Regulators of Iron Balance in Humans

By Clement Finch

Absorption in the human has been assumed to have a much larger role in regulating iron balance than excretion, and has been examined in more detail. In most instances, the evaluation of dietary iron has been based on the absorption of radioiron from a single meal. The amount absorbed is thought to be a function of the amount and availability of the dietary iron and the absorptive behavior of the intestine.

The extrapolation of such measurements to iron balance may not be justified because long-term studies have not always agreed with the prediction from radioiron absorption. That an increase in dietary iron may not always result in an increase in plasma ferritin of men with normal iron stores was shown in a 2-year study of iron fortification. A similar result was obtained when food iron availability was increased. Large doses of ascorbic acid were ingested with meals, enough to increase radioiron absorption from a test meal severalfold. This ascorbate fortified diet, administered over 2 years, showed no increase in plasma ferritin values of subjects with adequate body iron. One might suspect that in both these studies the intestinal mucosa had adjusted its absorption so as to hold iron stores at their initial level; yet the radioiron absorption from the ascorbate-fortified meal continued to be increased after 12 weeks of ascorbic acid ingestion. Perhaps excretion was altered. Whatever the reason, it appears unwise to consider that single absorption measurements can predict quantitative changes in iron balance of normal subjects. It is more appropriate to approach iron balance through measurement of iron stores.

One way of evaluating iron stores is by examining a marrow aspirate for hemosiderin within reticulo-endothelial cells, but this is only an approximation and may be misleading when iron stores are not normally distributed between reticulo-endothelial and parenchymal tissues. An elegant estimate of liver iron is provided by magnetic susceptibility measurements, but this procedure has limited availability and samples only a portion of storage iron. A quantitative measure of all body iron stores is found in the

*Iron depletion* refers to a decrease in iron stores from that expected for the age and sex of the individual but intact essential body iron. *Iron deficiency* refers to a virtual exhaustion of iron stores and a deficient plasma iron supply in an individual with no significant decrease in hemoglobin concentration. *Iron deficiency anemia* refers to the presence of iron deficiency and anemia due to iron deficiency. *Iron-deficient erythropoiesis* refers to a deficient marrow iron supply, regardless of the iron supply to other body tissues.
amount of iron mobilized by bleedings. Although most directly reflecting available storage iron, this is a demanding, "one-shot" procedure. Its other limitations are the time required to exhaust stores, particularly because hemosiderin iron may be mobilized more slowly than ferritin iron, and the difficulty of estimating the contribution of absorbed iron during the period of phlebotomy. Nevertheless, this remains the "gold standard" for determining storage iron.

More practical is the measurement of plasma ferritin, which reflects both reticulo-endothelial and parenchymal iron stores. It has been estimated that 1 μg/L plasma ferritin in the normal adult is equal to 8 to 10 mg tissue iron stores. However, when comparing individuals of widely differing body weight, the conversion to 120 μg/kg storage iron is preferable, because plasma ferritin is a concentration measurement. Reasonable agreement between phlebotomy and plasma ferritin measurements has been shown. Its convenience and the possibility of serial measurements make plasma ferritin the measurement of choice in studying the iron balance of normal subjects. The chief limitation of this method is that other conditions, particularly inflammation, will alter the relationship between the plasma ferritin level and iron stores.

THE STORE REGULATOR

The normal US adult male with no unphysiologic blood loss has iron stores of 1,000 ± 300 mg as derived from plasma ferritin and phlebotomy studies. Whereas it is not known whether excretion exerts any regulatory effect in the normal individual, it has been repeatedly shown by radioiron measurements, using radioiron salts or food labeled biosynthetically with radioiron, that non-heme iron absorption is inversely related to iron stores. Absorption from a test meal is high if iron stores are depleted and is suppressed if iron stores are enlarged. This regulation is so predictable in normal subjects that plasma ferritin measurements of iron stores have been used to predict absorption from a meal of known availability. The highly available heme iron is much less affected by the status of iron stores, but has seemed of secondary importance in considerations of iron deficiency because of its limited intake by most of the world's needy population.

Changes with age in plasma ferritin levels of normal subjects provide some evidence of the effect of changing iron requirements. A near-equilibrium state is found in the adult of secondary importance in considerations of iron deficiency population. Because of its limited intake by most of the world's needy population.

There are isolated observations on the rate of reconstitution of iron stores in iron-depleted but not iron-deficient subjects whose requirements are at a basal level. One individual, subjected to phlebotomies until his iron stores were exhausted and phlebotomized again after 1 year of normal diet, showed stores of 200 mg after the second bleeding. This represented a rate of increase of 0.5 mg/d during the preceding year. Another individual with low iron stores estimated at 280 mg, receiving an additional 25 mg of iron daily with his food for 500 days, had an increase in iron stores of 390 mg, as estimated from his plasma ferritin or a rate of increase of 0.8 mg/d. Another way of determining the capacity of the store-regulator would be to determine the increase in iron requirement at which iron deficiency would appear. Hallberg et al found that the prevalence of anemia increased when menstrual blood loss exceeded 1 mg of iron/d, indicating that the capacity of the store-regulator had been exceeded. These various observations, along with the rate of increase in iron balance as requirements for growth and menstruation cease, show that the build-up of depleted iron stores through diet in the nonanemic individual occurs at a rate of only about 1 mg/d.

The store-regulator has the additional function of preventing iron overload. Once the iron stores of the adult male are attained, there is a limitation to further increase. In surveys of healthy men up to the age of 90 years, the plasma ferritin at the 50th percentile shows no significant change after 30 years of age, despite some shift of iron to stores as lean body mass decreases.

Other observations support the stabilization of iron stores when levels of 800 to 1,200 are reached despite excess dietary iron. In one study, an adult male with normal stores received 10 mg of fortification iron as ferrous sulfate daily for 500 days, during which time there was no significant change in his plasma ferritin. As mentioned previously, fortification at a level of 7.5 mg of iron/d for 2 years produced no increase in plasma ferritin in a group of men with normal iron stores.

There is also evidence that the body acts to reduce absorption when iron stores are enlarged. Four subjects whose iron stores were enlarged by injection of 1,500 mg iron as iron dextran had a decreased absorption from a test meal over the following 563 days. An interesting animal model for the study of store reduction is the Besinge dog, with anemia caused by pyruvate kinase deficiency and spontaneously occurring iron overload. When the abnormal erythroid marrow was normalized by marrow transplant from a normal dog, the iron overload progressively decreased over the ensuing years. Similar data are now being obtained from thalassemic patients with iron overload who have been subjected to marrow transplant.

The extent to which iron balance can be maintained in the face of large amounts of oral iron is still a moot point. A modest increase in iron stores was found in Ethiopians consuming large amounts of iron from dirt-contaminated teff. A few reports in the literature describe presumably normal individuals receiving medicinal iron for long periods.
who eventually show massive iron overload.\textsuperscript{30} It is unclear at present whether these individuals are representative of the normal population or, more likely, that there is some as yet unidentified genetic or environmental factor that may have impaired their normal defense.

**THE ERYTHROID-REGULATOR**

There are situations in which larger amounts of dietary iron are absorbed than can be attributed to the store-regulator. For example, phlebotomized subjects on a normal diet have been shown by balance studies to replace 3 to 4 mg of iron loss in addition to their excretory loss.\textsuperscript{21,32} An equal or greater amount is absorbed by patients with thalassemia in the face of enlarged iron stores.\textsuperscript{33} Even more iron may be absorbed if available iron intake is increased. Patients with iron deficiency anemia receiving therapeutic doses of iron can absorb 20 to 40 mg/d as long as their anemia is still present,\textsuperscript{34} but the amount decreases as soon as the anemia is alleviated.\textsuperscript{35} Similar amounts are absorbed by individuals with normal iron stores whose marrow is stimulated by erythropoietin.\textsuperscript{20} Thus, there is a second regulator operating independently of iron stores.

It might be suspected that the location of this second regulator would be in a tissue highly sensitive to iron supply. The erythroid marrow qualifies because it uses more than 80% of plasma iron, yet receives only 5% to 10% of cardiac output, and is usually the first tissue to show the effects of iron deficiency when iron stores are exhausted. The association with erythropoiesis is illustrated by the decrease in absorption that occurs when the erythroid marrow hyperplasia in thalassemia is suppressed by transfusion.\textsuperscript{36} That this response is erythron-induced rather than caused by erythropoietin itself is shown by the absence of an absorptive response in aplastic anemia in which erythropoietin levels are extremely high but erythropoiesis is virtually absent.\textsuperscript{37}

The erythron-induced increase in iron absorption is in some way related to the increase in erythropoiesis, at least within a given disease state. However, for any given increase in plasma iron turnover, different anemic states differ in the magnitude of the absorptive response and its effect on iron balance.\textsuperscript{38} Some component of erythropoiesis other than simply its rate must explain the absorptive response. The common denominator would appear to be the unmet requirement of the erythroid marrow for iron. This imbalance between iron requirements of the erythroid marrow and its iron supply has been referred to as iron-deficient erythropoiesis.\textsuperscript{39} It occurs in iron deficiency caused by a decreased iron supply, but is also found with a normal or even increased iron supply in the face of increased marrow iron requirements.\textsuperscript{40,41}

Whereas a deficient iron supply can be detected in the iron-deficient subject by a transferrin saturation of less than 16%,\textsuperscript{42} the plasma iron and transferrin saturation needed increases as erythropoietic activity increases. Therefore, this indicator is of little use in the presence of increased erythropoiesis. Fortunately, there are other ways of demonstrating iron-deficient erythropoiesis in high-output states. It may be suspected in the anemic patient when the usual increase in RBC volume expected with erythropoietin does not occur.\textsuperscript{43} Unsaturated transferrin receptors in the marrow have been detected more directly by excessive increase in plasma iron turnover after elevation of the plasma iron.\textsuperscript{44} However, the most practical laboratory measurement, particularly in conditions in which there is no other defect in hemoglobin synthesis, is the RBC protoporphyrin concentration.\textsuperscript{39} There is an increase in protoporphyrin within the developing RBC as the iron supply for hemoglobin synthesis becomes deficient, regardless of the state of body iron stores. Although it was initially believed that reticulocytes had an elevated protoporphyrin, this was later shown to occur only in the presence of iron-deficient erythropoiesis.\textsuperscript{40} There is a close linkage between this increase in iron absorption and increased erythrocyte protoporphyrin, given sufficient time for the slower protoporphyrin response. It might be suggested that the implication could be transposed, ie, that an increased absorption of the magnitude seen in iron deficiency might be used to identify iron-deficient erythropoiesis in the individual with adequate or even increased iron stores.

In understanding the relation between erythropoiesis and iron supply, it is helpful to examine both in more detail. Stimulation of the erythroid marrow starts when anemia or hypoxia of the kidney increases the output of erythropoietin. That, in turn, acts on the erythroid marrow. The earliest discernible effect is the premature release from the marrow, within a matter of hours, associated with a shortened marrow radioiron transit time.\textsuperscript{45} In the absence of disease of the marrow stroma, these "shift reticulocytes" recognized in the Wright's-stained blood smear as basophilic macro-erythrocytes, may be assumed to result from an increased erythropoietin level.\textsuperscript{46}

The primary action of erythropoietin is to permit an increase in RBC production through apoptosis, preventing the early death of erythrocyte progenitors.\textsuperscript{47} The response depends on the functional integrity of the marrow and an adequate supply of iron. There are other supporting reactions in addition to the displacement of reticulocytes into circulation. Marrow blood flow increases as the marrow metabolic mass increases.\textsuperscript{44} With time, erythroid tissue may extend throughout the medullary cavities of the skeleton, leading to a further increase in blood supply. Thalassemia illustrates the extreme changes that may occur, ie, where the erythroid cells may use more than 150 mg of iron daily\textsuperscript{49} and may destroy a large portion of the skeleton in a futile attempt to sustain the circulating hemoglobin.\textsuperscript{50}

Stimulation of the erythroid marrow avails little if there is insufficient iron. Not only is iron necessary for hemoglobin synthesis, but is even more important for erythroid proliferation, as shown by the limited expansion of the erythroid marrow in iron deficiency anemia.

Animal studies show that a sudden increase in erythron iron requirements after exchange transfusion with immature erythroid cells can be met initially without any apparent decrease in plasma iron.\textsuperscript{51} This suggests that a labile pool of iron moves from tissue cells into the plasma, perhaps activating, in turn, the intracellular mobilization of storage iron. Continued marrow proliferation depends on the ability of iron stores to sustain the plasma iron level.\textsuperscript{52-54} Over a period of 2 or 3 weeks, the individual with adequate iron stores can mobilize 15 to 40 mg of storage iron daily, boost-
ing erythropoiesis to 2 to 3 times basal. At the same time, absorption is increased, but the amount absorbed from a normal diet is only a few milligrams a day. Thus, the size of iron stores will determine the early rate of RBC production, but stores will be progressively depleted.

Delivery of iron to marrow erythroid cells depends on marrow plasma flow and tissue penetration. It is easy to visualize that the massive erythroid proliferation, coupled with a decreased transferrin in thalassemia, can outrun its plasma iron supply. It is more difficult to understand why a normal supply cannot satisfy the needs of an acutely stimulated marrow. Nevertheless, the increase in RBC protoporphyrin after a single phlebotomy and the absorption of large amounts of iron in subjects with normal iron stores acutely stimulated by erythropoietin suggests that a normal plasma iron is not enough to satisfy an acutely stimulated erythroid marrow.

**INTERACTION OF THE TWO REGULATORS**

Unfortunately, it is not possible at the present time to characterize these regulators in other than physiologic terms. Although it is entirely possible that these two effects may operate through the same metabolic machinery in the intestinal mucosa, they do not appear to represent a continuum of a single regulator. It would be difficult to see how the erythroid-regulator could be involved in store effects because its own iron supply derived from the plasma iron or transferrin saturation is unaffected by changes in stores short of actual deficiency. Furthermore, the two regulators may respond in different sequence. In the case of gradual depletion, the store-regulator is activated when a negative balance develops, followed by activation of the erythroid-regulator when iron depletion infringes on the marrow’s iron supply. A different sequence is seen when erythropoiesis is stimulated by a blood donation. This results in an increase in the marrow iron requirement, creating imbalance between marrow needs and iron supply, as evidenced by an elevated RBC protoporphyrin, activating the marrow-regulator to achieve levels of absorption beyond those caused by the store regulator. This shortens the duration of erythropoiesis and alkalizes basal pH, leaving the store-regulator to slowly reconstitute the depleted stores. Regulation of iron balance breaks down when there are continued unmet erythron requirements caused by some proliferative RBC disorder such as thalassemia. In an attempt to maximize iron supply and alleviate the anemia, iron balance is continually positive despite the development of iron overload.

**CLINICAL IMPLICATIONS AND SPECULATIONS**

By far the most common problem with iron balance is iron depletion and iron deficiency. Increased requirements of 1 or 2 mg of iron are ordinarily met by the store-regulator, albeit at the expense of iron stores. Losses requiring the absorption of 3 or 4 mg, as illustrated by frequent blood donors, deplete stores, usually produce iron deficient erythropoiesis, and activate the erythron-regulator. At this point, the amount of iron in the diet may become critical. Losses of more than 4 mg require iron supplementation if anemia is to be prevented or treated. Subjects receiving medicinal doses of ferrous iron in the presence of iron deficiency anemia provide an additional iron supply sufficient to increase RBC production up to 4 times basal. After requirements have returned to basal levels, reconstitution of depleted iron stores from diet requires years unless supplemental iron is continued, and even then, many months.

The otherwise predictable behavior of the iron regulators does not hold when inflammation is present. The usual flow of iron from reticulo-endothelial and parenchymal cells as well as absorption is decreased, and the plasma iron level decreases within hours of onset. The deficient iron supply is evidenced within 1 or 2 weeks by an increase in RBC protoporphyrin. This generalized effect, which appears to act at the cellular level, can be reproduced by endotoxin. There is some evidence that injected erythropoietin can improve mobilization of iron in chronic inflammatory disease.

Hemolytic anemia dramatically increases plasma iron requirements. Fortunately, iron from RBCs catalyzed by reticulo-endothelial cells and from hemoglobin processed by hepatocytes can be rapidly returned to the erythroid marrow without greatly altering body iron balance. Such recirculating of iron far exceeds the rate at which iron stores can be mobilized. When the kinetics of hemolytic anemia are simulated by injecting nonviable RBCs into a subject subjected to bleedings, the subsequent rate of RBC production reaches 6 to 8 times basal.

Hereditary spherocytosis demonstrates the efficiency with which the body can manage a hemolytic process. Most individuals with this defect have little or no anemia despite an RBC turnover of 4 to 5 times normal. The compensated state includes a normal erythropoietin level, a normal marrow radioiron transit time, a normal or slightly elevated plasma iron and transferrin saturation, a normal RBC protoporphyrin, and iron stores usually maintained within the normal or near normal range. It may be that the unique role of the spleen in RBC destruction in this disorder accounts for the efficiency of iron recycling. At any rate, this condition and certain other compensated hemolytic processes attest to the fact that increased erythropoiesis in itself does not result in activation of the erythroid-regulator.

On the other hand, there are situations in which an increased RBC production associated with increased RBC destruction results in massive iron overload. This occurs in thalassemia, sideroblastic anemia, certain dyspoietic anemias, and hemolytic anemias in which the rate of erythropoiesis exceeds 5 times basal. However, it is not simply the rate of erythropoiesis because the iron-loading anemias have a much higher iron absorption than do patients with hereditary spherocytosis at the same plasma iron turnover. These iron-loading anemias are characterized by continued anemia despite increased erythropoietin stimulation and marked erythroid marrow expansion. Perhaps the cause of the iron overload is some inefficiency of iron recycling caused by the nature of RBC breakdown, leading to iron-deficient erythropoiesis and a resultant increased in iron absorption.

It might be expected that inborn abnormalities of the iron regulators might occur. The iron overload syndrome, HLA-related hemochromatosis, constitutes one of the more com-
mon genetic disorders. Typically, in the homozygous adult male there is a positive iron balance of 1 to 2 mg of iron daily, with ensuing tissue damage and clinical manifestations in the fourth to seventh decades. This rate of iron accumulation is validated by the phlebotomy requirements for maintenance after normal iron balance is regained. Although the increased absorption is probably present in childhood and adolescence, most of the excess at that time would be used for growth, and only later would appreciable overload develop. For the same reason, the adult homozygous female is protected by menstrual losses to the extent that clinical manifestations are aborted in most instances. In view of the rate of accumulation, it might be suggested that this is a disorder of the store-regulator. Although the great majority of families have late onset disease, there are a few instances in which multiple family members develop massive iron stores and iron toxicity in the first 2 or 3 decades of life. Whether these are the same genetic defect or, as the rate of iron accumulation suggests, represent a genetic defect of the erythroid-regulator remains to be determined.

There are conditions recognized as predisposing to enlarged iron stores, including alcoholism, cutaneous porphyria, and, perhaps, a large intake of heme iron. Of particular interest has been the overload seen in the iron overload seen in the South African Black consuming large amounts of home brew fermented in iron pots. It has been suggested that the severe overload in this instance is due to the combination of a large iron intake, alcohol, and a genetic defect. Cases have also been reported in which some disorder of RBC production in an individual heterozygous for HLA-related hemochromatosis is associated with iron overload.

Beyond this, the prevalence of more modest iron overload in the general population remains in doubt. One reason is that plasma ferritin is not very useful. Most of the plasma ferritin levels in the range of 200 to 2,000 μg/L are related to inflammation, tissue damage, neoplastic disease, and hyperthyroidism rather than to a simple increase in body iron. One way that inflammation may be distinguished is by its difficulty with which storage iron is mobilized by phlebotomy. Assuming a causative role for iron when a statistical association is found between a moderately elevated ferritin and some disease such as myocardial infarction is unjustified, particularly because other acute phase proteins such as fibrinogen show a similar association.

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REFERENCES


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