



Assessing iron status of a population^{1,2}

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ABSTRACT Reliable methods for assessing the iron status of a population are essential for developing effective public health measures to combat iron deficiency. The hemoglobin concentration, transferrin saturation, free erythrocyte protoporphyrin, and serum ferritin are all useful but they vary widely in their specificity and sensitivity for detecting iron deficiency. In applying these laboratory parameters, the usual approach in nutritional surveys is to determine the percentage of values outside the normal range. As an alternative, a model is presented here that uses these measurements to estimate the distribution of iron stores in a population. This approach may be particularly useful for evaluating the effectiveness of iron supplementation and fortification programs. *Am. J. Clin. Nutr.* 32: 2115-2119, 1979.

Iron lack is now recognized as the most common deficiency state in man, affecting 10 to 20% of the world population. Accordingly, methods for evaluating iron status in a given population segment have assumed increasing importance. Iron deficiency is usually identified in nutritional surveys by the anemia that accompanies its later stages. However, in certain geographic areas and especially in developing countries, other factors such as folate deficiency, protein-calorie malnutrition, chronic infection, and hemoglobinopathies may cause or enhance anemia. Using the hemoglobin levels as the only measure of iron status accounts for much of the difficulty in evaluating prevalence studies.

The level of circulating hemoglobin not only lacks specificity but is also relatively insensitive because of the wide scatter of values in normal subjects. Thus, the hemoglobin concentration, which averages about 14 g/dl in nonpregnant women, must fall by more than 2 g/dl to recognize anemia as defined by WHO criteria (1). As a result, errors occur frequently when one attempts to separate iron deficient and normal subjects on the basis of hemoglobin measurements. For example, Garby et al. (2) demonstrated by response or lack of response to oral iron therapy that about 20% of either normal or anemic women were classified incorrectly on the basis of the

initial hemoglobin concentration. Similar findings were obtained by analysis of the frequency distribution of hemoglobin levels in women in third trimester pregnancy (3). Roughly one-third of women belonging to the anemic population had hemoglobin levels in the normal range, while a similar proportion of normal women were incorrectly classified as anemic. It is clear from these studies that the hemoglobin alone is unsuitable as a screening method for detecting iron deficiency.

Fortunately, several laboratory parameters have become available in recent years that are more specific for iron deficiency than the hemoglobin. The best known of these are the serum iron and iron-binding capacity. Because uncomplicated iron deficiency produces a fall in serum iron and a rise in iron-binding capacity, the most sensitive param-

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² Supported in part by National Institute of Health Grants HL-06242 and AM 19011, and Contract 223-76-2112 from the Food and Drug Administration, United States Public Health Service.

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ter in screening for iron deficiency is the ratio of these measurements or the transferrin saturation (TS) (4). This measurement can detect about twice as many iron-deficient individuals as can hemoglobin measurements, but does have certain limitations. The method is time-consuming and subject to error from extraneous iron contamination. Precision is also limited by wide diurnal variations in normal individuals. Finally, a fall in TS can occur with a relatively mild or transient infection and is therefore not specific for iron deficiency.

Another parameter of iron status which provides roughly equivalent information to the TS is the free erythrocyte protoporphyrin (FEP). Since protoporphyrin is the complex that combines with iron to form hemoglobin, any limitation in iron supply to red cell precursors is reflected by an increase in unbound protoporphyrin of circulating red cells. Although the ability to detect iron deficiency by FEP measurements has long been recognized (5), earlier laboratory methods were too cumbersome for survey purposes. However, interest in this parameter has been renewed by the development of more simplified methods of analysis (5, 6). The sensitivity of FEP for detecting iron deficiency anemia is enhanced by expressing levels in relation to either packed red cell volume (8) or hemoglobin concentration (9).

An important advantage of FEP as compared to TS measurements is greater stability. While the TS can change in a matter of hours, the FEP increases only after several weeks of erythroid iron deprivation and returns to normal only slowly after treatment of iron deficiency. A recent study in 20 anemic and 20 normal infants was performed to compare the sensitivity and reliability of FEP and TS measurements in the detection of iron deficiency (9). The FEP produced a sharper distinction between the two groups and showed a more consistent and uniform response to iron therapy. However, the FEP may be less sensitive in detecting milder degrees of iron deficiency in the adult.

The most sensitive parameter of iron status is the serum ferritin (SF). Although ferritin has long been recognized as the major intracellular form of iron, the protein has only recently been detected in serum by sensitive immunoradiometric assays (10, 11). The SF

averages about 90 and 30 $\mu\text{g/liter}$ in normal men and women, respectively, a difference which accurately reflects the well-known sex difference in iron stores (12). High correlations have been demonstrated between SF and body iron stores as measured either directly by quantitative phlebotomy (13) or indirectly by iron absorption (12). In various clinical disorders SF correlates with marrow iron stores assessed histologically (14) suggesting that the reticuloendothelial cell is the immediate precursor compartment for the SF. An important feature of SF measurements is their ability to distinguish between true iron deficiency and the anemia of chronic infection; in contrast with the FEP and TS that are similarly affected by these two conditions, the SF falls with iron deficiency but rises with inflammation (14). In a recent study of patients with juvenile rheumatoid arthritis who may have anemia due to chronic inflammation, iron deficiency, or both, the SF could predict a response to oral iron therapy whereas the hemoglobin, FEP, and TS could not (15). Although the SF level that signifies iron deficiency may be altered by chronic inflammation, it is still a useful measure of iron status.

Each of these three parameters of iron status reflects change in different body iron compartments and is affected at different levels of iron depletion. It is convenient to define three stages of iron deficiency (Fig. 1). The least severe stage of iron depletion, defined as iron stores less than 100 mg, is identified by a SF level less than 12 $\mu\text{g/liter}$ mg; only the SF is affected at this stage of iron lack. With continued iron loss, iron stores become exhausted and the second stage of iron deficient erythropoiesis ensues which is recognized by a fall in the TS and/or a rise in FEP. In the final stage red cell production becomes further impaired and iron deficiency anemia can be identified by a significant fall in the circulating hemoglobin.

It is apparent from Figure 1 that no single iron parameter monitors the entire spectrum of iron status. Indeed the distinction between three levels of iron deficiency is entirely arbitrary since iron stores in a population undoubtedly form a continuum ranging from severe iron deficiency to iron overload. We wish to propose the following model by which the distribution of body iron in a population

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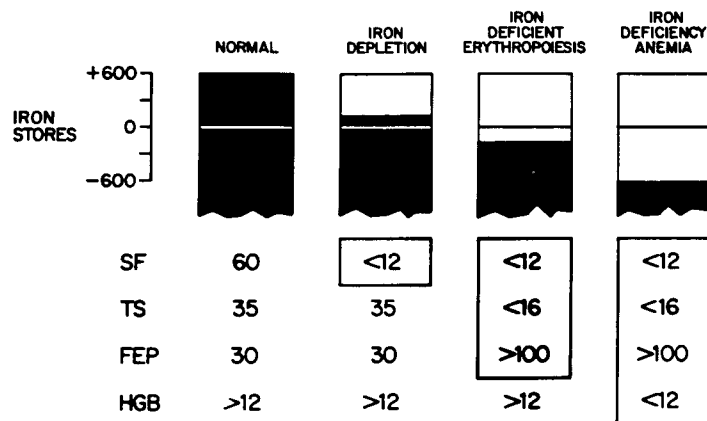


FIG. 1. Parameters of iron status in relationship to body iron stores (milligrams). Negative iron stores indi-

cate the amount of iron that must be replaced in circulating red cells before iron reserves can reaccumulate.

can be estimated from various parameters of iron status.

Except in geographic areas where iron deficiency is highly prevalent, the largest segment of a population has at least some body iron reserves and thus a SF above 12 $\mu\text{g/liter}$. In normal individuals, quantitative phlebotomy studies have shown that 1 $\mu\text{g/liter}$ SF is equivalent to between 8 and 21 mg iron stores (10, 13, 16) with a weighted mean of 9.9 mg. Thus, in individuals with a normal TS, FEP, and hemoglobin concentration, iron stores can be calculated by multiplying the SF by 10. Once iron stores are exhausted, the SF falls below 12 $\mu\text{g/liter}$ and then no longer reflects the true deficit in body iron.

At the other end of the spectrum, individuals with severe iron deficiency have a decrease in hemoglobin which is directly proportional to the deficit in essential body iron. In an adult woman weighing 70 kg for example, 1 g/dl circulating hemoglobin represents about 150 mg body iron. Assuming a mean hemoglobin in normal women of 14 g/dl, a deficit in essential body iron of at least 300 mg must occur before the hemoglobin falls below the cut-off level for anemia of 12 g/dl. Lower levels of hemoglobin can be converted directly; thus, a hemoglobin of 10 g/dl due to iron deficiency represents an iron deficit in relation to iron stores of 600 mg. It should be added that while these calculations lack precision in the individual subject, no systematic errors should occur when applied to the population at large.

As discussed previously, the hemoglobin

alone cannot be used to identify iron deficiency because other factors such as folate deficiency or chronic infection may cause anemia and even iron replete women may have a low hemoglobin. The TS, FEP, and SF must, therefore, be incorporated into the definition of iron deficiency anemia to exclude other causes of anemia. The use of these parameters to define iron deficiency was evaluated in a recent survey of 1564 subjects living in Northwest United States (8). It was observed that if only one of these parameters was in the iron deficient range and the other two normal, the prevalence of anemia was only slightly higher (10.9%) than in the population as a whole (8.3%). However, when any two of the three parameters were abnormal, the prevalence of anemia increased to 28% and when all three parameters were abnormal, to 63%. Thus, the cause of anemia can be reasonably attributed to iron deficiency only when at least two iron parameters fall within the iron deficient range.

To test the validity of these calculations at the extremes of iron status, we analyzed a survey of nutritional anemia in 426 adult women living in the United States (8). The cumulative frequency distribution of iron stores is shown in Figure 2. Assuming that 1 $\mu\text{g/liter}$ SF represents 10 mg storage iron, iron stores were less than 150 mg in 40%, less than 300 mg in 60%, less than 450 mg in 76%, less than 600 mg in 84%, and less than 750 mg in 91%. At the other extreme, 74 women (17.4%) had at least two abnormal iron parameters. Of these, 7.3, 3.3, and 1.2% had

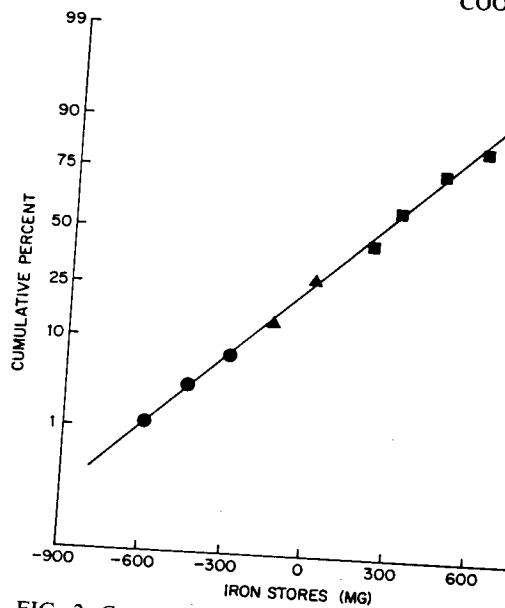


FIG. 2. Cumulative frequency distribution of iron stores in 426 women. In various regions of the curve, the points were derived from serum ferritin (■), transferrin saturation and/or free erythrocyte protoporphyrin (▲), and hemoglobin concentration (●) as described in text.

hemoglobin levels less than 12, 11, and 10 g/dl, respectively, representing deficits of 300, 450, and 600 mg.

To obtain the frequency distribution shown in Figure 2, two more points were added at intermediate degrees of iron deficiency using the TS and/or FEP levels. Because the exact point at which these parameters become abnormal during the development of iron deficiency has not been precisely defined, the following relationships were chosen empirically. In women with a normal hemoglobin, an abnormal TS or FEP was equated with absent iron stores, while an abnormal TS and FEP was assumed to represent a deficit of 150 mg. It can be seen from Figure 2 that iron stores in this total population of adult women approximate a Gaussian distribution, a finding that provides some tentative support for the model. At the same time there is evidence from other studies that iron stores in a population may not be normally distributed. For example, SF in normal men and women is log normally distributed (12) and this skewed distribution explains the fact that the points based on SF in Figure 2 are not strictly linear. Nevertheless, within the limits

depicted in Figure 2, iron stores approach a normal distribution as a first approximation.


An important question not answered by this analysis is the extent to which contributing factors such as folate deficiency or chronic infection might influence the results. This analysis of iron status was performed in a basically healthy population where iron deficiency is the major if not only determinant of iron parameters. Infection is particularly difficult to identify in population studies because the effect on TS, FEP, and hemoglobin levels is indistinguishable from true iron deficiency. In populations where chronic infection may contribute to anemia, the SF may have to be one of the two abnormal parameters required to diagnose iron deficiency anemia. Because the SF falls only with true iron deficiency, this refinement would exclude anemia due to chronic infection (14). Protein-calorie malnutrition may produce a similar distortion of the frequency distribution.

This model offers certain advantages over conventional methods for estimating iron status in prevalence surveys. An obvious advantage is the ability to estimate iron stores in the entire population rather than in a smaller segment of iron-deficient individuals. From Figure 2, for example, median stores were 220 mg iron with lower and upper 10 percentile of -240 and 700 mg iron, respectively. In fact, because iron stores were normally distributed in this population, iron status can be fully defined by the mean and SD.

The most useful application of this model for evaluating iron status will be to measure the impact of intervention strategies to combat iron deficiency. Because iron absorption is maximal in those with severe iron deficiency, fortification iron supplied to an entire population should have lesser effect in iron replete individuals and thereby produce a change in the slope of the probability curve shown in Figure 2. However, fortification might alter iron stores to the same extent in the entire population which would produce a parallel shift in the frequency distribution. The impact of fortification programs in iron replete individuals has been an important issue in developed countries and the model described here may provide an answer to this important question.

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An issue that often arises in iron supplementation or fortification trials is the choice of laboratory measurements of iron status. The frequency distribution in Figure 2 indicates that the best parameter depends largely on the basal iron status of the population. In populations where iron deficiency is highly prevalent, as in pregnant women, the critical parameter is the hemoglobin although other parameters are needed to establish that the anemia is due to iron deficiency. However, in a relatively iron replete population such as that shown in Figure 2, the SF monitors the largest segment of the population and is, therefore, the most useful measurement. At either extreme of iron status, the TS and FEP enhance the precision of estimates at intermediate levels of iron deficiency. Indeed, there is strong argument for including all four parameters of iron status because in doing so, the results of a field trial become less dependent on the basal iron status of the population. 

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