

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 among 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 lifelong 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 mass of 460 000 Daltons 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 $\mu\text{g/l}$. 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 ($\mu\text{g/l}$)	< 12	> 300
Iron transport		
Serum iron ($\mu\text{g/l}$)	< 600	> 1800
TIBC ($\mu\text{g/l}$)	> 400	< 2500
Transferrin saturation (%)	< 16	> 60
Red cell parameters		
Haemoglobin (g/l)	< 130 _♂ , 120 _♀	—
MCV (femtolitre)	< 80	—
Erythrocyte protoporphyrin ($\mu\text{g/l}$ 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 $\mu\text{g/l}$ 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 the 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 $\mu\text{g/l}$, 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. These 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 coexist. 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 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 produce 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 5.6 ± 1.2 mg/l in 82 normal male and female volunteers was sharply increased to 18.0 ± 11.4 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.* 1990b). When the serum ferritin fell below $12 \mu\text{g/l}$, 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

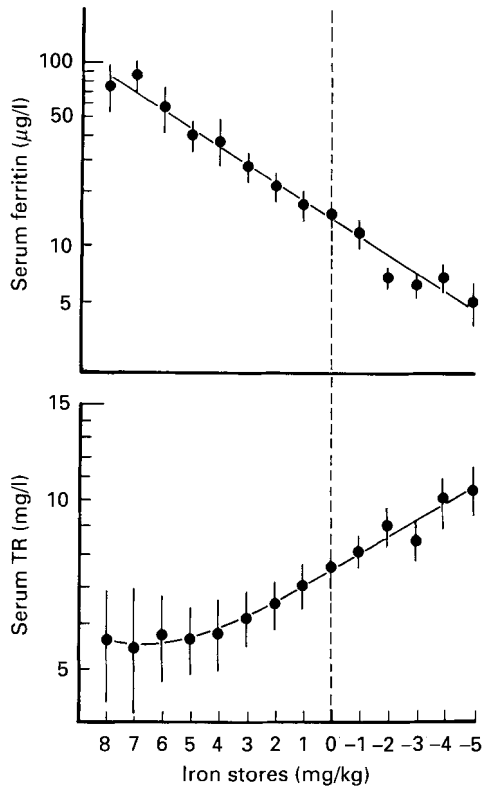


Fig. 1. Changes in body iron during repeated phlebotomy of healthy subjects (Skikne *et al.* 1990*b*). Serum ferritin measurements are shown in the upper panel and serum transferrin receptor (TR) measurements in the lower panel (mean \pm 1 SE). Negative values for iron stores represent the induced deficit 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. 1990a). 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 others using single cutoff 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.

This work was supported by NIH grant DK39246 and AID Cooperative Agreement DAN-5115-A-00-7908-00.

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