data collected will be confidential. The subjects privacy will be ensured by limiting the number of persons who have access to the raw data. In analysis and presentation of the results all subjects will be codified and no names will be revealed.

The study is funded by the Tanzanian government through loans from the World Bank, by World Health Organization, and by Scandinavian Africa Institute, Uppsala, Sweden.

X. COLLABORATING INSTITUTIONS

The project is part of the TFNC/WHO research collaboration in Nutrition. Personnel from TFNC will mainly be drawn from the Anemia programme and from WHO the nutrition officer is participating.

In Ilula villages, personnel from the Health Center will be part of the collaboration.

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XI. BUDGET

In Tanzania shillings if not otherwise stated.

Phase 1. Preparatory phase October - November 1991.

Transport:	164.000	
Per Diem & Salaries:	272.000	
Stationaries:	11.000	
Instrument & Eqiupment:	79.000	
total	526.000	≈(2300 US$)

Phase 2. Pilot test of questionnare, December- February 1992.

Transport:	306.000	
Per diem & salaries:	310.000	
Stationaries:	11.000	
Instrument & Equipment:	89.000	
total	716.000	≈(3100 US$)

Phase 3. Intervention study, March - March 1993.

Transport:	1176.000	
Per diem & Salaries	828.000	
Stationaries:	45.000	
Instrument & Equipment:	347.000	
total	2396.000	≈(10400 US$)

Phase 4, Analysis and dissemination of results, April -July, 1993.

Analysis:	86.000	
Seminar in Ilula:	278.000	
total	366.000	≈(1600 US$)

Lease of instruments for measure of adherence and purchase of hemoglobinometer.

Adherence instrument:	36.480 SEK	
Hemoglobinometer:	2.500 SEK	
total	38.980 SEK	≈(6300 US$)

Grand total 23.700 US$

The project is funded ~~through~~ by the Tanzania Government through loan from the World Bank (4000 US$), WHO/AFRO (5000 US$), WHO/Head quarters (applied for 12700 US$) and Scandinavian Africa Institute (2000 US$) applied for.

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APPENDIX B-6

Serum transferrin receptor and iron status in teenagers

by

To be completed:

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Running head: Serum transferrin receptor and iron status

Abstract

The concentration of transferrin receptor in serum (TR) was determined in a representative sample of 220 girls and 198 boys aged 15-16 years. TR was significantly higher in boys than in girls (6.14±0.088 and 5.72±0.085 mg/L, respectively; t=4,10, p<0.001). Two explanations were proposed: the larger red cell mass and greater number of normoblasts in boys, and an observed higher growth rate in boys.

Different methods were employed to study the relationship between TR and measurements describing iron status such as serum ferritin(SF), hemoglobin(Hb), mean cell volume(MCV), mean cell hemoglobin(MCH), transferrin saturation(TS) and total iron binding capacity(TIBC). TS showed the highest correlation with TR. Signs of an iron deficient erythropoiesis developed with increasing TR, appearing already within the range of TR observed in iron replete subjects and before SF reached levels associated with depleted iron stores. There was thus a marked overlap in the distributions of TR in iron deficient and iron replete subjects. The efficiency to diagnose mild iron deficiency was lower for TR than for SF. Common infections during the preceding month significantly increased SF but had no effect on TR. With decreasing TS there was an increasing TR also within the fully normal range. This observation suggests that the shedding of transferrin receptors to plasma is directly related to the number of receptors on cell surfaces not bound to the diferric-transferrin-complex. This hypothesis would explain the higher TR concentration both in states of increased erythropoiesis and in iron deficiency.

Introduction

Iron deficiency is often defined as an absence of iron stores. There are no evidence, however, suggesting that stored iron has any functional importance for health. In the diagnosis of iron deficiency it might therefore, at least from a theoretical point of view, be desireable to include further criteria for the diagnosis in addition to a depletion of iron stores. Such criteria would be various signs of a compromized supply of iron to the erythron. Classical laboratory signs are, for example, a reduction in transferrin saturation, hemoglobin concentration, and red cell indices. (For reviews see 1-3.)There are considerable difficulties in the practical interpretation of these laboratory measurements. The main reason is the wide range of these parameters in normal subjects which implies that there is a marked overlap in the distributions of these measurements in iron replete and iron deficient subjects, in turn making it almost impossible to identify subjects with mild iron deficiency using one or more of these methods (4-9).

Serum ferritin is considered to be a reliable measure of iron stores (10). Low serum ferritin values are associated with empty stores and would thus, according to current concepts, not necessarily be associated with an iron deficient erythropoiesis (11-17). Recent findings in a rather extensive material of 207 menstruating women indicated that iron stores were depleted at a serum ferritin concentration below 16μg/L, based on an absence of stainable iron in bone marrow smears (18). An unexpected observation in this study was that, at the point when iron stores were exhausted in bone-marrow smears, there were also unmistakable signs of an iron deficient erythropoiesis with a significant decrease of hemoglobin concentration, transferrin saturation and red cell indices. These findings thus indicated that the supply of iron to tissues was inadequate at the time

when iron stores were empty. Actually, the observations indicated that the release of iron from stores might even be impaired *before* iron stores were completely exhausted.

The uptake of iron in tissues is mediated by an active uptake of transferrin-bound iron by special transferrin receptors on the surface of the cells (19-21). It was recently observed that small amounts of transferrin receptors were detectable in plasma using sensitive immunological methods (22-24).The introduction of improved methods to determine the transferrin receptor (TR) in serum led to the important observations that in states with an expected increased density of transferrin receptors on cell surfaces there was also an increase in the concentration of TR in serum (25). Clinical and experimental studies suggested that in states of increased iron requirements with an increased formation of new cells, for example, an increased erythropoiesis, more receptors are formed and the concentration of TR in serum was increased. Moreover it was found that there was an increased concentration of TR in states of iron deficiency (26-29). These observations strongly suggested that determinations of TR might give new and independent information about the iron status which might have diagnostic importance both in clinical and epidemiological work. In a recent study in which iron deficiency was induced by repeated small phlebotomies in healthy volunteers careful analyses were made of the changes in TR, serum ferritin (SF) and several classical laboratory parameters of iron status (25). The findings suggested that iron status of a population might be fully assessed simply by using serum ferritin to measure iron stores, TR to measure mild tissue iron deficiency and hemoglobin concentration to measure advanced iron deficiency anemia (3). It was obvious, however, that there was a need to further evaluate the ability of serum ferritin and TR to establish the presence or absence of mild iron deficiency.

We had access to a representative material of teenagers comprising 198 boys and 220 girls, aged 15-16 years. This sample had been rather extensively examined hematologically and nutritionally (30). The prevalence figures of iron deficiency based on serum ferritin measurements were unexpectedly high both in boys and girls, 15 and 40%, respectively. The purpose of the present study was to further examine the diagnostic usefulness of TR in relation to other methods in this sample of teenagers. The purpose was also to get further information about the sources of variation of TR and some insight about factors determining the level of TR in order to facilitate an evaluation of possibilities and limitations in the practical diagnostic use of TR determinations in the diagnosis of iron deficiency.

Material

The present studies were made in a sample of 15-16 year old boys and girls in four schools in different parts of Göteborg to examine the prevalence of iron deficiency among adolescents and to try and clarify its causes and possible consequences. The sample was drawn from four schools in Göteborg selected to get a sample that should be rather representative of 15-16 year old boys and girls in Göteborg, attempting to cover different socio-economic and living conditions. Areas were chosen with a variation from high-income one-family housing to multi-apartment houses with lower income families. Göteborg is the second biggest town in Sweden with about 430.000 inhabitants. It is a center for trade, industry and higher education in Western Sweden and it has the main port in Sweden .

All boys and girls in the 9th grade in the schools selected were invited to participate.The study was done in 1990, in late spring when the incidence

of infections is usually low. In 1990 there were in total 5280 boys and girls in the 9th grade. Four schools in different areas were selected and all boys and girls in these schools were selected for the study comprising 260 boys and 255 girls. Details of the design of the study has been described previously (30). Blood samples were drawn in those girls and boys for whom permission was granted from their parents (girls N=220, 86% of original sample; boys N=207, 80% of original sample).

Methods

Serum transferrin receptor (TR)

To be completed by JIM!

Serum ferritin (SF)

The SF values used in the study were determined by a double-antibody polyethyleneglycol radioimmunoassay (Diagnostic Products Corp.,Los Angeles, CA, USA). The assay was calibrated against WHO 1st international standard, IS 80/602. The performance of the radioimmunoassay in our hands has been reported (31). Judging from results of quality control results (in-house controls and a large scale international immunoassay programme) there has been no change in bias or imprecision of that assay. Considering the importance of the accuracy and precision of the SF analysis in the present study all sera were also analyzed using an immunochemi-luminometric assay (MagicLite Ferritin, Ciba Corning Diagnostics Corp., Medfield, MA, USA) previously reported to correlate well with the radioimmunoassay (32).

Possibly a short summary of the results of the comparison of the two methods should be mentioned here?

Hematological methods

Hemoglobin concentration (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), and red cell distribution width (RDCW) were analyzed

with an automatic system, Technicon H2 equipment (Technicon Instruments Corporation, 511 Benedict Av. Tarrytown, New York, USA). Serum iron was determined according to Ischida et al (33) using bathophenantroline sulfonate as binder. Total iron binding capacity (TIBC) was determined by adding excess ferroammonium citrate to serum and removing excess iron with the ion-exchanger Amberlite IRA 400. Transferrin saturation (TS) was the ratio serum iron/TIBC expressed as a percentage.

Other methods

Body height and weight were measured at the present examination. Such measurements had also been made about one year earlier. The exact date was noted and the changes in weight and height were adjusted to correspond to a 365 day interval.

Statistics

All statistical analyses were made using a Statview II computer program (Abacus Concepts, Inc., Berkeley, Calif., USA). Graphical analysis of data (34) were made using a Kaleidagraph computer program - Mac II, version 2.1 [Synergy Software (PCS Inc.) Reading, PA, USA]

Results

Distribution of TR in boys and girls

The distribution of TR in the 220 girls and the 198 boys are graphed in figure 1. The mean values were 5.72 ±0.085 mg/L and 6.14 ±0.088 (M±SEM) in girls and boys, respectively. The difference between the means was statistically highly significant ($t=4.10$; $p<0.001$).

Figure 1.

As reported in a previous study (30) the prevalence of iron deficiency defined as SF<16µg/L was 40% in these girls and 15% in these boys. This fact

would be expected to give the opposite result with higher concentrations of TR in girls and lower in boys. Nevertheless, it was considered important to study the effect of differences in iron status on TR. Comparisons were made within girls between those with SF <16μg/L and those with SF≥16μg/L. The mean values were 5.97±0.142 and 5.56±0.142 mg/L (M±SEM), respectively. The number of subjects in the two groups were 88 and 132, respectively. The difference between the mean values was statistically significant ($t=2.38; p<0.02$).

For boys the corresponding figures were 6.63±0.301 and 6.04±0.086 mg/L , respectively. The number of subjects were 31 and 166, respectively. The difference between the means was also statistically significant ($t= 2.46; p<0.02$).

A comparison was also made between the TR values in girls and boys with SF≥16μg/L, in order to include only iron replete subjects. The difference between these means (5.56 and 6.04 mg/L) was still highly significant ($t=3.71; p<0.001$). The 95th percentile ranges (mean±2 SD) were 3.16 - 7.96 and 3.82 - 8.27 mg/L in these girls and boys, respectively (N= 132 and 166, respectively).

Growth can be expected to influence iron requirements and thus TR and SF. Table 1 shows body height and body weight in the girls and the boys and the absolute and relative changes in height and weight during the last 365 days (see section on methods). The correlation coefficients for the relationships between these growth parameters and the concentration of TR are also shown in the table which also contains the correlation coefficients for the corresponding relationships with SF. Growth expressed as change in absolute or relative body height had a significant effect on the concentration of TR in boys. The greater the growth the higher the concentration of TR and the lower the concentration of SF. In the girls no correlation was seen

between TR and the growth parameters. For SF there was a significant correlation between increase in body weight and reduction in SF.

Table 1

Relationships between TR and other indices of iron status

The relationships between the concentration of TR in serum and hemoglobin concentration (Hb), serum iron (PI), transferrin saturation(TS) transferrin concentration (TIBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and serum ferritin (SF) were studied in different ways. Simple regression studies were made in the girls and in the boys and the correlation coefficients for these relationships are shown in table 2.

Table 2

To facilitate comparisons table 2 also contains the correlation coefficients fo the corresponding relationships with serum ferritin (SF). The correlation coefficients between the hematological parameters and TR and SF, respectively, were consistently higher for SF than for TR. After a z-transformation of the correlation coefficients for SF and TR they were separately compared in the boys and in the girls using a t-test. In boys the correlation coefficients were significantly different for Hb and TIBC. In girls a significant difference was only seen for TIBC.

To get further information about these relationships the materials of boys and girls were divided into decentiles based on their TR values (Figur 2). The relationships between TR and the different hematological parameters were also examined using a locally weighted least square based graphical method (34). These results are shown in figure 3.

Figure 2. Figure 3.

In the girls the decentile values for Hb showed a significant decrease in relation to TR (r=0.51). In boys no significant decrease in Hb was seen. Using the locally weighted least square based graphical method it was evident as shown in Figure 3, however, that there was a decrease in Hb both in boys and girls starting at a concentration of TR at about > 7 mg/L. A similar pattern was seen for MCH (figure 2 and 3) but the decrease at a TR value of about 6.5 mg/L both in boys and girls. MCV also decreased with increasing concentration of TR and started at a about the same TR level as for MCH, about 6.5 mg/L. As was also reported in a previous paper analyzing the present material, MCH and MCV values are higher in girls than in boys. The relationship between TR and TS show a different pattern. With both methods (figure 2 and 3) there was a continuous decrease in TS with increasing concentration of TR. The parallell displacement of the curves are probably related to the mentioned sex difference in TR distributions. A similar pattern was observed for TIBC, but with a continuous increase with increasing TR and with a parallell displacement of the curves for boys and girls.

The relationship between the concentrations of SF and TR was examined in different ways. The material was divided into decentiles based on the concentrations of both SF and TR . Within each decentile the mean values for SF and TR concentrations were then calculated for the boys and the girls as shown in figure 4 and figure 5. Each figure contains two panels: one panel describing the relationship based on decentile values and one panel a graph calculated with the locally weighted least square error method.

The upper panel of figure 4 showed that the TR values were consistently higher in boys than in girls for all SF decentiles. The difference was even more evident in the lower panel comparing the relationship between SF and TR using the locally weighted least square error method to

calculate the TR values corresponding to different SF values. The graph shows the consistently higher TR concentrations in boys. Figure 4 also shows that the trend towards higher TR values at lower SF values started already at log SF 1.4 (25 µg/L) in the boys and even earlier in the girls.

Figure 5 shows, as was also seen in figure 4, that in girls the concentration of SF successively decreased with increasing TR decentile values. This decrease was also evident in the lower panel showing the same relationship but based on the locally weighted least square error method. It should be emphasized that the differences between figure 4 and figure 5 are due to the fact that the dependent variables in the analyses are different. In boys the different pattern in the relationship between SF and TR was more obvious in figure 5 than in figure 4. It was evident that SF remained constant until the TR concentration had reached a level of about 7 mg/L. After that, the rate of decrease in the concentration of SF with increasing TR was about the same in boys and in girls.

Usefulness of determinations of TR in the diagnosis of iron deficiency

In order to evaluate the practical usefulness of TR measurements in the diagnosis of iron deficiency calculations were made of the sensitivity, specificity, diagnostic efficiency and predictive value of TR determinations using different cutoff values. Commonly employed definitions and methods were used for this purpose (35,36). The results for girls and boys are given in table xcxc. As expected the specificity of TR determinations increases the higher the cutoff value. The "price" however is the low sensitivity. The differences in the distributions of TR in girls and boys are also reflected in this table.

Effect of a preceding infection on the concentration of TR

Subjects who had a history of an upper respiratory infection during the preceding month were recorded. The mean values of TR in boys and girls are shown in Table 2 for those with and without a preceding infection. As a control the corresponding figures for SF are also included.

Table 2

Discussion

The main diagnostic difficulty in the evaluation of iron status is to establish the presence of a mild iron deficiency with an iron deficient erythropoiesis but with hemoglobin values within the normal range of the population. This problem is common both in clinical practice and in epidemiological studies and is thus important to solve. The recent results, mentioned in the introduction, showing that there is a close relationship between the degree of iron depletion and the concentration of TR in serum strongly suggested that TR might be a valuable new tool in the diagnosis of iron deficiency (3).

Studies reported so-far indicate that the concentration of TR in serum in some way is related to erythropoiesis, for example, with high concentrations in hemolytic anemia and low levels in aplastic anemia (22-25, 27-29). Moreover high concentration of TR was also seen in iron deficiency. Even if the mechanisms behind the shedding of transferrin receptors to plasma are not fully understood, measurements of TR may be a valuable index of erythropoiesis and iron status. The present studies were performed to get further information about the relationship between TR and other indicators of iron status and to learn more about the pathophysiological meaning and signification of TR. Several unexpected observations were made which may have importance for the understanding of different factors influencing the concentration of TR in plasma and thus for the use of TR in the diagnosis of iron deficiency

Differences in TR concentration between girls and boys.

Iron requirements are much higher in teenaged girls making their iron balance situation more critical than for boys. The prevalence of iron deficiency in the present sample of girls was also much higher, 40% of the girls had SF < 16μg./L compared with 15% of the boys. Since the concentration of TR is known to increase in iron deficiency one would therefore have anticipated that the TR values would have been higher in girls than in boys. The present, opposite finding with statistically significantly higher TR values in boys than in girls was thus surprising.

A main part of the delivery of iron to tissues is used for the production of hemoglobin. The higher Hb values in boys thus implies that boys have a higher red cell production and more normoblasts per unit body weight than girls and that they would thus have a greater number of transferrin receptors and a higher concentration of TR in plasma. The mean Hb values in boys and girls in the present study were 146.8 g/L and 133.5 g/L, respectively.The ratio of the mean Hb values was thus 1.10. The ratio of the mean TR values for all boys and all girls was 1.07 (6.14 and 5.72 mg/L, respectively). To reduce the effect of the higher prevalence of iron deficiency on the TR values in the girls, the TR values in girls and boys were compared only in those considered to be iron replete (with SF ≥ 16 μg/L). The ratio of the mean TR values was then 1.09 (6.04 and 5,56 mg/L, respectively). The mean Hb values in these girls and boys were 134.8 and 147.7 g/L, respectively, and the ratio of mean Hb values boys/girls was 1.10. The correlation coefficient for the relationship between TR and Hb in these iron replete 299 subjects (163 boys and 129 girls) was 0.214. This means, however, that only about 5% of the variation in TR can be explained by the variation in Hb.

It may seem hard to understand why the correlation between TR and Hb is not higher since Hb would be a good average measure of the rate of

erythropoiesis. One possibility would be that, for example, the hour-to-hour variation in erythropoiesis is great and that TR , possibly having a small time-constant, rather reflects the momentary varying erythropoiesis

Differences in growth might be another contributory cause of the differences in TR between boys and girls.There were no significant correlations in girls between TR and the different growth parameters. In boys, however, changes in height were significantly correlated to TR. Only about 5% of the variation in TR within the group of boys was explained by the variation in change in height. The difference in rate of growth between boys and girls, however, was very marked and is related to the well known fact that girls have their growth spurt almost two years earlier than boys.

Table 1 also shows the correlation between SF and growth. It was quite evident that the higher the rate of growth in boys the lower was the SF. A reasonable interpretation would be that much of the available iron was used to cover the requirements for growth and that less iron was available for the formation of iron stores in boys growing rapidly. It is thus evident that the iron balance situation is more critical in boys growing rapidly. The fact that there was a significant correlation between SF and the increase in weight, both in boys and girls, suggests that the limiting factor in the diet was not a lack of energy but rather a lack of iron.

In previous reports no significant differences were seen in the concentration of TR between adult men and women (23, 29). One confounder in such comparisons, however, might be the higher prevalence of iron deficiency in adult women which would be expected to result in higher TR levels in women than in men. Another confounder acting in opposite direction would be the higher red cell mass per unit body weight in men. Comparisons of TR in iron replete adult men and women have not been reported.

Differences in TR in relation to iron status

According to current concepts about the relationship between iron status and TR one might have anticipated that TR would be about the same in all iron replete subjects irrespective of the amounts of stored iron. Thus, the concentration of TR would not be expected to increase until the supply of iron to the erythron was insufficient. Under such conditions TR would potentiallybe an ideal parameter (indicator?) of iron status and be more or less specific for an iron deficient erythropoiesis.

In a previous study in adult women it was found that 98% of women with stainable iron in bone-marrow smears had SF ≥16µg/L (or, a log value ≥1,2) and that 84% of women with no stainable iron were below this cutoff value. Moreover it was found that absence of iron stores was associated with signs of an iron deficient erythropoiesis (18). Using this cutoff value for SF in studies on the relationship between SF and TR it was evident that the concentration of TR started to increase already at SF concentrations above 16 µg/L. In the girls there was an almost continuous increase in TR from the lowest to the highest SF values (figure 4). In the boys the TR values started to increase from a SF concentration of about 25µg/L (log 1.4).

Most of the girls and boys with SF ≥16µg/L can be considered to be iron replete. An analysis of the distribution of the TR values was made in these girls and boys and the mean values + 1 SD were 6.76 and 7.15 mg/L, respectively.The corresponding limits for + 2 SD were 7.96 and 8.27mg/L, respectively. Using these latter limits as the upper normal values for TR in iron replete subjects, all hematological parameters examined were changed before this point was reached. The results thus indicated that there were clear signs of an iron deficient erythropoiesis within the range of TR values observed in subjects with normal levels of SF. It is thus reasonable to assume that there is a more or less parallell increase in the concentration of

TR and the appearance of signs of an iron deficient erythropoiesis - i.e. a compromized supply of iron to the erythron.

The parallell reduction in transferrin saturation and increase in concentration of TR suggest that they are inversely related and that the supply of iron bound to transferrin influences the rate of shedding of transferrin receptors to plasma and thus the concentration of TR. This conclusion is valid even though the correlation coefficients for the relationship between TR and TS, both in girls and boys, were rather low, explaining only 5-7% of the variation. The r-values, however, were higher than for the relationships between TR and other hematological parameters.

In a recent study in man the relationship between iron status and TR was examined in a group of normal volunteers in whom iron deficiency was induced by repeated phlebotomies (26). In the initial phase of iron depletion there was a successive decrease in SF and rather unchanged concentrations of TR. Before iron depletion was complete, however, as judged from the SF determinations,TR started to increase. During the continued iron depletion with decreasing concentrations of SF, the concentration of TR progressively increased. The findings in that study, with an increase in TR starting before iron stores were fully depleted, were thus consistent with the present findings. The difference in experimental design between the previous study, in which each subject served as its own control, and the present study, which is crossectional, should be remembered in the comparison of results. A main point in the comparison between the two studies is that TR values in both studies were increased also in the iron replète state. It was also true, that there were signs of an iron deficient erythropoiesis even before iron stores were completely exhausted.

Putting together results from the present study on TR with results in a previous study on the same material of teenagers (30), mainly focused on the prevalence of iron deficiency and changes in SF and in hematological

parameters,the following comprehensive picture is obtained. During a continuous and slow negative iron balance, i.e. when iron requirements exceed iron absorption, iron stores will be gradually reduced. Initially, serum ferritin is the only laboratory parameter that is successively reduced as was observed in the phlebotomy study (26). At some point during the development of iron depletion, but before iron stores are completely exhausted, the rate of release of iron from stores will be reduced. Less iron is mobilized from the stores than is needed to cover the deficit between iron losses and dietary iron absorption. The supply of iron to tissues then becomes insufficient, or in other words, the supply of transferrin-iron to transferrin receptors becomes inadequate. For some reason, when less transferrin-iron binds to the surface receptors, more receptors seem to be released from these surfaces to plasma and the concentration of TR increases.

An insufficient supply of iron from stores, and from absorption, will result in a reduction in plasma iron and transferrin saturation. Owing to the well known marked diurnal variation in serum iron, in turn mainly due to differences in the rates of formation and destruction of red cells, the reduction in transferrin saturation is difficult to establish in the single individual . The inadequacy in the supply of iron to tissues will lead to an increase in the formation of transferrin and thus toan increased concentration of transferrin in plasma.

A reduced formation of hemoglobin and a reduction in the size of the red cells (MCV) and their hemoglobin content (MCH) will then be consequences of the insufficient supply of iron to the transferrin receptors of the erythropoietic cells .

Considering the outlined, hypothetical, pathophysiological course of events it is reasonable to assume that the hematological changes and the

changes in concentration of TR induced by a negative iron balance will be rather simultaneous.

The present findings are consistent with this interpretation. The observations may also give an indication about factors expected to influence the concentration of TR in plasma. Firstly it might be assumed that the concentration of TR in plasma is a function of the number of receptors on the surface of developing cells, in turn being a function of the need for iron transport to these cells i.e. iron requirements of tissues. The increased erythropoiesis in boys compared with girls and the higher rate of growth would then give a greater number of receptors on cell surfaces. An insufficient supply of iron to cell surfaces, in a state of iron deficiency, will result in the release of a greater number of "free" receptors that are not bound to iron-transferrin complexes, and a higher TR, and again assuming that such "free" receptors might be more prone to be shedded to plasma. TR in plasma might thus be a function of the number of unoccupied receptors on cell surfaces. - In states with increased erythropoiesis the number of receptors would be increased due to an increased formation of cells with receptors. In iron deficiency, with this simple outlook, more receptors would be available due to a reduced utilization of existing receptors.

Efficiency of TR in the diagnosis of iron deficiency

Two circumstances reduce the diagnostic value of TR determinations. It was obvious that during the depletion of iron stores the concentration of TR increased also in fully iron replete subjects. This was observed both in the present study and in a previous study (30). It was most striking in the girls but it was also clearly seen in the boys. Similarly, there were apparent signs of an iron deficient erythropoiesis with reductions in Hb, MCH and MCV within the range of TR seen in iron replete subjects. The changes seen in the concentration of TR during the development of iron deficiency thus

show great similarities to the changes seen in SF in previous studies ( ). The present observations are also consistent with the findings in the previously published phlebotomy study where there was a rather wide transitional zone covering both the state of iron repletion and the state of iron deficiency with decreasing SF and increasing TR. All these findings fit with a previous conclusion that the release of iron from stores to the erythron is diminished already before iron stores are completely exhausted.

A critical evaluation of the diagnostic value of a method with calculations of its specificity and sensitivity would require that one had access to a key correctly classifying the subjects under study. In the present investigation independent measures of iron status such as presence or absence of stainable iron in adequate bone marrow smears were not available. Results in a recent study comparing serum ferritin and iron stained bone-marrow smears indicate, however, that measurements of serum ferritin may be validly used as a single criterion on the presence/absence of iron deficiency. Using a cutoff value for SF of <16μg/L it was found that 98% of iron replete and 84% of iron deficient subjects were correctly classified. With a prevalence of iron deficiency of 15% in boys only 2.4% (16% out of 15%) of the whole sample of boys would be incorrectly classified as iron replete. For the girls with a prevalence of iron deficiency of 40% the corresponding figure would be 6.4% (16% out of 40%).

This fact was used in the classification of girls and boys as iron deficient or iron replete in table 4 analyzing the efficiency of determinations of TR for the diagnosis of iron deficiency. The differences in the distributions of TR in girls and boys are obvious. Sensitivity and specificity are not influenced by the prevalence of iron deficiency which, however, influences the calculations of both diagnostic efficiency and predictive value of a positive result. This fact should also be considered when comparing the results in girls and boys. In the previous study based on bone-marrow

analyses to establish the iron status of the women, serum ferritin was outstanding to correctly classify the iron status of a subject compared with Hb, TS, MCV, MCH and a standardized iron absorption test. The diagnostic efficiency for SF was 93%.

Table 4

As mentioned above there is a marked overlap in the distributions of the classical laboratory parameters describing iron status between iron replete and iron deficient subjects. Therefore, one cannot expect that the relationships between TR and SF on the one hand and different hematological measurements on the other would show high correlation coefficients. This was evident from the rather low correlation coefficients seen in Table 1. Even though several of the correlation coefficients (r) were statistically highly significant, the r^2 values indicate that the variations in SF or TR would only explain a minor part (2-15%) of the observed variation in the hematological variables. The correlation coefficients were consistently higher for SF compared with TR and some of the differences between SF and TR were statistically significant using a t-test after z-transformation. By and large these results suggest that the predictive value to establish an iron deficient erythropoiesis would be higher for SF than for TR. A reasonable explanation would be that in addition to iron status, several other factors, such as growth, may influence the concentration of TR whereas SF is mainly influenced by the iron status.

A problem in the practical use of serum ferritin is the fact that common infections, such as common cold with fever, may increase the SF level (38-40) and lead to an underestimation of the prevalence of iron deficiency. The present study was made in April- May which is a period in Sweden with a low prevalence of upper respiratory infections. In spite of that the rate was sufficiently high to significantly increase serum ferritin

both in girls and boys. It was of great practical interest that the concentration of TR was not at all influenced by the preceding infections.

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Figure legends

Figure 1 Distribution of serum transferrin receptor concentration in 220 girls and 198 boys aged 15-16 years. The difference between the means was statistically highly significant.

Figure 2. Relationship between serum transferrin receptor concentration (TR) and different hematological parameters. The materials of boys and girls were diveded into decentiles based on their TR concentration. Mean values calculated for each decentile are graphed in the figure.

Figure 3. Relationship between serum transferrin receptor concentration (TR) and different hematological parameters. A locally weighted least square based graphical method was used (see text).

Figure 4. Relationship between serum transferrin receptor concentration (TR) and serum ferritin. In the upper panel the materials of boys and girls had been divided into decentiles based on their serum ferritin concentration. Mean values of TR calculated for each decentile are graphed in the figure. In the lower panel the relationship was analyzed using the locally weighted least square based graphical method (see text).

Figure 5. Relationship between serum ferritin (SF) and serum transferrin receptor concentration (TR). In the upper panel the materials of boys and girls had been divided into decentiles based on their TR concentration. Mean values of SF calculated for each decentile are graphed in the figure. In the lower panel the relationship was analyzed using the locally weighted least square based graphical method (see text).

Table 1 Changes in weight and height in 15-16 year old girls and boys during the last 12 months and their relationship to the concentration of transferrin receptor and ferritin in serum.

	Number of subjects	Weight (kg)		Height (cm)		Body mass index (BMI)		Weight change (kg)		Height change (cm)		Weight change %		Height change %	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Boys	206	63.6.	9.39	175.7	7.08	20.6	2.33	5.97	3.47	4.78	2.90	9.5	5.4	2,7	1,7
Girls	207	58.2	8.31	166.6	6.76	20.9	2.02	3.35	2.91	1.78	1.70	5.8	5.3	1.1	1.0
Correlation coefficients Relationship of changes to serum transferrin receptor (TR)															
Boys						0.089		0.089		0.214**		0.117		0.218**	
Girls						0.025		0.027		0.029		0.053		0.039	
Correlation coefficients Relationship of changes to serum ferritin (SF)															
Boys						0.151*		0.202**		0.383***		0.300***		0.398***	
Girls						0.012		0.191**		0.017		0.193**		0.024	

* $p<0.05$
** $p<0,01$
*** $p< 0.001$

Table 2 Correlation coefficients for the relationships between concentration of transferrin receptor in serum and different hematological parameters in boys and girls and for the corresponding relationships between serum ferritin and the same parameters. As there are about 200 boys or girls in each comparison the critical values for $p<0.05$, $p< 0.01$, and $p< 0.001$ are 0.138; 0.181, and 0.23, respectively. Correlation coefficients for transferrin receptor(TR) and serum ferritin (SF) were compared for boys and girls respectively using a t-test after a z-transformation of the correlation coefficients

	Transferrin receptor in serum		Serum ferritin		Comparison of correlation coefficients for TR vs SF t-test	
	Boys	Girls	Boys	Girls	Boys	Girls
Transferrin receptor conc.	-	-	0.220	0.312		
Serum ferritin	0.220	0.312	-	-		
Hemoglobin conc	0.051	0.181	0.340	0.316	**3.03**	**1.44**
Transferrin saturation	0.267	0.220	0.308	0.334	**0.45**	**0.25**
Plasma iron	0.097	0.108	0.170	0.141	**0.34**	**0.11**
TIBC	0.138	0.181	0.322	0.394	**2.01**	**2.33**
MCH	0.160	0.198	0.276	0.286	**1.22**	**0.94**
MCV	0.137	0.168	0.172	0.173	**0.36**	**0.05**
MCHC	0.004	0.085	0.107	0.238	**1.03**	**1.57**

Table 3. Effect of an upper respiratory infection with fever during the month before the study on the concentrations of serum ferritin and serum transferrin receptor.

	Number of subjects	Serum ferritin (SF)µg/L	log SF		Serum transferrin receptor mg/L	
			Mean	SD	Mean	SD
Girls						
Girls with a preceding infection	47 (21.2% of total)	29.9	1.3606	0.3123	5.82	1.04
Girls with no infection	175	20.1	1.2313	0.288	5.70	1.32
Difference		9.9	0.1293	0.293	0.12	
t-value			2.69			
p			<0.01		NS	
Boys						
Boys with a preceding infection	42 (20.2% of total)	39.9	1.5548	0.2123	6.13	1.08
Boys with no infection	164	29.9	1.4164	0.2419	6.14	1.26
Difference		10	0.1384	0.234	0.01	
t-value			3.42			
p			<0.001		NS	

Table 4. Sensitivity, specificity, predictive value of a positive test and diagnostic effeciency of different cutoff values for serum transferrin receptor concentration (mg/L) to diagnose iron deficiency in 220 girls and 196 boys. Calculations were based on serum ferritin <16µg/L (=iron deficiency) and ≥16µg/L (=iron repletion)
Sensitivity: Percent of iron deficient subjects above cutoff value; Specificity: Percent of iron replete subjects below cutoff value. Predictive value of positive result: Percent of subjects with positive test who are iron deficient. Diagnostic efficiency: Percent of subjects correctly classified as iron deficient and iron replete.

Cutoff value for TR	Girls				Boys			
	Sensitivity	Specificity	Predictive value of positive result	Diagnostic efficiency	Sensitivity	Specificity	Predictive value of positive result	Diagnostic efficiency
<mg/L	%	%	%	%	%	%	%	%
4	99	6	41	44	97	2	15	16
4.5	89	19	42	43	90	8	15	20
5	76	31	42	50	83	14	15	29
5.5	57	52	44	54	70	33	16	38
6	38	76	51	60	57	49	17	59
6.5	27	79	60	64	47	73	24	69
7	12	94	60	62	43	84	33	78
7.5	8	97	64	61	27	92	38	82
8	7	99	75	62	17	96	42	84
9	5	99	80	61	7	99	67	85
10	1	99	-	60	3	99	-	85

Fig 1.

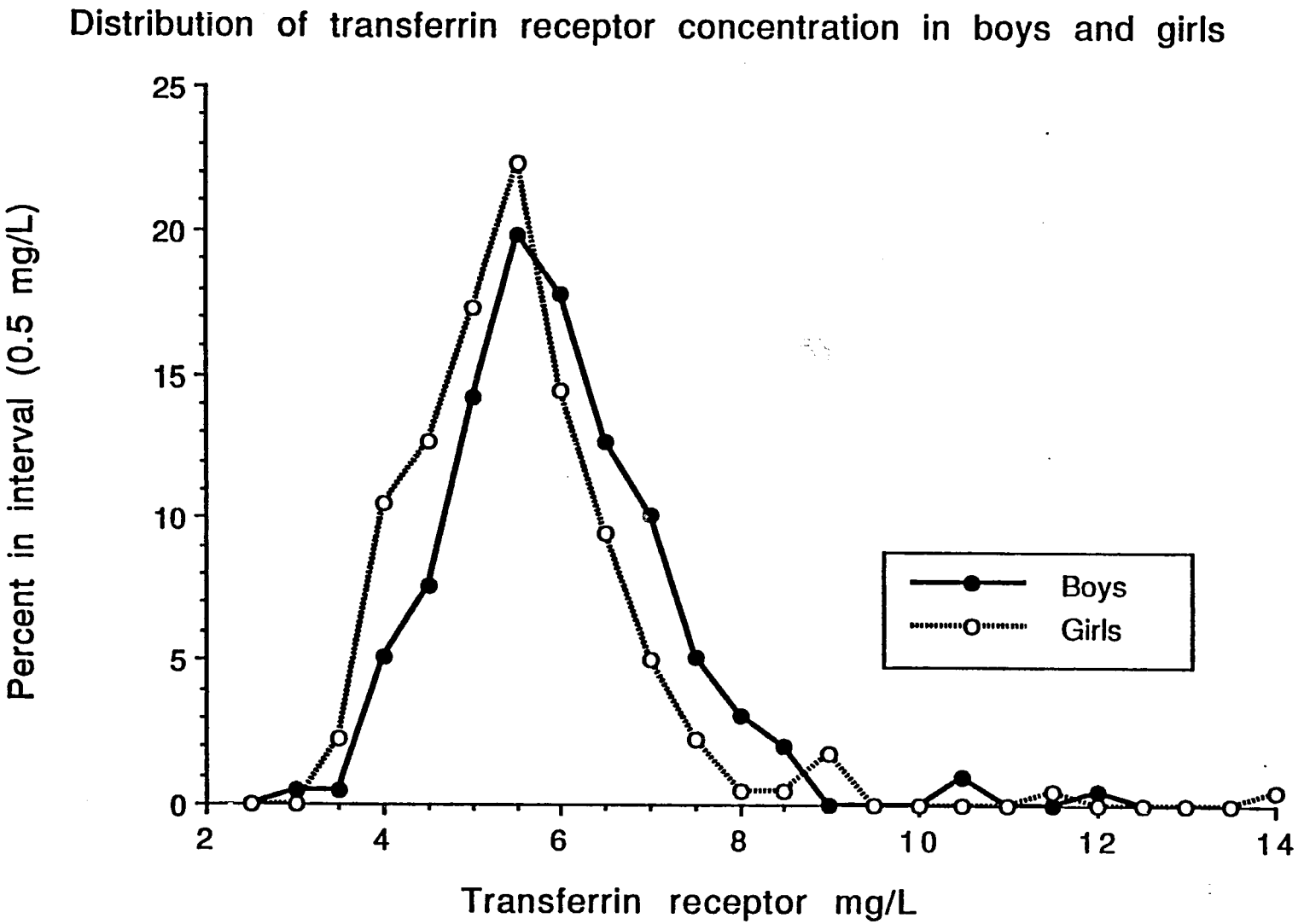

Distribution of transferrin receptor concentration in boys and girls
Percent in interval (0.5 mg/L)
25
20
15
10
5
0
2
4
6
8
10
12
14
Transferrin receptor mg/L
Boys
Girls

Fig 2A

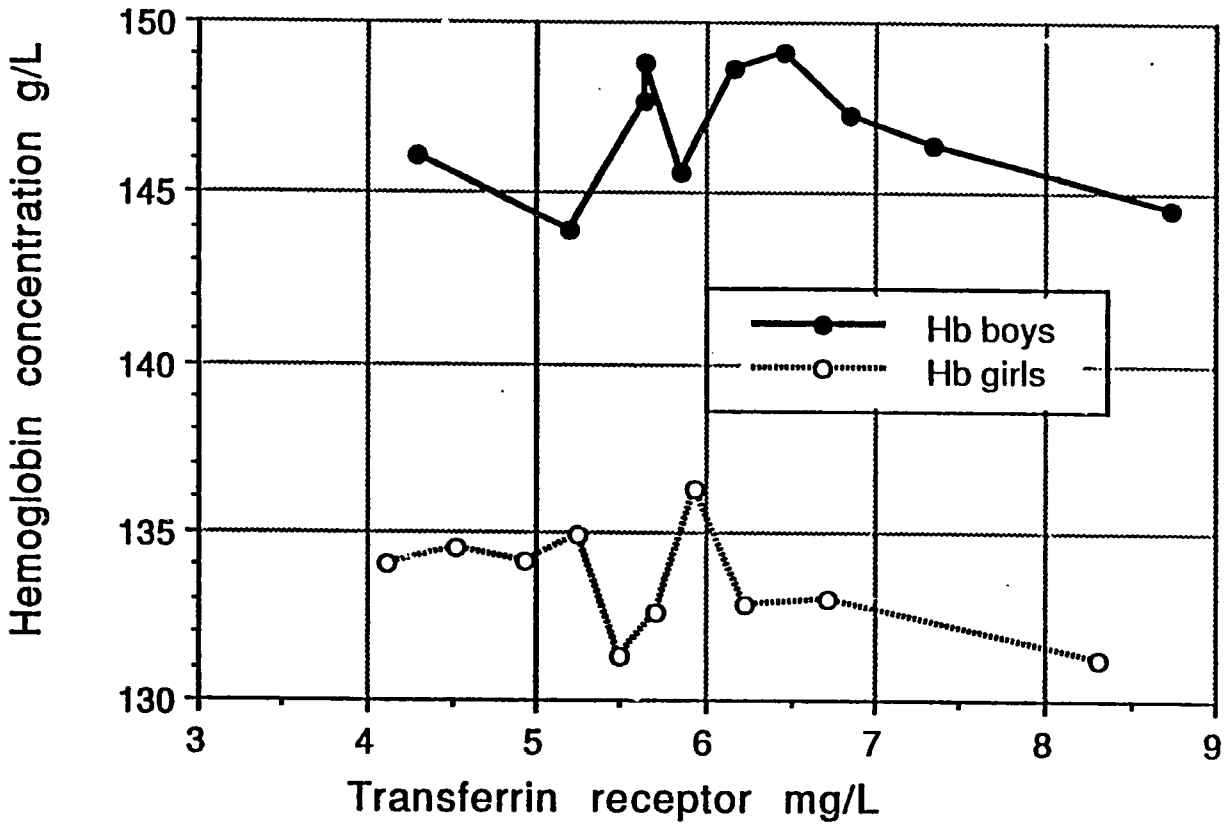
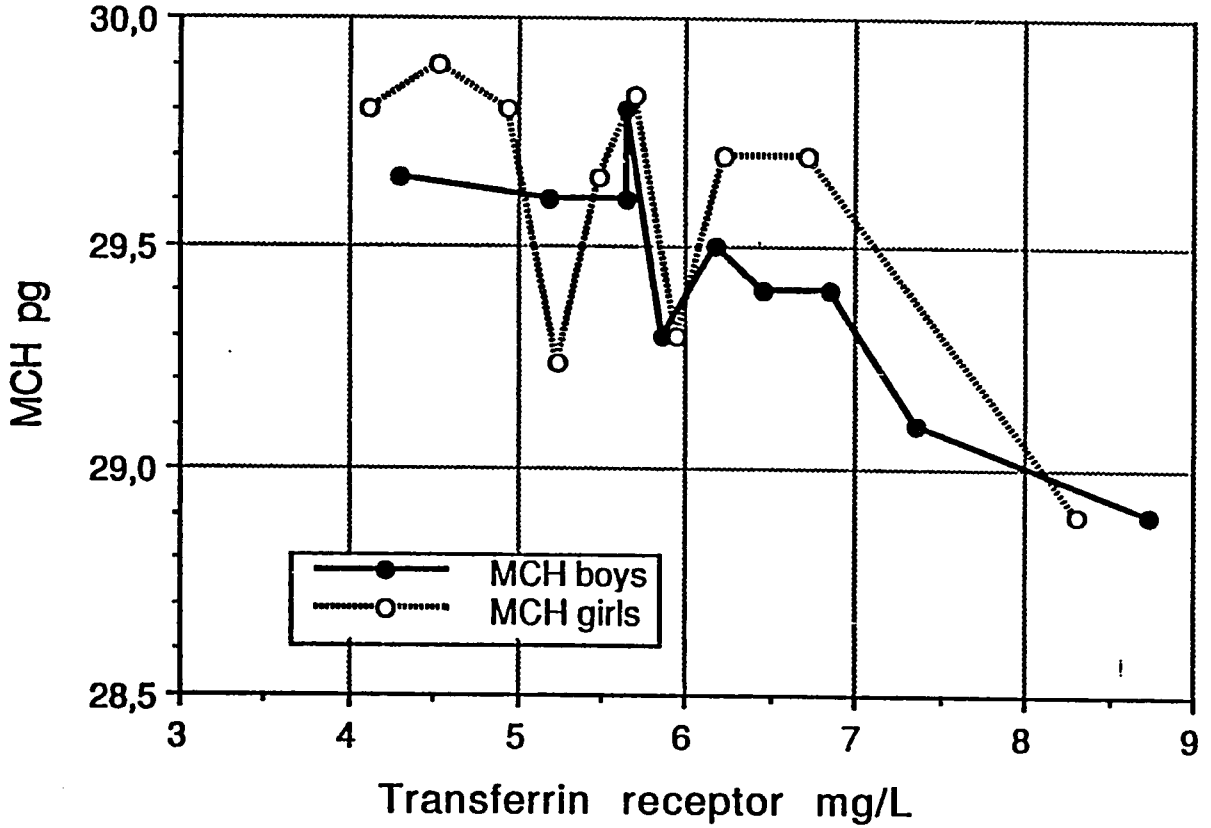
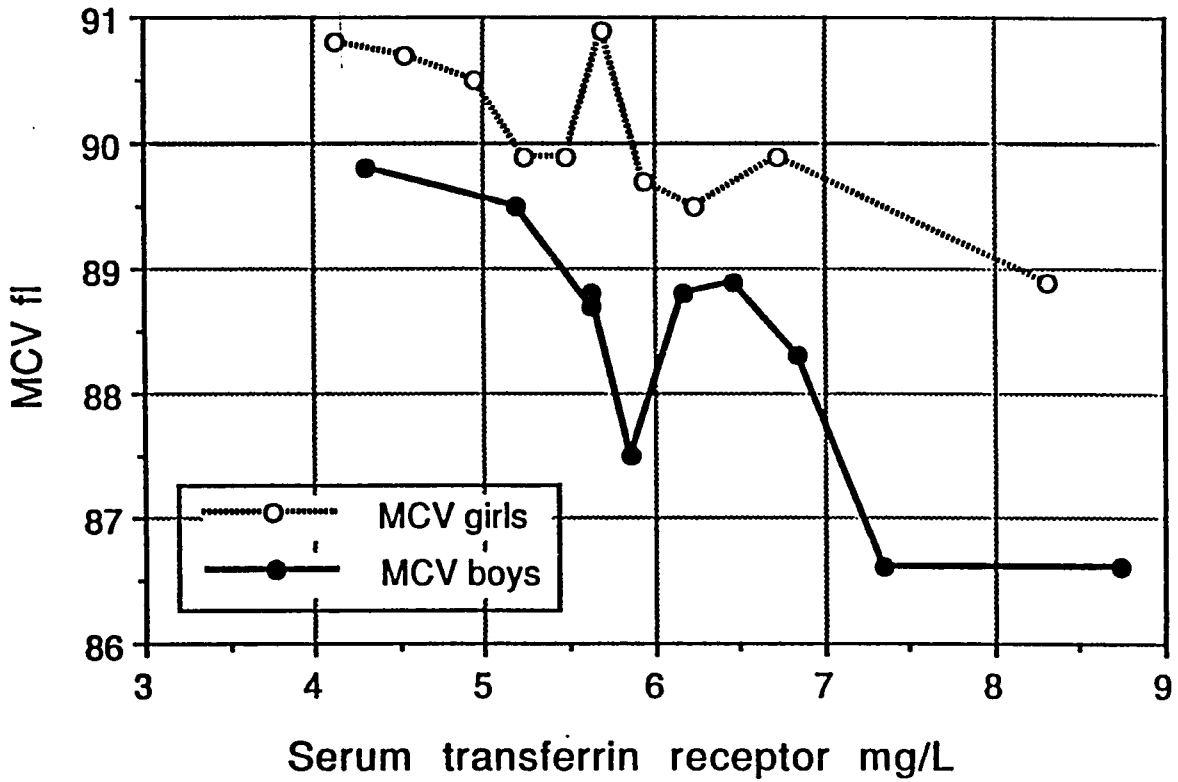
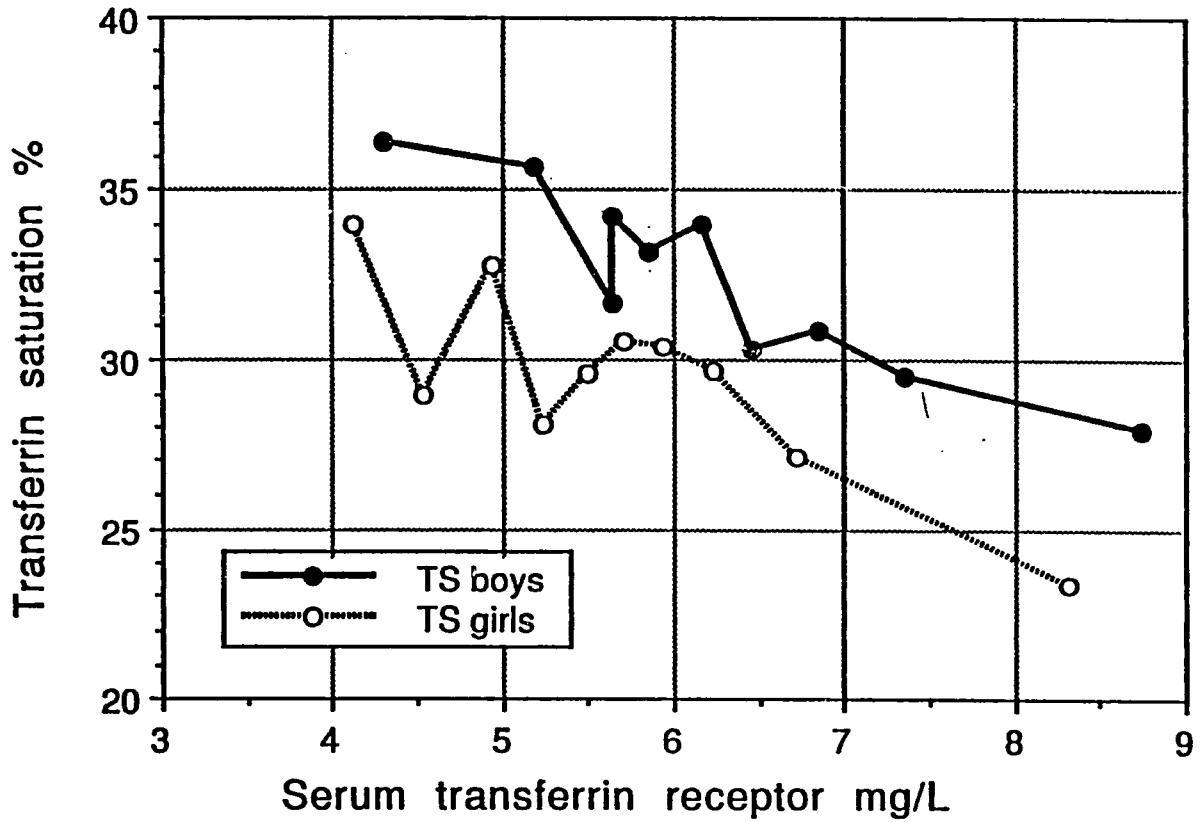
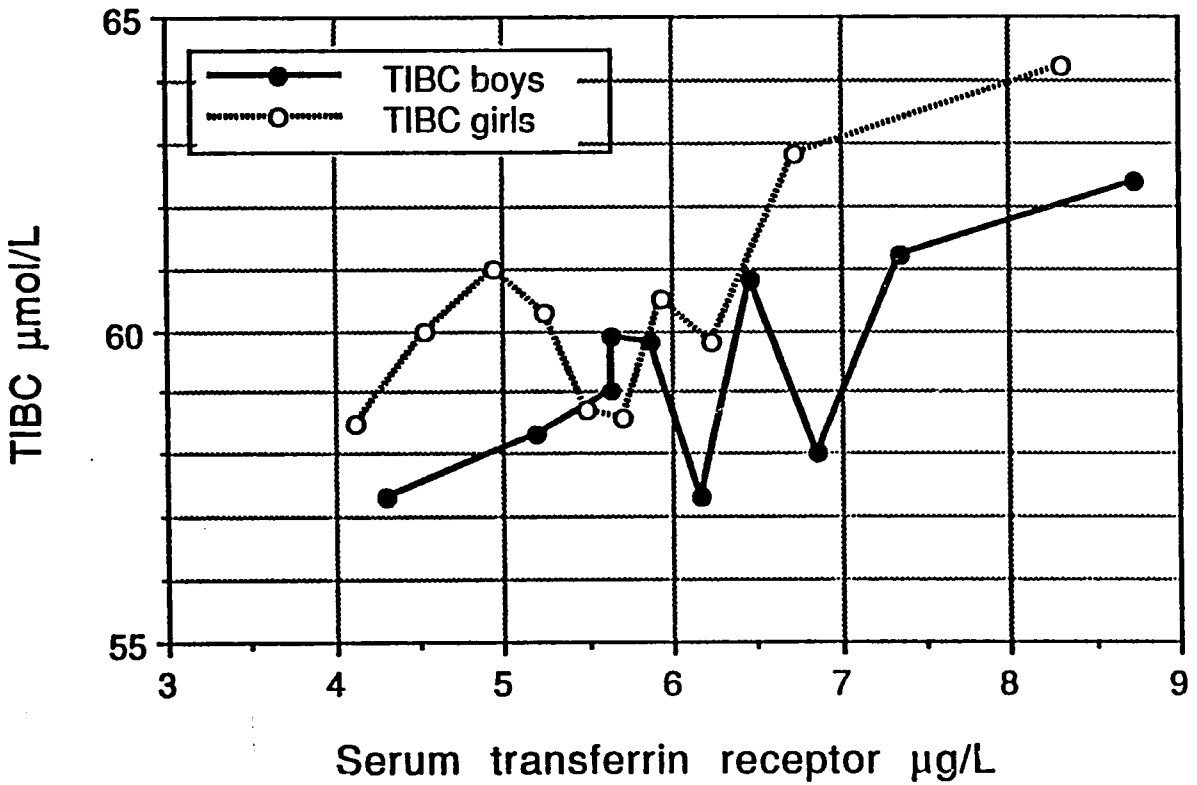

Hemoglobin concentration g/L
150
145
140
135
130
3
4
5
6
7
8
9
Transferrin receptor mg/L
Hb boys
Hb girls

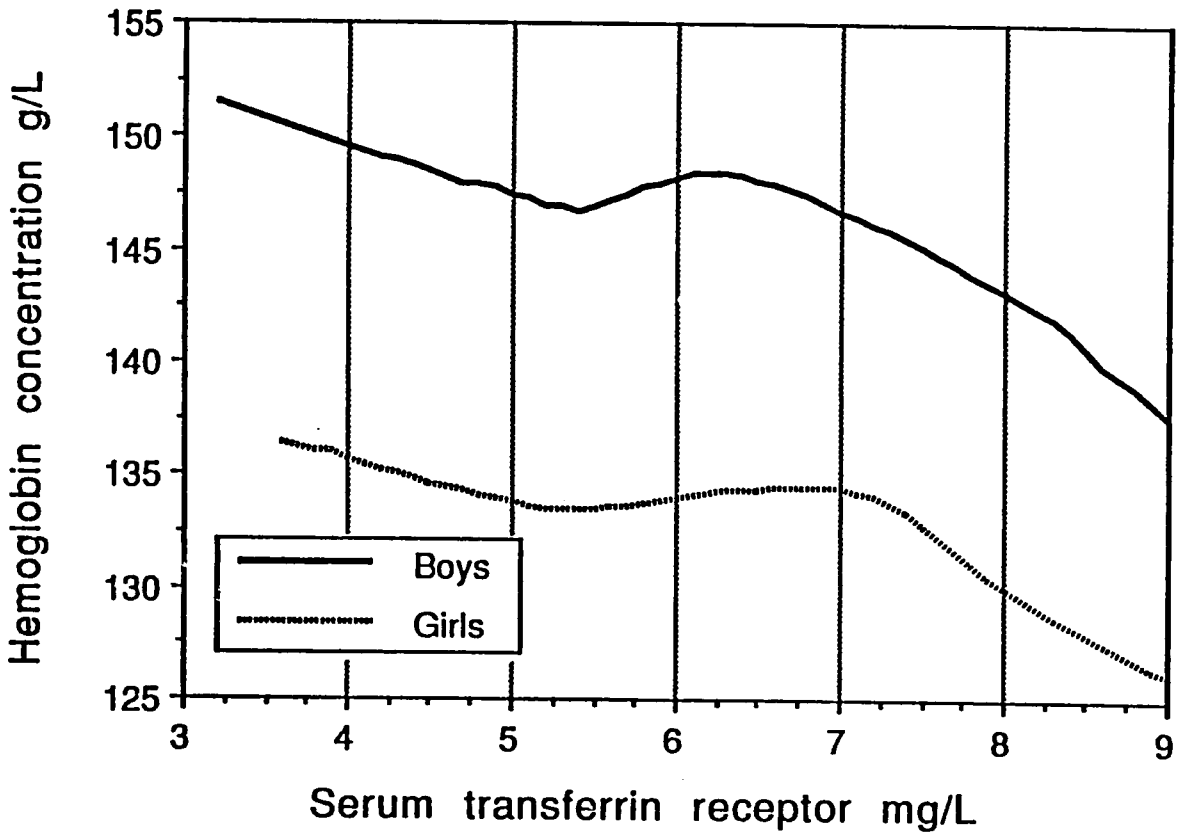
Fig 2B


30,0
29,5
29,0
28,5
MCH pg
3
4
5
6
7
8
9
Transferrin receptor mg/L
MCH boys
MCH girls


91
90
89
88
87
86
MCV fl
3
4
5
6
7
8
9
MCV girls
MCV boys
Serum transferrin receptor mg/L


Transferrin saturation %
40
35
30
25
20
3
4
5
6
7
8
9
Serum transferrin receptor mg/L
TS boys
TS girls


TIBC boys
TIBC girls
TIBC µmol/L
65
60
55
3
4
5
6
7
8
9
Serum transferrin receptor µg/L


Hemoglobin concentration g/L
155
150
145
140
135
130
125
3
4
5
6
7
8
9
Boys
Girls
Serum transferrin receptor mg/L

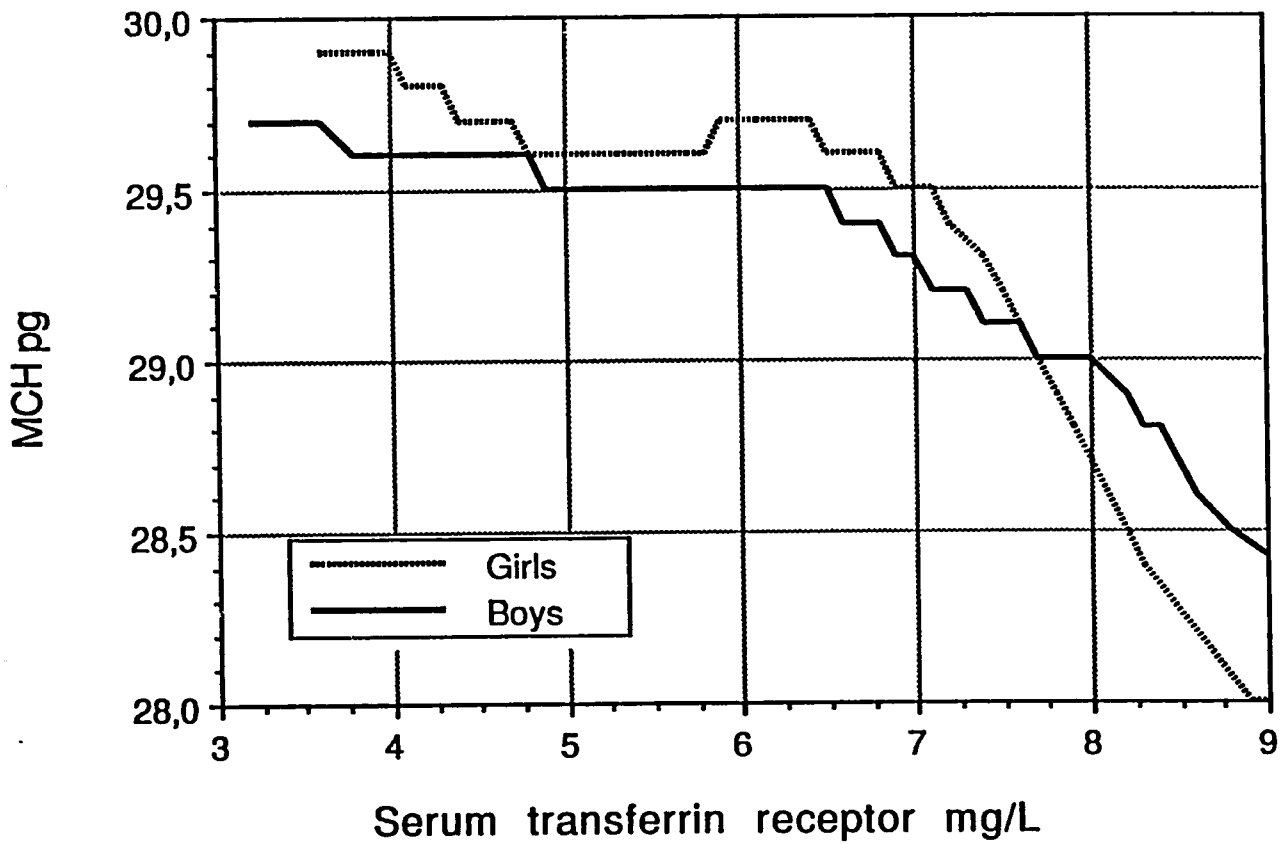

30,0
29,5
29,0
28,5
28,0
MCH pg
3
4
5
6
7
8
9
Girls
Boys
Serum transferrin receptor mg/L

Fig 4,C

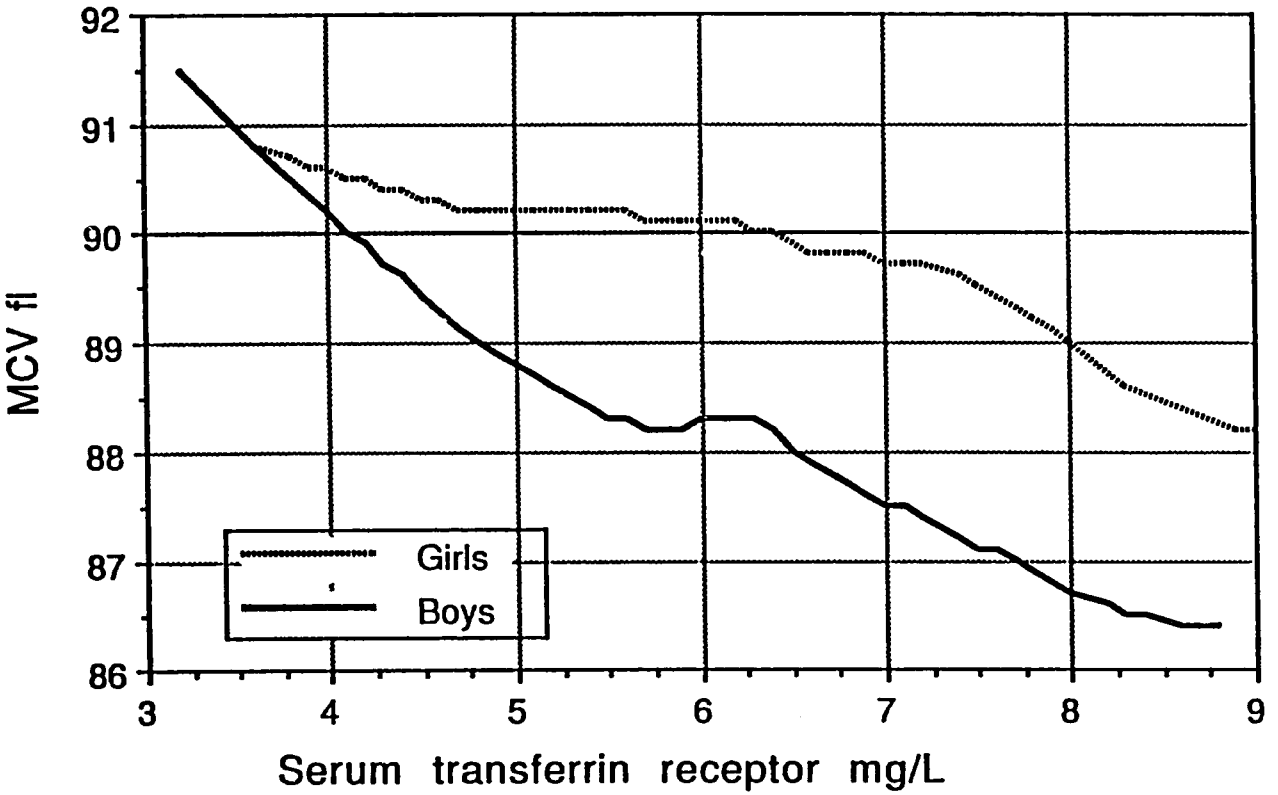

92
91
90
89
88
87
86
MCV fl
3
4
5
6
7
8
9
Girls
Boys
Serum transferrin receptor mg/L

Fig 3b

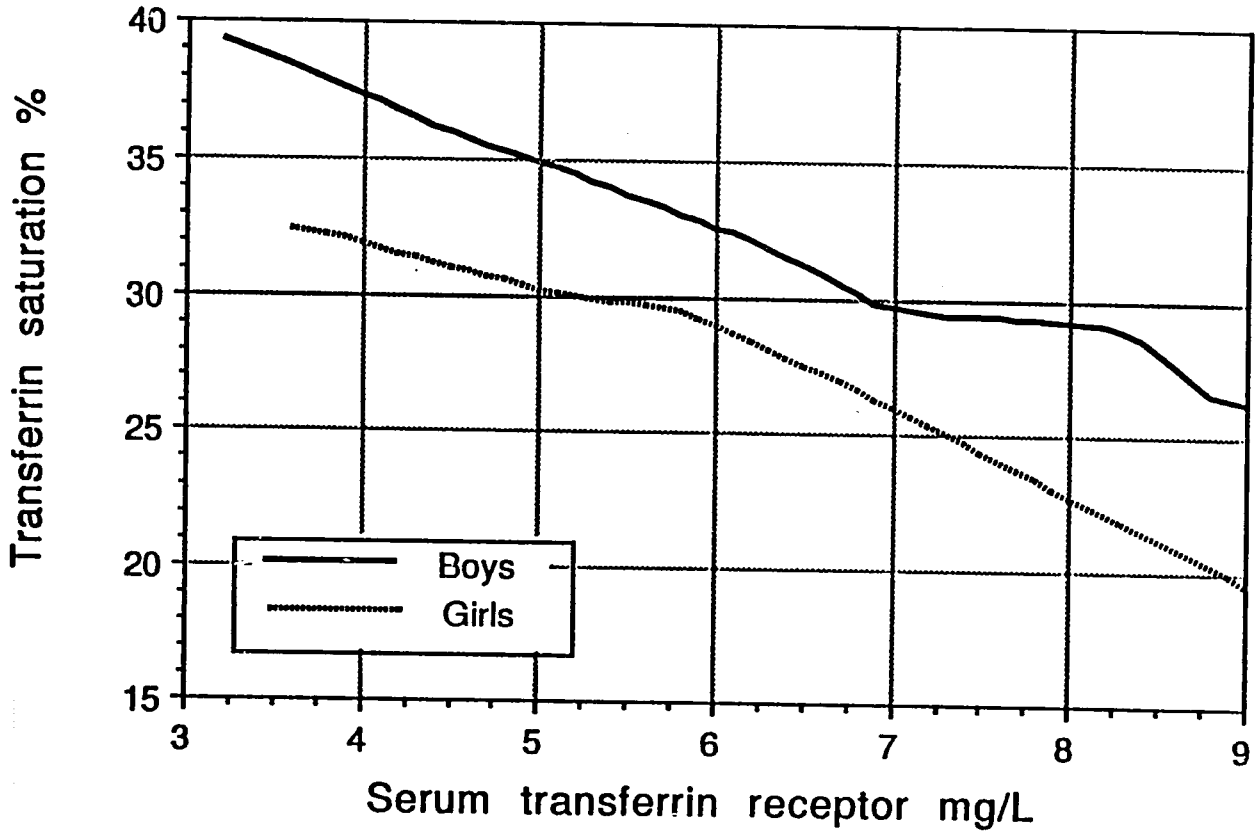

Transferrin saturation %
40
35
30
25
20
15
3
4
5
6
7
8
9
Boys
Girls
Serum transferrin receptor mg/L

Fig 5E

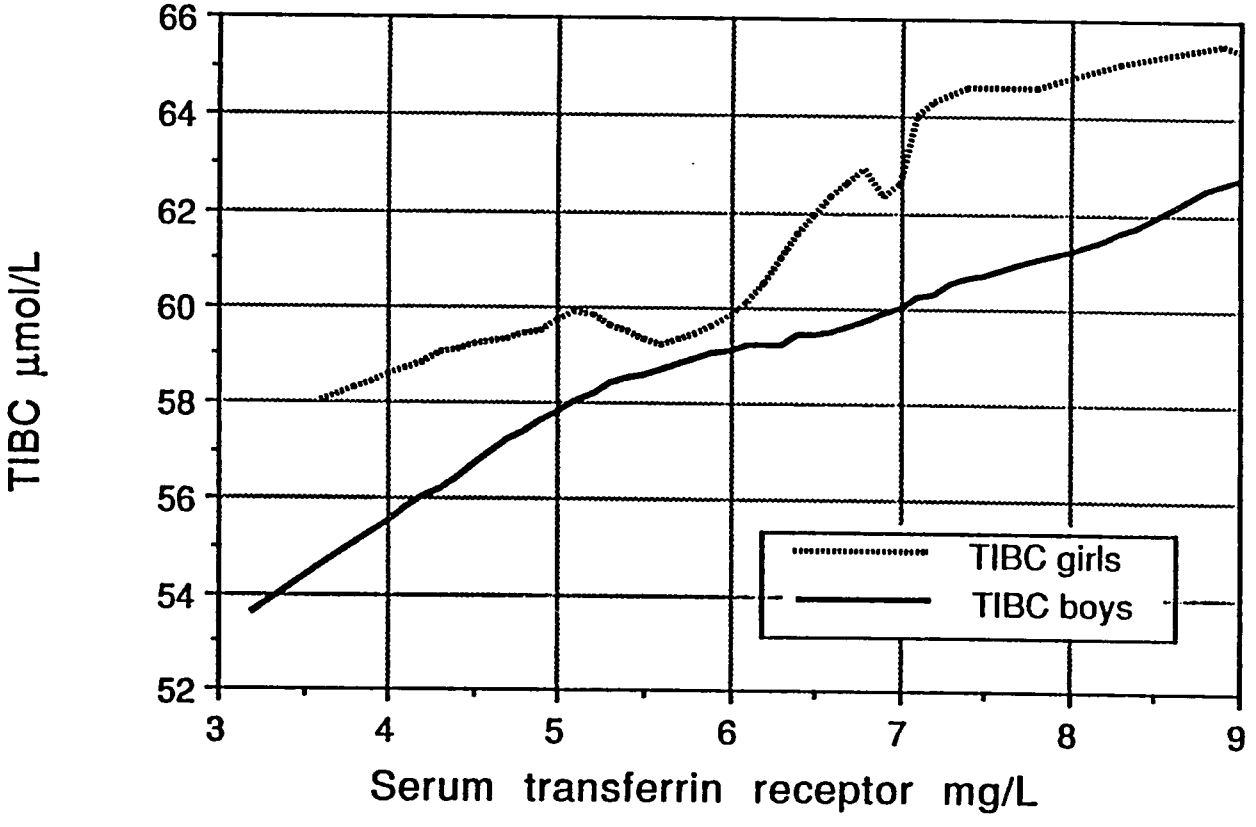

66
64
62
60
58
56
54
52
TIBC µmol/L
3
4
5
6
7
8
9
Serum transferrin receptor mg/L
TIBC girls
TIBC boys

Fig 4A

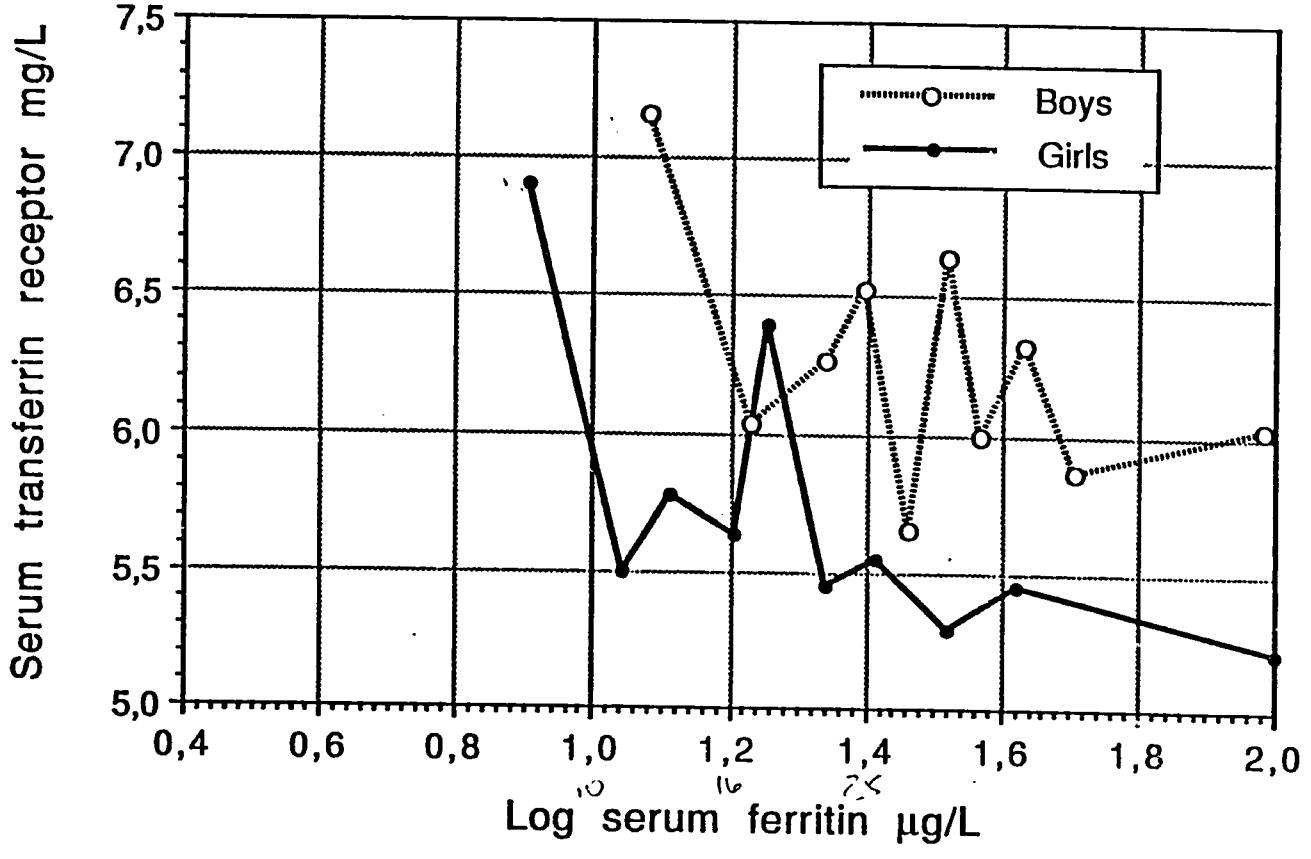

Serum transferrin receptor mg/L
7,5
7,0
6,5
6,0
5,5
5,0
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
2,0
Log serum ferritin µg/L
Boys
Girls

Fig 4B

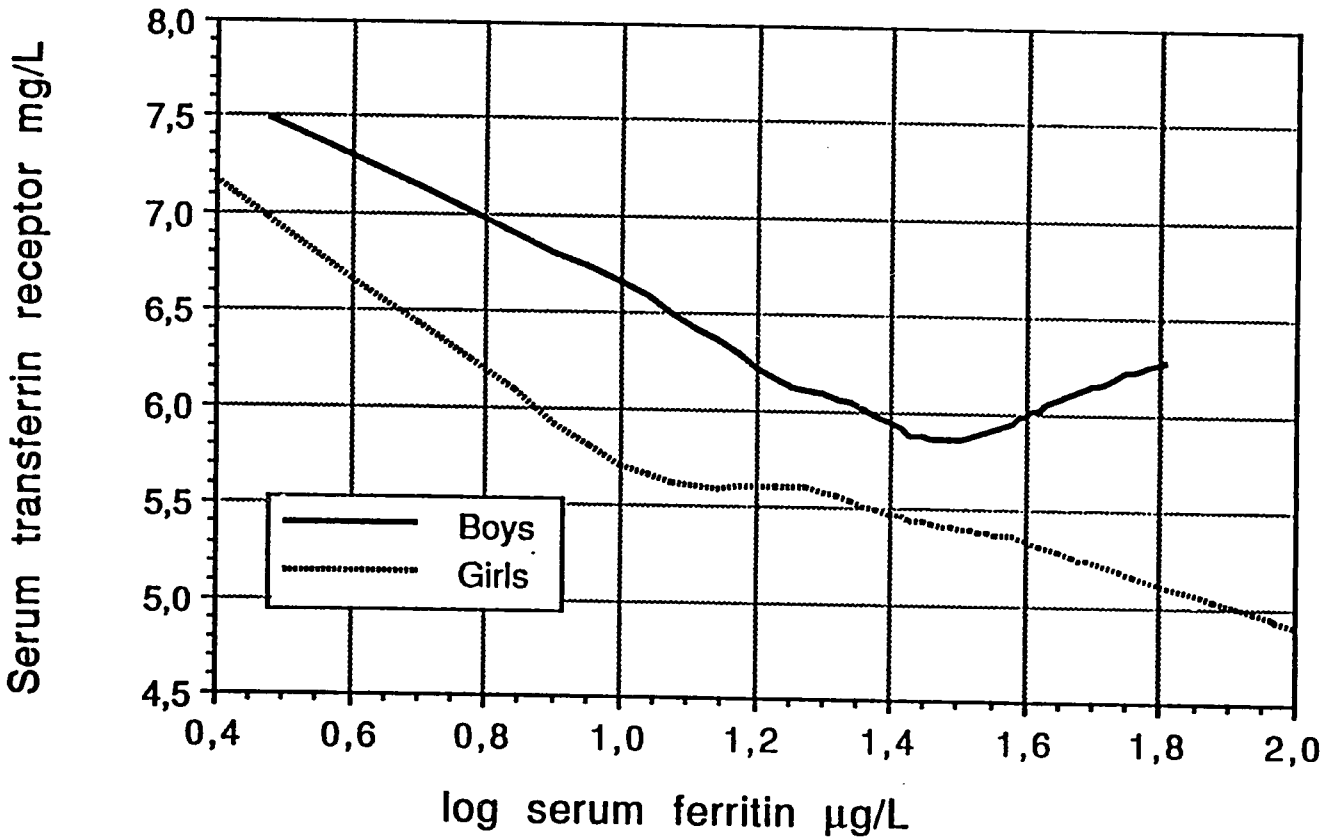

Serum transferrin receptor mg/L
8,0
7,5
7,0
6,5
6,0
5,5
5,0
4,5
0,4
0,6
0,8
1,0
1,2
1,4
1,6
1,8
2,0
log serum ferritin µg/L
Boys
Girls

Fig 5 A

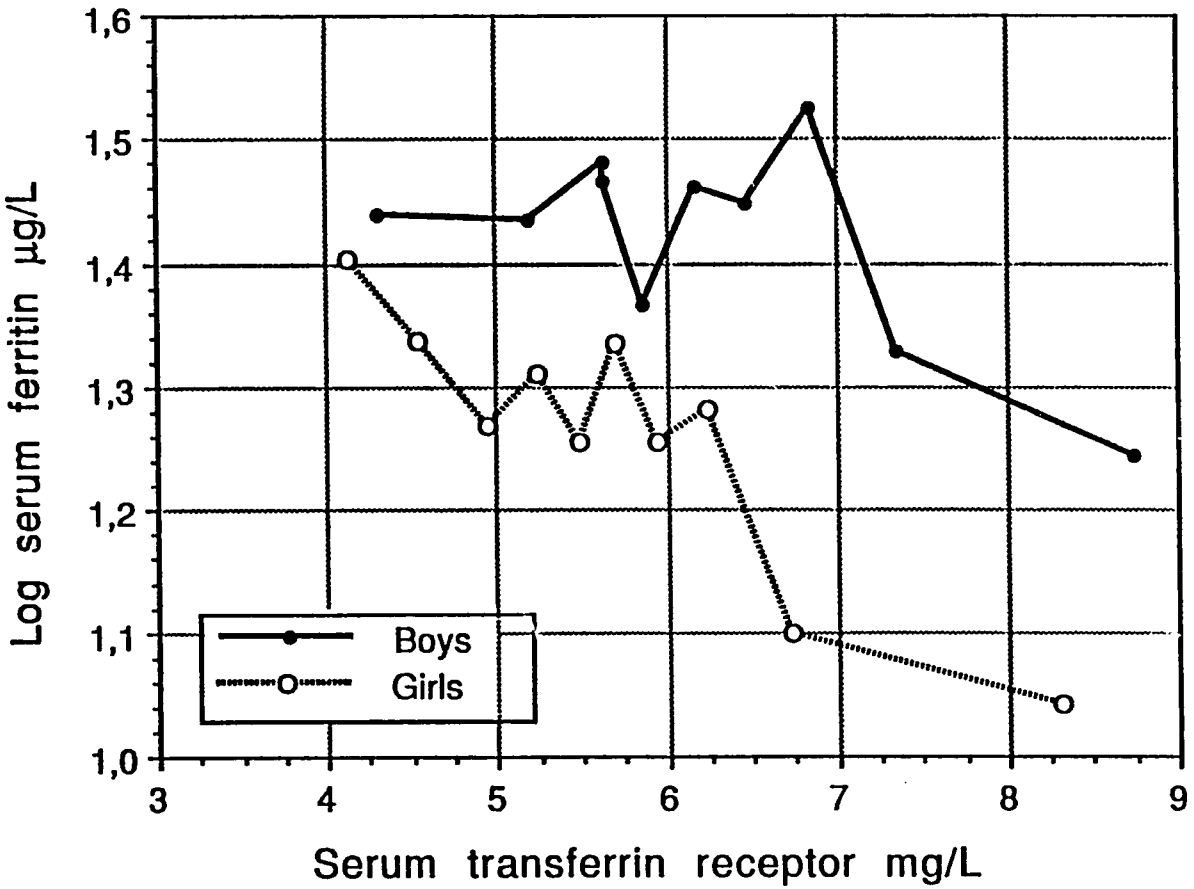
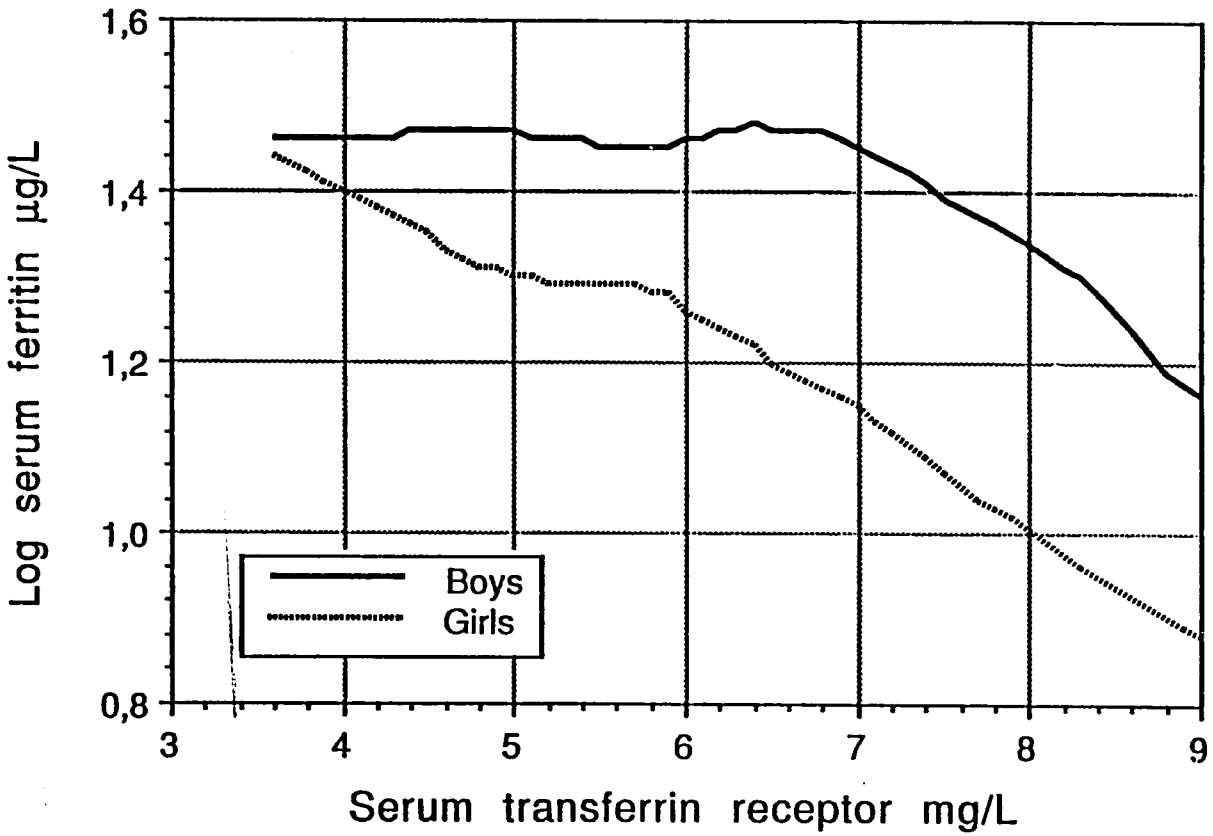
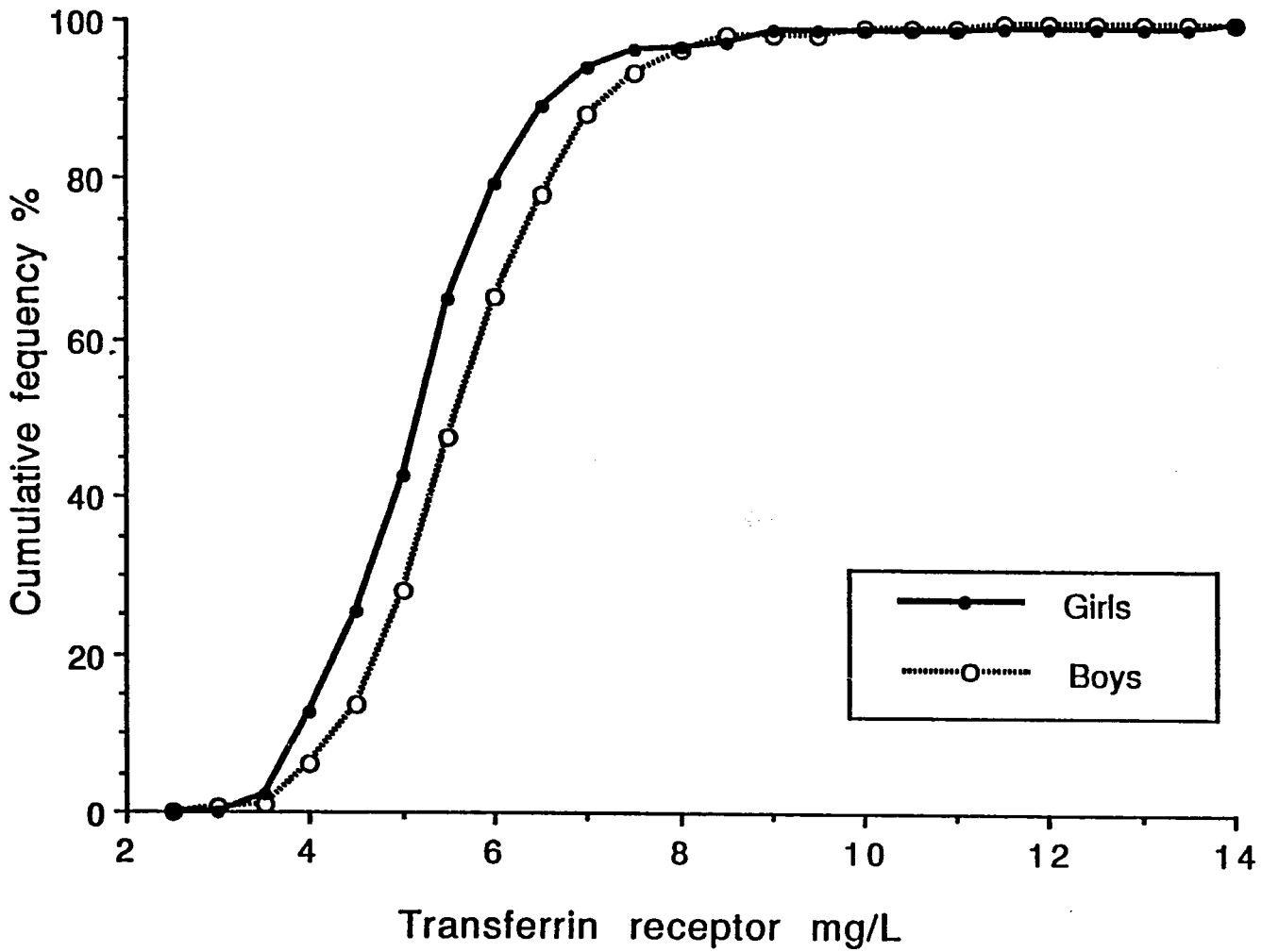

Log serum ferritin µg/L
1,6
1,5
1,4
1,3
1,2
1,1
1,0
3
4
5
6
7
8
9
Serum transferrin receptor mg/L
Boys
Girls

Fig 5b

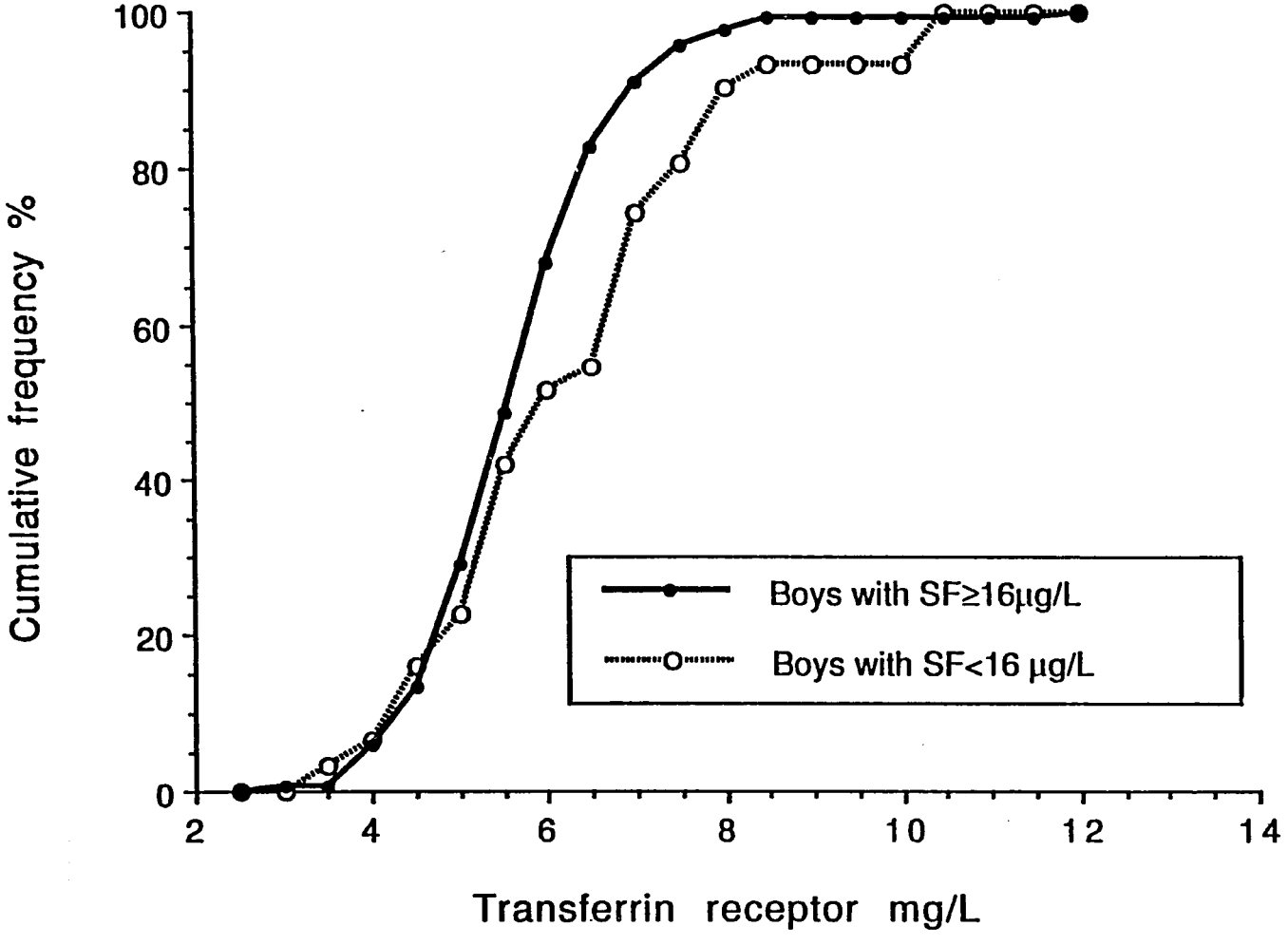

Log serum ferritin µg/L
1,6
1,4
1,2
1,0
0,8
3
4
5
6
7
8
9
Boys
Girls
Serum transferrin receptor mg/L

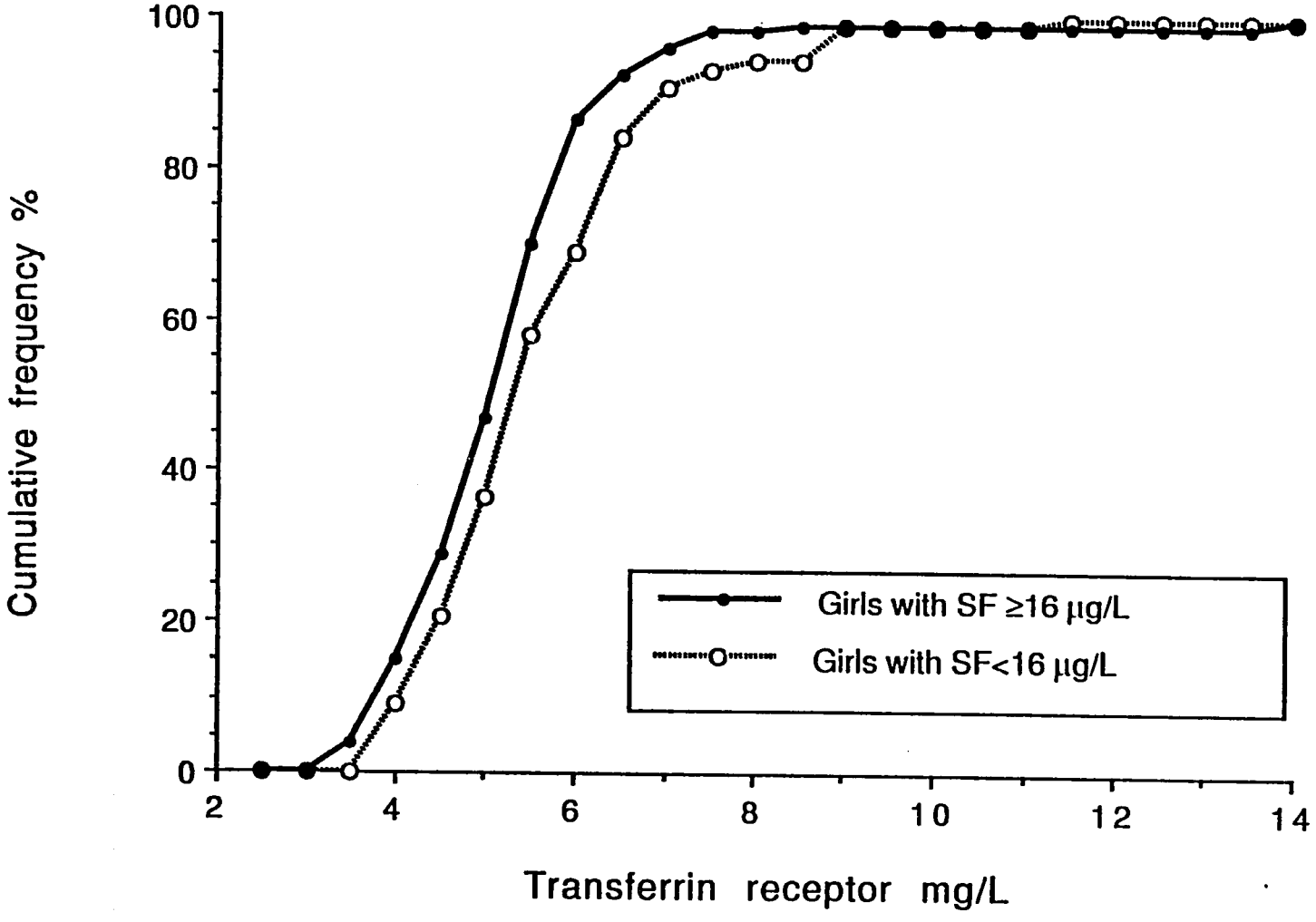
For information only

Cumulative distribution of transferrin receptor concentration in boys and girls



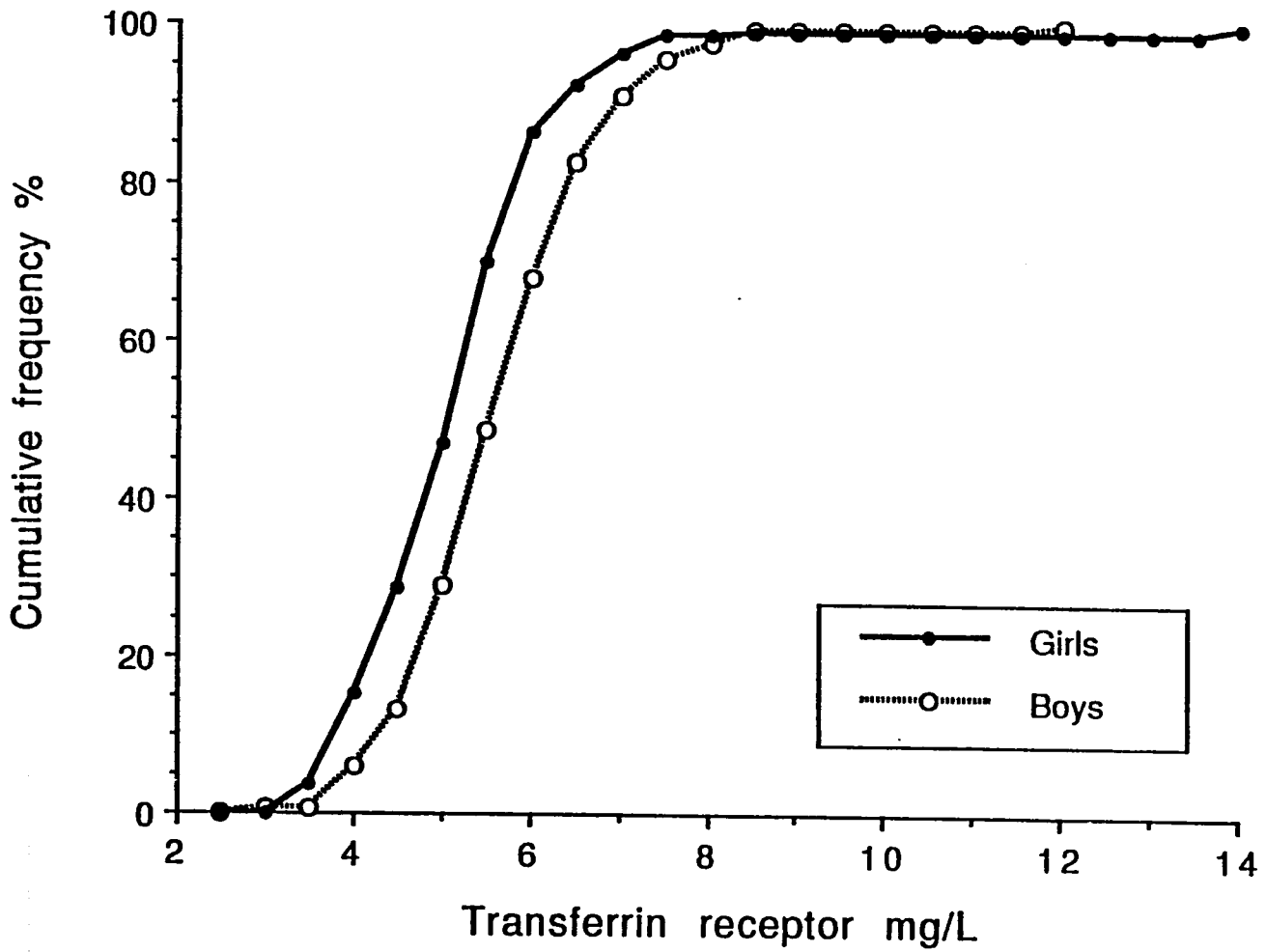
Cumulative distribution of transferrin receptor concentration in boys




Cumulative distribution of transferrin receptor concentration in girls
Cumulative frequency %
100
80
60
40
20
0
2
4
6
8
10
12
14
Transferrin receptor mg/L
Girls with SF ≥16 µg/L
Girls with SF<16 µg/L

For information only

Cumulative distribution of transferrin receptor concentration in boys and girls with serum ferritin ≥16 µg/L



APPENDIX B-7

EVALUATION OF THE IRON CONTENT OF NORMAL DIETS AND THE RESULTS OF IRON SUPPLEMENTATION IN SCHOOL CHILDREN IN JAMAICA

Prepared by

THE GOVERNMENT OF JAMAICA

in collaboration with

THE CARIBBEAN FOOD AND NUTRITION INSTITUTE

Caribbean Food and Nutrition Institute
Kingston 7, Jamaica

Pan American Health Organization
Pan American Sanitary Bureau, Regional
Office of the World Health Organization

1992

INTRODUCTION

Existing data indicate that anaemia exists in all countries of the English-speaking Caribbean. It is most common in pregnant, lactating women and in pre-school children, with country prevalence (using World Health Organization [WHO] criteria) between 27-100 percent and 15-85 percent respectively.

Severe anaemia, with a haemoglobin concentration in the blood below 8 g/dl is found in around 6 percent of pregnant and lactating women and 11 percent of pre-school children in some of the countries.

There are three major causes of anaemia in the Caribbean:

1. Inadequate iron nutrition (major cause);
2. Specific parasitic infestation (in pockets in some countries); and
3. Haemoglobinopathies (Genotype SS [Sickle Cell Disease] in about 0.9 percent).

All three causes are important from a public health point of view and all three may interact.

The major (dietary) nutritional cause of anaemia is iron deficiency, probably due to a low absorption in the intestine and an inadequate dietary intake. A dietary deficiency of folate is sometimes also responsible. Changes in food habits of some sections of the population are associated in some instances with a deficiency of vitamin B12. This appears to be currently a minor problem in the English-speaking Caribbean but it could increase.

Hookworm and whipworm (trichuris) are the two main parasites associated with anaemia. However, in the English-speaking Caribbean, infestations of hookworm are isolated in certain areas of the population in some countries. The major haemoglobinopathy causing anaemia is sickle cell disease and studies have shown that about 0.5-0.9 percent of West Indians suffer from sickle cell diseases (Genotype SS) (1-11).

Studies conducted in antenatal clinics and surveys conducted in the Caribbean from 1972-1982 show that there are an estimated 76,086.5, 65,769.0 and 6,422.5 antenatals with haemoglobin levels below 11.0, 10.0 and 8.0 g/dl respectively, from an estimated population in the English-speaking Caribbean of 5,670,000. This anaemia is probably caused from a deficiency of iron, folate and vitamin B12 (12).

Anaemia has been recognized as a public health problem in Jamaica since 1950. A review of these studies from 1950 to 1974 has been made and summarized (1).

It was concluded that iron deficiency was the most common cause of this anaemia in Jamaica. The anaemia appeared to affect mostly infants 0-18 months of age and pregnant and lactating women. Only one study even mentioned school age children.

In an island wide anaemia survey conducted in 1980 on pre-school age children and pregnant and lactating women, it was found that 61.6 percent, 58.7 percent and 69.1 percent of pregnant women, lactating women and pre-school age children, respectively, had haemoglobin levels below WHO Recommended Standards (3).

In 1987 an island wide anaemia survey of 2,716 females and 2,334 males aged up to nineteen years, 209 pregnant and 250 lactating women, showed that 65% boys and 56% girls less than two years old, and 32% of the fifteen to nineteen year old females, were iron deficient. Among pregnant and lactating women, the prevalence of iron deficiency was 52% and 42% respectively. The iron intake was 60.7 and 86.1% below RDA values for males and females 13-15 year old, respectively. It was concluded that the social and economic costs to the countries in terms of the loss of productivity, mortality and medical care have

reached alarming proportions and are worsening in the face of economic adjustments (13).

Since numerous studies have indicated that iron deficiency in pregnancy is a serious public health problem in Jamaica as well as the entire English-speaking Caribbean. When the pregnant women first present themselves at the ante-natal clinics they are already iron deplete. The problem is how to reach the adolescent females who are the ones getting pregnant or soon will be the ones facing adulthood.

This proposal seeks to address a possible means of reducing that iron deficiency in adolescent females as well as school age children both male and female through studying the effect of iron fortified beverages on the anaemia status of school age children.

OBJECTIVES OF THE RESEARCH

The Overall Objective:

To evaluate a programme for reducing iron deficiency and anaemia in school age children in Jamaica by supplementing the diet with fortified food products.

A. To determine the nutrient intake of school age children in Jamaica.

B. To determine a typical diet and determine the iron intake and iron absorption from that diet.

C. To evaluate a system of iron supplementation of normal diets with an iron fortified food for school age children.

D. To evaluate the iron status of Jamaican school age children receiving both school feeding programmes.

VEHICLES FOR IRON SUPPLEMENTATION

Milo dissolved in skimmed milk powder, the traditional Jamaican school feeding programme, and the school feeding programme which consists of milk and bun are being considered for the investigation.

Milo is a chocolate-based drink that is widely used throughout the Caribbean. It is sold in crystal form in cans and a 250 gram package milo drink and is made by Trinidad Foods Ltd. which is a subsidiary of Nestle, Switzerland. In the latter, crystals are dissolved in 250 ml of milk and sealed. Iron in the form of saccharated ferric oxide is added to milo at the rate of 3.5 mg/gram. The absorption of ferric oxide was questioned. Newer data indicated an adequate absorption from saccharated ferric oxide (19).

A new type of iron in the form of ferrous succinate has been added to milo. The absorption of this new type of iron must be tested in the context of the normal dietary patterns of the children.

The School Feeding Programme was agreed on in 1984 between the World Food Programme (WFP), and the Government of Jamaica. By the agreement, 25,000 children attending Primary and All-age Schools in Jamaica receive a snack at lunch-time, between 11:00 a.m. and 1:00 p.m. The snack consists of a bun (solid) and one-half pint of milk (liquid), and children are required to make a contribution of 20 cents daily.

The objectives of the project are as stated below:

1. By helping to provide a daily dietary supplement, food aid is intended to:
   a) encourage greater and more regular school attendance;
   b) assist in satisfying the minimum daily dietary needs of participating children; and

c) serve as a source of income transfer to participating families.

Production and distribution of the snacks - buns and milk - are undertaken by a local factory, Nutrition Products Limited (NPL) and its subsidiaries. All commodities are provided by WFP. The responsibilities of NPL include the following:

i) production of sufficient quantities on a daily basis

ii) maintenance of an agreed standard for the products

iii) daily delivery to the respective schools.

The distribution route, and approximate times of delivery, are established by the NPL, and communicated to the schools. This generally represents regular delivery times.

The process of distribution at the school level also involves those teachers designated with such responsibility. The teachers receive the allotted number of snacks, and distribute to participating children within their institution. The Food Issue and Utilization Statements (FIUSs), which are designed to monitor internal distribution of the snacks, are also completed by the teachers. The terms and restrictions which apply to distribution include the following:

i) children should indicate to their teacher/school their interest in receiving a daily snack from the SFP;

ii) older children have the option of receiving two (2) buns and one-half pint of milk per day, while the younger ones receive one (1) bun and one-half pint of milk per day;

iii) children who cannot afford the 20 cents on a given day(s), as indicated by their parent/guardian, should receive a free snack;

iv) there is a maximum allowable number of free snacks permitted for distribution daily.

The bun and milk snack is intended to provide participating children with 30 percent of their daily energy (calorie) and of protein requirements. The composition of the respective products is as follows:

Bun	-	60g wheat flour
	-	15g brown sugar
	-	15g milk powder
	-	5g butteroil
1/2 pt. Milk	-	25g dried skimmed milk
	-	10g butteroil

There are marginal variations in product composition, given the variation in product offering. Substitutes for the original 'nutribun' include a bulla or spice cake.

Some schools in Jamaica still use the traditional school feeding programme. This consists of a cooked meal at lunch time and is usually served to most children in that school. The lunch usually consists of a dumpling (made from wheat flour), yam and chicken neck and back. It may also consist of a dumpling, rice and chicken neck and back.

METHODOLOGY

Preliminary Investigations

Jamaica is divided into fourteen parishes. Two parishes will be chosen to evaluate a typical diet of school age children and their iron status. These parishes will be chosen from previous nutrition studies indicating the areas in Jamaica that are most likely to be seriously iron deficient.

Food Consumption and Analysis

A random sample of school age children from several schools will be chosen for the food consumption study and dietary data collected by two 24 hour recalls (one mid-week day eg. Thursday and one weekend day eg. Sunday). This will provide a good average range for a day's intake.

A sub-sample of these children will be chosen for a more detailed dietary study. The foods eaten by these children for a day will be weighed for purposes of a comparison with the 24 hour recall and a more accurate determination actual intakes.

The data will be sent to CFNI, Kingston, Jamaica, where the nutrients will be calculated on the AST Premium 386/25 computer.

Two to three typical diets will be postulated from the above data The foods which comprise these diets will be freeze-dried at CFNI, Kingston, .maica and sent to Nestle, Switzerland for composite analysis. Total calories, protein, dietary fibre, iron, copper, zinc, vitamins and phytate content will be analyzed.

Food samples will also be sent to the University of Kansas Medical Center where iron absorption studies will be undertaken.

Hematology

A finger prick blood sample will be taken from each school age child. The hemoglobin level will be determined by the Hemocue method (15). Additional blood will be collected in a microcuvette collecting tube. The blood samples will be stored in a ice container and taken to the Iron Nutrition Monitoring Laboratory, CFNI, Kingston, Jamaica.

The blood samples will be centrifuged, the plasma removed and frozen. The ferritin and transferrin receptor level will be performed at the Iron

Nutrition Monitoring Laboratory in Jamaica and some samples will be re-determined at the University of Kansas as a quality control system (16-18).

Final Investigation

The schools chosen for the final investigation should be taken from the schools in the preliminary study. The schools should be in an iron deficient area. Depending upon the iron deficit the amount of milo given to each child will be determined. It could be one or two glasses daily. Also the amount of iron added to the milo could be changed.

Four schools will be chosen for the study. The four schools will be divided as follows:

Control	Test Group A	Test Group B	Test Group C
One glass of skim milk containing 25.0 g of unfortified milo.	One glass of skim milk containing iron fortified milo.	The traditional school feeding.	The school feeding consisting of milk and bun.

Two hundred and forty school age children aged 6-14 years of age will be chosen for the study. This will be 60 children from each shcool.

A blood sample will be taken from each child and the haemoglobin, plasma ferritin and transferrin receptor level will be determined as described above.

Each child in the Control and Test Group A will be given milo under careful supervision. An individual will be assigned to each school to prepare the milo daily. A careful record will be kept on the amount of milo consumed by each child per day. The children receiving the traditional school feeding (Group B) and those receiving the milk and bun (Group C) will also be carefully monitored as to the amount of food they receive daily.

The feeding trial will continue for a period of ten months. At the end of the ten month period another blood sample will be taken from each school age

child and the haemoglobin, ferritin and transferrin receptor level will be determined.

ANALYSIS OF DATA

The data will be sent to CFNI for analysis. Correlations will be done on the various haemological parameters. Comparisons will be made before and after implementation of the new fortification scheme. A final report will be written at CFNI and presented to the Government of Jamaica and the donor organization.

Responsibilities

The Government of Jamaica

The Government of Jamaica will be responsible for supplying the traditional school feeding programme and the school feeding programme consisting of milk and bun.

The University of Kansas

The University of Kansas will be responsible for technical advise and collaborating on quality control on the haematology determinations. They will also be responsible for conducting the iron absorption studies.

Nestle Research Center

The Nestle Research Center will be responsible for technical advise. They will also be responsible for providing the unfortified and fortified milo and the skim milk powder. They will conduct a composite analysis on a typical Jamaica diet.

The Caribbean Food and Nutrition Institute

The Caribbean Food and Nutrition Institute will be responsible for conducting the survey, analyzing the blood samples, doing the statistical analysis and writing the final report.

ETHICAL ISSUES

The project proposal will be presented to the Ethics Committee of the University of the West Indies.

Iron Added to Milo

The central assumption in this study is that the use of iron fortified milo will have a positive effect on blood indicators.

Quantification of this model requires several requirements:

1. We assume no significant dietary changes and that any beneficial effects are largely due to the consumption of iron fortified milo.
2. Twenty five grams of milo will be dissolved in dried skim milk powder and served to each school age child.
3. Each 25 grams of milo will contain 5 mg of iron.
4. For the average anaemic adult (70 Kg body weight):
   a) 150 mg extra iron absorbed will yield a 1.0 g/dl increase in haemoglobin;
   b) 10 mg extra iron stored will yield 1.0 ug increase in ferritin;
   c) 10% of iron ingested will be absorbed, if the person is anaemic (5.0% if the person is not anaemic) [Dr. Cook, personal communication].

   We assume that each school age child weighs about 30-40 kgs.

40 kg child = 4/7 of 150 = 85.7

i.e. 85.7 mg extra iron absorbed will yield a 1.0 g/dl increase in haemoglobin.

If anaemic will absorb 0.5 mg of iron per day, a nine month school year of 180 days, 180 x 0.5 = 90 mg of iron absorbed per school year.

Per school year the Hb should increase about 1.0 g/dl.

Per school year the ferritin should increase 9.0 ug/l.

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BUDGET - 1992
Preliminary Investigation

	CFNI	REQUESTED
PERSONNEL		
Jamaica		
Stipend for Field Supervisor ($400 per month for 3 months)		1200
Jamaica - CFNI		
Public Health Nutritionist	20% of time (No cost)	
Nutrition Educator	20% of time (No cost)	
Biochemist - $1,000 per month (two months)		2,000
Laboratory Helper - $150 per month (two months)		300
Secretarial Assistance, phone calls		1,000
University of the West Indies		
Statistician/Systems Analyst (one month)		1,000
Data Entry Clerk (one month)		500
Supplies and Equipment		
Lancets Blood Collecting Tubes Hemocue microcuvettes		2,000
Hemocue instruments (2 @ $600 each)		1,200
Transportation and Per Diem		
The type of transportation is to be determined.		2,000
		$10,200

BUDGET - 1993
Main Investigation

	CFNI	REQUESTED
PERSONNEL		
Jamaica		
Stipend for Field Supervisor ($400 per month for 12 months)		4,800
Stipend for four persons to prepare and record the intake of milo and the two school feeding programme. ($50 per month per person for 10 months)		2,000
Jamaica - CFNI		
Public Health Nutritionist	20% of time (No cost)	
Nutrition Educator	20% of time (No cost)	
Biochemist - $1,000 per month (2 months)		2,000
Laboratory Helper - $150 per month (2 months)		300
Secretarial assistance, phone calls, stationery		1,000
University of the West Indies		
Statistician/Systems Analyst - two months ($1,000 per month)		2,000
Data Entry Clerk (2 months) ($500 per month)		1,000
SUPPLIES AND EQUIPMENT		
Jamaica		
Lancets, blood collecting tubes, Hemocue microcuvettes		1,000
Paper and Office Supplies		500
Jamaica - CFNI		
Chemicals, glassware, etc.		1,500

	CFNI	REQUESTED
Transportation and Per Diem		
		4,350
Transportation Costs The type of transport is to be determined.		
		22,450
FINAL STUDY		
		11,200
PRELIMINARY STUDY		
		21,450
FINAL STUDY		
		32,200
	PAHO Programme Support costs	4,186
		36,386

APPENDIX B-8

GASTRIC DELIVERY SYSTEM IRON TRIAL

Introduction:

Maternal mortality in Indonesia is high (MMRatio=250-718/100,000 LB). As in most developing countries, the major causes of maternal mortality are hemorrhage, sepsis and toxemia. Maternal anemia is a recognized risk factor for maternal death due to hemorrhage. In one study in Indonesia, the maternal mortality ratio for anemic women was substantially higher (700/100,000) than for non-anemic women (190/100,000). In a study noted in World Health Statistics 1982, pregnant women in Indonesia had the lowest average hemoglobin levels among women in 5 countries in the region. The National Household Health Survey of 1985/86 showed a prevalence rate of anemia over 70% in women of childbearing ages.

There has not been sufficient research to document the etiology of the problem of anemia in Indonesia; however, poor absorption of dietary iron due to rice-based diets appears to be an important contributing factor. In other countries in the region, supplementation of dietary iron with iron sulfate has achieved reductions of anemia of fifty percent. However, there appears to be many obstacles to widespread acceptance of iron sulfate supplementation as a public health strategy for reduction of the prevalence of anemia. Among the problems are the lack of benefit from these iron tablets among women, and the problems of continued compliance where the tablets are available. Many women who have access to the tablets simply do not want to take them because of the perception that the side-effects (nausea, constipation, etc.) outweigh the potential benefits of taking them.

In Indramayu in West Java, for example, formative research into the factors influencing compliance with iron supplementation of pregnant women found that:

- Maternal anaemia is not perceived as priority health problem by pregnant women, their families, or traditional and modern maternal care providers.

- Factual knowledge of maternal anaemia, its relationship to maternal and neonatal health, and the need for and benefits of iron supplementation for pregnant women is low at the community level and among traditional and modern maternal care providers.

- Side effects of iron supplementation (constipation, nausea, change in color of stools) and undesirable tablet characteristics (smell, taste) are common causes of discontinuation of iron tablet use. Social support from family influentials and maternal care providers can increase continuation of therapy.

Recently, a new formulation and delivery system for iron has been tested, and in trials in Jamaica, appears to significantly lower the rate of adverse effects while still reducing anemia. If true, this method of iron delivery would significantly decrease problems with compliance. This new delivery

system is called the Gastric Delivery System for Iron (GDS Iron). This study proposes to examine two questions:

Is the GDS Iron pill as effective in anemic pregnant women at raising hemoglobin levels as the standard Ferrous Sulfate tablet, and is it better tolerated?

Is compliance less of a problem with the GDS Iron tablet when compared to a standard ferrous sulfate formulation and placebo that contains no iron?

These questions will be answered by two separate trials in different study populations. The studies will be done in Surabaya, Indonesia by the research group at PS Dr. Sutomo Hospital. Additional laboratory support will be provided by experts from the University of Kansas. Logistic support and technical assistance will also be provided by the MotherCare Project through the USAID/Jakarta TAACS Adviser.

TRIAL 1

Summary:

The first trial will take place in a group of anemic pregnant women and will involve two groups of women. The first group will be given the GDS Iron pill (50 mg. elemental iron) for a period of 120 days during the last four months of their pregnancy. The second group will be given the standard ferrous sulfate therapy (60 mg. elemental iron) for the same time period during the last 4 months of their pregnancy.

After obtaining informed consent, the women in the two groups will be randomly allocated to each group following screening to determine that they meet the criteria for gestational age and anemia (8.0 - 10.9 g/dl hemoglobin). Both groups will be treated identically, seen every 30 days and given pills in packets of 30 pills for a total of 120 days (5 visits prior to birth, at birth and a final time 30 days following birth). They will have hemoglobin, ferritin and transferrin levels measured at three points during the pregnancy (entry, 60 days and at 120 days), and at birth (both mother and infant), and 30 days (mother and infant). The analysis will compare biochemical parameters with reports of compliance and side effects, through interviews with women in the two groups, to determine if the GDS Iron formulation is as efficacious as ferrous sulfate, and if it has a higher compliance rate.

Sample size and study length:

Two groups of pregnant women with hemoglobin between 8.0 and 10.9 g/dl will be enrolled, with 300 women in each group. The women will be recruited from 2 prenatal care clinics. Each clinic sees an average of 20 women per day, and estimates are that each week 25 anemic pregnant women will be enrolled per clinic, for a total of 50 women per week. All the women will be enrolled by the end of the 6th week of the study. Allowing 120 days for the duration of

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the pregnancy, and 30 days for the required follow-up period, two weeks for data entry and cleaning, plus four weeks for analysis and report writing, the study will be completed by the end of the 8th month.

Enrollment:

Pregnant women aged 15-45 attending the prenatal clinics will be enrolled. The goals of the study will be explained to them, and they will be asked to participate in a screening process to determine their eligibility. They will have a fingerstick hemoglobin determination done, and if anemic (8.0-10.9 gm/dl) will be asked to participate in the study. Following receipt of written informed consent, two vacutainers of blood will be taken. Baseline social, demographic, nutritional, family planning and previous pregnancy/birth data will be taken. Women screened and found to be anemic but not choosing to enter the study will be told that they are anemic and counseled regarding taking iron.

Data gathering:

Following the first clinic visit where baseline data will be gathered, the women will be seen at intervals of 30 days. A social worker will interview the women and record answers to questions concerning compliance and side effects. Following the interview, women will be given a 30 day supply of pills and asked to return in 30 days. Women in the study will have transportation paid for when returning to the clinic for visits. Women who do not return for the clinic visits will be visited by the social workers at home if possible. At the third, fifth, sixth and seventh return visit (60 days, 120 days, birth and 30 days after birth) blood will be taken for hemoglobin, transferrin receptor and ferritin. Weight and MUAC will be taken also. The hemoglobin and ferritin will be determined at Dr. Sutomo Hospital. Frozen samples will be sent to the University of Kansas for determination of transferrin receptor. Ten percent of samples will also have hemoglobin and ferritin checked for quality control. Data will be entered in a database using Epi-Info by a data entry person.

Data Analysis:

The data will be analyzed first with descriptive statistics and then by stratified analysis. Following this, logistic regression will be done if the stratified analysis is not sufficiently powerful. Leaving Hb as a continuous variable, an ordinary least squares multiple regression will also be done. Socio-demographic variables (age, religion, education, occupation, number of persons in household, number of children); reports of compliance and side effects (diarrhea, nausea, loss of appetite, heartburn; abdominal cramps, constipation, color of stool); biochemical and physical parameters (height, weight, MUAC, hemoglobin, ferritin, transferrin receptor) and others (abbreviated nutritional history, menstrual history, etc.) will be determined for the two groups to see if there are significant differences between the GDS

Iron group, and the ferrous sulfate group, the control group. Data for infants will be compared using birth (cord blood) and 30 day samples.

Ethical considerations:

The study does not expose any of the women, fetuses or babies to health risks. Rather, it provides the pregnant women with a major health benefit as it identifies them as being anemic during their pregnancy (which they would not have known) and offers therapy for this. Any women who are anemic at the end of the study (non-responders) will be given a supply of iron sulfate and counseled regarding its appropriate use. Women whose biochemical parameters indicate that the anemia is probably not the result of iron deficiency will be counseled regarding seeking the appropriate treatment. The major risks in the study are those resulting from the drawing of blood for biochemical tests. These risks are minimal.

TRIAL 2

Summary:

The second trial will take place in anemic non-pregnant women and will involve three groups of non-pregnant, anemic women. The first group will be given the GDS Iron pill for a period of 90 days. The second group will be given ferrous sulfate therapy in an identical appearing capsule for the same time period. The third group will receive an identical appearing placebo containing biologically inert ingredients for the same time period. The study will be double blinded with neither the women nor the researchers knowing who is in which group. After obtaining informed consent, the women in the three groups will be randomly allocated to each group following screening to determine that they meet the criteria for anemia. All three groups will be treated identically, seen every 30 days and given pills in packets of 30 pills for a total of 90 days (3 visits including final). They will have hemoglobin, ferritin and transferrin levels measured at the three points and the analysis will compare biochemical parameters with reports of compliance and side effects in the three groups. This data will be used to draw conclusions as to whether the GDS Iron pill is significantly better (fewer side effects, higher degree of compliance, decreases rates of anemia) than the standard ferrous sulfate pill or the placebo.

Sample size and study length:

Three groups of non-pregnant women with hemoglobin between 8.0 and 10.9 g/dl will be enrolled, with 100 women in each group. The women will be enrolled from two busy family planning clinics. Each clinic sees approximately 50 patients per day and its estimated that each week 25 women meeting the criteria will be enrolled, for a total of 50 women from the two clinics. Thus, the three groups of 100 women will require 6 weeks to enroll. Allowing 90 days for the duration of the study, 2 weeks for data entry/cleaning, four weeks for analysis and report writing, it will require 6 months to finish the study.

Enrollment:

Menstruating women aged 15-45 attending the family planning clinics who are not pregnant or breast-feeding will be enrolled. The goals of the study will be explained to them, and they will be asked to participate in a screening process to determine their eligibility. They will have a fingerstick hemoglobin determination done, and if anemic (8.0-10.9 gm/dl) will be asked to participate in the study. Following receipt of written informed consent, two vacutainers of blood will be taken, baseline social, demographic, nutritional, family planning and previous pregnancy/birth data will be taken. Women screened and found to be anemic but not choosing to enter the study will be told that they are anemic and counseled regarding taking iron.

Data gathering:

Following the first clinic visit when baseline data will be gathered, the women will be seen at intervals of 30 days. A social worker will interview the women and record answers to questions concerning compliance and side effects. Women in the study will have transportation paid for when returning to the clinic for visits. Women who do not return for the clinic visits will be visited by the social workers at home if possible. At the second and fourth follow-up visits (45 days and 90 days) blood will be taken for hemoglobin, transferrin receptor and ferritin. Weight and MUAC will be taken at each visit also. The hemoglobin and ferritin will be determined at Dr. Sutomo Hospital. Frozen samples will be sent to the University of Kansas for determination of transferrin receptor. Ten percent of samples will also have hemoglobin and ferritin checked for quality control. Data will be entered in a database using Epi-Info by a data entry person.

Analysis

The data will be analyzed first with descriptive statistics and then by stratified analysis. Following this, logistic regression will be done if the stratified analysis is not sufficiently powerful. Socio-demographic variables (age, religion, education, occupation, number of persons in household, number of children, etc.); reports of compliance and side effects (diarrhea, nausea, loss of appetite, heartburn, abdominal cramps, constipation, color of stool, menstrual history, etc.,); biochemical and physical parameters (height, weight, MUAC, hemoglobin, ferritin, transferrin receptor) and others (abbreviated nutritional history, menstrual history, history of other drugs/medicines, etc.) will be determined for the three groups to see if there are significant differences between the GDS Iron group, the ferrous sulfate group and the placebo group.

Ethical considerations:

The study does not expose any of the women to health risks. In reality, it provides them with a major health service, as it identifies them as being anemic (which they would not have known) and offers therapy for this. Any women who are anemic at the end of the study (placebo group, non-responders) will be given a supply of iron sulfate and counseled regarding its appropriate use. Women whose biochemical parameters indicate that the anemia is probably not the result of iron deficiency will be counseled regarding seeking the appropriate treatment. The major risks in the study are those resulting from the drawing of blood for biochemical tests. These risks are minimal.

Personnel and logistics:

Both studies will be done by the Research Group at RS Dr. Sutomo Hospital. The studies will be done concurrently, but with staggered starting points to avoid logistic and personnel problems. The Research Group will have a Study

Coordinator, and each study will have a Principal Investigator and Co-investigators. The studies will share ancillary personnel who are two social workers (one for each clinic), and a data entry/secretary/computer operator.

A computer with hard disk, monitor and printer will be purchased for data entry and analysis. The specialized software for data input and analysis will be provided. Two Hemocue machines with sufficient cuvets for all samples will be provided. Other equipment such as vacutainers/bleeding equipment, laboratory supplies, computer paper and diskettes will be purchased locally with money provided from the budget. The budget will provide funds for transport of the women to the clinic appointments, and for the social workers to visit women who have missed appointments. Funds will also be provided for training, for communications and mailing, and for publication of the final report.

Samples will be taken in duplicate (two vacutainers). One will be used to determine hemoglobin and ferritin at RS Dr. Sutomo. The other will be frozen and periodically collected by the AID TAACS Adviser and sent to the University of Kansas for determination of transferrin receptor. Ten percent of those sent to Kansas will have hemoglobin and ferritin values checked for quality control purposes.

Data analysis will be done by the principal investigators with the help of the AID TAACS Adviser.