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PERSPECTIVES ON NUTRITIONAL IRON DEFICIENCY

Leif Hallberg
Department of Clinical Nutrition, Göteborg University, Sahlgrenska, University Hospital, Annedalsklinikerna, SE-413 45 Göteborg, Sweden; e-mail: hallberg@medtak.qu.se

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■ **Abstract** Nutritional iron deficiency (ID) is caused by an intake of dietary iron insufficient to cover physiological iron requirements. Studies on iron absorption from whole diets have examined relationships between dietary iron bioavailability/absorption, iron losses, and amounts of stored iron. New insights have been obtained into regulation of iron absorption and expected rates of changes of iron stores or hemoglobin iron deficits when bioavailability or iron content of the diet has been modified and when losses of iron occur. Negative effects of ID are probably related to age, up to about 20 years, explaining some of earlier controversies. Difficulties in establishing the prevalence of mild ID are outlined. The degree of underestimation of the prevalence of mild ID when using multiple diagnostic criteria is discussed. It is suggested that current low-energy lifestyles are a common denominator for the current high prevalence not only of ID but also of obesity, diabetes, and osteoporosis.

CONTENTS

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INTRODUCTION

Iron deficiency (ID), the most common deficiency disorder in the world, affects millions of people (26). ID develops when absorption of dietary iron cannot cover the physiological losses and requirements of iron. This review deals with nutritional ID and focuses on new information, new approaches, and new interpretations of available information.

PATHOGENESIS OF NUTRITIONAL IRON DEFICIENCY

Iron metabolism is unique in several aspects. The body is economical in its handling of iron in the body. When a red cell dies, its iron is reutilized. Iron absorption is in some ways controlled by the requirements of the body. Extra iron can be stored by a specially designed protein (ferritin), which is utilized at times of increased iron requirements. The highly reactive properties of iron are balanced by unique control and transport systems. In spite of these ingenious mechanisms, ID is the most common deficiency disorder in the world and the main remaining deficiency in the industrialized, developed world. To understand this paradox, and to find effective ways to combat the deficiency, it is necessary to examine incomes (absorption) and expenditures (requirements) of iron, as well as current knowledge of the various control systems in the body responsible for maintenance of iron balance. An important observation was made by McCance & Widdowson in 1937 (70). In their studies, they found that iron was not excreted from the body, which implies that iron balance was maintained by a regulation of iron absorption.

PHYSIOLOGICAL IRON REQUIREMENTS

Basal iron losses from the exterior and internal surfaces of the body, menstrual iron losses, and iron needed for growth, including pregnancy, determine physiological iron requirements.

Basal Iron Losses

Iron lost from exterior and interior surfaces of the body constitute basal iron losses. A collaborative study showed that basal iron losses in men were of a magnitude of 14 μg/kg of body weight/day (38). The study was based on the rate of decrease of the specific activity of the long-lived radioisotope ^{55}Fe administered intravenously. The dilution of the tracer by the absorption of iron to cover these losses was followed for several years. This ingenious principle was developed in 1959 by Finch (30). Further studies indicated that about half of these losses represented "physiological" blood losses (12).

Loss of iron from sweating was once considered marked, especially in the tropics (33). However, indirect studies comparing total iron losses in those living

in hot and humid environments with those living in nontropical environments showed no differences (38). Direct studies of the iron content of sweat under controlled conditions showed that sweat iron losses are negligible (15).

Menstrual Iron Losses

An early study showed that menstrual iron losses varied between 6 and 179 milliliters in 100 healthy women (8). The intraindividual variation was examined in 13 women and was considered marked. This finding was in contrast to later observations that for each individual, iron losses were almost constant (54). A probable reason for the different results was the great care taken in the latter studies to ensure a complete sampling of the menstrual blood. These unexpected observations had important consequences for understanding iron balance in menstruating women. By studying a random sample of women at a time before the introduction of contraceptive pills and intrauterine devices, the basal variation in iron requirements in women could be established (47). Later studies in a random sample of Swedish women confirmed the distribution of iron requirements, and the effect of contraceptive pills could be examined (55). Studies in nonidentical and identical twins (83) indicated that menstrual blood losses were genetically controlled and that this control was mediated by the contents of plasminogen activators in the uterine mucosa (82). Several studies in geographically widely separated countries strongly suggest that menstrual iron losses are the same worldwide and, thus, that iron requirements have been the same for very long time, probably many thousands of years. It could thus be concluded that differences in prevalence of ID is mainly related to differences in absorption of dietary iron, disregarding differences in iron losses related to parity and birth spacing and degree of infestation with, mainly, hookworm.

Growth Requirements

The Newborn, Full-Term Infant Iron requirements during the first 4–6 months of life are negligible, especially if late clamping of the umbilical cord has occurred. This unique situation for iron is explained by the excess of circulating hemoglobin (Hb) the infant is born with. This is due to the high affinity of fetal Hb for oxygen. The fetus thus "wins" over the mother in the struggle for oxygen at the placental interface. However, as a direct consequence, the delivery of oxygen from fetal Hb to tissues is lowered. The fetus thus needs more Hb to deliver a certain amount of oxygen to its tissues (regulated by erythropoietin). At delivery, the production of fetal Hb is exchanged for the production of normal Hb A, and oxygen is more readily available from the lungs. Successively, much iron is thus released to build up iron stores of the infant. This extra iron covers iron needs for the infant during the first 4–6 months of life. After about 6 months, when iron stores are exhausted, the iron requirements are very high, especially during the following 18 months, the weaning period. Iron requirements may amount to about 100 μg/kg/day, which is about four times more than for an average adult menstruating woman. After about the age of 2 years, iron requirements per unit of body weight are reduced.

Adolescence The growth spurt during adolescence is another period of high iron requirements. For boys, puberty is associated with both considerable growth and a marked increase in Hb concentration and mass. Iron requirements for boys are about 20% higher than average iron requirements for menstruating women. For girls, growth is not completed at menarche and thus total iron requirements are high. For 14-year-old girls, for example, median iron requirements can be about 30% higher than for their mothers (79).

Pregnancy

Iron requirements in pregnancy are very high, as discussed in previous reviews (41, 42). A main problem is the uneven distribution of the requirements over the duration of pregnancy. Because of the absence of menstrual iron losses and the negligible needs of the fetus, iron requirements in the first trimester are very low. They get successively higher as the pregnancy continues, reaching a maximum in the third trimester. The ability to absorb dietary iron increases as the iron requirement increases. Despite the increased propensity to absorb iron, however, even with a highly bioavailable diet, iron needs during pregnancy cannot be met by diet alone, especially during the second half of pregnancy. In addition, during the second half of pregnancy, when requirements are high, the actual absorption of iron from the diet is far below the need. This is true also for a highly bioavailable Western-type diet. Thus, to a great extent, iron balance during pregnancy is dependent on the amount of stored iron. It may be the main physiological role of iron stores. The problem is the low iron stores in present-day women in both developed and developing countries. It can be estimated that in our early ancestors, who consumed high-meat diets, iron stores may have amounted to about 500 mg, which is approximately the amount of stored iron needed to cover iron requirements during pregnancy. This is the reason for the paradoxical, unphysiological necessity of supplying pregnant women with iron supplements during the later half of pregnancy. The very high iron requirement of pregnancy is a special problem in teenage pregnancies because girls in their teens may not have reached their full growth. The anemia that is seen in early pregnancy (the physiological anemia of pregnancy) (41) is due primarily not to ID but to an increased plasma volume combined with the increased capacity of red blood cells to deliver oxygen to the placenta, which is probably mainly due to an increased concentration in red blood cells of 2,3-diglycerophosphate (41). This is a mechanism similar to that seen in "sports anemia," where the change is less well adapted to its purpose. (53).

DIETARY IRON ABSORPTION

The first studies to estimate the absorption of dietary iron were chemical balance studies. Some of these early, meticulous studies gave good information about the magnitude of iron absorption from the diet. No information was obtained, however,

about the variation in iron absorption related to different meal compositions or about the variation between subjects.

When radioiron isotopes became available, they were added to several "standard meals" of different composition to assess the variation in iron absorption between iron-deficient and iron-replete subjects. A detailed review of this early phase of iron absorption studies was published by Moore (74). It was known early on (45, 57, 58, 96) that heme and nonheme iron were absorbed differently. The iron porphyrin ring of heme iron is absorbed by utilization of a special receptor on the mucosal cell surface (37, 94). Heme iron is then degraded within the mucosal cell by heme oxygenase (77) and then enters the same iron pool in the intestinal mucosal cells as the absorbed nonheme iron.

In 1951, the technique of using biosynthetically radioiron-labeled foods to study nonheme iron absorption was introduced by Moore & Dubach (75). Several studies of labeled single-food items were made by several investigators and summarized by Moore in 1968 (74) and later by Martinez-Torres & Layrisse (69). There was a marked variation in nonheme iron absorption from different foods and more iron was absorbed by iron-deficient subjects. Another important observation was that there was an interaction between foods in the absorption of iron, e.g. meat enhanced the absorption of nonheme iron (66). The results of these new studies on iron absorption from single, biosynthetically labeled foods did not, however, allow estimations of iron absorption from the total diet. To be able to understand the paradoxical relationship between dietary iron intakes, which were often quite high, and iron status in different populations, which was often low, there was an obvious need for this knowledge.

At this time it was known that there were two kinds of dietary iron—heme iron, found mainly in meat and constituting up to 15% of the dietary iron, and nonheme iron, found in cereals fruits, roots, vegetables, etc, and constituting the remaining part and sometimes all of the dietary iron. At a 1969 joint meeting of the International Atomic Energy Agency and the World Health Organization, four participating groups agreed to examine the validity of measuring iron absorption from an inorganic iron salt with radiolabeled iron and taken as a drink with a specific food radiolabeled with another iron tracer. This became the starting point for the development of the extrinsic tag method, which made it possible to measure iron absorption not only from single foods but from different meals simply by adding a radio-labeled iron tracer to a food or a meal. This methodological development then suddenly made it possible to identify various dietary factors that enhanced or inhibited iron absorption. It also allowed quantitative studies on interactions between different foods. The currently recognized factors are outlined in Table 1.

The information about these factors was reviewed in the first volume of this series 20 years ago (40). The main new factors that have been identified since then deal with the strongly inhibiting iron-binding polyphenols and their widespread occurrence in nature (14, 16), and with the inhibition of both heme and nonheme iron by calcium, which probably acts within the mucosal cells (46, 56). Contradictory results about the effect of calcium on iron absorption have been obtained by different groups. The reasons for the discrepancies have been discussed (43).

TABLE 1 Factors influencing dietary iron absorption

Determinant	Factors
Absorption	
Heme iron	Iron status of subject
	Amount of heme iron as meat
	Calcium content
	Food preparation (time, temperature)
Nonheme iron	Iron status of subject
	Amount of potentially available nonheme iron (adjustment for fortification and contamination iron, which may be only partially available)
Dietary factors	
Enhancing	Ascorbic acid
	Meat, chicken, fish and other seafood
	Fermented vegetables and soy sauces (not all items)
Inhibiting	Phytate and other inositol phosphates
	Iron-binding phenolic compounds
	Calcium
	Soy proteins

Several attempts have been made to develop an algorithm to predict the absorption of dietary iron (21, 72, 73, 95). One algorithm was recently published based on only three dietary factors: meat, ascorbic acid, and phytate (78). Recently, a more comprehensive algorithm was developed to predict the absorption of iron from composite meals based on the content of eight dietary factors known to influence iron absorption (48). The algorithm is based on analyses of "dose-response" relationships between these factors influencing iron absorption and the interaction between the factors. The validity of the algorithm to predict iron absorption from single meals was examined by direct comparisons of observed and predicted absorption of iron from several meals. It was also shown that the algorithm could also be used to predict iron absorption from a whole varied diet over several days.[1]

It has been suggested that the iron absorption from a single meal given in a fasting state does not correctly describe the effect of various factors in a meal when given as part of the whole diet (21). The variation in iron absorption between different single meals is more marked than the variation between diets composed of

[1]In Equation 2 in the published algorithm (78), two parentheses were incorrectly inserted. The correct Equation 2 should be as follows: absorption ratio $= 1 + 0.01\,\mathrm{AA} + \log(\mathrm{P} + \mathrm{l})^* 0.01^* 10^{0.8875^*\log(\mathrm{AA}+1)}$, where AA is ascorbic acid (in milligrams) and P is phytate phosphorus (in milligrams).

many different meals. It might be possible, for example, that the absorption from one single meal is influenced by the absorption of iron from preceding meals. However, in direct studies, this was not observed (36, 93). Moreover, the fact that the sum of the absorption of iron from many single meals (48) was the same as the total absorption from the diet over several days strongly contradicts such a supposition.

CONTROL OF DIETARY IRON ABSORPTION

Iron status is determined by the balance between iron requirements and iron absorption. The total amounts of iron in the body cannot be controlled by excretion of iron but only by a regulation of the absorption, as mentioned in the introduction. Two factors have been considered to control the absorption of dietary iron: the amount of iron in stores, and the erythropoietic activity (13, 29). The erythroid regulator mainly responds to acute marrow iron needs whereas, by influencing the absorption of dietary iron, the store regulator would be mainly responsible for the maintenance of iron balance. Nothing is known, however, about the nature of these regulators, as outlined in a critical review by Finch (29).

Recently, interest has been focused on molecular biological structures and components in the intestinal mucosal cells that seem to control the absorption of iron to meet bodily needs at the mucosal level. What is not known, however, is how and where actual information about iron status and iron requirements of the body are evaluated and mediated to the intestinal mucosal cells, wherein reside the physiological mechanisms involved in preventing ID and iron overload. In other words, how is the absorption of iron controlled? Recently, new observations about the relationships between iron absorption, iron requirements, and iron stores have thrown new light on the capacity and ability of this control (50, 51).

Several studies in humans have shown a straight-line relationship between log iron absorption from reference doses of inorganic iron (3 mg of Fe^{2+}) and log serum ferritin (SF) (11, 22, 88, 92). Similar relationships between log iron absorption from whole diets and log SF (35, 52, 60) have been shown and have provided a possible new interpretation of the relationship between log total daily dietary iron absorption and log SF. Furthermore, a reevaluation and recalculation of the relationship between log SF and iron stores (50) has made it possible to validly estimate iron stores from log SF.

We found straight-line relationships between log daily iron absorption per unit of body weight (micrograms of Fe per kilogram of body weight per day) and iron stores (micrograms of Fe per kilogram of body weight) for different diets. Moreover, diets with different iron bioavailability formed parallel regression lines (50). Corresponding parallel regression lines were actually observed previously between log SF and log iron absorption from single meals with different bioavailability, including the reference dose (92).

A diagram showing the linear relationship between log dietary iron absorption and iron stores is shown in Figure 1 to illustrate the new principle for analyzing the

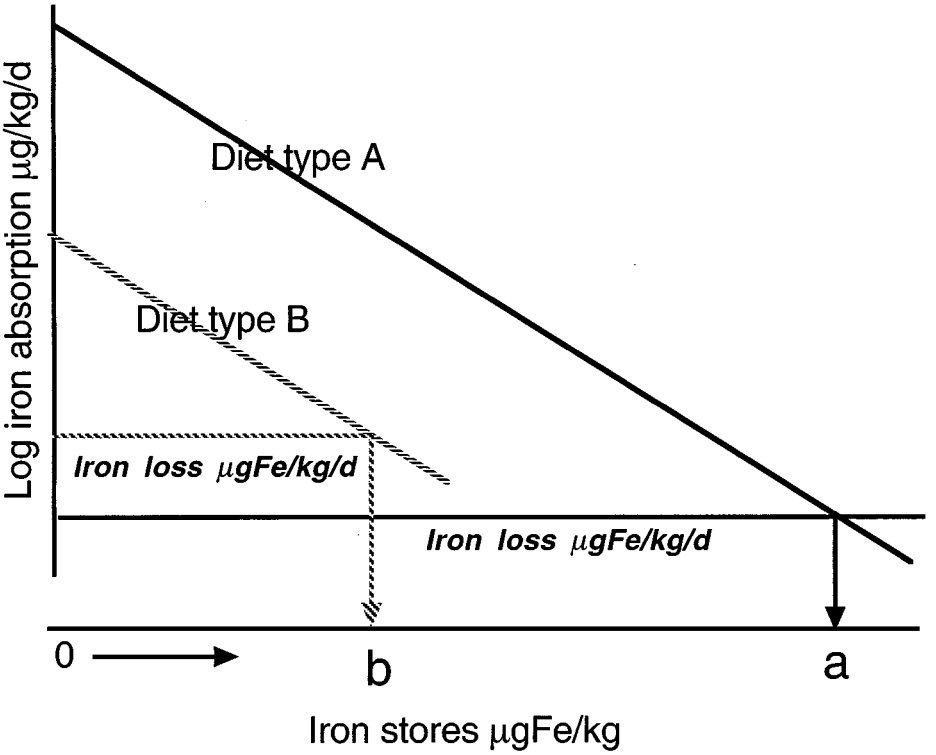

Figure 1 The relationship between iron absorption, iron stores, and iron losses—two examples with two diets (*A* and *B*) with different bioavailabilities and with two different iron losses. For a subject consuming a diet of type *A* and having the low daily iron loss (the lowest horizontal line), the balance point where absorption equals losses is projected to point *a* on the iron store scale. A subject consuming a diet of type *B* with a lower bioavailability shows a lower, parallel regression line (log iron absorption vs iron stores). The balance point, point of intersection between absorption, and loss lines corresponds to an iron store of point *b*.

relationship between total daily iron absorption, iron stores, and total daily iron requirements (50). A horizontal line, corresponding to the actual iron losses (iron requirements—micrograms of Fe per kilogram of body weight per day), is inserted. At the point of intersection between these two lines, absorption equals losses and corresponds to the maximal amount of stored iron that can be present under the conditions prevailing. Figure 1 shows two examples with different bioavailability, different iron losses, and different resulting iron stores.

These relationships can be described mathematically with no model assumptions in an exponential equation.

$$dM/dt = k_1 e^{Mk_2} - L, \qquad 1.$$

where k_1 is absorption at zero iron stores (micrograms of Fe per kilogram of body

weight per day), *M* is the amount of stored iron (micrograms of Fe per kilogram of body weight), and k_2 a constant (see below). Steady states for iron stores can be calculated for different amounts of absorbed and lost iron by setting $dM/dt = 0$. The constant k_2 describing the slope of the relationship between absorption and iron stores is calculated from actual absorption studies (35, 52, 60). The constant k_2 was found to be 0.00024 in three separate studies. The absolute value of the constant k_2 depends on the units used. It is probable that the constant is an expression for a biological, fundamental property describing the control of iron absorption in relation to iron stores that may also be found in other animals besides humans.

By integrating the exponential equation, describing the relationship between absorption, losses, and iron stores, the rates of change in iron stores over time can also be calculated, e.g. the time needed to reach a certain iron store when changing iron losses or changing the bioavailability of the dietary iron.

Unexpectedly, it was observed that when modifying losses or absorption of iron, a main part (about 90%) of the changes in iron stores occurred within 1 year, approaching asymptotically a new steady state after about 2–3 years. In practice, this means that the main effects on iron status by iron fortification or by improvements in dietary bioavailability are expected to be observed after about 1 year. Inversely, it can be concluded that the main changes in iron status (e.g. in SF) observed in a population sample at a later date can not be ascribed to dietary modifications made. A later increase in SF may, for example, be due to a higher prevalence of infections. The strict control of iron absorption is only valid for dietary iron and is not valid for iron taken as supplements.

The basis for the calculations is that at any steady-state absorption equals losses. The recently described inertia or adaptation in absorption (62) when changing the diet to a lower or higher bioavailability does not contradict this fact. The current observations and calculations have other important implications. With increasing iron stores, the absorption of iron is reduced to the level needed to cover the daily iron requirements. This explains the observations that SF remained unchanged in men after 2 years of iron fortification with 7.5 mg of highly available iron given daily (7), and that the same was seen when 10 mg of iron as ferrous sulphate was given to an iron-replete man for 500 days (85). Another example of the effective control of iron stores is the observation in iron-replete men that 2 g of ascorbic acid given with meals for 2 years had no effect on SF. It may seem paradoxical that in that study, ascorbic acid given with meals still had an effect on the absorption of small iron doses. The amounts of iron absorbed, however, were well within the range of iron absorption required to cover the basal iron losses (20). All these observations are consistent with the observations that iron stores remain constant in men after about the age of 20–25 years, as illustrated by rather constant median SF values in the N-HANES II studies (76). The unchanged iron content in iron stores with age was also observed in US autopsy studies of liver iron content in subjects who suffered traumatic deaths (91). An important implication of the effective control of iron absorption is that it would be impossible to induce iron overload by consuming diets with even very high

iron contents due to, for example, high intake of red meat, or excessive iron fortification in iron-replete men. Exceptions would be the few subjects who are homozygotes for hereditary hemochromatosis and those who have thalassemia major.

In the N-HANES II studies in the United States (76), higher SF values were observed with increasing age, especially at ages above 45 years, not only in postmenopausal women but also in men. The autopsy studies mentioned above (91) directly measuring iron stores showed no corresponding increase in iron stores with age. Observations of a very effective control of iron absorption preventing iron overload are also inconsistent with any "physiological growth" of iron stores with age (50). Because SF is a sensitive, acute-phase reactant increasing, for example, after simple infections (61) or with regular use of alcohol (67, 71, 90), it is possible that some unknown external factor may increase with age—a kind of an unknown aging factor? No sufficiently valid explanation has been found. One implication of these observations is that great care must be taken in simple translations of SF into iron stores, especially in middle-aged or older people. It has been suggested that high iron stores (SF > 200 μg/liter) are associated with coronary artery disease (84) and cancer (89). A more obvious explanation, that a high SF may be the effect of different pathological conditions related to the development of coronary heart disease and not a cause of it, seems not to have been carefully considered, especially because other studies have not confirmed such a hypothesis (2–4, 87).

In an extensive study of 203 women, all aged 38 years, the relationships between iron absorption, iron requirements, and various parameters of iron status, including examination of bone marrow smears, were studied (49). It was evident that SF and transferrin saturation showed a continuous decrease with increasing iron requirements, based on measurements of menstrual iron losses and body weight, indicating successively lower iron stores. Iron absorption increased with increasing iron requirements, but it could only balance the increasing iron losses up to a certain point when the stainable iron in the bone marrow suddenly disappeared at the same time as Hb dropped and transferrin concentration in plasma increased. These comprehensive observations fit well with the predicted changes using the new equations (50). It is thus evident that menstruating women with high iron requirements, and/or consuming diets with low iron bioavailability, will be iron deficient (have no iron stores) and may even develop an ID anemia (IDA). It is also evident that the average woman with average menstrual iron losses and consuming a "typical" Western diet with low-to-moderate meat intake will have iron stores rather below than above 100 mg. This implies that it is impossible for the present-day Western woman, even those consuming a good diet, to build up iron stores of the magnitude needed (about 500 mg) to fully cover the extra iron requirements for pregnancy. It is interesting, however, that according to the new algorithm presented above (48), and information about a probable composition of the diet in early women (27), iron stores of such a magnitude (about 500 mg) were probably present in early women, who had a very high intake of meat and ascorbic

acid. This fact probably also balanced the estimated high intakes of phytate and calcium in the diets of early man (50).

HOW IS THE IRON BALANCE COMMUNICATED TO THE INTESTINAL MUCOSA?

When iron requirements increase and iron balance becomes negative, for example due to hookworm infestation, the body tries to prevent the development of ID by increasing the absorption of iron. Studies with both humans and animals indicate that the up-regulation of iron absorption in some way, as mentioned above, is related to the amount of iron present in iron stores (13, 29, 51). When studying the relationship between iron absorption and SF as a measure of iron stores, Hallberg et al (51) unexpectedly observed that the relationship between iron absorption and iron stores (SF), noted in iron-replete subjects, was also valid in subjects with ID—with "negative iron stores," suggested by SF < 15 μg/liter, and with subnormal Hb (51). This implied that it is not only the empty iron stores per se but also a Hb iron deficit that in some way controls the absorption of dietary iron. Analyzing more closely the relationships between iron absorption on the one hand and the iron stores on the other, and the relationship between iron absorption and Hb deficit, it was evident that the two relationships formed a continuum. As discussed in a recent paper (51) on the physiological control of iron absorption, including the effect of various hematological disorders, the balance of evidence including the observed continuum suggest that the hepatocyte may be a probable cell that registers both the amounts of stored iron and a deficit of Hb iron. This interpretation is also supported by observations from rat liver transplantation studies, which suggest that the hepatocyte iron stores are a major determining factor controlling iron absorption (1). How the hepatocyte mediates the iron status to the intestinal mucosa is still, however, unknown.

WHAT IS IRON DEFICIENCY AND WHAT IS IRON DEFICIENCY ANEMIA?

How is the internal transport of iron to tissues controlled? The observation that transferrin was a specific transport protein for iron in the body was an important discovery (65). The extremely strong binding of iron to transferrin at physiological pH was required to prevent the toxic effect of iron ions. It remained a mystery, however, how iron could be released and delivered from transferrin to tissues. The existence of specific cell membrane receptors for transferrin was suggested about 40 years ago by Jandl & Katz (63). Intense research resulted in the isolation and characterization of the receptor [for a review, see Baker & Morgan (5)]. The important discovery that all cells had specific receptors on their surface, to bind the transferrin-iron, explained the mystery. The entire complex of transferrin-iron

and transferrin receptor was shown to be taken up by the cells by invagination of a part of the cell wall to form a vacuole, thus transferring the entire complex from the exterior to the interior of the cell. Iron is then released in the vacuole by lowering the pH within the vacuole. Each cell expresses its actual need for iron by the number of receptors on the cell surface. This ingenious system controls the transport of iron within the body from plasma to the cells in different tissues requiring iron, thus also preventing the presence of free iron extracellularly. Within the cells, other mechanisms prevent the formation of free iron radicals. The chemical structure of the transferrin receptor is well known and is the same for cells in different tissues. This implies that if the transfer of iron to one kind of cell needing iron is shown to be insufficient, for example, to produce Hb in red blood cells in the erythron, then it can be predicted that the transfer of iron to all cells in other tissues needing iron at the same time would also be insufficient. In practice, it means that if erythropoiesis is observed to be limited because of a lack of iron, then it can be deduced that the transfer of iron to such tissues as brain and muscle is also insufficient. A demonstration of an iron-deficient erythropoiesis thus implies that a true, functionally important ID is present even if it may be technically difficult to demonstrate symptoms from various organs.

LIABILITIES OF IRON DEFICIENCY

Negative effects of ID are not directly related to a reduction in Hb because the body has several effective mechanisms to compensate for an insufficient supply of oxygen to tissues caused by a lowered Hb. The most immediate compensatory step is an increased cardiac output delivering more oxygen per unit of time to tissues. The next step is an increase in the concentration of 2,3-diglycerophosphate in red blood cells causing a shift in the form of the Hb-oxygen dissociation curve, thus facilitating oxygen delivery to tissues. This latter change needs about 2–3 days to be fully effective. With more longstanding problems in oxygen delivery, there will be an increased production of erythropoietin to increase the concentration of Hb if possible. There are thus a series of consecutive mechanisms to ensure an adequate supply of oxygen to tissues. It follows that symptoms of ID are probably directly caused not by a lowered Hb level but by an insufficient supply of iron to the tissues. During the development of a negative iron balance and when iron stores are exhausted, the supply of iron to all tissues, including the erythron in the bone marrow, will be compromised. The concentration of Hb will then start to decrease. This stage is the beginning of an iron deficient erythropoiesis. Theoretically, there is no intermediate stage between normality and ID. Sometimes the term iron depletion has been used with other laboratory criteria of ID. This concept might be a paraphrase of ID that is still not detectable with current laboratory methods. The distribution of the Hb in healthy, iron-replete subjects is very wide, e.g. in adult women, roughly between 120 and 160 g/liter. This variation is due mainly to a variation in Hb between subjects and only to a small extent to the day-to-day

variation within each individual because each subject has a markedly constant Hb. This means that a woman with no iron stores, who normally has her homeostatic Hb level set at 160 g/liter, can lose about 25% of her Hb mass and still have a Hb remaining within the normal range of a population of healthy women. ID anemia (IDA), usually defined as an ID combined with a Hb < l20 g/liter (a Hb value below the mean minus two standard deviations of the population), is thus a statistical cutoff value in a population with no specific biological meaning. The main use of IDA is to compare iron status in different populations and not in different subjects, not even in clinical practice except when Hb < l20 g/liter.

During a negative iron balance in an iron-replete subject, iron stores will at first be successively depleted. At a certain point, when the rate of release of iron from stores is lower than the amount of iron needed for all tissues, including the erythron, a functional ID is reached—the delivery of iron to all tissues is then compromised (is insufficient). The first thing easily noted is a successive decrease in Hb concentration. With a continued negative iron balance, the decrease in Hb will continue and may pass below the mean Hb minus two standard deviations of the distribution of Hb of the population. This is the established statistical concept of IDA in a population. Negative effects of ID in different tissues may be expected to occur before the Hb in an individual has passed below the lowest normal level of the population. The severity of symptoms related to ID may of course be expected to be more pronounced the more severe the deficiency and the longer the time the deficiency has existed. Symptoms of ID from various tissues may thus be expected to be more severe if the deficiency has led to a reduction in Hb below the subnormal level of the population. IDA is sometimes a practical level in both clinical and epidemiological work.

Including the presence of anemia in the definition of ID, however, will underestimate the true prevalence of ID. In clinical practice, knowledge of previous Hb levels of a patient is extremely valuable in the diagnosis of ID, for example, in patients with pathological gastrointestinal bleedings due to cancer in the gastrointestinal tract and may lead to early detection of a bleeding cancer.

The nestor in hematology, Maxwell M. Wintrobe, once chairing an expert WHO meeting on nutritional anemias, defined ID, didactically, as a state (*a*) when an otherwise healthy individual has a Hb below the optimal value for that specific individual, (*b*) when there is no infection or other disorder present, (*c*) when there is no lack of other nutrients required for an optimal hematopoiesis, and (*d*) when laboratory signs are compatible with ID. These considerations are important for understanding that ID without anemia, in its classical definition, has been shown to have various negative effects (see below). Another factor to consider when evaluating negative effects of ID is related to the fact that symptoms of nutritional ID probably have developed over a long period of time and therefore might be considered as a normal state in most subjects. This is a well-established clinical fact in many patients with various disorders having symptoms that have developed over a long period of time. General symptoms that may be related to ID, such as lowered physical work capacity or mental

changes, must therefore be analyzed according to changes in symptoms after double-blind treatments with iron and placebo over a sufficiently long time period.

There are many conflicting reports about symptoms of ID without anemia [for a review, see Beutler et al (10)]. Other important reviews about negative effects of ID can be found elsewhere (9, 24, 86).

Studies on negative effects of ID are often more easily studied in animals. A real breakthrough in the understanding of effects of ID on rats on work performance was made by Edgerton et al in 1972 (28) and Finch et al in 1976 (31). In the latter study, for example, it was shown that the reduced working performance in iron-deficient rats was due not to the induced anemia but to tissue ID and probably to lack in the muscles of certain enzymes related to phosphorylation. Several studies were made confirming these results. The studies were all made using growing, iron-deficient rats. One study, however, was made using adult rats where, contrary to previous findings in weanling rats, there was no decrease in muscle enzymes in spite of a moderate anemia (64). These data strongly suggest that the age when ID is induced is important for its effect on muscle enzymes. In a detailed study of nine healthy adult men with a mean age of 29 years, an IDA induced by repeated venesections and maintained for 9 weeks was not associated with a reduction in maximal muscle enzyme activities. After retransfusion, normal endurance was reestablished (18). In contrast, maximal muscle enzyme activities in 17 Indonesian subjects, both normal and iron deficient, and studied by the same group, showed significantly lower maximal muscle enzyme activities unrelated to their present iron status (19). These observations might suggest that the significantly reduced maximal muscle enzyme activities in this sample were an effect of ID earlier in life, as a parallel to the different findings in weanling and adult rats. These results suggest that it may be difficult to induce negative effects of ID in adult subjects and possibly that it may be impossible to treat potential symptoms of ID induced when young.

Most negative effects of ID are thus expected in growing individuals from infancy and childhood up through adolescence. The three tissues that are early on finally differentiated and developed in the body are brain, muscle, and the eye lens. The brain develops up to the age of about 20 years and the brain iron content also increases up to this age (59). Animal experiments indicate that to ensure an adequate supply when different structures are being formed, it is important that the iron be delivered to the brain in sufficient amounts and at the correct time (25). The huge number of studies made on effects of ID on mental performance was extensively reviewed by the British Nutrition Foundation (13a). Studies of infants and animals suggest that it may be difficult to repair negative effects of ID that had developed earlier. Therefore, it may be assumed that iron adequacy should be optimal at least during the first 20 years of life. It should be remembered that there are several well-designed studies, all of adolescent girls, showing that there are negative effects of ID related to both mental symptoms and physical activity that are unrelated to anemia (6, 17, 80, 98).

HOW TO ESTABLISH A DIAGNOSIS OF IRON DEFICIENCY?

The wide variation in the concentration of Hb in healthy, iron-replete subjects makes it difficult to establish whether a specific person has a normal or a subnormal level of Hb. The traditional indicators of iron status, e.g. mean cell Hb (MCH), mean cell volume (MCV), and transferrin saturation, have the same problem with wide interindividual variations in both iron-replete and iron-deficient subjects. Thus, very marked overlaps are observed in the distributions of all these parameters in normal and iron-deficient subjects. Thus, any cut-off value chosen will have either a low specificity or a low sensitivity. The use of technically perfect, stained bone marrow smears is still the gold standard to evaluate iron status and thus also to examine the validity of different laboratory methods. In this way, the sensitivity and specificity of different laboratory methods has been evaluated (44). So far, SF seems to best discriminate between iron-deficient and iron-replete subjects. A SF $\leq$ 15 μg/liter always means ID. Also, above this limit ID may be present, especially in subjects with even a simple, recent infection (61). In the actual study (44), however, this information was unfortunately not available. There are reasons to believe that even a regular moderate use of alcohol may induce an increase in SF (67, 71). Because SF seems to be a very sensitive acute-phase reactant, associations between high SF and other diseases must be interpreted with great caution, e.g. iron overload, coronary heart disease, and cancer. The concentration of transferrin receptors in serum has also been used to establish a diagnosis of ID (23, 88). The main advantages are that it allows separation between ID and chronic anemia and that it is not influenced by infections. Its disadvantages are the lower sensitivity to detect mild ID and the fact that there is still no material available to standardize the different methods used. All these facts imply that it is difficult to establish a diagnosis of mild ID, especially in epidemiological studies, where, for example, the interpretation of a therapeutic trial with iron supplements and placebo is impossible in mild ID due to the "regression toward the mean" using repeated Hb measurements.

In some studies, therefore, combined diagnostic criteria have been used, for example in the N-HANES II studies in the United States. In all kind of studies and for all diseases, use of combined criteria will improve the specificity but decrease the sensitivity. This will automatically lead to an underestimation of the true prevalence of ID. This was demonstrated in a study of 203 women, where iron-stained bone marrow smears were used as a gold standard (44) and different criteria could be validly tested. In the N-HANES II studies, performed between 1976 and 1980 and using the multiple criteria, the prevalence of ID in women aged 20–49 years, for example, was estimated at about 10% (39) (calculated 95% confidence limits were 8%–12%) and at 11% in the more recent N-HANES III studies (68). As stated elsewhere (39), these cut-off values were arbitrarily chosen. It is of great interest, however, that at about the same time as the N-HANES II studies were made, there was a more direct, but much smaller, study on iron status in the United States

based on measurements of the content of iron in liver samples in 259 subjects who died traumatic deaths (91). Very low iron contents in the liver was used as an indicator of ID and was found in 10 out of 28 women aged 20–50 years. The observed prevalence of ID in the autopsy material had a 95% confidence interval of 18%–56%.

In a random sample of 38-year-old women in Sweden studied at about the same time, the prevalence of ID, based on absence of stainable iron in bone marrow smears, was observed in 90 out of 286 women (32%) in the original sample (81). The 95% confidence range was 26%–37%. It should be mentioned that the distribution of SF in women at this age was almost identical in the N-HANES II 1976–1980 study in the United States (76), in the 1976 Nutrition Canada National Survey (97), and in the study in Sweden (44). When calculating the prevalence expected in this Swedish sample, using combined criteria similar to that used in the N-HANES II study, the prevalence was likewise reduced from about 32% to around 10% (44). This latter study was made in a subsample of 203 women out of the total 286, where all measurements needed were available.

Although the amount of autopsy material was small compared with the N-HANES II material, using Fisher's exact test, the difference for the two prevalences representing the United States was significant ($p < 0.001$). This important observation suggests that the use of multiple criteria in the N-HANES II study probably led to a considerable underestimation of the true prevalence of ID in the United States, and to the same extent as was noted in the Swedish studies using combined criteria. The balance of evidence would suggest that the 10% prevalence of ID in 20- to 44-year-old women reported in the United States is probably much too low and would be about two to three times higher had not the combined criteria been used.

CONCLUDING COMMENTS

The balance of current evidence strongly indicates that to ensure optimal health and development, it is important to prevent and treat even mild ID in growing individuals, at least up to the age of around 20 years, and in pregnancy. Considering the ingenious systems available that control the absorption and metabolism of iron, it is paradoxical that ID is the most common deficiency disorder in the world and the main remaining deficiency in the developed world. A reasonable explanation may be the marked changes that have occurred in human nutrition. Before about 10,000 years ago, humans lived as nomads and a main part of the diet was obtained by hunting and fishing (27, 34). The introduction of cereals at about this time, first wheat and later on rice and maize, formed the basis for formation of societies, but it was also associated with a diminution in body size. The diet was thus radically changed. Another factor influencing human nutrition was the rapid growth of populations during the past millenium and especially during the past few centuries. Industrialization, automation, and other developments have

successively reduced energy requirements and thus the need for food. The current low-energy lifestyle and the dramatic increase in the number of cars and in the number of hours spent watching television and using computers have further led to a situation where the risk of nutrient deficiencies, especially ID, has increased. At the same time, the risk of obesity, diabetes, coronary heart disease, and osteoporosis have also increased. It is important to prevent ID in infants, children, adolescents, and women of childbearing age, especially those who are pregnant, to ensure optimal development of muscles and brain. The weaning period in infants is especially critical because of the extremely high iron requirements and the importance of adequate iron nutrition during this crucial period of development.

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