REVIEW ARTICLE

Nagoya J. Med. Sci. **86**. 361–369, 2024
doi:10.18999/nagjms.86.3.361

Nature of storage iron turnover

Hiroshi Saito

Formerly of Division of Hematology, First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan

ABSTRACT

Despite recent advance in the study of the nature of storage iron turnover, a comprehensive analysis remains lacking. This study aimed to clarify the nature of storage iron turnover. Ferritin-hemosiderin iron transformation rate and the standard normal storage iron turnover rate were utilized in this study to describe the mechanism of iron absorption in relation to ferritin and hemosiderin iron turnover. The synchronization of radioiron uptake peaks by bone marrow and liver indicates that the distribution of radioiron is proportional to the pre-existing iron levels in organs at 24 h after radioiron injection. Moreover, the synchronization indicates the independence of iron mass from red cell precursors in acquiring plasma iron. Thus, the erythron does not dominate the radioiron uptake process. The inverse correlation between transformation rate and the amount of pre-existing iron storage implies that the intra-storage iron turnover is active in iron deficiency, but inactive in iron overload. The decreased ferritin/hemosiderin iron ratio in chronic hepatitis C (CHC) with normal iron storage suggests a trend of iron transformation from ferritin into hemosiderin. The correlation between the pretreatment iron storage and the speed of rebound in CHC implies that the vacant iron-storing rooms in iron-removed cells have a potential to increase iron absorption. This study presents new insights into the turnover of stored iron to enhance our understanding of iron metabolism in various hematologic disorders.

Keywords: quantitation of ferritin and hemosiderin iron, computer-assisted serum ferritin kinetics, radioiron reflux from storage iron, storage iron turnover rat, iron-attracting potential of iron-storing cells

Abbreviations:
Ft: ferritin
Hs: hemosiderin
SIT: storage iron turnover rate
CHC: chronic hepatitis C
IDA: iron deficiency anemia

INTRODUCTION

In 1946, Granick[1] proposed the theory of automatic iron absorption blockage after iron administration. In 1950, Huff et al[2] developed ferrokinetics and elucidated the dynamic feature

Received: December 4, 2023; accepted: December 27, 2023
Corresponding Author: Hiroshi Saito, MD, PhD
1314 Otokikiyama, Tenpaku-ku, Nagoya 468-0063, Japan
E-mail: eise@beetle.ocn.ne.jp

of erythropoiesis. However, they could not determine storage iron turnover rate (SIT). In 1950, Finch et al[3] revealed in detail the storage iron metabolism in relation to erythropoiesis. In 1952, Haskins et al[4] determined the total amount of storage iron by measuring the total blood removed through phlebotomy to the level of iron deficiency. In 1953, Shoden et al[5] conducted the first animal experiment investigating the ferritin (Ft) and hemosiderin (Hs) iron metabolism. However, the iron exchange between Ft and Hs remained unclear due to the limitations of biochemical method. In 1958, Saito[6] demonstrated the iron turnover of Ft and Hs in the liver and spleen, as well as the blockage of iron absorption through the saturation of Ft formation and the incomplete blockage of iron absorption by the unsaturation of Hs formation by animal experiment. In 1960, Pollycove et al[7] estimated normal SIT of negligible amount (0 to 1 mg/day) by observing the plasma radioiron disappearance curve in normal participants. In 1972, Addison et al[8] developed a ground-breaking immunoradiometric method for determining serum ferritin in humans. Subsequently, clinicians began using serum ferritin as an index of storage iron further. However, the turnover of Hs iron in connection with Ft iron remained unclear since 1958.[6] In 2012, Saito et al[9] developed a computer-assisted method "serum ferritin kinetics", which enabled us the quantitative determination of Ft and Hs iron simultaneously. This article aims to reveal the nature of storage iron metabolism by analyzing previous research data.

METHODS

Two indices were employed in this study: the iron transformation rate (TR),[10] which was derived from serum ferritin kinetics[9]; and SIT with a standard normal value of 5.8 mg/day,[11] which was calculated as a sum of turnover rates in the three major iron-storing cells namely hepatocytes, macrophages, and enterocytes.[12-15]

RESULTS AND DISCUSSION

Storage iron turnover in normal participants

The normal range of whole-body storage iron typically falls between 100 mg and approximately 2500 mg, with most individuals having levels within the range of 600–1000 mg.[12] The upper limit of normal storage iron levels is not precisely defined. The normal range for serum ferritin levels is approximately 12 ng/mL to around 250 ng/mL.

Notably, the changes in the ratio between Ft and Hs in total iron stores alter the shape of the serum ferritin decreasing curve. The ratio of Ft to Hs fluctuates in response to the total iron storage level, reflecting the balance between Ft and Hs in iron homeostasis.

Iron pathways in iron-storing cells in iron deposition and mobilization. In the process of iron deposition, transferrin-bound iron is internalized into iron-storing cells. The absorbed iron is initially converted into Ft, while some is transformed into Hs.

During iron mobilization, a decrease in plasma iron levels results in a reduction of Ft. To replenish the removed Ft during iron removal,[9] new Ft is synthesized by utilizing Hs as the sole available source for Ft synthesis in iron-storing cells. Figure 1 shows the serum ferritin kinetics process.

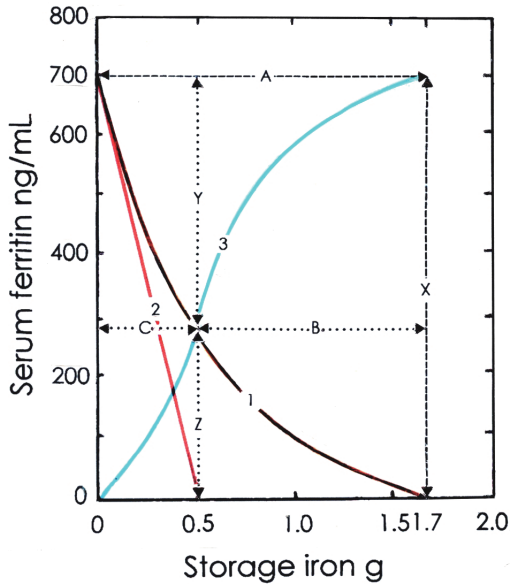

Fig. 1 Decrease of serum ferritin in a treated iron deficiency anemia patient with constant blood loss
Dark-brown curve No. 1 shows the exponential decrease of pre-existing serum ferritin and total iron stores.
Red line No. 2 shows the linear decrease of pre-existing serum ferritin and ferritin iron.
Blue curve No. 3 shows the recovery of serum ferritin and ferritin iron.
The horizontal axis shows the amounts of iron stores:
Line A indicates the 1.7 g of total iron stores removed.
Line B indicates the 1.2 g of pre-existing hemosiderin iron removed.
Line C indicates the 0.5 g of pre-existing ferritin iron removed.
A = (B + C)
The vertical axis shows the levels of serum ferritin:
Line X indicates the 700 ng/mL of pre-existing serum ferritin removed.
Line Y indicates the 430 ng/mL of recovered-serum ferritin removed.
Line Z indicates the 270 ng/mL of recovered-serum ferritin remained (not removed).
X = (Y + Z)

Turnover of Ft and Hs in duodenal epithelium. The stored iron in the cell can either move into the plasma or be absorbed into the intestinal lumen through epithelial exfoliation within 10 days.[16-18]

Granick[1] proposed the hypothesis of automatic blockage of iron absorption after oral iron administration. Saito[6] demonstrated not only the blockage through Ft formation, but also the incomplete blockage of iron absorption through Hs formation.[6] Iron absorption is regulated by hepcidin-ferroportin axis,[19] which is also regulated by storage iron level.

Difference in iron turnover between radioiron and pre-existing body iron. The red cell radioiron utilization (RCU) ranges from 80–100%.[7,20-23] However, the non-radioiron ratio of red cell iron (RCI) to whole-body iron (WBI) is approximately 67%.[12,18] The difference in red cell utilization between radioiron and non-radioiron indicates that RCU is enlarged by the additional red cell fixation of radioiron released from non-erythron tissue in 14 days due to radioiron recycling the same pathways (Fig. 2).

The red cell iron turnover rate (RCIT) is also increased, as it is calculated by multiplying the increased RCU with the plasma iron turnover rate (PIT). Conversely, the red cell iron renewal

rate (RCIR), obtained by dividing RCI with mean red cell life span, indicates the red cell non-radioiron turnover rate.[11] The difference between the increased RCIT and RCIR corresponds to the turnover rate of radioiron released from non-erythron tissue (TITr; Fig. 3). TITr is determined as the turnover rate of extra red cell fixed radioiron refluxed from non-erythron tissue in 14 days (Fig. 3). The red cell fixed iron does not exchange with plasma iron.[24]

Researchers in ferrokinetics[7,12,20-23] regarded the increased RCIT as a net value. Consequently, the ferrokinetic SIT were underestimated less than the half of the standard SIT of 5.8 mg/day.[11,13]

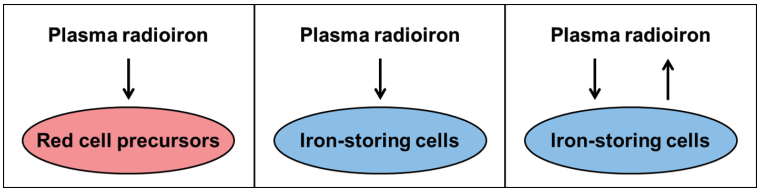

Fig. 2 Three pathways of intravenously injected radioiron in normal individuals
Fig. 2 Left: Fixed to red cell precursors.
Fig. 2 Mid: Fixed to iron-storing tissue cells.
Fig. 2 Right: Taken up and subsequently released by iron-storing tissue cells.

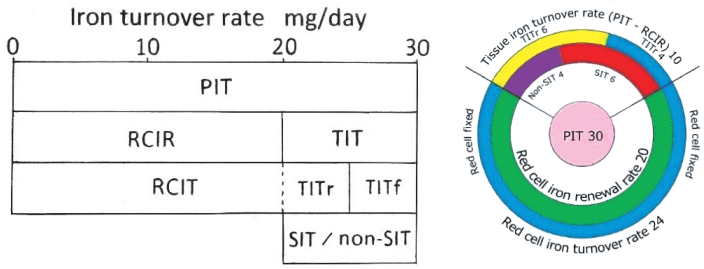

Fig. 3 Average body iron turnover rates of a standard normal adult male
Fig. 3 Left: The first bar indicates the plasma iron turnover rate (PIT).[2] The second bar indicates the red cell iron renewal rate (RCIR) and the non-erythron tissue iron turnover rate (TIT). The third bar indicates the red cell iron turnover rate (RCIT), tissue fixed iron turnover rate (TITf), and tissue released iron turnover rate (TITr), which is measured as the turnover rate of additionally red cell fixed radioiron in 14 days. The broken line indicates a proportion of TITr in RCIT. The border line between TITr and TITf shifts toward right or left according to their amount in TIT. The bottom box indicates the storage iron turnover rate (SIT) and non-SIT. SIT/non-SIT ratio in TIT increases according to the increase of iron storage.
Erythyro-ferrokinetic indices:
PIT = Total body plasma iron turnover rate[2]: 30 mg/day (100%).
RCIR: 20 mg/day (67% of PIT).
TIT: 10 mg/day (33% of PIT) = (PIT – RCIR).
RCIT: 24 mg/day (80–100% of PIT) = PIT x RCU/100.
TITr: 4 mg/day (13% of PIT) indicates the turnover rate of tissue released and then red cell fixed radioiron in 14 days.
TITf: 6 mg/day (20% of PIT).
TIT = (TITf + TITr).
SIT = 6 mg/day (20% of PIT).
non-SIT = 4 mg/day (13% of PIT).
Fig. 3 Right: Supplement to the left diagram.

Fig. 4 Iron-attracting potentials of iron-removed cell and iron-deposited cell
Fig. 4 Left: Vacant iron-storing rooms in an iron-removed cell with iron admitting capacity.
Fig. 4 Right: Iron-filled iron-storing rooms in an iron-deposited cell with a magnet-like force for attracting plasma iron.

Synchronization of radioiron uptake peaks in bone marrow and liver. The process of radioiron fixation by red blood cells is unidirectional. This one-way process may lead to confusion as the erythron dominates over the non-erythron tissue in acquiring iron from plasma. However, the erythron does not dominate the radioiron uptake process. The synchronization of radioiron uptake peaks in the bone marrow and liver after 24 h indicates competition between red cell precursors and tissue iron-storing cells for acquiring plasma iron.

The distribution of radioiron in the body in normal individuals after 24 h, not after 14 days, is proportional to the pre-existing body iron distribution.[25,26] The larger the iron storage, the more radioiron is fixed to iron-storing tissue cells.[6,25,26]

Thus, we can infer that the iron mass in each cell possesses an iron-attracting potential similar to a magnet (Fig. 4 Right).

A large labile iron pool in bone marrow and liver. Labile iron pool (LIP) refers to non-protein-bound, loosely iron-bound, and redox-active complexes. Pollycove et al[7,22] emphasized the presence of a large LIP in bone marrow and liver. Finch[22] raised concern about this, stating that the speed of radioiron uptake by red cell precursors and hepatocytes is considerably rapid to allow such an involvement before synthesizing hemoglobin and Ft. This type of LIP is unlikely to exist, as it can be cytotoxic to bone marrow and liver cells.

Storage iron turnover in iron deficiency states

Iron deficiency indicates the state of storage iron exhausted to a level < 100 mg, and a serum ferritin < 12 ng/mL.

In blood depleted rats, the maximal radioiron uptake by liver and spleen Ft appeared 24 h after radioiron injection,[6] similar to that observed in humans.[11]

The radioiron uptake by rat liver Ft was greater than that in other organs,[6] indicating that the main site of tissue iron turnover is the liver Ft. The release of radioiron from liver Ft was significantly higher in rats with depleted blood compared to normal rats. The release of radioiron stopped within 2 days[6] after the appearance of radioiron uptake peak at 24 h in normal and blood depleted rats. This indicates that the liver Ft iron turnover is more active in iron deficiency than that in normal rats.

In patient with normal iron storage after the treatment of iron deficiency anemia (IDA), Ft initially decreased rapidly and slowly later, while Hs decreased slowly initially and then rapidly[26] in constant iron removal. The prompt response of Ft to iron mobilization indicates the active turnover of Ft. The delayed response of Hs indicates its passive turnover. A small amount of Hs remains to coexist with Ft in the level close to the exhaustion of iron stores.[9,26] This evidence is indicative of the cooperation of Ft and Hs in iron mobilization in all the levels of iron stores from pre-iron deficiency state to iron overload. Accordingly, the existing morphology-based definition of Hs, as the aggregates of denatured Ft,[12] requires modification while considering the

functional feature of Hs in combination with Ft turnover.

The rapid uptake and release of radioiron by liver Ft in iron-depleted rats indicate active iron turnover in the liver Ft.[6] The iron transformation[10] between Ft and Hs increases in iron deficiency. Conversely, the storage iron turnover between hepatocyte and plasma decreases according to the decrease of iron storage.

Prior to the discovery of serum ferritin assays, Heinrich et al[27] identified the state of iron deficiency with normal hemoglobin, referred to as "pre-latent, and latent iron deficiency." However, with the availability of serum ferritin assays, this state is no longer latent but can manifest. Therefore, it is appropriate to refer to this state as "iron deficiency without anemia (ID)."[17,28]

In ID, despite the lack of stored iron, serum iron levels remain as high as possible to support hemoglobin synthesis. Moreover, the increased iron absorption in ID prevents it from falling into IDA.[17] Ft/Hs iron ratio becomes highest in IDA.

Approximately 30% of the menstruating individuals are ID.[17] During the last trimester of pregnancy most pregnant women develop IDA. Meanwhile, a woman loses around 0.4 to 1 g of iron concurrently during pregnancy and delivery.[29]

Iron refractory iron deficiency anemia (IRIDA) is a form of IDA caused by genetic mutations.[30] Iron absorption and utilization in IRIDA are decreased. The response of erythropoiesis and storage iron turnover to oral and parental iron administration is not active as compared with ordinary IDA.

Storage iron turnover in chronic hepatitis C

Increased hepatic Hs iron deposition is a characteristic finding in chronic hepatitis C (CHC).

We observed a prompt response of Ft and delayed response of Hs to iron removal in CHC, similar to that observed in IDA. Moreover, we found a minimal inverse correlation between TR[10] and pre-existing Ft, and no inverse correlation between TR and pre-existing Hs iron in CHC.[10] Hs was greater than Ft in all cases of CHC with the normal storage iron level. These findings suggest the transformation of Ft into Hs in CHC, with mild increase of iron absorption due to the change of intracellular environment.

Shiono et al[31] elucidated the correlation between the amount of iron stores before phlebotomy therapy and the rate of iron restoration (absorbed iron per day) while maintaining storage iron level within normal range in CHC. This result implies that the larger the vacant iron-storing room in the iron-removed cells (Fig. 4 Left), the stronger the iron-attracting potential for increasing iron absorption.

In terms of iron deposition, the conversion of active Ft into passive Hs reduces iron toxicity to iron-storing cells. The iron reduction therapy developed by Hayashi et al[32] has proven effective in reducing disorders in CHC by removing iron.

Needle biopsy may not be necessary if its purpose is to assess the degree of Hs deposition in CHC, as Ft and Hs can be determined safely by serum ferritin kinetics.[9]

Storage iron turnover in iron overload

Iron overload is the state of increased whole-body storage iron larger than around 2500 mg, and a serum ferritin levels approximately 250 ng/mL indicates iron overload. The border between normal and overload iron remains unclear.[12]

In iron overloaded rats, radioiron deposited gradually in liver and spleen. Meanwhile, the release of deposited radioiron did not occur in 3 days.[6] This could be attributed to the following: the burying (dilution) of a minute amount of radioiron in the massive storage iron pool, and hindrance of radioiron release due to a magnet-like iron-attracting effect by the huge storage iron mass in accordance with the law of mass action (Fig. 4 Right).

In iron addition, Hs increases along with the increase of iron stores by converting Ft into Hs. Hs increases linearly and limitlessly in the iron overload range.[5,9] This prevents iron toxicity by limiting the increase of Ft with active iron turnover.

The increase of SIT indicates the increase of iron turnover between iron-storing cells and plasma in proportion to the increase of iron stores. Conversely, non-SIT, which refers to the sum of iron turnover rates of myoglobin and enzymes, does not increase in proportion to the increase of iron stores.

The inverse correlation between TR[10] and pre-existing storage iron observed in hereditary hemochromatosis (HH) implies the decrease of storage iron turnover between Ft and Hs in iron overloaded HH.[33] However, the storage iron turnover was more active in HH than that in transfusion dependent patients. The difference could be related to the levels of erythropoietic activity.

In patients with HH, whose iron storage level was maintained within normal range after phlebotomy therapy, iron absorption increased higher than that in normal individuals.[34] This suggests the iron-attraction by the vacant iron-storing rooms in iron-removed cells, as observed in CHC,[31] in addition to the effect of uncontrolled iron absorption in HH.

ACKNOWLEDGEMENTS

The author expresses cordial thanks to Dr Tomita H and Professor Naoe T, Department of Hematology and Oncology, Nagoya University Hospital; to Dr Ohashi H, Department of Hematology, National Nagoya Medical Center; to Dr Maeda H, Department of Internal Medicine, Kawamura Hospital and Professor Hayashi H, Department of Medicine, Hokuriku University School of Pharmacology for providing precious clinical data. The author would like to extend cordial thanks to Mr Utsumi K, former staff of Sony Corporation, for his cooperation in computer simulation and making illustrations.

STATEMENT OF ETHICS

The author has no ethical conflicts to disclose.

DISCLOSURE STATEMENTS

The author has no conflicts of interest to declare.

No funds, grants, or other support was received.

INFORMED CONSENT

The informed consent of all participants was obtained orally in the study.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

1 Granick S. Ferritin; its properties and significance for iron metabolism. *Chem Rev*. 1946;38(3):379–403. doi:10.1021/cr60121a001.

2 Huff RL, Hennessy TG, Austin RE, Garcia JF, Roberts BM, Lawrence JH. Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders. *J Clin Invest*. 1950;29(8):1041–1052. doi:10.1172/JCI102335.

3 Finch CA, Hegsted M, Kinney TD, et al. Iron metabolism; The pathophysiology of iron storage. *Blood*. 1950;5(11);983–1008. doi:10.1182/BLOOD.V5.11.983.983.

4 Haskins D, Stevens AR Jr, Finch S, Finch CA. Iron metabolism; iron stores in man as measured by phlebotomy. *J Clin Invest*. 1952;31(6):543–547. doi:10.1172/JCI102639.

5 Shoden A, Gabrio BW, Finch CA. The relationship between ferritin and hemosiderin in rabbits and man. *J Biol Chem*. 1953;204(2):823–830.

6 Saito H. Studies on storage iron: the dynamic behaviors of ferritin and hemosiderin iron under various experimental conditions. *Nagoya J Med Sci*. 1958;21(4):288–300.

7 Pollycove M, Mortimer R. The quantitative determination of iron kinetics and hemoglobin synthesis in human subjects. *J Clin Invest*. 1961;40(5):753–782. doi:10.1172/JCI104310.

8 Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Llewellin P. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *J Clin Pathol*. 1972;25(4):326–329. doi:10.1136/jcp.25.4.326.

9 Saito H, Tomita A, Ohashi H, Maeda H, Hayashi H, Naoe T. Determination of ferritin and hemosiderin iron in patients with normal iron stores and iron overload by serum ferritin kinetics. *Nagoya J Med Sci*. 2012;74(1–2):39–49. doi:10.18999/nagjms.74.1-2.39.

10 Saito H, Hayashi H. Transformation rate between ferritin and hemosiderin assayed by serum ferritin kinetics in patients with normal iron stores and iron overload. *Nagoya J Med Sci*. 2015;77(4):571–583. doi:10.18999/nagjms.77.4.571.

11 Saito H. Problems in ferrokinetics: extra radio-iron fixation to red cells. *Nagoya J Med Sci*. 2018;80(4):475–485. doi:10.18999/nagjms.80.4.475.

12 Bothwell TH, Charlton R, Cook JD, Finch CA, eds. *Iron metabolism in man.* Oxford: Blackwell Scientific Publication; 1979.

13 Saito H. Storage iron turnover from a new perspective. *Acta Haematol*. 2019;141(4):201–208. doi:10.1159/000496324.

14 Gonzales-Mejia ME, Doseff AI. Regulation of monocytes and macrophages cell fate. *Front Biosci (Landmark Ed)*. 2009;14(7):2413–2431. doi:10.2741/3387.

15 CORDIS. Life span of human cells defined: most cells are younger than the individual. CORDIS Europa news. https://cordis.europa.eu/article/id/24286-life-span-of-human-cells-defined-most-cells-are-younger-than-the-individual. Published Aug 12, 2005, Accessed Oct 17, 2017.

16 Green R, Charlton R, Seftel H, et al. Body iron excretion in man: a collaborative study. *Am J Med*. 1968;45(3):336–353. doi:10.1016/0002-9343(68)90069-7.

17 Saito H. *Clinical disorders of iron metabolism: from iron deficiency to iron overload*. Entire book in Japanese. Osaka: Iyaku Janarusha; 1999.

18 Saito H, Sargent T 3rd, Parker HG, Lawrence JH. Whole body iron loss in normal man measured with a gamma spectrometer. *J Nucl Med*. 1964;5(8):571–580.

19 Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophs Acta*. 2012;1823(9):1434–1443. doi:10.1016/j.bbamcr.2012.01.014.

20 Pollycove M. Iron kinetics and Discussion. In: Gross F ed. *Iron metabolism: international symposium sponsored by CIBA at Aix-en-Provence, 1st–5th July, 1963*. Springer; 1964:148–177.

21 Hosain F, Marsaglia G, Finch CA. Blood ferrokinetics in normal man. *J Clin Invest*. 1967;46(1):1–9. doi:10.1172/JCI105501.

22 Cook JD, Marsaglia G, Eschbach JW, Funk DD, Finch CA. Ferrokinetics: a biologic model for plasma iron exchange in man. *J Clin Invest*. 1970;49(2):197–205. doi:10.1172/JCI106228.

23 Saito H, Yamada H. Studies on red cell production and destruction in various hematological disorders in view of ferrokinetics. *Acta Haematol Jpn*. 1973;36(5):681–709.

24 Hahn PF, Bale WF, Ross JF, Hettig RA, Whipple GH. Radio-iron in plasma does not exchange with hemoglobin iron in red cells. *Science*. 1940;92(2380):131–132. doi:10.1126/science.92.2380.131.

25 Van Dyke D, Anger H, Pollycove M. The effect of erythropoietic stimulation on marrow distribution in man, rabbit and rat as shown by Fe59 and Fe52. *Blood*. 1964;24(4):356–371. doi:10.1182/blood.V24.4.356.356.

26 Saito H, Hayashi H, Tomita A, Ohashi H, Maeda H, Naoe T. Increasing and decreasing phases of ferritin and hemosiderin iron determined by serum ferritin kinetics. *Nagoya J Med Sci*. 2013;75(3–4):213–223. doi:10.18999/nagjms.75.3-4.213.
27 Heinrich HC. Prelatent, latent and manifest iron-deficiency states in blood-donors. Pathogenesis, diagnosis, prevention and treatment [in German]. *Munch Med Wochenschr*. 1968;110(33):1845–1852.
28 Ning S, Zeller MP. Management of iron deficiency. *Hematology Am Soc Hematol Educ Program*. 2019;2019(1):315–322. doi:10.1182/hematology.2019000034.
29 Georgieff MK. Iron deficiency in pregnancy. *Am J Obstet Gynecol*. 2020;223(4):516–524. doi:10.1016/j.ajog.2020.03.006.
30 Finberg KE, Hennesy MM, Campagna DR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). *Nature Genet*. 2008;40(5):569–571. doi:10.1038/ng.130.
31 Shiono Y, Hayashi H, Wakusawa S, et al. Body iron stores and iron restoration rate in Japanese patients with chronic hepatitis C as measured during therapeutic iron removal revealed neither increased body iron stores nor effects of C282Y and H63D mutation on iron indices. *Nagoya J Med Sci*. 2001;64(1–2):51–57. doi:10.18999/nagjms.64.1-2.51.
32 Hayashi H, Takikawa T, Nishimura N, Yano M, Isomura T, Sakamoto N. Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. *Am J Gastroenterol*. 1994;89(7):986–988.
33 Milder MS, Cook JD, Stray S, Finch CA. Idiopathic hemochromatosis: an interim report. *Medicine (Baltimore)*. 1980;59(1):34–39. doi:10.1097/00005792-198001000-00002.
34 Sargent T, Saito H, Winchell HS. Iron absorption in hemochromatosis before and after phlebotomy therapy. *J Nucl Med*. 1971;12(10):660–667.