

METABOLISM OF IRON STORES

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ABSTRACT

Remarkable progress was recently achieved in the studies on molecular regulators of iron metabolism. Among the main regulators, storage iron, iron absorption, erythropoiesis and hepcidin interact in keeping iron homeostasis. Diseases with gene-mutations resulting in iron overload, iron deficiency, and local iron deposition have been introduced in relation to the regulators of storage iron metabolism. On the other hand, the research on storage iron metabolism has not advanced since the pioneering research by Shoden in 1953. However, we recently developed a new method for determining ferritin iron and hemosiderin iron by computer-assisted serum ferritin kinetics. Serum ferritin increase or decrease curves were measured in patients with normal storage iron levels (chronic hepatitis C and iron deficiency anemia treated by intravenous iron injection), and iron overload (hereditary hemochromatosis and transfusion dependent anemia). We thereby confirmed the existence of two iron pathways where iron flows followed the numbered order (1) labile iron, (2) ferritin and (3) hemosiderin in iron deposition and mobilization among many previously proposed but mostly unproven routes. We also demonstrated the increasing and decreasing phases of ferritin iron and hemosiderin iron in iron deposition and mobilization. The author first demonstrated here the change in proportion between pre-existing ferritin iron and new ferritin iron synthesized by removing iron from hemosiderin in the course of iron removal. In addition, the author disclosed the cause of underestimation of storage iron turnover rate which had been reported by previous investigators in estimating storage iron turnover rate of normal subjects.

Key Words: ferritin and hemosiderin, serum ferritin kinetics, storage iron turnover, iron deficiency and overload

INTRODUCTION

Iron is an essential element for the living body. The human body stores iron in the form of ferritin and hemosiderin in liver, spleen, marrow, duodenum, skeletal muscle and other anatomic areas. Hemosiderin has been known as yellow-brownish granules that can be stained by Prussian blue in the tissue cells. On the other hand, ferritin is invisible by photomicroscopy or may be faintly visible and stained diffusely in the tissue cells by Prussian blue, if concentrated. Hemosiderin and ferritin are iron-containing proteins with magnetic susceptibility. Hemosiderin is water-insoluble and thermally denatured, but ferritin is water-soluble and heat-resistant up to 75°C. These characteristic differences were used for the fractionation of ferritin and hemosiderin.

The total amount of body iron stores is around 600 to 1000 mg in the normal adult male and around 200 to 300 mg in the normal adult female.¹⁾

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The amount of ferritin iron is slightly larger than hemosiderin iron in the range from iron deficiency to normal.^{2, 3)} However, the amount of hemosiderin iron becomes larger than ferritin iron in the iron overload range.²⁻⁴⁾ Ratio of hemosiderin iron per ferritin iron increases along with the progress of iron deposition.²⁻⁴⁾

The prevalence of iron deficiency anemia in the menstruating female is less than 10%.⁵⁻⁷⁾ However, that of iron deficiency without anemia is around 20 to 40% in the menstruating female.^{7, 8)} The amount of storage iron in the normal female increases gradually after menopause, but it is still lower than the level of the normal male even after 20 years.⁸⁾ The main cause of iron deficiency is increased blood loss.

Storage iron behaves as if resisting change in the iron density gradient⁴⁾ in accordance with an iron homeostasis.⁹⁾ The transformation of ferritin into hemosiderin^{10, 11)} might be the second best evolutionary step to reduce iron toxicity, compensating for the human body's lack of an iron excretion function.

The increase of non transferrin-bound iron, both in whole body iron overload and in localized iron deposition^{12-14, 17)}, produces hazardous free radicals causing various disorders. To reduce iron toxicity, phlebotomy is performed for treating hereditary hemochromatosis and chronic hepatitis C. Also, an iron chelating agent, deferasirox¹⁸⁾, is now in use for treating anemic patients with transfusional iron overload.

Ferrokinesis is no longer performed due to the regulation of in vivo application of radioactive isotopes and the development of other measures, although it yielded a large amount of important information on iron metabolism and erythrokinetics. However, detailed storage iron turnover studies have not been conducted.

Recent advance of molecular biology and genetics revealed many factors regulating iron metabolism. Hepcidin, discovered in 2000 by Krause *et al.*¹⁹⁾, was initially recognized as an antimicrobial peptide produced in the liver. However, it was also found to be a regulator of iron metabolism in 2001 by Nicolas *et al.*²⁰⁾

The characteristic behaviors of ferritin iron and hemosiderin iron in iron deposition and mobilization were clarified through serum ferritin kinetics by Saito *et al.*^{4, 21)} in 2012–13 after 60 year-long blank period.

The above-mentioned knowledge of the metabolism of iron stores seems essential not only for understanding the basis of the iron metabolism, but also for studying the vast field of medicine.

The criteria of author's terminology are indicated in Table 1.

Table 1 Classifications of iron status according to storage iron levels

The term "Normal iron stores" (abbreviated to Normal in the table) means the level of iron stores between iron deficiency and iron overload regardless of disease and iron distribution in the body.

The border between the state of iron decrease within normal iron stores and iron deficiency is clear cut. However, that of iron increase within normal iron stores and iron overload is not so clear; a transitional zone between normal iron increase and iron overload was assigned.

| Iron deficiency ±symptom | <decrease | Normal | increase< | Iron overload ±symptom |
|--------------------------|-----------|--------|-----------|------------------------|
| Iron stores | g | <0.1 | 2.5~5.0< | |
| Serum ferritin | ng/ml | <12 | 250~500< | |
| TIBC* | μg/dl | >360 | ~200> | |

*Total iron-binding capacity of serum.

CLINICAL METHOD FOR DETERMINING IRON STORES

Quantitative determination of iron stores

The total amount of iron in the blood removed by phlebotomy^{15, 22-25)} or that in the transfused blood for patients with transfusion-dependent anemia can be determined by using the ratio of hemoglobin iron to hemoglobin. The total amount of iron stored after intravenous iron injection of patients with iron deficiency anemia can be obtained by subtracting the iron fixed in the increased hemoglobin in red cell mass²⁶⁾ from the total amount of iron injected. The total amount of iron stores divided by the term of non-iron deficiency state after iv iron infusion until the recurrence of iron deficiency gives the storage iron decrease rate^{26, 27)} in patients with iron deficiency anemia with constant blood loss.

Semiquantitative estimation of iron stores

The correlation between the total amount of storage iron and hemosiderin grain counts from biopsy sample has been used for the estimation of iron stores. Total iron binding capacity and transferrin receptor are helpful for the diagnosis of iron deficiency states.

Body surface monitoring methods such as dual-energy X-ray CT, superconduction quantum interference device susceptometry (SQUID), and magnetic resonance imaging (MRI)²⁸⁾ were introduced. However, other than for MRI, these methods are not adopted for clinical settings. Although MRI is useful for the estimation of localized iron deposition, it has limitations regarding the quantitative determination of total amounts of storage iron in iron overload²⁹⁾, and is especially unreliable in mild iron overload range such as serum ferritin below 1500 ng/ml.

Radioimmunoassay of serum ferritin developed by Addison *et al.* in 1975³⁰⁾ was a breakthrough for studies on the iron metabolism. For example, the differential diagnosis of hypochromic hypoferrremic anemia from iron deficiency anemia became possible by assaying serum ferritin.³⁰⁻³³⁾

Serum ferritin may render a value higher than the actual storage iron level in patients with various inflammations, malignancies, or hereditary hyperferritinemia-cataract syndrome.³⁴⁾ Therefore, appropriate examinations are needed for excluding suspected cases of overestimation. Despite its disadvantages, serum ferritin has been regarded highly for the diagnosis and treatment of patients with iron deficiency anemia and iron overload.^{4-8, 15, 23-27, 30-33)}

Since Addison *et al.*³⁰⁾ revealed that serum ferritin concentration reflects the iron stores of the body, a rate³³⁾ and a formula⁶⁾ were proposed for converting serum ferritin into iron stores. However, such conversion methods do not always reflect the amount of iron stores because serum ferritin cannot reflect hemosiderin iron. Furthermore, serum ferritin and the amount of storage iron are not correlated linearly as shown in Fig. 1a and Fig. 1b. The ratio of serum ferritin to iron stores was lower in the level below 1 g than that above 1 g. This may indicate the iron insufficiency for ferritin synthesis in patients with decreased but within the normal level of storage iron (Fig. 1a) (Table 1).

Determination of ferritin iron and hemosiderin iron by serum ferritin kinetics

Saito *et al.*⁴⁾ developed a clinical method for the simultaneous determination of ferritin and hemosiderin iron by using a serum ferritin decrease curve measured in the course of iron removal therapy.⁴⁾ The method is based on the fact that the serum ferritin decrease curve is composed of the sum of two components, one decreasing and the other recovering. The decreasing component reflects the decrease of pre-existing tissue ferritin iron, and the recovering component reflects the increase of tissue ferritin iron by removing iron from hemosiderin.⁴⁾ The best fit decrease curve of serial serum ferritin assay dots was selected by computer simulation with a spreadsheet program. By the above-described processing, the amount of ferritin iron and hemosiderin iron

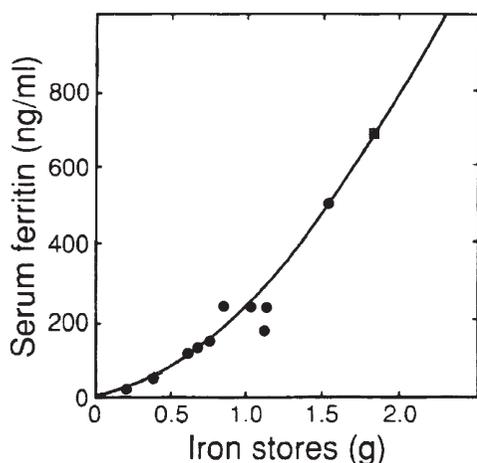


Fig. 1a

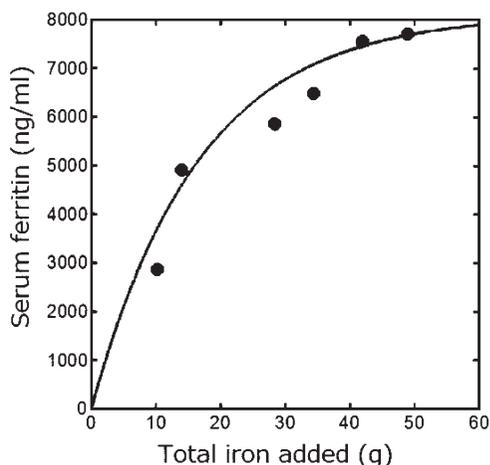


Fig. 1b

Fig. 1 Correlation between serum ferritin and the amount of storage iron.

Fig. 1a: The ratio of serum ferritin to iron stores was lower below 1 g than above 1 g as observed in 9 cases with chronic hepatitis (round dot) and 1 case with treated iron deficiency anemia (square dot).⁴⁾
 Fig. 1b: The serum ferritin increase rate declined gradually along with the progress of iron addition by transfusion in a case with transfusion-dependent anemia before the treatment by deferasirox. The combination of Fig. 1a with Fig. 1b will be a top-heavy sigmoid correlation curve covering storage iron levels from low normal to iron overload.

among total iron stores was determined.⁴⁾ In addition, a method of proportional allotment was applied²⁾ for displaying the increasing and decreasing phases of ferritin iron and hemosiderin iron, and also for determining the change in proportion between pre-existing old ferritin and new ferritin synthesized by removing iron from hemosiderin in the course of iron removal. The proportion of old and new ferritin iron in the mixture of both were calculated by the formula: percent new ferritin iron = $100 \times \text{new ferritin iron} / \text{total (new + old) ferritin iron}$.

Iron balance was determined by the serum ferritin value returned to the previous level.⁴⁾

INCREASE OF IRON STORES

Iron overload

Long-term positive iron-balance (absorption > loss) leads to iron overload (Table 1).

Iron contained in decreased hemoglobin in anemia, except for iron deficiency anemia, is added to storage. However, if storage iron is less than 1 g before the development of anemia, the total amount of storage iron after its development will stay within the normal range.

The incidence of iron overload is higher in males than in females with menstrual blood loss. Generally, iron overload symptoms appear in patients with serum ferritin levels above 1000 ng/ml.

The main causes of iron overload are blood transfusion, alcohol consumption, mistreatment and uncontrolled increase of iron absorption in patients with hereditary hemochromatosis.³⁵⁻³⁷⁾ However, iron absorption is not always increased in iron overloaded hereditary hemochromatosis. Sargent *et al.*³⁷⁾ demonstrated an increase of iron absorption in patients with hereditary hemochromatosis whose storage iron level was kept within the normal range after phlebotomy therapy.

Uncontrolled increase of iron absorption due to gene mutation results in iron overload in

patients with the C282Y mutated hemochromatosis (*HFE1*)^{35, 36}, hemojuvelin (*HJV*) mutated hemochromatosis (*HFE2*)³⁸, hepcidin antimicrobial peptide (*HAMP*) mutated juvenile hemochromatosis³⁹, transferrin receptor 2 (*TfR2*) mutated hemochromatosis (*HFE3*)⁴⁰⁻⁴², solute carrier family 40 member 1 (*SLC40A1*) mutated ferroportin disease (*HFE4*)⁴³⁻⁴⁵, thalassemia major⁴⁶, aceruloplasminemia¹⁶, congenital atransferrinemia^{47, 48} and solute carrier family 11 member 2 (*SLC11A2*) mutated divalent metal transporter 1 (*DMT1*)-associated hypochromic anemia.^{49, 50} *DMT1* is a duodenal apical iron transporter regulated by body iron stores.⁵¹ It is known as natural resistance-associated macrophage protein 2 (*NRAMP 2*).

Iron removal therapy is effective not only for hereditary hemochromatosis²²⁻²⁵, transfusional iron overload and chronic hepatitis C¹⁵, but also for nonalcoholic steatohepatitis and type 2 diabetes.⁵²

Iron stores in erythrocyte precursors

Transferrin-bound iron (TfBI) binds transferrin receptor 1 (TfR1) on the surface of erythrocyte precursors. Then, the TfBI-TfR1 complex is internalized, iron is released and utilized for hemoglobin synthesis or stored. TfR1 expression and intracellular iron concentration are inversely correlated.

Bessis *et al.*⁵³ observed erythroblasts surrounded by ferritin by electronmicroscopy. They speculated that such ferritin was supplied from “nursing cells” (marrow macrophages) to erythroblasts for hemoglobin synthesis. However, it seemed difficult to confirm the direction of ferritin movement from marrow macrophages to erythroblasts morphologically. Iron stores in red cell precursors are not an index of the body storage iron level, but are affected by the serum iron level. Bessis *et al.*⁵³ and Zail *et al.*⁵⁴ reported that ferritin had disappeared from the mature erythrocytes. Later, Cazzola *et al.*⁵⁵ detected ferritin in erythrocytes by radioimmunoassay. However, erythrocyte ferritin is not worth assaying clinically.

Sideroblasts appear in iron overload or in anemia along with disorders of hemoglobin synthesis, with excessive accumulation of iron in mitochondria. Refractory anemia with ring sideroblasts is characteristic of bone marrow erythrodysplasia and ring sideroblasts.

Iron stores and iron absorption

Food iron is absorbed through the intestine mostly via duodenum mediated by *DMT1*^{49, 50}. Iron absorption is 1 mg/day in normal males and 1.2 mg/day in normal females.^{56, 57} Iron overload is mostly caused by the increase of iron absorption.

In 1948, Granick⁵⁸ proposed the mucosal block theory, which implied an automatic control of iron absorption by the saturation of ferritin formation in the mucosal epithelial cells following oral iron administration. Saito⁵⁹ revealed that hemosiderin formation was not saturated, although ferritin formation was saturated in rats after oral iron administration. He then thought that incomplete blockage of iron absorption could occur by way of hemosiderin formation in the enterocytes.

Iron can enter the duodenal epithelial cells from both blood circulation and intestine. The total body storage iron level is reflected in the storage iron level in enterocytes.^{56, 57, 60} Iron entering the enterocytes via intestine becomes intracellular labile iron⁶¹ temporarily and is synthesized into ferritin and hemosiderin in the enterocytes. Thus stored iron in the enterocytes becomes intracellular labile iron temporarily, and enters blood circulation (absorbed) sooner or later in negative iron balance, or it is lost by exfoliation.^{56, 57, 62}

Iron absorption is inversely correlated to the amount of iron stores. However, iron absorption normalizes before the storage iron level normalizes, along with the recovery of hemoglobin in the course of treatment of iron deficiency anemia.⁶³

Shiono *et al.*⁶⁴⁾ revealed that the larger the iron stores before iron removal, the faster the rebound by acceleration of iron absorption after phlebotomy therapy for patients with chronic hepatitis C.¹⁵⁾ Such a trend of rebound^{36, 37)} is also observed in hereditary hemochromatosis after phlebotomy therapy.

The increase of hemosiderin per ferritin ratio in hereditary hemochromatosis is not as marked as that of chronic hepatitis C.²¹⁾ The relative increase of hemosiderin iron in hepatocytes of chronic hepatitis C with a normal level of iron stores^{4, 21)} suggests the difficulty of storing iron in the form of ferritin in the hepatocytes suffering from chronic hepatitis C.

A mild anemia observed in iron-overloaded patients with hereditary hemochromatosis disappears after phlebotomy therapy. Similarly, anemia in transfusion-dependent patients is improved after iron removal by deferasirox.

Nicolas *et al.*²⁰⁾ revealed the relationship between hepcidin deficiency and iron overload. Nemeth *et al.*⁶⁵⁾ and Ganz *et al.*⁶⁶⁾ clarified that iron release from enterocytes and macrophages was regulated by hepcidin-ferroportin axis. Although Hadley *et al.*⁶⁷⁾ reported that they observed no correlation between serum prohepcidin concentration and iron absorption in normal female subjects, Fraser *et al.*⁶⁸⁾ doubted the reliability of the prohepcidin assay kit they used. Trombini *et al.*⁶⁹⁾ suggested that hepcidin production was regulated by the combination of the marrow's iron requirements and iron supply from transferrin. Hepcidin production is also regulated by the storage iron level⁷⁰⁾ that is controlled by erythropoietic activity. Increase of erythropoietic activity suppresses hepcidin production⁷⁰⁾ and the decrease of hepcidin and storage iron promotes iron absorption.

Thus, iron absorption, erythropoietic activity, hepcidin and storage iron interact as the main regulators of iron homeostasis.

On the other hand, the mechanism of uncontrolled increase of iron absorption in patients with gene mutations⁷¹⁾ cannot be explained simply by the action of hepcidin. Low hepcidin in hereditary hemochromatosis indicates the derangement of the sensing system to the storage iron level due to hemochromatosis (*HFE*) gene mutations. The mechanisms of uncontrolled iron absorption in gene mutated patients are investigated extensively in relation with iron sensing proteins of HJV^{38, 39, 72, 73)}, transferrin receptor 2⁴⁰⁻⁴²⁾, HFE⁷⁴⁾, iron regulatory protein (IRP)⁷⁵⁾ and others, but much remains to be elucidated. Beutler⁷⁶⁾ pointed out an important issue regarding factors those causing the dissociation between clinical manifestations and gene mutations.

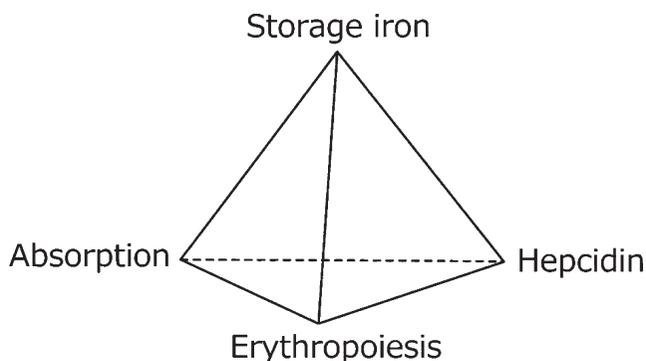


Fig. 2 Interactions among the major factors regulating iron homeostasis. These factors; iron absorption, erythropoiesis, storage iron and hepcidin, are regulated by erythropoietin, transferrin saturation, interleukins, divalent metal transporter 1, iron regulatory proteins, iron responsive element, hemojuvelin, hypoxia-inducible factor¹¹⁰⁾, growth differentiation factor 15⁹¹⁾ and others.

Iron entering the body via parenteral routes

Iron entering the body through parenteral routes follows different courses from the iron absorbed via the enteric route.

The transfused senescent red cell or intravenously injected colloidal iron is captured by the reticuloendothelial (RE) phagocytes. There, red cell or colloidal iron is decomposed and stored temporarily. Then, the stored iron in RE is released and redistributed by transferrin to whole body tissues in proportion to the ratio of distribution of pre-existing iron.¹⁾

Most of the iron enters the body via respiratory tract deposits in pulmonary tissue.^{17, 77)}

Generally, hemosiderin iron per ferritin ratio increases along with the increase of iron deposition.^{1, 4)} However, after the restoration of the normal level of hemoglobin and iron stores by intravenous colloidal iron therapy to iron deficiency anemia, an increase of hemosiderin iron was higher than ferritin iron^{4, 21)} due to the increased iron concentration in RE phagocytes.

Brain iron deposition

Brain iron deposition contributes to the tissue damage of neurodegenerative diseases such as aceruloplasminaemia¹⁶⁾ with iron overload due to ceruloplasmin gene mutation, Parkinsonian syndromes⁷⁸⁾, Hallervorden-Spatz syndrome {Pantotenate kinase-associated neurodegeneration (PKAN) due to pantothenate kinase 2 (*PANK2*) gene mutation⁷⁹⁾}, neuroferritinopathy⁸⁰⁾ due to ferritin light polypeptide gene mutation, Alzheimer's disease and amyotrophic lateral sclerosis with gene mutations.

DECREASE OF IRON STORES

Iron deficiency

Long-term negative iron-balance (absorption<loss) results in iron deficiency. Iron deficiency implies the state of storage iron exhaustion (Table 1). It can be classified into 3 stages: iron deficiency without anemia, iron deficiency anemia without tissue damage, and iron deficiency anemia with tissue damage.^{1, 63)} Iron deficiency tissue damage, so-called Paterson-Kelly or Plummer-Vinson syndrome, occurs mostly in middle-aged female patients with chronic iron deficiency anemia. Iron deficiency in the developing brain in the early stage of life seems to be a risk factor.⁸¹⁾

Iron deficiency limits hemoglobin synthesis⁸²⁾ and this knowledge is applied for the treatment of polycythemia vera. Hayashi¹⁵⁾ revealed that iron deficiency sedated chronic hepatitis C. Furthermore, Kato *et al.*⁸³⁾ observed that iron deficiency inhibited the incidence of hepatoma in chronic hepatitis C, and Saito⁸⁴⁾ found the inhibition of hyperthyroidism by iron deficiency.

Finberg *et al.*⁸⁵⁾ discovered iron refractory iron deficiency anemia with high hepcidin levels and suppressed iron absorption resulted from a defect in the *TMPRSS6* gene. Moreover, the utilization of intravenously infused iron in iron refractory iron deficiency anemia is inferior to that of ordinary iron deficiency anemia.⁸⁵⁾

Helicobacter pylori can survive in strong acidic circumstance by producing alkaline substance and receiving nutrient iron from lactoferrin. Hershko *et al.*⁸⁶⁾ suggested that *Helicobacter pylori* infection triggered autoimmune gastritis resulting in iron deficiency. *Helicobacter pylori* infection seems to be related to iron deficiency by decreasing iron absorption and by increasing iron loss.

Storage iron and hypoferrinemia

Hypoferrinemia is seen not only in iron deficiency anemia, but also in non-iron deficiency states with the normal level of iron stores and iron overload.

The human body reserves iron probably because the supply of a sufficient amount of iron is difficult by iron absorption alone when there is an urgent need for erythropoiesis as in the case of a large amount of blood loss. It is also difficult to mobilize storage iron fast enough to meet the acute need for iron, as in the case with erythropoiesis elevated up to 6 to 8 times the normal⁸⁷⁾ even when having a sufficient amount of iron reserve. The shortage of iron supply from storage at the time of an acute increase of erythropoiesis results in hypoferrremia, by which the erythropoiesis is suppressed. Suppressed erythropoiesis by hypoferrremia was called "iron-restricted erythropoiesis" by Hillman.⁸⁸⁾

Hypoferrremia occurs immediately after the supplementation of an essential element, such as vitamin B12, folate, erythropoietin, etc. in patients with element-deficiency anemia, even if patients have a sufficient amount of storage iron for recovering their previous normal hemoglobin level.

Heilmeyer *et al.*⁴⁷⁾ first reported a 7 year-old-girl with congenital atransferrinemia with hypoferrremia, microcytic hypochromic anemia and iron overload. Later, this condition was recognized to be associated with transferrin (*TF*) gene mutations.⁴⁸⁾

Hypoferrremia occurs in inflammation anemia, a representative of anemia of chronic disease with iron-restricted erythropoiesis.⁸⁸⁾ Chronic kidney disease is also regarded as an anemia of chronic disease affected by interleukin-6. Hypoferrremia in inflammation anemia is caused by the suppression of iron efflux from iron storing cells into plasma due to the down regulation of ferroportin by hepcidin inducing internalization and degradation of hepcidin-ferroportin complex.⁸⁹⁾ Dallalio *et al.*⁹⁰⁾ suggested the existence of erythropoiesis suppressing factors other than hepcidin in anemia of chronic disease. Theurl *et al.*⁹¹⁾ reported that the hepcidin expression in anemia of chronic disease was regulated by growth differentiation factor 15. These studies disclosed the multilayered regulation of iron metabolism in anemia of chronic disease.

Hypoferrremia due to inflammation can be differentiated from iron deficiency anemia by assaying serum ferritin, total iron-binding capacity, C-reactive protein and other indices, as well as by clinical manifestations. Detection of iron deficiency anemia co-existing with inflammation anemia is possible if serum ferritin is decreased below 12 ng/ml.⁶³⁾ Iron administration to inflammation anemia is a contraindication; even if iron supply elevates serum iron and reticulocyte counts only temporarily, it is fraught with danger and may result in iatrogenic iron overload.

Iron loss

The amount of iron loss is around 1 mg/day, around 3% of the total plasma iron turnover rate in a normal male, and 1.2 mg/day in a normal female^{56, 57)} It is balanced with the amount of iron absorption in normal subjects. Most iron deficiencies are caused by increased blood loss. Iron is lost daily by intestinal hemorrhage and exfoliation of enterocytes in normal subjects.^{56, 57, 60)} Iron loss in sweat is not a significant amount, but it may be overestimated by external iron contamination. Storage iron level in the enterocytes is proportional to the total storage iron level in iron equilibrium. Therefore, the amount of iron loss by the exfoliation of enterocytes is increased in iron overload, and that is decreased negligibly in iron deficiency state. Iron is also lost by menstruation, ulcer, trauma, hemodialysis, operation and intravascular hemolysis. In intravascular hemolysis, haptoglobin-bound hemoglobin and hemopexin-bound heme are rapidly cleared from circulation, stored and reutilized, or lost by hemoglobinuria and hemosiderinuria.

TURNOVER OF IRON STORES

Intracellular iron turnover

Iron is not only needed for ferritin synthesis, but also iron accelerates its synthesis⁹²⁾, which

results from selective translational activation by iron responsive element⁹³⁾ (IRE), and iron removal from ferritin protein shell results in the degradation of ferritin.^{94, 95)} Iolascon *et al.*⁴⁹⁾ and Beaumont *et al.*⁵⁰⁾ state that DMT1 is a key mediator of iron absorption and iron transfer from endosomes into the cytosol following the uptake of the transferrin receptor complex. Above-mentioned intracellular iron turnover is under the coordinate regulation of IRPs⁷⁵⁾ and IREs.⁹⁶⁻⁹⁸⁾

IREs are thought to be associated with iron regulated amyloid precursor protein transcript in Alzheimer's disease.⁹⁹⁾

High serum ferritin without the increase of body storage iron level and early onset of bilateral cataract in patients with hereditary hyperferritinemia-cataract syndrome^{34, 95, 100, 101)} are caused by mutations in the IRE segment of the ferritin light chain (*FTL*) gene. The slower degradation of light (L)-chain ferritin than heavy (H)-chain ferritin^{95, 100, 101)} accelerates the accumulation of ferritin in lens.

Storage iron turnover at red cell sequestration

Storage iron turnover in RE cells was not elucidated until the time Fillet *et al.*¹⁰²⁾ determined radioiron release from RE cells after the injection of radioiron labeled nonviable red cells to normal dogs. Radioiron released from RE cells in the early phase with a 34 minute half time originated from labile iron in RE cells, but that in the late phase with 7 day half life originated from iron stores in RE cells. In this study, it took 3 to 4 weeks for virtually all radioiron to be released from RE cells and utilized, while it took 2 weeks for the red cell utilization of transferrin-bound radioiron. The utilization speed of radioiron was delayed by the digestion process of non-viable red cells in RE cells. Iron stores and hemoglobin iron in the erythroblasts destroyed in ineffective erythropoiesis seem to follow the same process as red cells. Total red cell iron divided by mean red cell life span gives red cell iron renewal rate, which indicates net amount of red cell iron turnover rate.

Body iron turnover

Body iron turns over from plasma to plasma via bone marrow and spleen mostly in accordance with the erythron iron recycling. On the other hand, a small circuit exists between plasma and iron compounds in parenchymal tissues.

Finch¹⁰³⁾ determined total body iron loss by following up red cell radioactivity for 4.5 years. He revealed that it took 3 red cell life spans to mix radioiron with pre-existing body iron completely and that the body's iron turnover rate was 0.61 mg/day in a normal male. Saito *et al.*⁵⁶⁾ determined body iron loss rate average 0.89 mg/day of 2 normal subjects by whole body counting for 300 days, and revealed that iron was lost mostly by bleeding and epithelial exfoliation into the intestinal tract. Storage iron loss by the exfoliation of enterocytes was around 0.1 mg/day, and that loss from the urinal tract was around 1/10 of the loss in feces in an average two normal subjects.⁵⁶⁾

Saito⁶⁰⁾ observed that radioiron incorporated into ferritin and hemosiderin in liver and spleen with a peak uptake at 24 hours and then majority of it was released to plasma by 48 hours after intraperitoneal radioiron injection to normal rats. Such an iron turnover seemed to take place in humans. However, the uptake and release of radioiron by liver and spleen could not be displayed by ferrokinetic body surface monitoring^{56, 105-107)}, being veiled by a high background of radioactivity in plasma and bone marrow within 2 days after radioiron injection.

After the pioneering study by Shoden *et al.*^{2, 3)}, it took 60 years until the clarification of ferritin and hemosiderin iron turnover^{4, 21)} as follows.

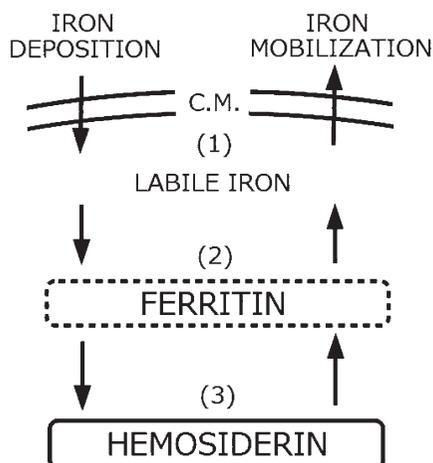


Fig. 3 Iron pathways of ferritin and hemosiderin in iron deposition and mobilization.⁴⁾
 The numbers in quotation indicate the order of tracing in the same lap route.
 Abbreviation: C.M., cell membrane.

Iron pathways of ferritin and hemosiderin

Iron deposition: Shoden *et al.*²⁾ fractionated ferritin and hemosiderin iron in the liver and spleen biochemically and proposed 5 iron pathways in iron deposition: an iron pathway #1 from plasma to ferritin, a direct iron pathway #2 from plasma to hemosiderin bypassing ferritin synthesis, an iron pathway #3 from plasma to hemosiderin glancing off ferritin, #4 from ferritin to hemosiderin, and #5 from hemosiderin to ferritin.²⁾ However, the only one pathway in combination with #1 and 4 was confirmed by Saito.⁴⁾ The other 3 pathways (#2, 3 and 5) are inconceivable and contradictory considering the direction of iron flow and saturation of ferritin formation in iron deposition.⁴⁾

Thus, one intracellular iron pathway exists in iron deposition where iron flows follow the numbered order \rightarrow (1) labile pool \rightarrow (2) ferritin \rightarrow (3) hemosiderin⁴⁾

Iron mobilization: Shoden *et al.*²⁾ advanced an uncertain proposition regarding iron pathways: an as yet unproven iron pathway from hemosiderin to ferritin or ferritin to hemosiderin, and a direct iron pathway from plasma to ferritin or hemosiderin to plasma. The only pathway as a set of pathways from ferritin to plasma and from hemosiderin to ferritin was confirmed by Saito *et al.*⁴⁾ Two pathways from ferritin to hemosiderin and a direct pathway from hemosiderin to plasma are inconceivable and contradictory considering the direction of iron flow and active ferritin synthesis in iron mobilization.⁴⁾

Thus, one intracellular iron pathway exists in iron mobilization where iron flows follow the numbered order \leftarrow (1) labile pool \leftarrow (2) ferritin \leftarrow (3) hemosiderin⁴⁾ in iron mobilization (Fig. 3).

The above-mentioned 2 pathways in iron deposition and mobilization indicate the iron flow through the same simple route according to the iron density gradient.

Serum ferritin kinetics revealed that the iron turnover of ferritin is active, but that of hemosiderin is passive mediated by ferritin.^{4, 21)}

Phases of ferritin and hemosiderin iron turnover

Iron deposition: The increase of ferritin iron is larger than that of hemosiderin iron initially, but it is smaller later in the course of the linear increase of total iron stores in iron addition.

In contrast, the increase of hemosiderin iron is smaller than that of ferritin iron initially, but it is larger later.²¹⁾

The reduction of the increasing rate of ferritin iron and the linear increase of hemosiderin iron along with the increase of storage iron²⁻⁴⁾ is caused by the transformation of ferritin iron into hemosiderin iron in iron deposition.^{10, 11)} This implies the limitation of iron storing capacity in the form of ferritin.⁴⁾ The linear increase of hemosiderin^{4, 21)} in higher storage iron level implies the limitlessness of iron storing capacity in the form of hemosiderin.^{4, 21)} The limitless iron storing capacity of hemosiderin is caused not only by the transformation of ferritin into hemosiderin, but also by the expansion of tissue cell space as seen by hepatomegaly in patients with hereditary hemochromatosis along with the increase of iron deposition.

Iron mobilization: The decrease of ferritin iron is larger than the decrease of hemosiderin iron initially, but it is smaller later in the course of the linear decrease of total iron stores in iron removal.²¹⁾ In contrast, the decrease of hemosiderin iron is smaller than that of ferritin iron initially, but it becomes larger later in the course of the linear decrease of total iron stores in iron removal.²¹⁾

The increasing (Fig. 4b) and decreasing (Fig. 5a and 5b) curves of ferritin and hemosiderin iron do not show a mirror image, but rather an image reversed upside down in the opposite side; convex ferritin and concave hemosiderin iron increasing curves versus concave ferritin and convex hemosiderin iron decreasing curves.

The percentage of ferritin iron decreased and that of hemosiderin iron increased in the course of iron removal is shown in Fig. 6a. The percentage of old ferritin decreased and new ferritin iron increased in the course of iron removal is shown in Fig. 6b. The proportion of old and new ferritin iron in the mixture of both was reflected to the proportion of old and new ferritin iron remaining in iron removal as shown in Fig. 6c.

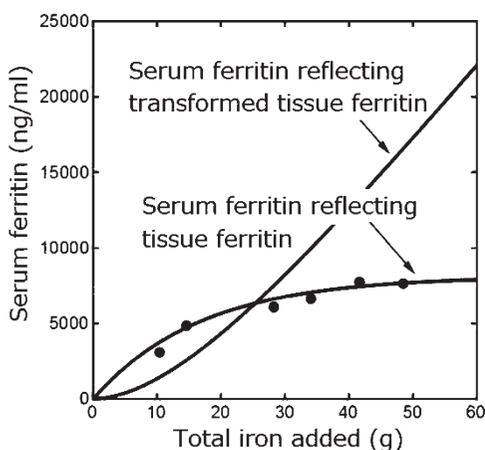


Fig. 4a

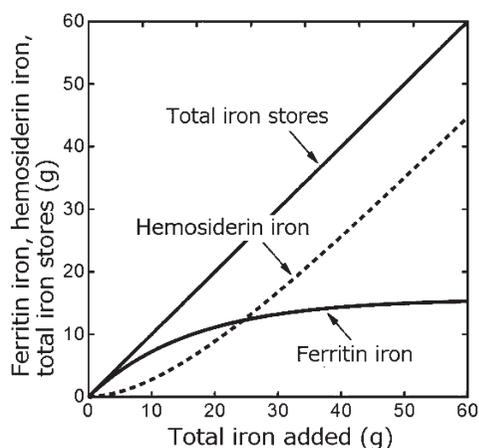


Fig. 4b

Fig. 4 Fig. 4a: Increasing phases of serum ferritin in patient with transfusion-dependent anemia.²¹⁾ Declination of serum ferritin increase in iron addition reflects the transformation of ferritin into hemosiderin.

Fig. 4b: Increasing phases of ferritin iron (solid curve) and hemosiderin iron (dotted curve) along with the linear increase of total iron stores in transfusion-dependent anemia.²¹⁾

The crossing point of increasing curves of ferritin iron and hemosiderin iron appears at a certain storage iron level in iron addition, when the amount of initial hemosiderin iron exceeds that of initial ferritin iron. The shifting of the crossing point toward a higher storage iron level in iron addition implies the increase of iron storing capacity of ferritin. Fig. 4b was made from the data of Fig. 4a.

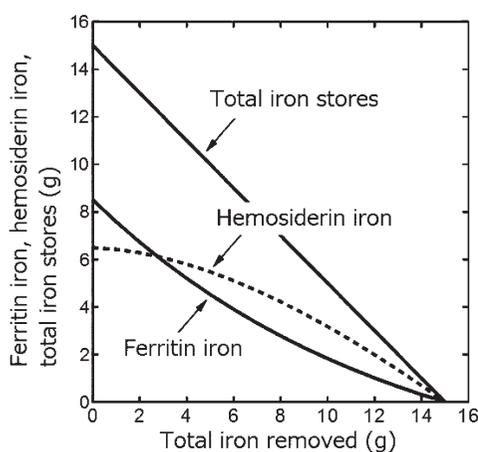


Fig. 5a

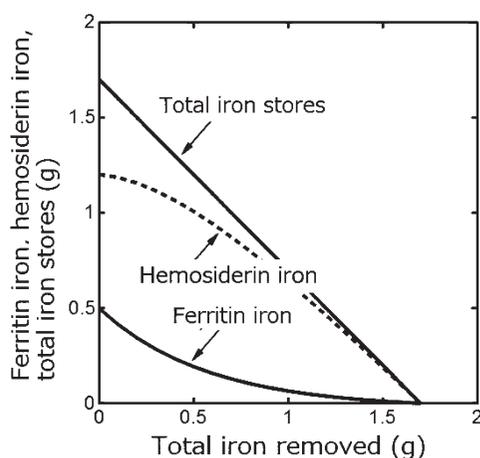


Fig. 5b

Fig. 5 Decreasing phases of ferritin iron (solid curve) and hemosiderin iron (dotted curve) along with the linear decrease of total iron stores.

Fig. 5a shows a case of hereditary hemochromatosis.

Fig. 5b shows a case of treated iron deficiency anemia with constant blood loss.²¹⁾

The crossing point of decreasing curves of ferritin iron and hemosiderin iron does not appear in cases with the initial hemosiderin iron larger than the initial ferritin iron.

Storage iron turnover rate

The plasma iron turnover rate (PIT) multiplied by the iron distribution ratio of an iron component gives the iron turnover rate. Intravenously injected radioiron is initially distributed in proportion to pre-existing body iron.¹⁾ Therefore, the initial iron turnover rate of an iron component is proportional to the ratio of the pre-existing body iron distribution.

Two weeks after the radioiron injection in a normal subject, red cell radioiron utilization (RCU) reaches the levels from 80% to 100%.^{1, 56, 106-109)} When RCU was determined elaborately by Hosain *et al.*¹⁰⁸⁾, it ranged from 78% to 84% in 6 normal men. Consequently, the same authors thought that higher RCU determined by previous authors might have been overestimated. Any of these results, RCU exceeds 2/3 ratio^{1, 109)} of net red cell non-radioactive iron utilization due to the cumulative one-way red cell fixation of plasma radioiron reflux from parenchymal tissue iron components for two weeks. So, the red cell iron turnover rate¹⁰⁴⁾ (RCIT) obtained from $(PIT \times RCU/100)^{104)}$ becomes larger than the net red cell iron turnover rate. Thus, it does not seem appropriate to call the value $(PIT \times RCU/100)$ "red cell iron turnover rate (RCIT)."¹⁰⁴⁾ However, the name "red cell iron turnover rate (RCIT)" is already used generally. Accordingly, the name RCIT is used here as it is. For this reason, the red cell iron renewal rate (RCIR), obtained from red cell iron divided by mean red cell life span, was adopted as the index of the net red cell iron turnover rate. Then, the ratio (RCIR/PIT) indicates net red cell non-radioactive iron utilization in contrast to RCU with excess. RCIR corresponds to around 2/3 of PIT, and the rest of RCIR in PIT, $(PIT - RCIR)$ indicates the parenchymal tissue iron turnover rate, which corresponds to around 1/3 of PIT in normal male (Fig. 7). The same ratio of the total radioiron reflux was determined by Cook *et al.*¹⁰⁹⁾ in normal subjects.

The ratio of storage iron to total parenchymal tissue iron in normal male seems to be reflected in the iron turnover rate. Although the ratio may vary according to the levels of iron stores, even within the normal range, a common ratio 0.7 is used for approximation in estimating the storage iron turnover rate from the parenchymal tissue iron turnover rate in normal male due to

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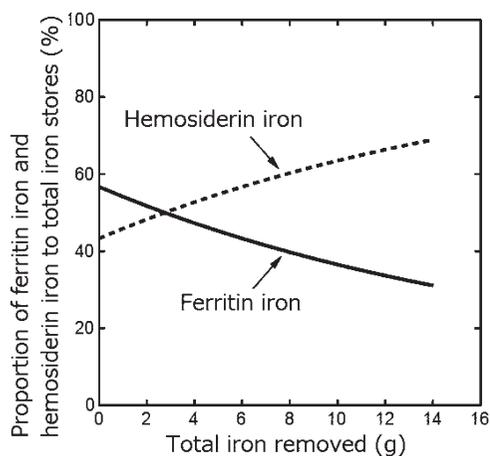


Fig. 6a

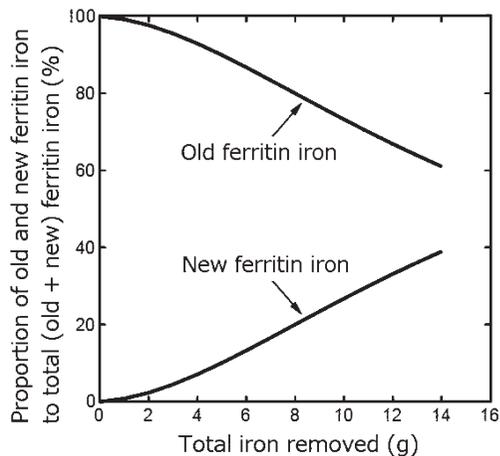


Fig. 6b

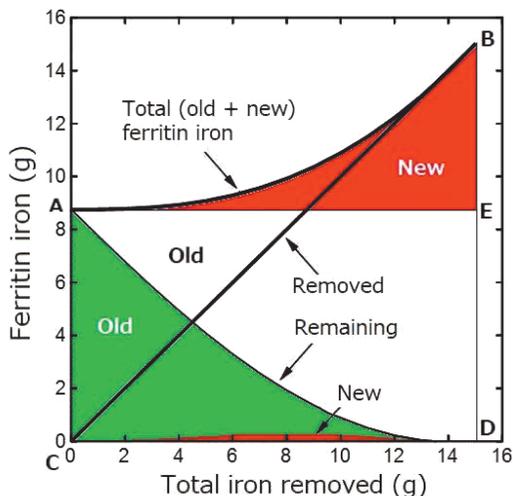


Fig. 6c

- Fig. 6** Fig. 6a, 6b and 6c are made from the data of the same patient with hereditary hemochromatosis.
- Fig. 6a: Proportion of pre-existing ferritin iron and hemosiderin iron to total iron stores in the course of iron removal.
- Fig. 6b: Proportion of pre-existing (old) ferritin iron and new ferritin iron synthesized by taking iron from hemosiderin in the course of iron removal. Since, iron stores are composed of the sum of ferritin iron and hemosiderin iron, the only iron source available for the ferritin synthesis (recovery) is hemosiderin iron in iron removal under dietary iron-restriction.
- Fig. 6c: Proportion of old and new ferritin iron remaining in the course of iron removal.
- The area enclosed by points A, B, D and C indicates the total amount of ferritin iron, which is composed of old ferritin iron (square area ACDE) and new ferritin iron (quasi-triangular red area ABE). The area ABC is shifted to the equivalent area ACD to show the decreasing phase of total ferritin iron. Diagonal line CD indicates the amount of ferritin iron removed. Proportions of old and new ferritin iron in the area ACD are zoned green and red, respectively. The proportions of new ferritin iron (areas ABE) and old ferritin iron (area ACDE) are reflected by those in color zones, although old and new ferritin iron cannot be separated due to mixing.

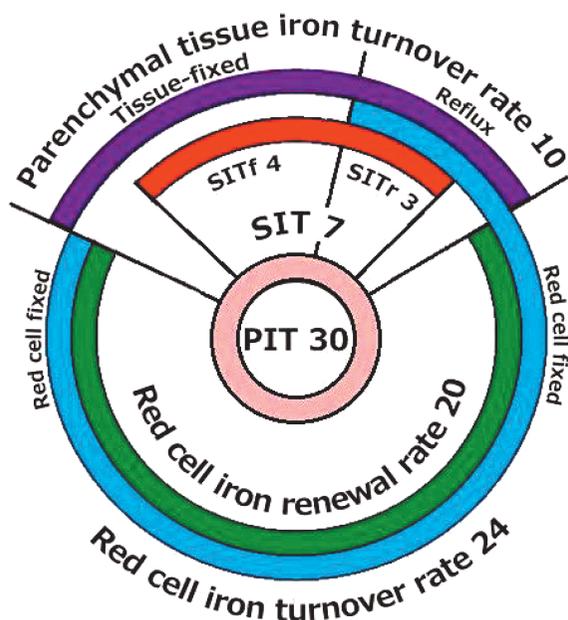


Fig. 7 Iron turnover rates in the model of a typical normal male.

The subject is under the conditions; red cell iron: 2400 mg (15 g/dl hemoglobin, and 4800 ml blood volume), total body iron: 3600 mg, red cell radioiron utilization (RCU): 80% and PIT: 30 mg/day (cf. Table 2 and Table 3).

Abbreviations / Color / Arc angles ($^{\circ}$ = degree): PIT, plasma iron turnover rate / pink / 360 $^{\circ}$; Red cell iron turnover rate (not abbreviated) / blue / 288 $^{\circ}$; Red cell iron renewal rate (not abbreviated) / green / 240 $^{\circ}$; Parenchymal tissue iron turnover rate (not abbreviated) / purple / 120 $^{\circ}$; Tissue-fixed, parenchymal tissue iron turnover rate due to tissue-fixed radioiron / purple / 72 $^{\circ}$; Reflux, parenchymal tissue iron turnover rate due to refluxed and red cell-fixed radioiron / purple / 48 $^{\circ}$; SIT, storage iron turnover rate / red / 84 $^{\circ}$; SITf, storage iron turnover rate due to storage-fixed radioiron / red / 50.4 $^{\circ}$; SITr, storage iron turnover rate due to refluxed and red cell-fixed radioiron / red / 33.6 $^{\circ}$.

the difficulty of measuring an individual ratio.

Parenchymal tissue iron turnover rate is composed of two components; one is the tissue-fixed radioiron and the other is the refluxed and red cell-fixed radioiron beyond the net red cell iron turnover rate. The term “tissue-fixed radioiron” means the radioiron remaining in tissue after releasing a labile fraction of tissue radioiron once taken up. Storage iron turnover rate (SIT) is also composed of two components; the storage-fixed radioiron (SITf) and the refluxed and red cell-fixed radioiron (SITr).

To explain the above-described contents, an example of storage iron turnover rate estimated in the model of a typical normal male is shown in Table 2 and Fig. 7 (Table 3 in part).

Bothwell *et al.*¹⁾ proposed about a 9 mg/day parenchymal tissue iron exchange in normal male. Although they did not refer to the method, they proposed 5 to 7 mg/day storage iron exchange (i. e. turnover). The storage iron turnover rate estimated from their parenchymal tissue iron exchange by the present method was 6.3 mg/day (23% of PIT). This datum was similar to the value estimated in the model of a typical normal male as shown in Table 2 and Table 3.

On the other hand, Huff *et al.*¹⁰⁴⁾ supposed a 2.7 mg/day (10% of PIT) storage iron turnover rate in normal male. However, the storage iron turnover rate estimated here from the parenchymal tissue iron turnover rate was 4.9 mg/day (18% of PIT). Pollycove *et al.*¹⁰⁷⁾ reported that storage

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Table 2 Storage iron turnover rate (SIT) and SIT-related indices estimated in the model of a typical normal male with 70 kg body weight, 2400 mg red cell iron at 15 g/dl hemoglobin and 4800 ml blood volume, 120 day mean red cell life span, 80% red cell radioiron utilization (RCU), and 30 mg/day plasma iron turnover rate (PIT)
(cf. Table 3 and Fig. 7)

| | |
|--|-----------------------------|
| RCIT; red cell iron turnover rate: $PIT \times RCU/100$ | $30 \times 0.8 = 24$ mg/day |
| RCIR; red cell iron renewal rate: (red cell iron/mean red cell life span) | $2400/120 = 20$ mg/day |
| PTIT; parenchymal tissue iron turnover rate: $(PIT - RCIR)$ | $30 - 20 = 10$ mg/day |
| SIT; storage iron turnover rate: $(PIT - RCIR) \times 0.7^*$ | $10 \times 0.7 = 7$ mg/day |
| PTIT due to tissue-fixed radioiron: $PIT \times (1 - RCU/100) = PIT \times 0.2$ | $30 \times 0.2 = 6$ mg/day |
| SIT due to storage-fixed radioiron (SITf): $(PIT - RCIT) \times 0.7 = PIT \times 0.2 \times 0.7$ | $6 \times 0.7 = 4$ mg/day |
| PTIT due to refluxed and red cell-fixed radioiron: $(RCIT - RCIR)$ | $24 - 20 = 4$ mg/day |
| SIT due to refluxed and red cell-fixed radioiron (SITr): $(RCIT - RCIR) \times 0.7$ | $4 \times 0.7 = 3$ mg/day |

0.7* is the approximation ratio of storage iron to total parenchymal tissue iron in the normal male.¹⁾

SIT/PIT/100 = $7/30/100 = 23\%$, RCIR/PIT = $2/3$, and PTIT/PIT = $1/3$.

SIT = SITf + SITr. PIT = RCIR + PTIT.

Table 3 Parenchymal tissue iron turnover rate (PTIT) estimated from $(PIT - RCIR)$ and storage iron turnover rate (SIT) estimated from $\{(PIT - RCIR) \times 0.7\}$ in normal male
RCU given by Huff R *et al.* was too low as a normal value. Moreover, red cell iron turnover rate (RCIT) was lower than red cell iron renewal rate (RCIR) contradictorily.
(cf. Table 2 and Fig. 7 about a model case)

| | BW | PTIT | SIT | SIT* | RCU | RCIT | RCIR | PIT | RCIR/PIT | SITf | SITr |
|-----------------------------|------|--------|--------|--------|-----|--------|--------|--------|----------|--------|--------|
| | kg | mg/day | mg/day | mg/day | % | mg/day | mg/day | mg/day | % | mg/day | mg/day |
| Bothwell TH ¹⁾ | 70 | 9 | 6.3 | 5~7 | 80 | 19 | 15 | 24 | 63 | 3.4 | 2.9 |
| Huff R ¹⁰⁴⁾ | 77 | 7 | 4.9 | 2.7 | 74 | 20 | 24** | 27 | 89 | 3.9 | 1.0 |
| Pollycove M ¹⁰⁷⁾ | 71.5 | 14 | 9.8 | neg | 91 | 32 | 21 | 35 | 61 | 2.1 | 7.7 |
| A model case | 70 | 10 | 7.0 | - | 80 | 24 | 20 | 30 | 67 | 4.2 | 2.8 |

Abbreviation: BW, body weight; RCIR, red cell iron renewal rate; SITf, storage iron turnover rate due to tissue-fixed radioiron; SITr, storage iron turnover rate due to refluxed and red cell-fixed radioiron; neg, negligible.

Tissue-fixed/Refluxed and red cell-fixed = $(PIT - RCIR)/(RCIT - RCIR) = SITf/SITr$.

$(RCIR/PIT/100)$ indicates net non-radioactive iron utilization (%).

SIT* indicates proposed storage iron turnover rate by authors.

24** = red cell iron (2866 mg) \times fraction of red cell iron renewal/day (0.0085).¹⁰⁴⁾

iron turnover was negligible in normal subjects assuming that the reflux mostly originated from erythroblasts. However, the storage iron turnover rate estimated here from parenchymal tissue iron turnover rate was 9.8 mg/day (28% of PIT) (Table 3).

Thus, storage iron turnover rate can be estimated appropriately by using net red cell non-radioactive iron turnover rate (RCIR). However, it will be underestimated by using red cell iron turnover rate (RCIT) with surplus.

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