Iron availability for erythropoiesis

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BODY IRON EXCHANGE The balance of iron in humans is controlled by the rate of iron absorption. Iron excretion is not regulated and is limited to obligatory losses caused by shedding of epithelial cells from the skin, intestine and urinary tract, and to menstruation and inapparent intestinal bleeding. A number of intraluminal and mucosal factors modulate iron absorption. The level of erythropoiesis and body iron stores are important determinants of the rate of transfer of iron from the mucosa to the plasma. A high rate of erythropoiesis associated with hypoxia, ineffective erythropoiesis, hemolysis or a transfusion of blood with a high reticulocyte count increases the rate of iron absorption. An increase in the level of iron stored in the body is followed by a reduction in absorption, while depletion of body iron stores enhances absorption. However, the mechanism by which erythropoiesis and iron storage can influence iron absorption remains unknown.

INTERNAL IRON EXCHANGE The major pathways of internal iron exchange have been identified (Figure 1)[1,2]. The erythron-RE pathway consists of iron uptake by immature erythroid cells, which incorporate most of the iron into hemoglobin, the subsequent circulation of iron-containing red blood cells through the vascular

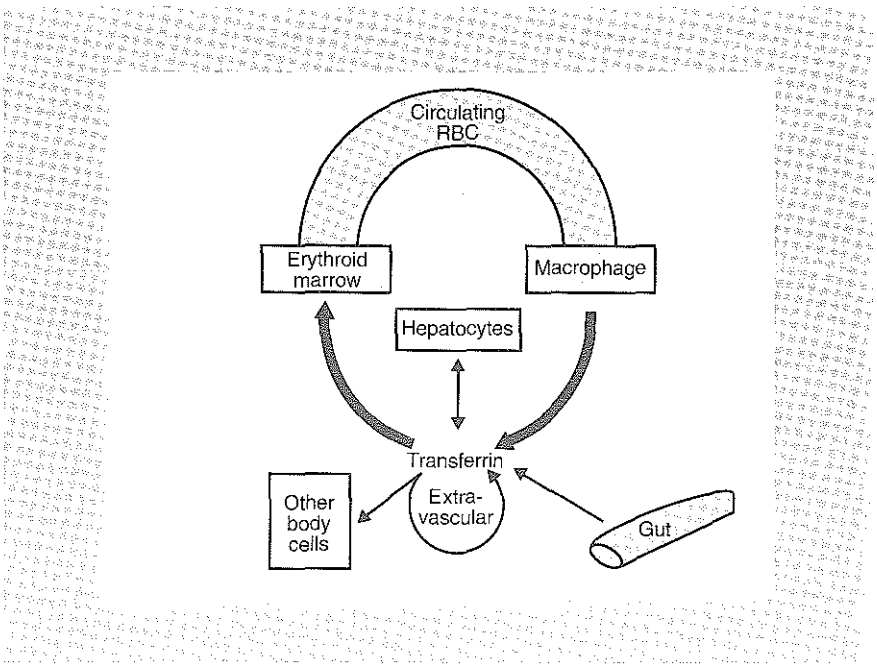

Figure 1. Internal iron metabolism[5].

system, the eventual processing of senescent or defective red blood cells by the reticuloendothelial (RE) system (macrophages and Kupffer cells) and the return of iron to circulating transferrin.

A second pathway is parenchymal iron uptake directly from circulating transferrin by various tissues, predominantly hepatocytes. Exchange between hepatocyte and plasma transferrin is bidirectional, in contrast to the unidirectional iron flow from RE cells to the plasma[3]. The daily transport of iron through the plasma can be calculated from the plasma iron level and the radiolabeled iron disappearance curve after intravenous injection of a tracer dose, to give the plasma iron turnover (PIT). This parameter has been used extensively as a quantitative measure of red blood cell production[4]. This relationship is meaningful because approximately 80% of iron passing through the plasma is delivered to the erythroid marrow, while iron exchange in the other tissues remains relatively constant. Thus, changes in PIT relate primarily to changes in erythroid activity. An important prerequisite for the use of this measurement of marrow function is that transferrin receptors are saturated with iron-bearing transferrin[5]. If the receptors are not fully saturated, iron supply rather than marrow function limits erythropoiesis. This situation can be suspected when $t_{1/2}$ for clearance of radiolabeled iron is less than 25 minutes or transferrin saturation is <15%, or <40% when erythropoiesis is stimulated.

STORAGE OF IRON In adults, approximately two-thirds of the body's iron stores are in RE cells and one-third in hepatocytes. RE cells and hepatocytes may either store iron taken up as ferritin-hemosiderin or return it to circulating transferrin[6]. Iron transport via the RE cells represents approximately 70% of the plasma iron supply, thus the rate of RE iron release determines plasma iron levels[7]. When more iron is required for red blood cell production, an increased amount of red blood cell iron is immediately returned to the circulating transferrin and, in addition, storage iron is mobilized. Hepatocytes also act as regulators of plasma iron levels by taking up increased amounts of iron when the body is in a hyperferremic state or by mobilizing stores to release more iron to the circulating transferrin in situations when increased iron levels are required.

Western adult males have iron stores of approximately 600 - 1000 mg of iron while in women iron stores are in the range of 200 - 300 mg. The most practical measure of iron depletion is the serum ferritin concentration, which closely parallels body iron reserves. Each 1 µg/l serum ferritin corresponds to approximately 8 mg storage iron. A serum ferritin concentration <12 µg/l indicates virtual exhaustion of the body's iron stores. It must be noted that a single donation of 500 ml of blood for transfusion each year imposes an iron requirement of over 0.5 mg/day, and has been shown to reduce the serum ferritin concentration, and therefore presumably the iron stores, by 50%.

Ferrokinetics have been used to characterize iron release from storage cells, ie RE cells and hepatocytes. Two phases of iron release have been identified: an early phase seen after initial processing of recently obtained iron, which is completed within hours, and a late phase associated with the release from body iron stores,

which develops over a period of days and weeks (Figure 2). The RE system appears to determine the diurnal fluctuations of serum iron levels by varying the immediate output of heme iron, independent of the available binding sites on transferrin[7,8]. However, for iron to be released from the hepatocytes, free binding sites on transferrin are necessary to avoid direct recycling of iron to the hepatocyte. The partitioning of iron between the early and late phases, as well as the rate of radiolabeled iron release in the late phase, depends on the size of the iron stores.

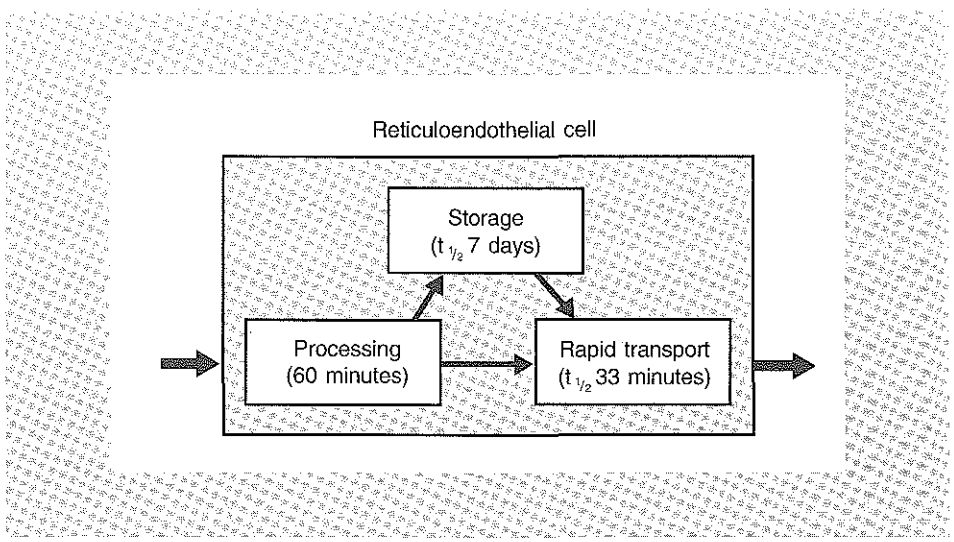

Figure 2. The processing of iron by RE cells. Iron is freed from hemoglobin during the first hour and is either stored as ferritin or returned to the plasma[1].

Iron release is also considerably modified in parallel to changes in the rate of erythropoiesis. Although a higher turnover of iron occurs in the RE cells compared with the hepatocytes, a similar model of storage iron mobilization can be proposed for both. The total amount of iron leaving the cell remains constant in normal conditions. When body (marrow) requirements are increased, more iron is donated and given its more rapid turnover, augmenting the proportion of early release is the most efficient mechanism. When body (marrow) requirements are decreased, iron supply is diminished. The plasma iron levels decrease when marrow requirements cannot be matched by iron mobilization, and increase when marrow activity is not sufficient to take up the minimum amount of iron, which the RE cells are not able to retain. When erythropoiesis is stimulated, an increase is observed in iron absorption and release of iron from hepatocytes, as well as RE cells, and these decrease when marrow activity is depressed. Iron mobilization from the gut and from the body's iron stores intensify when an iron deficiency develops, and slow when iron stores are excessive. Inflammation produces a blockade in iron absorption and the release of iron from the body's stores. Hyposideremia in inflammatory states is mainly due to defective RE release of iron. It occurs within hours of inflammation. Early RE release is decreased

because ferritin production is enhanced in inflammatory RE cells. The inflammatory block in RE iron release is seen not only in infections but also in cancer and rheumatoid arthritis, and following trauma, surgery and myocardial infarction.

SERUM TRANSFERRIN RECEPTOR AS A CLINICAL TOOL FOR EVALUATING MARROW RESPONSE TO RECOMBINANT ERYTHROPOIETIN

Iron transport in the plasma is carried out by transferrin, which donates its iron to cells via the transferrin receptor. Transferrin binds to its receptor on the cell membrane and the complex is then internalized in a vacuole; iron is released to the cytoplasm, apotransferrin is returned to the plasma and the receptor returns to the cell surface. Virtually all cells have transferrin receptors on their surface, but the largest numbers of receptors are located in the erythroid marrow (80 - 95% of body transferrin receptors). A soluble form of the transferrin receptor is also found in the plasma. As the concentration of serum transferrin receptor correlates very closely with the total mass of receptors in the bone marrow, this parameter provides an excellent quantitative measurement of total erythropoietic activity in humans, when iron deficiency anemia is ruled out[9]. It correlates well with the standard ferrokinetic measurement and has many advantages because it is simpler, more precise, and does not require injection of radiolabeled iron into the patient. Clinical measurement of the transferrin receptor level has been used to quantify erythropoiesis in anemias and polycythemias of various origins, bone marrow transplantation and pregnancy.

The transferrin receptor level is particularly useful in clinical studies using recombinant human erythropoietin (r-HuEPO) to treat anemias of various origin, including the anemia of chronic renal failure or in many examples of the anemia of chronic disease. It is useful to monitor the erythropoietic response to r-HuEPO therapy because it has been shown to be more reliable and quantitative than the reticulocyte count. The transferrin receptor level can help determine if a certain dose of r-HuEPO is effective in enhancing erythropoietic activity before any change in the hemoglobin levels can be detected. The serum transferrin receptor level could also be a powerful predictor of patient response to r-HuEPO. All patients responding to r-HuEPO treatment show an early increase in circulating transferrin receptor, while non-responders show no change[10]. These modifications occur within 10 days of therapy, long before any change in the hemoglobin level can be detected. If a patient receiving r-HuEPO shows no increase in serum transferrin receptor levels within 2 weeks, there are two possible reasons for the lack of response: there is either a medical reason (iron deficiency, inflammation, marrow damage etc) or the dose is insufficient and should be increased.

ERYTHROPOIETIC RESPONSE TO BLOOD LOSS

Recently, the quantitative value of a simple model of erythropoiesis based on the fact that the red blood cell mass determines erythropoietin production, which in turn stimulates erythropoiesis, was validated[11]. The response to acute hemorrhage in a normal patient provides a good example of timing of the marrow response to erythropoietin. There is an

immediate response to erythropoietin with stimulation of marrow precursors and the appearance of shift cells on the peripheral smear. This response occurs within hours of any significant reduction in red blood cell mass. At that time, the reticulocyte count will not have changed from the basal level. Proliferation of red blood cell precursors, as shown by an increase in the ratio of erythroid to granulocyte elements in the marrow, occurs over the next 48 -72 hours. An increase in the reticulocyte production index is not seen until 5 days after an increase in erythropoietin levels.

Numerous studies have demonstrated that there is a threshold hemoglobin level (hemoglobin = 10.5 g/dl, hematocrit = 32%) below which erythropoietin production increases significantly outside the normal range. Because of this threshold, a single unit weekly phlebotomy usually fails to enhance erythropoietin production significantly but does cause anemia. Thus, r-HuEPO therapy has been found to be beneficial in autologous blood donation.

Iron plays a key role in determining the level of response of the bone marrow to blood loss. The overall level of marrow production in a patient with hemorrhagic anemia is influenced by the amount of available iron stores and the level of serum iron. In this situation the normal cycle of hemoglobin catabolism and iron reutilization has been interrupted, and the RE cell stores must be called upon to supply iron for the increased production in the marrow. There is an upper limit to this iron supply mechanism. Regardless of the severity of the anemia, the RE system can only provide sufficient iron to support a red blood cell production level of approximately three times the normal level. In addition, iron stores are not inexhaustible. Most adults have only enough stores to replace 20 - 50% of the circulating red blood cell mass. Therefore, as iron stores are depleted, the plasma iron level will fall, the marrow becomes less proliferative and the reticulocyte response fails. In contrast, patients with hemolytic anemias and high levels of red blood cell destruction are capable of much higher levels of sustained red blood cell production, reflecting the higher level of iron supply made available by the increased levels of hemoglobin breakdown.

IRON SUPPLEMENTATION It is essential to recognize the important role of iron supply in determining the rate of marrow response to erythropoietin. Iron is obviously required for hemoglobin synthesis and an inadequate iron supply affects the ability of red blood cells to differentiate normally. Iron also plays a key role in controlling the proliferative response of the marrow to erythropoietin stimulation. Thus, an inadequate iron supply resulting in low plasma iron levels will result in a poor proliferative response, despite high levels of erythropoietin. It is important therefore to monitor the patient's iron status closely. If there is any evidence of iron deficiency at any stage in r-HuEPO-treated patients, it must be treated appropriately with oral and if necessary parenteral iron. In view of the importance of an adequate supply of iron, it is advisable to place all patients on oral iron supplements routinely during r-HuEPO treatment.

The tolerance of oral iron has been carefully studied in 5000 blood donors[12]. For a dose of approximately 200 mg of total elemental iron per day, 25% of subjects complained of symptoms of gastric intolerance. If the dose was doubled to 400 mg/day, 42% of patients noted severe gastric intolerance (Figure 3). At the same time, increasing the daily dose from 200 mg to 500 mg resulted in a modest increase in the amount of iron absorbed. Therefore, while a lower oral dose is slightly less efficient, it increases compliance.

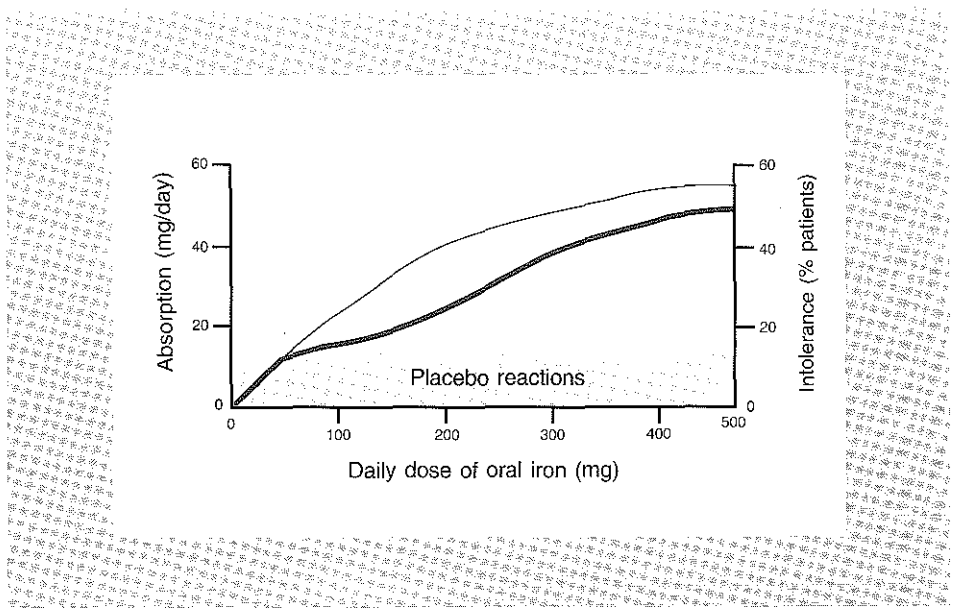

Figure 3. The relationship of increasing doses of oral iron to (——) absorption of oral iron, and to (— —) prevalence of side effects [12].

Many preparations of iron are available. It is obvious that the recovery of a patient with uncomplicated iron deficiency is not helped by vitamin supplements or minerals. The position has been succinctly summarized by Crosby[13]: 'The choice of iron to prescribe has been made needlessly difficult. Almost everything works and the claims for superiority of the many proprietaries are usually based on evidence which is incompetent, irrelevant and immaterial'. Similarly, the rate at which the hemoglobin concentration increases after parenteral iron therapy is of the same order as that following oral iron so long as the patient is not iron deficient relative to the rate of erythropoiesis desired. In some situations, such as in infection or in severe inflammation, a defect in iron supply may markedly inhibit the response to erythropoietin. Both the reticulocyte response and the rise in the hemoglobin level are suppressed. Therapy with either oral or injectable iron may not correct the defect in these situations, since iron absorption and RE iron release can both be impaired.

References

1. Bothwell Th, Charlton RW, Cook JD, Finch CA (1979) Iron metabolism in man, Blackwell Scientific Publications, Oxford.

2. Finch CA, Huebers H (1982) Perspectives in iron metabolism. *N Engl J Med* 306: 1520-1528.

3. Finch CA, Deubelbeiss K, Cook JD et al (1970) Ferrokinetics in man. *Medicine* 49: 17-53.

4. Cook JD, Marsaglia G, Eschbach JW, Funk DD, Finch CA (1970) Ferrokinetics : a biological model for plasma iron exchange in man. *J Clin Invest* 49: 197-205.

5. Beguin Y, Stray SM, Cazzola M, Huebers HA, Finch CA (1988) Ferrokinetic measurement of erythropoiesis. *Acta Haematol (Basel)* 79: 121-126.

6. Hershko C (1977) Storage iron regulation. In : Progress in hematology, Brown EB ed. Grune and Stratton, New York: pp. 105 -148.

7. Fillet G, Cook JD, Finch CA (1974) Storage iron kinetics VII. A biological model for reticulo-endothelial iron transport. *J Clin Invest* 53: 1527-1533.

8. Fillet G, Beguin Y, Baldelli L (1989) Model of reticuloendothelial iron metabolism in humans: abnormal behavior in idiopathic hemochromatosis and in inflammation. *Blood* 74: 844-851.

9. Huebers HA, Beguin Y, Pootrakul P, Einspath D, Finch CA (1990) Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood* 75: 102-107.

10. Beguin Y, Pootrakul P, Haley R et al (1987) Plasma transferrin receptor levels as a monitor of erythropoiesis in man: correlation with ferrokinetics and stimulation of recombinant human erythropoietin. *Blood* 70 (Suppl. 1): 51a.

11. Beguin Y, Clemons GK, Pootrakul P, Fillet G (1992) Quantitative assessment of erythropoiesis and functional classification of anemia and polycythemia based on measurements of serum transferrin receptor and erythropoietin. *Blood* (in press).

12. Sölvell L (1970) Oral iron therapy - side effects. In : Iron deficiency. Pathogenesis. Clinical aspects. Therapy. Hallberg L, Harwerth HG, Vannotti A eds. Academic Press, London : pp. 573-583.

13. Crosby WH (1966) Iron and anemia. In: Disease-a-Month. Year Book Publishers Inc., Chicago: pp 1-72.