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Body iron metabolism and pathophysiology of iron overload

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Abstract Iron is an essential metal for the body, while excess iron accumulation causes organ dysfunction through the production of reactive oxygen species. There is a sophisticated balance of body iron metabolism of storage and transport, which is regulated by several factors including the newly identified peptide hepcidin. As there is no passive excretory mechanism of iron, iron is easily accumulated when exogenous iron is loaded by hereditary factors, repeated transfusions, and other diseased conditions. The free irons, non-transferrin-bound iron, and labile plasma iron in the circulation, and the labile iron pool within the cells, are responsible for iron toxicity. The characteristic features of advanced iron overload are failure of vital organs such as liver and heart in addition to endocrine dysfunctions. For the estimation of body iron, there are direct and indirect methods available. Serum ferritin is the most convenient and widely available modality, even though its specificity is sometimes problematic. Recently, new physical detection methods using magnetic resonance imaging and superconducting quantum interference devices have become available to estimate iron concentration in liver and myocardium. The widely used application of iron chelators with high compliance will resolve the problems of organ dysfunction by excess iron and improve patient outcomes.

Keywords Hemochromatosis · Hepcidin · Iron metabolism · Iron overload · Non-transferrin-bound iron (NTBI)

1 Introduction

Iron is an essential metal for hemoglobin synthesis of erythrocytes, oxidation–reduction reactions, and cellular proliferation, whereas excess iron accumulation causes organ dysfunction through the production of reactive oxygen species (ROS). The total amount of body iron is approximately 3–4 g, two-thirds of which is composed of red blood cell (RBC) iron and recycled iron by RBC destruction; the remainder is stored in ferritin/hemosiderin, while only 1–2 mg of iron are absorbed in the intestinal tract and circulated in the blood [1]. Body iron metabolism is a semi-closed system, and is critically regulated by several factors including the newly identified peptide hepcidin. In the circulation, iron is usually bound to transferrin (Tf), and most of the Tf-bound iron is utilized for bone marrow erythropoiesis [1]. As there is no active mechanism to excrete iron from the body, a progressive accumulation of body iron easily occurs as a result of long-term transfusions in patients with anemia of genetic disorders such as thalassemia, sickle cell disease (SCD), and Diamond Blackfan syndrome, and of bone-marrow failures such as aplastic anemia (AA) and myelodysplastic syndromes (MDS). In order to consider pathophysiological mechanisms of organ injury by iron overload, an understanding of molecular mechanisms of body iron metabolism is essential.

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Table 1 Molecules involved in body iron metabolism

Molecules for intestinal iron absorption
Divalent metal transporter 1 (DMT1)
Duodenal cytochrome *b* (Dcytb)
Heme carrier protein (HCP)
Hemeoxygenase-1
Ferroportin
Hephaestin
Transferrin
Molecules for bone marrow iron uptake
Transferrin receptor 1
Transferrin
Molecules for reutilization of senescent red blood cells
Hemeoxygenase-1
Ferroportin
Transferrin
Molecules for hepatic iron storage
Ferritin
Hemosiderin
Transferrin
Transferrin receptor 1
Transferrin receptor 2
Non-transferrin-bound iron
HFE
β2-microglobin
Divalent metal transporter 1
ZIP14
Hemojuvelin
Molecules for systemic iron regulation
Hepcidin
(Unknown erythroid regulator?)

2 Molecular mechanisms of body iron metabolism

Table 1 shows a list of molecules involved in body iron metabolism, categorized as functions including intestinal absorption, erythroid iron uptake, reutilization of senescent RBCs, hepatic iron storage, and systemic regulation.

2.1 Intestinal iron absorption

Ingested iron is classified as non-heme iron and heme iron. Non-heme iron derived from plants is mainly composed of inorganic ferric Fe(III) iron, and is absorbed into enterocytes through the divalent metal transporter 1 (DMT1) after reduction of Fe(III) to Fe(II) by duodenal cytochrome *b* [2, 3]. In contrast, heme-iron derived from meat is absorbed through a heme carrier protein into enterocytes, where it is degraded by hemeoxygenase-1 (HO-1). Iron within enterocytes is then transferred from the luminal to the vascular site of the cell, and released into the circulation via the metal transporter, ferroportin in the form of Fe(II). Excreted Fe(II) is thereafter oxidized to Fe(III) by hephaestin, a homolog of ceruloplasmin, and the resulting ferric iron is bound to serum Tf [4].

2.2 Red blood cell iron reutilization in the reticuloendothelial system (RES) and iron load by blood transfusion

The average life span of circulating RBCs is approximately 120 days, indicating that 20 mg of iron derived from 20 ml of RBCs are processed by RES/macrophages on a daily basis. Within macrophages, heme derived from phagocytized RBCs is catabolized by HO-1, and free iron is released.

Intra-cellular iron is released into the circulation via ferroportin, and the iron is donated to Tf and reutilized for bone marrow erythropoiesis.

In patients with genetic anemias and bone marrow failures, regular transfusion is required in order to overcome the intractable symptoms. Transfused RBCs are taken up and degraded by RES/macrophages, in which the recycled iron is overloaded and the excess iron saturates the binding capacity of Tf. This excess iron appears in the circulation as a form of non-Tf-bound iron (NTBI) [1, 5], and causes organ dysfunction by the production of ROS. One milliliter of blood contains approximately 0.5 mg of iron, and there is no active mechanism for excretion of this excess iron. In Japan, one unit of blood corresponds to 200 ml of whole blood or 140 ml of concentrated RBCs, both of which contain approximately 100 mg of iron. As the critical level of iron overload at which organ dysfunction occurs in the liver is approximately 7 mg/g dry liver weight [6], according to the formula derived by Angelucci [body iron accumulation (mg/kg) = liver iron concentration (LIC; mg/g dry weight × 10.6)] [7], only 40 Japanese units of transfusion are required to reach this level.

2.3 Iron uptake and utilization in liver

The liver is a major storage organ of iron, in which excess iron is stored as ferritin and hemosiderin. In addition to these proteins, an additional fraction of free iron is present in the form of the labile iron pool (LIP) within cells. The LIP is biologically active in intracellular metabolism via oxidation–reduction reactions, cell proliferation, and cell signaling, but is toxic if present in excess. As shown in Fig. 1, hepatocytes have essentially two pathways for uptake of iron from the circulation: Tf-bound iron (Fe_2-Tf) at physiological iron concentrations, and NTBI in iron overload conditions [3].

Concerning the uptake of Fe_2-Tf, there are three pathways involved: two are dependent on and one is independent of transferrin receptor (TfR) recycling.

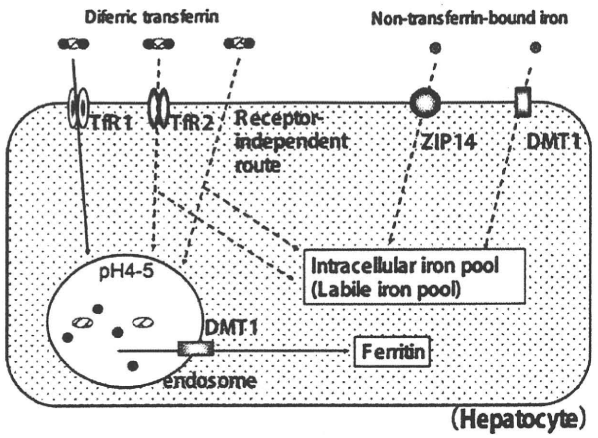

Fig. 1 Routes for iron uptake by hepatocytes. Hepatocytes have several pathways for iron uptake from the circulation. Concerning uptake of Tf-bound iron (Fe_2-Tf) at physiological concentrations, there are three pathways involving TfR1, TfR2, and TfR-independent mechanisms. The pathway via TfR1 is a classical one and is well elucidated. When serum Fe_2-Tf binds to TfR1, the Fe_2Tf-TfR1 complex is internalized by endocytosis, and iron is released within the endosome when endosomal pH is acidic. The resulting apotransferrin-TfR1 complex is then recycled back to the cell surface for re-utilization. Released iron into the endosome is transferred to the cytoplasm by DMT1; the resulting cytoplasmic free iron is used for iron-related biological functions, and the rest of the iron is stored as ferritin. In addition to TfR1, TfR2 and the mechanism that is independent of TfR1 and TfR2, are also considered to be important routes for iron uptake in hepatocytes, but the details of these routes remain to be elucidated. Concerning the hepatic uptake of NTBI, which is present in the serum during conditions of iron overload, DMT1 and ZIP14 are considered to be involved

Transferrin receptor 1 (TfR1) is a classical functional receptor, expressed highly in erythroblasts, but less so in hepatocytes. When serum Fe_2-Tf binds to TfR1, Fe_2-Tf is internalized by endocytosis. Internalized Fe_2Tf-TfR1 complexes within the endosome release iron when endosomal pH is acidified. The resulting apotransferrin-TfR1 complex is then recycled back to the cell surface for re-utilization. Transferrin receptor 2 (TfR2), a new homolog of TfR1, is ubiquitously expressed on hepatocyte surfaces and possesses a similar mechanism of recycling, but the binding affinity is rather weak: the functional role of TfR2 for cellular iron uptake is still obscured. In hepatocytes, there is another Fe_2-Tf uptake mechanism that is independent of TfR recycling, which is also considered to be important [8].

In iron-overloaded conditions, NTBI appears in the circulation and is taken up through two molecules such as DMT1 and ZIP14 on hepatocytes [9].

2.4 Bone marrow iron metabolism and erythropoiesis

Bone marrow erythroblasts require large amounts of iron for hemoglobin synthesis. TfR1 is strongly expressed in erythroblasts and functions as the uptake system of extracellular Fe_2-Tf. Within erythroblasts, iron is transferred to mitochondria and is incorporated into the center of the heme ring, which is synthesized by condensation of δ-aminolevulinic acid, a product made by erythroid δ-aminolevulinic acid synthase (eALAS). It is noteworthy that the synthesis of eALAS is also regulated by an iron-responsive-element binding protein (IRP) as well as TfR1 [10]. It is well known that genetic abnormalities of this pathway cause the phenotype of ringed sideroblastic anemias [11].

2.5 Systemic regulation of body iron metabolism

It has been postulated for a long time that a soluble factor acts to synchronize body iron metabolism between different organs. Recently, a basic peptide called hepcidin, an antimicrobial purified from urine, was found to have this role [12]. Hepcidin is considered to be a negative regulator that inhibits both intestinal iron absorption and reticulo-endothelial iron release. It is mainly synthesized in the liver, in which production is enhanced during iron overload and inflammation [13]. In some patients with genetic hemochromatosis, an abnormality of *hepcidin* gene has been reported. In these patients, hepcidin production was suppressed and iron absorption increased [14]. Furthermore, hepcidin expression is also down-regulated even in patients without a genetic abnormality of hepcidin. These reports strongly suggest that hepcidin plays an important role in tissue iron deposition in many iron-overloaded conditions including HFE hemochromatosis [15]. Currently, several additional molecules such as TfR2 and hemojuvelin (HJV) are also known to be involved in its regulation [16]. Furthermore, it is becoming clear that there is a role for hepcidin even in secondary iron overload. In a mouse model of β-thalassemia, representing ineffective erythropoiesis, there is an upregulation of hepcidin and a down-regulation of ferroportin, explaining how hepcidin also contributes to the formation of secondary hemochromatosis associated with ineffective erythropoiesis [17].

3 Forms of iron in serum and tissue

As free iron is extremely toxic to cells, the body has a number of protective mechanisms with which to bind iron in various tissue compartments. In serum, iron is usually bound to Tf, but some is present as NTBI when iron concentration exceeds the iron binding capacity of plasma Tf. It is also noted that ferritin is present in serum, although its biological role in iron transport is unclear.

3.1 Iron in plasma: Tf-bound iron and non-Tf-bound iron (NTBI)

It is well known that plasma Tf is capable of binding and transporting ferric iron to cells via TfRs. The binding capacity of Tf to inorganic iron is very strong, and this characteristic behavior prevents iron from existing in its free form under normal physiological conditions. As the Tf saturation in normal physiological conditions is up to 35%, this suggests that there is sufficient capacity to prevent the release of free toxic iron into the circulation [18]. However, when the iron binding capacity of Tf is saturated in the iron-overloaded state, an additional iron compartment NTBI, appears in the circulation. This compartment is biologically more toxic than Tf-bound iron. Among the NTBI fractions, labile plasma iron (LPI) is the most toxic. Unlike Tf-bound iron, the cellular uptake of NTBI is not dependent on the TfR, and therefore the resulting iron is diffusely distributed throughout the organs, independent of the presence of the TfR [5, 19]. Unlike serum iron, TIBC and percent-Tf-saturation measurements, the inter-institutional difference of NTBI and LPI measurements are too great and these parameters have not been standardized.

3.2 Iron in tissue: tissue ferritin and labile iron pool (LIP)

Within cells, iron is stored in the proteins ferritin or hemosiderin. Ferritin is a cytoplasmic protein consisting of 25 heterodimeric subunits of H and L that stores iron as ferric hydroxide phosphate in a controlled manner. Each molecule can store up to 4,500 Fe(III) within the protein shell [20], and release greater quantities of iron when the body is iron deficient. Most ferritin is present in liver, spleen, and bone marrow, and a trace amount is found in the blood as serum ferritin. It is noteworthy that the synthesis of ferritin is post-transcriptionally regulated by the cytoplasmic transacting factor IRP. IRP activates ferritin synthesis when iron is excess in the cell [21]. This adaptive response is important for preventing cells from free iron toxicity.

In addition to ferritin iron, LIP is present within cells in order to facilitate biological actions involving iron atoms, and can become cytotoxic or carcinogenic when the concentration exceeds the protective capacity of ferritin. Most of the LIP is free ferric iron bound to citrate or adenosine diphosphate, and a small amount of LIP is reduced to ferrous iron, which is responsible for oxidation–reduction reactions and the Fenton reaction. Iron toxicity is developed thorough the production of ROS.

3.3 Serum ferritin

In 1972, Jacobs et al. [22, 23] in the UK reported that ferritin was also present in serum, although its amount was very low. By quantitative phlebotomy, it was found that serum ferritin (SF) correlated with total body iron stores. Although it is still not clear how SF is produced, it is the most convenient laboratory test available to estimate body iron stores at the present time. However, the level of SF is also affected by acute and chronic inflammation and infections. Therefore, data should be interpreted carefully when using SF as a biological marker for evaluation of body iron stores, as shown in Table 2. There is a difference between the standard values of SF concentration in males and females (normal range 10–220 μg/L in males; 10–85 μg/L in females). It is clear that low SF values less than

Table 2 Considerations needed to use serum ferritin as a biological marker for the evaluation of body iron store

•	**There is a sex difference of standard values of serum ferritin concentration**
✓	Male: 10–220 μg/L, Female: 10–85 μg/L
•	**Serum ferritin will be increased in various clinical conditions other than iron overload**
✓	Chronic inflammation (effect of inflammatory cytokines)
✓	Chronic liver damage (release from destroyed hepatocytes)
✓	Malignancies (release from destroyed tumors)
✓	The conditions needed to be considered for differential diagnosis dependent on the value of serum ferritin
	Slight elevation (250–500 μg/L)
	Malignancies, chronic liver damage, chronic inflammation, mild iron overload
	Mild elevation (500–1000 μg/L)
	Early stage of iron overload, ineffective erythropoiesis (thalassemia, etc)
	The frequency of the conditions except iron overload decreases
	Moderate elevation (1000–5000 μg/L)
	Iron overload, Adult Still's disease, hemophagocytic syndrome
	Severe elevation (more than 5000 μg/L)
	Iron overload (hemochromatosis)

12 μg/L are usually representative of body iron deficiency. On other hand, patients with SF levels that are higher than the normal range may be indicative of conditions such as iron overload, inflammation, collagen disease, malignancy, and hepatic diseases [24]. This characteristic feature of the SF assay is considered to be a disadvantage for monitoring iron overload. Especially in Japan, the significance of SF as an inflammation marker has been over-stressed because there are few patients with hereditary hemochromatosis showing significantly high values of more than a couple of thousand or ten thousand microgram per liter.

Systemic measurements of SF in various diseases were conducted mainly in the late 1970s, just after the development of this assay, and it was found that AA and sideroblastic anemia patients who had received blood transfusions had SF levels of more than 1,000 μg/L, whereas patients without transfusions had lower levels. These old data have suggested previously that anemic patients who had ineffective erythropoiesis without transfusion support could maintain their SF levels at values less than 1,000 μg/L, even though adaptive increases in intestinal iron absorption were noted [25]. Therefore, the interpretation of the value of SF for the assessment of body iron status is simplified if other clinical conditions such as inflammation and malignancy are excluded by other modalities. The clinical studies concerning the relationship between blood transfusion and SF have been conducted mainly in the Europe and US, showing that there is a clear-cut positive correlation between the amount of chronic blood transfusion and the elevation of SF in patients with β-thalassemia [26, 27]. Furthermore, the concentration of heart iron is increased when SF levels become greater than 1,800 μg/L, and the prevalence of cardiac events is significantly increased when SF levels are more than 2,500 μg/L [6, 28]. Similar results concerning the relationship between SF and organ dysfunction of liver and heart were shown in a Japanese retrospective study in transfusion-dependent patients with bone-marrow-failure syndromes [29]. In this study, 90% of patients with either cardiac or hepatic complications had high SF levels of more than 1,000 μg/L. Coincidentally, this level of SF also represents the threshold of the target value at which iron chelation therapy should be initiated in patients with transfusion iron overload, according to the guidelines of the International MDS Symposium [30].

4 Measurement of body iron stores: comparison with serum ferritin

Direct and indirect methods are available for the estimation of body iron. As previously mentioned, the measurement of SF is the most convenient and cost-effective technique, although other factors can also influence its value. There is no argument that the gold standard for iron determination is direct tissue iron determination. Notably, other methods that are becoming increasingly important include physical methods such as the superconducting quantum-interference device (SQUID) and magnetic resonance imaging (MRI).

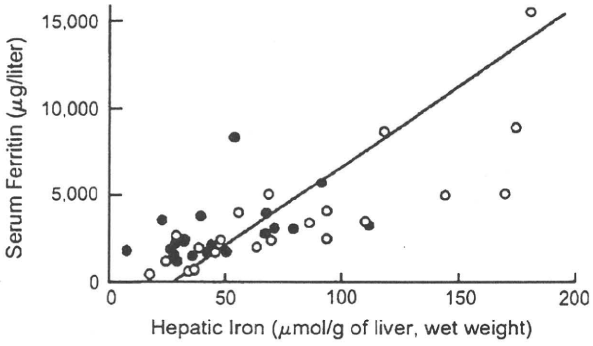

Fig. 2 Comparison of hepatic iron and serum ferritin concentrations. Indirect estimation is compared with the reference method, based on the direct measurement of hepatic iron levels by chemical analysis or magnetic-susceptibility studies. *Open circles* denote the values at the start of the trial (before deferiprone therapy), and *solid circles* the values at the time of the final analysis. The *diagonal line* denotes the simple linear least-squares regression between the two variables. (From [31]. Reproduced with permission. Olivieri NF et al. N Engl J Med. 1995;332:918–22. Copyright ©1995 Massachusetts Medical Society. All rights reserved)

4.1 Direct measurement

Liver is the major organ for iron storage and has the largest capacity to store excess iron. The measurement of hepatic iron concentration by liver biopsy is the most reliable means to assess body iron storage; however, this procedure is invasive and cannot be used in all cases [7]. Figure 2 compares the indirect estimation of body iron based on serum ferritin and LIC. Open circles denote the values at the start of the trial (before treatment with deferiprone), and solid circles denote the values at the time of the final analysis. The correlation between these measurements was significant ($R = 0.73$; $P < 0.005$) [31]. Concerning the determination of cardiac iron deposition, myocardial biopsy can be used; however, this procedure is not often conducted without special experimental reasons due to its high technical risk.

In patients with β-thalassemia, there is a correlation between LIC and cumulative amounts of RBC transfusions [26] and the risk of organ dysfunction is enhanced when LIC values are greater than 7 mg/kg wet tissue, and LIC levels of over 15 mg/kg wet tissue increase the risk of early cardiac death due to iron deposition in the myocardium [6]. Studies in the deferasirox clinical development program in β-thalassemia also demonstrated a correlation between the reduction in LIC and SF values ($R = 0.63$).

4.2 Physical measurement of body iron

As iron is one of the heavy metals, an increased concentration of biological iron consisting of ferritin and hemosiderin can be detected by body imaging procedures. Until recently, abdominal echograms and computed tomography (CT) produced images at high iron concentrations, although these two modalities are not quantitative and are only capable of detecting iron overload under conditions of extremely high iron deposition [32]. Recently, quantitative procedures such as SQUID [33] and MRI have been introduced, which use the physical characteristics of iron. However, SQUID apparatus is only available in a couple of institutions in the Europe and US because of its cost. On other hand, LIC determinations by MRI are widely available. This method utilizes the specific characteristic of iron that shortens T1, T2, and T2* relaxation times. The measurable range of iron concentration by R2 (in a 1.5-T MRI magnet) is 0.3–42.7 mg Fe/g dry tissue, which covers the concentrations observed in iron-overloaded livers.

In addition to LIC measurement, the determination of cardiac iron concentration is clinically important because one of the major causes of death in iron overload is sudden cardiac arrest. The most reliable non-invasive method of cardiac iron is MRI R2*, which was developed by Anderson et al. [34]. The advantage of MRI R2* is the shorter time period required to acquire an image as only one breath period is necessary by this procedure.

Of the patients with LIC values below 350 μmol/g, all but one had myocardial iron within normal (≤8 μmol/g) or nearly normal ranges. When liver iron levels reached a threshold of 350 μmol/g, iron deposition became evident in the myocardium. At the same time, there was a proportional increase in urinary iron excretion, indicating raised levels of labile iron. SF levels of >1,800 μg/L were also associated with myocardial deposition.

5 Toxic effect of iron overload on organ function

Iron overload induces organ damage in liver, heart, pancreas, thyroid, and the central nervous system. The main cause of this organ damage is due to the overproduction of ROS in the presence of excess iron.

5.1 Mechanism of iron toxicity

The production of ROS by iron is mainly through the Fenton reaction, which eventually forms hydroxyl radicals from superoxide or hydrogen peroxide [35]. Among ROS, the hydroxyl radical is the most toxic fraction and it targets carbohydrate, protein, and nucleic acids. It is known that the reaction of hydroxyl radicals with the nucleic acid base 8-hydroxyguanine (8-OHG) is highly correlated with teratogenicity and carcinogenicity by oxidative stresses. Another powerful ROS showing similar reactivity as the hydroxyl radical is lipid hydroxyl-peroxide: ROOH. In iron overload, lipid peroxidative products such as malondialdehyde and 4-hydroxy-2-nonenal are increased, which form the radicals ROO-(alkyl oxyradical) and RO-(alkoxy radical). These lipid-based radicals possess longer half lives than hydroxyl radicals, and also have a stronger capacity for chronic cell toxicity and DNA damage.

5.2 Iron overload syndrome

Pathological conditions representing body iron overload are designated as iron overload syndromes, and iron deposition causes organ dysfunction including cell death, fibrosis, and carcinogenesis. Iron overload syndromes are classified as genetic or secondary as shown in Table 3.

Hereditary hemochromatosis is the most common genetic disorder in Western countries [36], and its clinical

Table 3 Classification of iron overload

Hereditary hemochromatosis and related disorders	
Hereditary hemochromatosis	Type 1
	HFE gene (6p21.3) mutation
	Type 2
	Subtype A: *hemojuvelin* gene (1q21) mutation
	Subtype B: *hepcidin* gene (19q13) mutation
	Type 3
	Transferrin receptor 2 gene (7q22) mutation
	Type 4
	Ferroportin gene (*2q32*) mutation
Ferritin gene mutation	*H-ferritin* gene mutation (mRNA iron-responsive-element mutation)
DMT1 gene mutation	
Ceruloplasmin gene mutation	
Atransferrinemia	*Transferrin* gene mutation
Secondary iron overload	
Ineffective erythropoiesis	Thalassemia, sideroblastic anemia, myelodysplastic syndromes
Administration of iron for long periods	Take orally or intravenous injection
Transfusion for long periods	
Dietary iron overload	
Liver dysfunction	Alcoholic liver injury, chronic hepatitis (type C), non-alcoholic steatohepatitis
Others	Porphyria

manifestation is systemic iron deposition mainly in liver, heart, brain, and endocrine organs. This organ damage is considered to be a result of tissue injuries by iron-induced oxidative stresses [37]. In 1996, the causative gene was identified as *HFE* in the human chromosome 6 [38], and approximately 85% of patients with hereditary hemochromatosis in Western counties have a homologous mutation of C282Y in their *HFE* gene. Thereafter, other genes such as *hemojuvelin* (*HJV*), *TfR2*, *ferroportin*, and *hepcidin* (*HAMP*) gene were identified [39]. In spite of the lack of genetic background, iron overload is commonly observed as a secondary condition. The most common condition occurs in patients who require long-term blood transfusions due to severe anemias. This condition includes genetic disorders such as thalassemia and SCD, and anemia refractory to conventional treatments. In these patients, ineffective erythropoiesis and continuous accumulation of exogenous iron by transfusion are considered to be responsible for the iron overload. The resulting organ failures such as liver failure, cardiac failure, and severe diabetes mellitus affect patients' outcome [1]. In addition to these classical conditions, there are many diseases that show mild iron deposition or dysregulation of body iron distribution. Such conditions include chronic hepatitis C, alcoholic liver disease, non-alcoholic steatohepatitis, and insulin resistance, and iron is an important cofactor that modifies these disease conditions. Furthermore, it is becoming clear that excess iron is also hazardous as it promotes atherosclerosis, carcinogenesis, diabetes, and other lifestyle-related disorders [40].

5.3 Organ dysfunction by excess iron

The liver is the most important organ for iron storage with the largest capacity to sequester excess iron. The periodical change of organ dysfunction by long-term transfusions has been studied in patients with homozygous β-thalassemia. Usually, within 2 years of transfusion, abnormalities of liver function tests (LFTs) such as transaminase are not prominent; LFTs are within the normal range or slightly elevated. During these periods, the liver biopsy examination shows a slight fibrosis with mild inflammation and iron deposition. Clinically, the liver is hardened and palpable, and serum transaminase levels are moderately elevated, while other LFTs are within the normal range or slightly elevated. Therefore, it is important for transfusion-dependent patients that clinicians make a correct staging in order to confirm whether any liver lesions are fibrotic or cirrhotic by examining CT, MRI, and biochemical analyses including serum transaminase determinations.

The most important adverse event of long-term transfusion is a sudden death due to cardiac failure. It was reported that approximately 70% of deaths in patients with β-thalassemia are cardiogenic [41]. Signs of cardiac dysfunction include cardiac hypertrophy, arrhythmia, and endocarditis, which eventually cause cardiac failure. Left ventricular disturbance is prominent and is represented as the decrease of ventricular ejection fraction (VEF) by cardiac echogram. As this decrease of VEF appears prior to the clinical signs of cardiac failure and the enlargement of cardiac shadow in chest X-rays, the cardiac echogram is the most useful modality for the follow-up of myocardial damage by iron overload [42]. MRI is also useful to assess the ventricular function, and the deposition of iron in cardiac muscles is detectable by an increase in signal intensity. Furthermore, MRI calculation of T2* or R2* allows the possibility of semi-quantitation of iron concentrations, even at relatively low concentrations [43].

According to a follow-up study in patients with β-thalassemia, organ dysfunction by iron overload appears firstly in the liver when serum ferritin exceeds 1,000 μg/L, and other organ involvements including heart follow in accordance with the further development of iron deposition. Significant cardiac iron deposition is usually observed when LICs are more than 15 mg/g dry weight or serum ferritin levels are more than 1,800–2,500 μg/L [6].

Clinically, in order to detect organ dysfunctions, serum ferritin determinations should be conducted once every 1–3 months. When serum ferritin levels exceed 1,500 μg/L, patients should be examined for the symptoms of cardiac failure or arrhythmias [44], and periodical cardiac echograms may also be useful in diagnosis.

In addition to iron deposition in the liver and heart, pancreatic beta cells are another important target of iron toxicity, which cause glucose intolerance and diabetes mellitus. An additional factor leading to the development of glucose intolerance is hepatic disturbance of insulin utilization, which accelerates beta cell depletion due to hyperinsulinemia [45]. From a clinical perspective, serial determinations of blood glucose, urine sugar, and glycoalbumin are useful, whereas glycohemoglobin is not as useful owing to the effect of transfusions. Endocrinopathies by long-term transfusion include developmental disturbances, incomplete puberty, and thyroid dysfunctions [46]. In patients with thalassemia and SCD, special attention should be paid to early onset symptoms such as disturbances of development and sexual immaturity.

6 Conclusion

Iron is essential for the body, but extremely toxic when excess amounts are present. As the body has no active excretion pathways for iron, a continuous load of iron exceeding 1–2 mg/day will result in iron overload, and organ failures including liver and heart. The recent

understanding of body iron metabolism at a molecular level enables us to elucidate the mechanism of iron toxicity more precisely. Improvement of patients' outcomes is becoming promising if a correct early diagnosis is made, and suitable management of these intractable conditions using iron chelation with high compliance is conducted.

References

1. Andrews NC. Disorders of iron metabolism. N Engl J Med. 1999;341:1986–95.
2. McKie AT, Latunde-Dada GO, Miret S, et al. Molecular evidence for the role of a ferric reductase in iron transport. Biochem Soc Trans. 2002;30:722–4.
3. Trinder D, Fox C, Vautier G, Olynyk JK. Molecular pathogenesis of iron overload. Gut. 2002;51:290–5.
4. Sargent PJ, Farnaud S, Evans RW. Structure/function overview of proteins involved in iron storage and transport. Curr Med Chem. 2005;12:2683–93.
5. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol. 2005;18:277–87.
6. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997;89:739–61.
7. Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med. 2000;343:327–31.
8. Ikuta K, Zak O, Aisen P. Recycling, degradation and sensitivity to the synergistic anion of transferrin in the receptor-independent route of iron uptake by human hepatoma (HuH–7) cells. Int J Biochem Cell Biol. 2004;36:340–52.
9. Liuzzi JP, Aydemir F, Nam H, Knutson MD, Cousins RJ. Zip14 (Slc39a14) mediates non-transferrin-bound iron uptake into cells. Proc Natl Acad Sci USA. 2006;103:13612–7.
10. Schranzhofer M, Schifrer M, Cabrera JA, et al. Remodeling the regulation of iron metabolism during erythroid differentiation to ensure efficient heme biosynthesis. Blood. 2006;107:4159–67.
11. Fleming MD. The genetics of inherited sideroblastic anemias. Semin Hematol. 2002;39:270–81.
12. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276:7806–10.
13. Inamura J, Ikuta K, Jimbo J, et al. Upregulation of hepcidin by interleukin-1beta in human hepatoma cell lines. Hepatol Res. 2005;33:198–205.
14. Ganz T. Hepcidin in iron metabolism. Curr Opin Hematol. 2004;11:251–4.
15. Bridle KR, Frazer DM, Wilkins SJ, et al. Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet. 2003;361:669–73.
16. Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology. 2007;46:1291–301.
17. Gardenghi S, Marongiu MF, Ramos P, et al. Ineffective erythropoiesis in β-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin. Blood. 2007;109:5027–35.
18. Cazzola M, Huebers HA, Sayers MH, MacPhail AP, Eng M, Finch CA. Transferrin saturation, plasma iron turnover, and transferrin uptake in normal humans. Blood. 1985;66:935–9.
19. Breuer W, Hershko C, Cabantchik ZI. The importance of non-transferrin bound iron in disorders of iron metabolism. Transfus Sci. 2000;23:185–92.
20. Koorts AM, Viljoen M. Ferritin and ferritin isoforms I: structure-function relationships, synthesis, degradation and secretion. Arch Physiol Biochem. 2007;113:30–54.
21. Harrison PM, Arosio P. The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta. 1996;1275:161–203.
22. Jacobs A, Beamish MR, Allison M. The measurement of circulating ferritin. J Clin Pathol. 1972;25:1003.
23. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Br Med J. 1972;4:206–8.
24. Piperno A. Classification and diagnosis of iron overload. Haematologica. 1998;83:447–55.
25. Saito H, Hayashi D, Ohya T, Ohya F, Yamada H. Clinical evaluation on serum ferritin (author's transl). Rinsho Ketsueki. 1979;20:1317–25.
26. Galanello R, Piga A, Forni GL, et al. Phase II clinical evaluation of deferasirox, a once-daily oral chelating agent, in paediatric patients with β-thalassaemia major. Haematologica. 2006;91:1343–51.
27. Cappellini MD, Cohen A, Piga A, et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood. 2006;107:3455–62.
28. Jensen PD, Jensen FT, Christensen T, Eiskjaer H, Baandrup U, Nielsen JL. Evaluation of myocardial iron by magnetic resonance imaging during iron chelation therapy with deferrioxamine: indication of close relation between myocardial iron content and chelatable iron pool. Blood. 2003;101:4632–9.
29. Takatoku M, Uchiyama T, Okamoto S, et al. Retrospective nationwide survey of Japanese patients with transfusion-dependent MDS and aplastic anemia highlights the negative impact of iron overload on morbidity/mortality. Eur J Haematol. 2007;78:487–94.
30. Gattermann N. Guidelines on iron chelation therapy in patients with myelodysplastic syndromes and transfusional iron overload. Leuk Res. 2007;31(Suppl 3):S10–5.
31. Olivieri NF, Brittenham GM, Matsui D, et al. Iron-chelation therapy with oral deferiprone in patients with thalassemia major. N Engl J Med. 1995;332:918–22.
32. Long JA Jr, Doppman JL, Nienhus AW, Mills SR. Computed tomographic analysis of beta-thalassemic syndromes with hemochromatosis: pathologic findings with clinical and laboratory correlations. J Comput Assist Tomogr. 1980;4:159–65.
33. Brittenham GM, Farrell DE, Harris JW, et al. Magnetic-susceptibility measurement of human iron stores. N Engl J Med. 1982;307:1671–5.
34. Anderson LJ, Westwood MA, Holden S, et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. Br J Haematol. 2004;127:348–55.
35. Crichton RR, Wilmet S, Legssyer R, Ward RJ. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J Inorg Biochem. 2002;91:9–18.
36. Yen AW, Fancher TL, Bowlus CL. Revisiting hereditary hemochromatosis: current concepts and progress. Am J Med. 2006;119:391–9.
37. Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Engl J Med. 2004;350:2383–97.
38. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13:399–408.
39. Franchini M. Hereditary iron overload: update on pathophysiology, diagnosis, and treatment. Am J Hematol. 2006;81:202–9.

40. Bonkovsky HL, Lambrecht RW, Shan Y. Iron as a co-morbid factor in nonhemochromatotic liver disease. Alcohol. 2003;30:137–44.
41. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al. Survival and causes of death in thalassaemia major. Lancet. 1989;2:27–30.
42. McGowan JH, Cleland JG. Reliability of reporting left ventricular systolic function by echocardiography: a systematic review of 3 methods. Am Heart J. 2003;146:388–97.
43. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J. 2001;22:2171–9.
44. Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. Br J Haematol. 2000;110:971–7.
45. Olivieri NF. The β-thalassemias. N Engl J Med. 1999;341:99–109.
46. Fung EB, Harmatz PR, Lee PD, et al. Increased prevalence of iron-overload associated endocrinopathy in thalassaemia versus sickle-cell disease. Br J Haematol. 2006;135:574–82.

NAFLDにおける非トランスフェリン結合鉄（NTBI）測定意義に関する検討

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1．はじめに

鉄は生体にとって必須の金属栄養素であるが，過剰状態になると反応性に富む自由鉄が増え，活性酸素種（reactive oxygen species: ROS）を産生することによって細胞毒性を呈し，肝障害，心不全，糖尿病，甲状腺，副腎などの内分泌機能障害，神経障害などの種々の臓器不全を引き起こす．もともと生体鉄代謝における鉄供給はごく微量が消化管から吸収されるが，そのほとんどがマクロファージにおける老廃赤血球のヘモグロビン鉄の再利用による半閉鎖的な代謝系からなり，絶妙な調節のもとにその恒常性が保たれている[1]．そして血中の鉄のほとんどがトランスフェリン（Tf）に結合したTf結合鉄として存在し輸送されている．通常，Tfは30%程度しか飽和されておらず，血中に細胞毒性の強い非トランスフェリン結合鉄（non-transferrin-bound iron: NTBI）はほとんど存在しない．しかし，大量輸血，鉄剤の過剰投与，慢性炎症，慢性肝疾患などの病態で鉄過剰症になるとTfの鉄結合能を超えて，血中にNTBIが出現してくる．NTBIの細胞内への取り込みは制御機構がなく，非選択的に全身の実質臓器の各種細胞内に取り込まれ，Fenton反応・Haber-Weiss反応を介してラジカルを産生し，臓器障害をもたらす[2]．

血中のNTBIの測定は困難で実用化されておらず，測定結果に対する臨床的意義に関しても確立していない．非アルコール性脂肪性肝疾患（nonalcoholic fatty liver disease: NAFLD）でも軽度から中等度の肝内鉄過剰蓄積が認められ，肝細胞内の過剰な不安定自由鉄が酸化ストレスを増強し，炎症，線維化，肝発癌に関与することが知られている[3]．今回，我々はNAFLD患者の血清NTBI濃度の測定し，その臨床的意義を検討した．

2．対象と方法

対象患者：1999年から2008年までに旭川医科大学病院で診断されたNAFLD患者37名と健常ボランティア40名で，血清ALT値が男性30IU/L以下（19名），女性20IU/L以下（14名）をコントロールとした．

血清NTBI測定法：Metal free HPLC systemを用いた[1]．概要を以下に述べる．

① 血清にコバルト溶液を添加し，コバルトイオンでApo-Tfをブロックする．

Clinical importance of serum NTBI in patients with NAFLD.
Takaaki Ohtake, Katsuya Ikuta, Koji Sawada, Masami Abe, Shigeki Miyoshi, Yasuaki Suzuki, Yutaka Kohgo, Katsunori Sasaki. Asahikawa Medical College.

② NTA（Nitrilotriacetic acid）溶液を添加し，Tfに結合していない不安定鉄（NTBI）を捕捉する．
③ NTBIを捕捉したNTAを限外濾過で，Tf，フェリチンなどから分離する．
④ 分離したNTBIをNTAからさらに高親和性鉄キレート剤CP-22に置換する．
⑤ Metal free HPLC systemで測定し，標準試料から作成した標準曲線から鉄イオン濃度を算出する．

3．結　果

NAFLD症例の血清ALT（中央値）は男性49IU/L，女性64IU/Lで，健常ボランティアの男性18IU/L，女性11.5IU/Lに比べて有意に上昇していた（$p<0.0001$）（**図1A**）．鉄代謝に関連して赤血球ヘモグロビンは両性ともNAFLDと健常者とでは差を認めなかった（**図1B**）．しかし，貯蔵鉄のマーカーである血清フェリチン（中央値）はNAFLD症例の男性316.3ng/mL，女性90.3ng/mLで，健常者の男性136ng/mL，女性15.3ng/mLに比べて有意に上昇していた（$p=0.001$）（**図1C**）．血清トランスフェリン飽和度（%Tf）は上昇傾向を認めるが有意ではなかった（**図1D**）．NAFLD症例では軽度から中等度の高トランスアミナーゼ血症と軽度の鉄過剰症があることが確認された．

これらの対象に対して血清非トランスフェリン

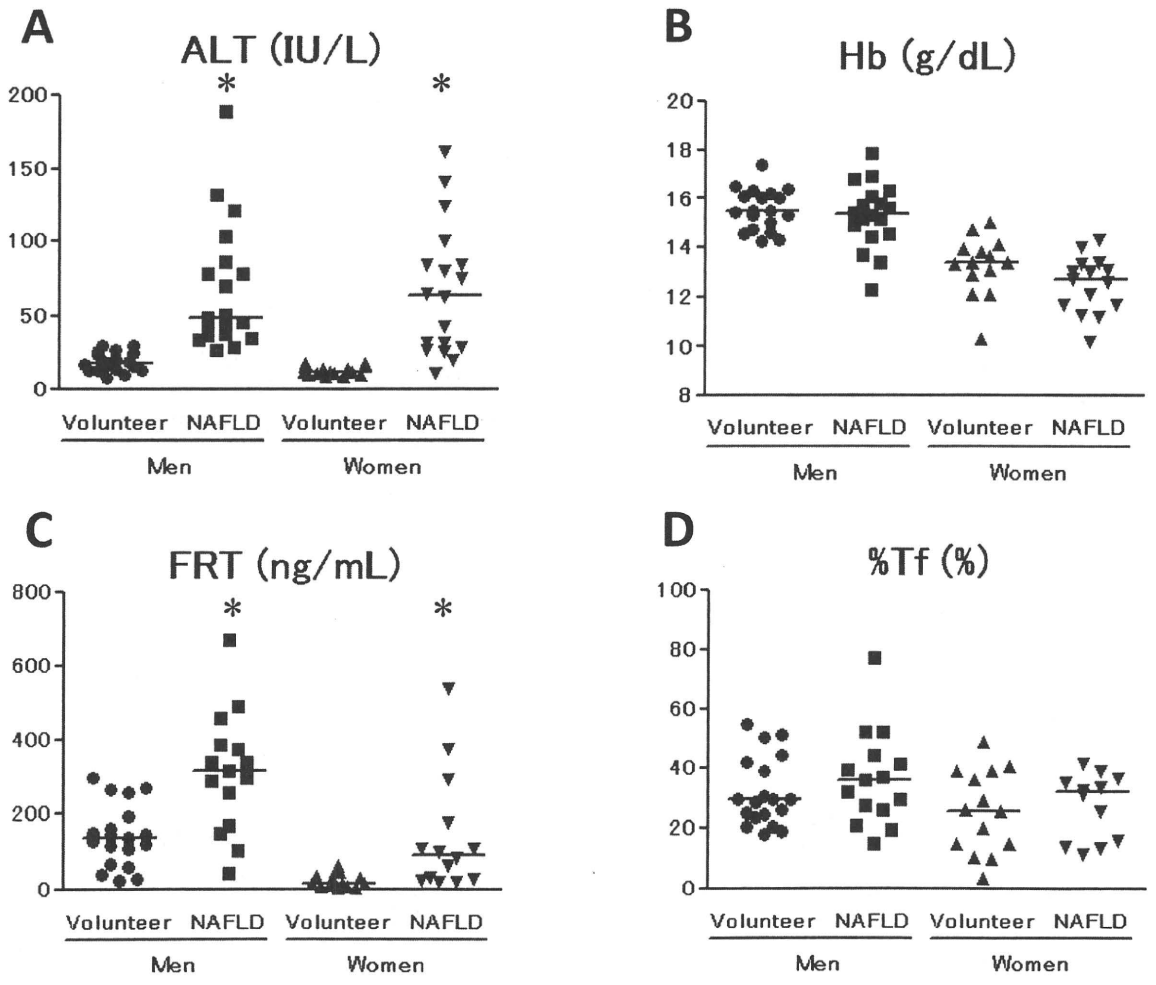

図1　健常ボランティアとNAFLD症例の血液生化学検査および鉄関連マーカー．（A）血清ALT値．*$p<0.0001$（Mann-Whitney）．（B）ヘモグロビン値（Hb）．（C）血清フェリチン値（FRT）．*$p=0.001$（Mann-Whitney）．（D）血清トランスフェリン飽和度（% Tf）．

結合鉄濃度（NTBI）を測定した．健常者が0.20±0.14μM，NAFLD症例が0.27±0.24μM，男性が健常者0.22±0.15μM，NAFLD症例0.31±0.26μM，女性が健常者0.18±0.12μM，NAFLD症例0.22±0.22μMであった（**図2A**）．NAFLD男性例では健常ボランティアの75%パーセンタイル以上（0.31μM）を示す症例が47%存在し，NTBIが高い傾向があった（**図2C**）．

健常ボランティア，NAFLD症例に関して血清フェリチンまたは% TfとNTBIとの相関を見ると健常者，NAFLD症例ともに血清フェリチンとNTBIとに相関関係はない．一方，健常者では明らかではないが，NAFLD症例においては% TfとNTBIが正の相関を認めた（r^2＝0.3381，p＝0.0015）（**図3D**）．

【症例提示】

60歳，女性．糖尿病，高血圧症，非アルコール性脂肪性肝炎（NASH）でフォローされている．降圧剤の投薬をされているが，糖尿病は食事療法のみで血糖コントロールは良好である．1993年頃初めて肝機能異常を指摘され，2003年，当院に受診する．身長152.7cm，体重54.6kg，Body Mass Index 23.4で明らかな肥満はない．肝生検で肝細胞の脂肪沈着，風船様変性，架橋形成所見，鉄沈着を認めNASHと診断した（**図5A，C**）．その後，食事療法，運動療法を開始し，体重の減少とともに血清ALT値は改善し30 IU/L以下で経過しているが，血清フェリチン高値は持続している（**図4A，B，C**）．2008年の2回目の肝生検では脂肪沈着は改善しているが，風船様変性，鉄

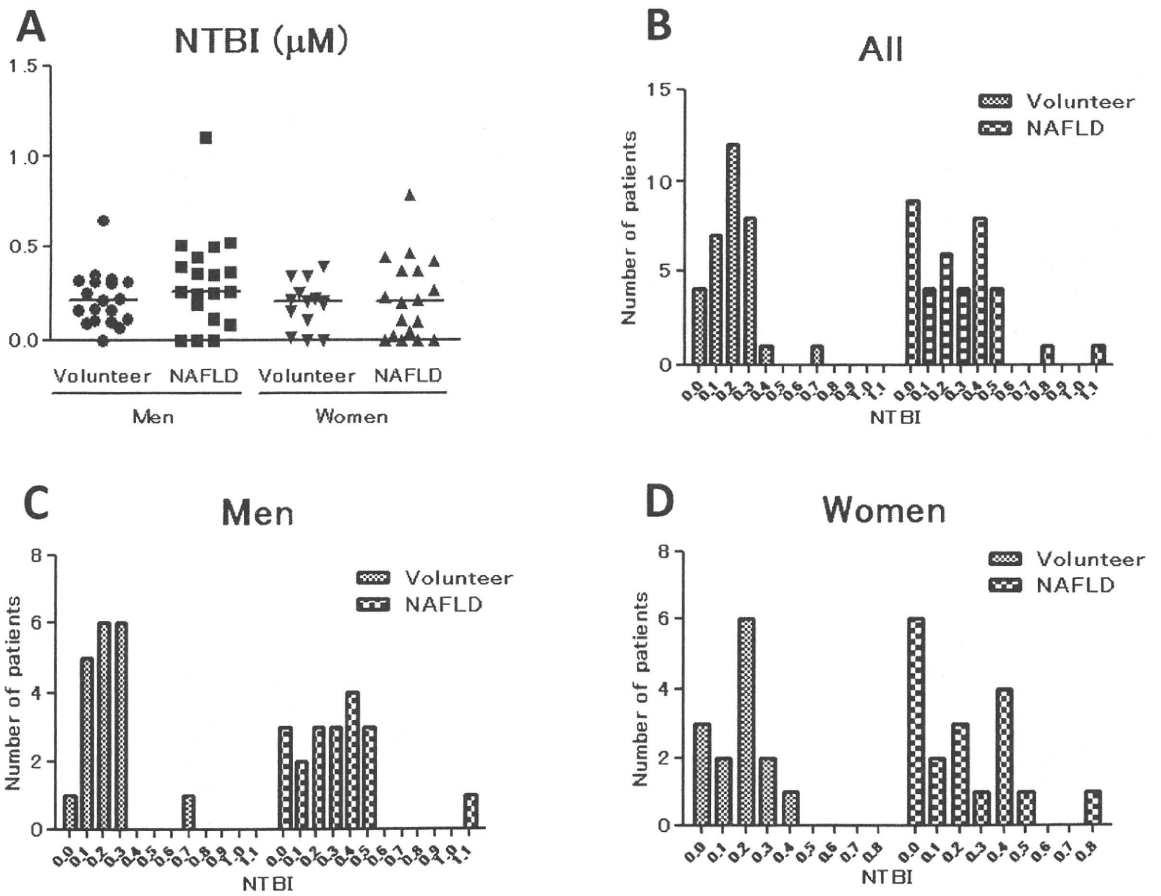

図2　血清NTBI濃度．（A）散布図．（B）ヒストグラム（全例）．（C）ヒストグラム（男性）．（D）ヒストグラム（女性）．NAFLD男性例では健常ボランティアの75%パーセンタイル以上（0.31μM）を示す症例が47%存在し，NTBIが高い傾向があった．

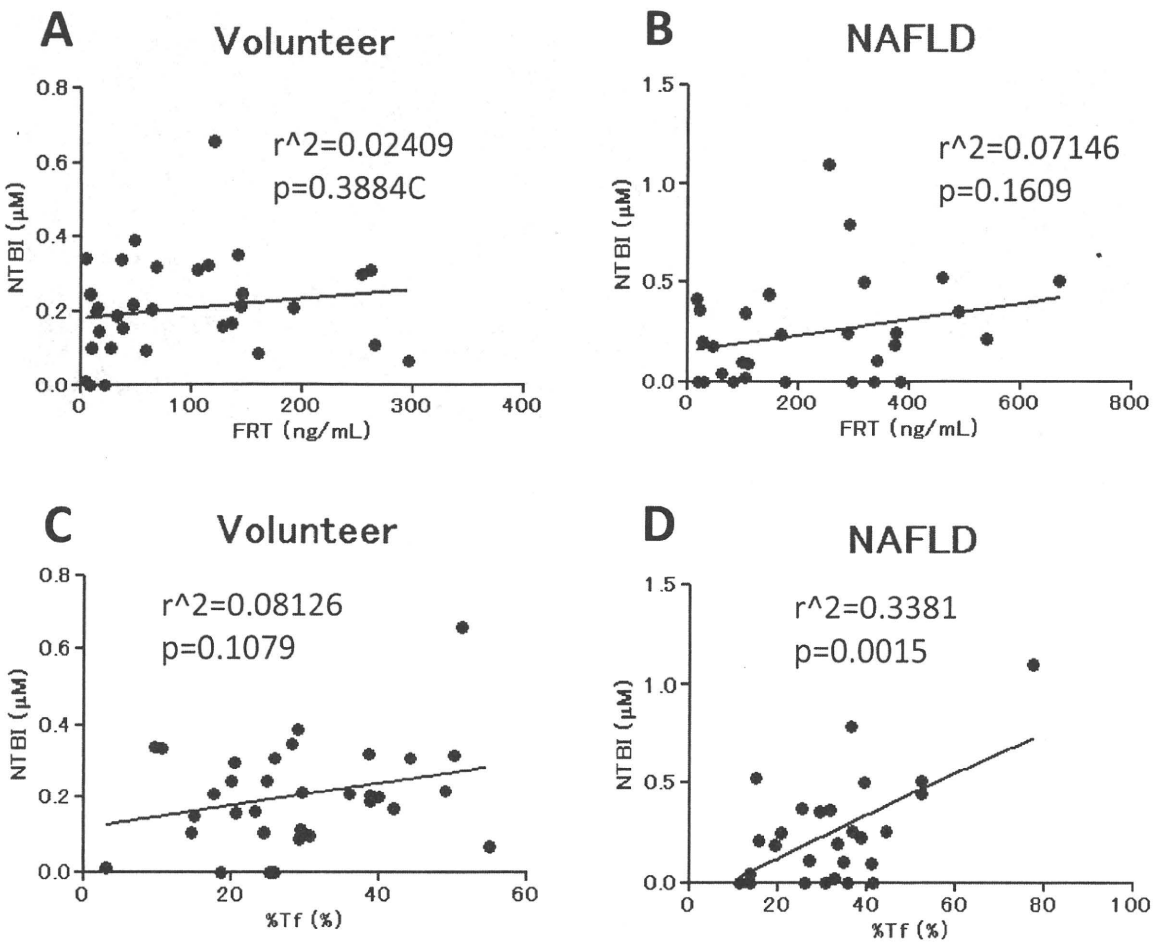

図3　血清フェリチンまたは% Tf と NTBI との関連．(A)健常者の血清フェリチンと NTBI．(B)NAFLD 症例の血清フェリチンと NTBI．(C) 健常者の% Tf と NTBI．(D) NAFLD 症例の% Tf と NTBI．NAFLD 症例において% Tf と NTBI は正の相関を認める（r^2＝0.3381，p＝0.0015）．

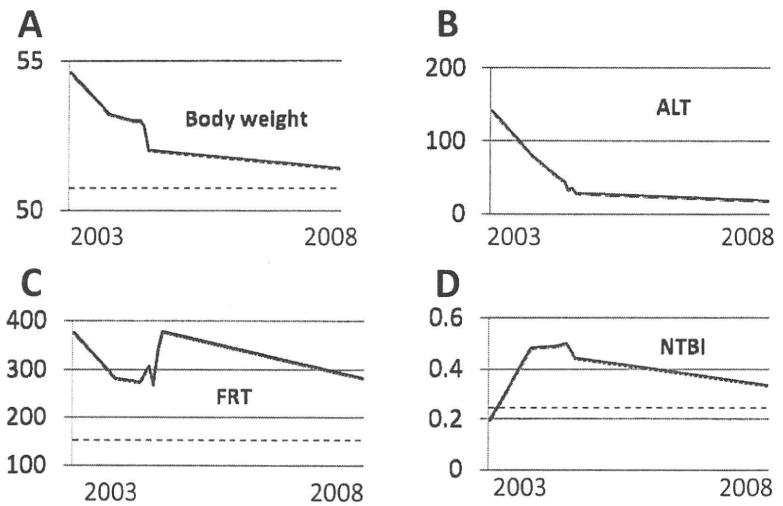

図4　提示症例の臨床経過．(A) 体重（kg）．(B) 血清 ALT 値（IU/L）．(C) 血清フェリチン値（ng/mL）．(D) 血清 NTBI 濃度（μM）．

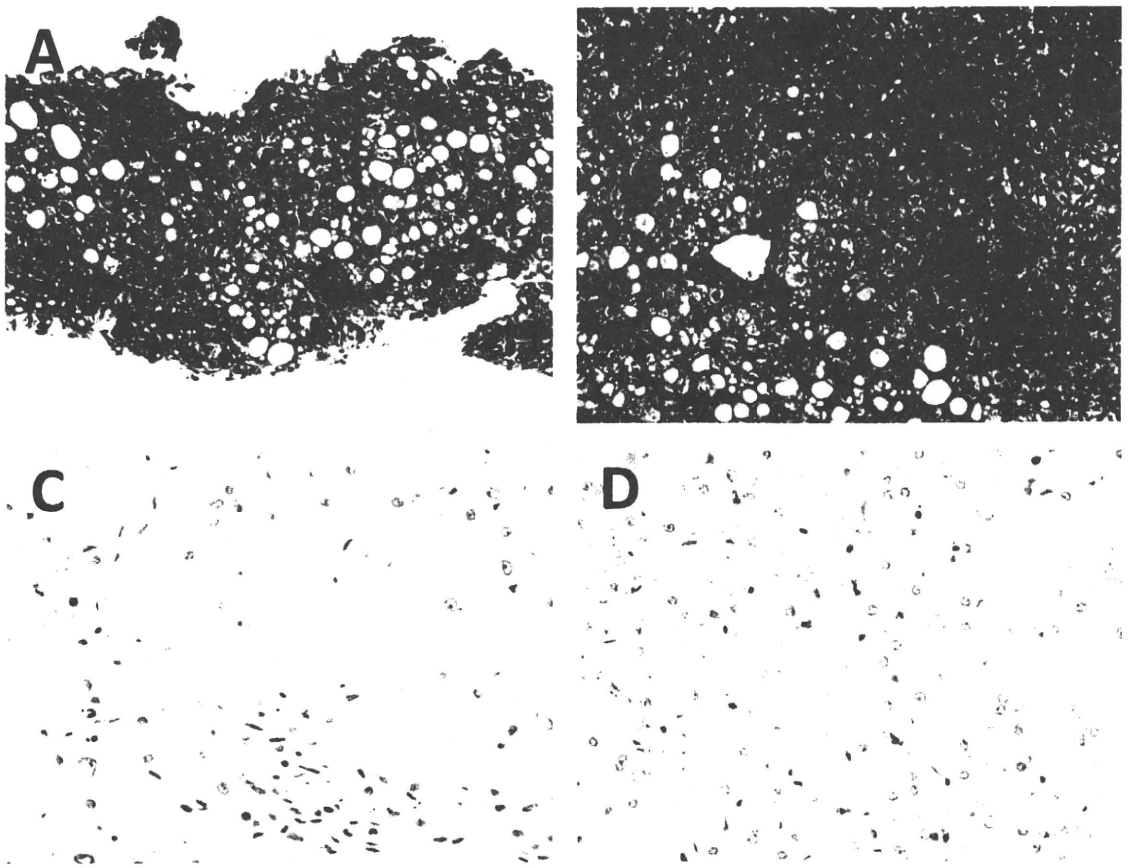

図5　（A）2003年初診時肝生検（Azan染色）．（B）2008年2回目の肝生検（Azan染色）．（C）初回肝生検（鉄染色）．（D）2回目肝生検（鉄染色）．倍率：100倍（A，B），400倍（C，D）．

沈着の改善はない（**図5B, D**）．血清NTBIは0.4 μM前後の軽度高値を持続している（**図4D**）．

4．考　　察

NAFLDの病理は肥満に伴う肝細胞への中性脂肪の沈着から始まり，脂肪酸による脂肪毒性，インスリン抵抗性が病態の基盤をなしている．病態を進展させる因子としてサイトカイン，エンドトキシン，酸化ストレスが提唱されている．そして，C型慢性肝炎[5]，アルコール性肝障害[6),7)]と同様にNAFLDにおいても二次性の肝内鉄沈着が高頻度に認められ，肝細胞障害，肝線維化，肝発癌に寄与する可能性が指摘されている[3)]．鉄沈着による肝細胞障害は自由鉄の増加によるラジカル産生増加を介した酸化ストレスである．NAFLD症例では有意に血清フェリチンが上昇し，鉄過剰症であることが確認された．

血清NTBI測定の検討はもっぱら遺伝性ヘモクロマトーシス[8)]や鎌状赤血球，サラセミアなど[9)]の輸血依存性の難治性貧血患者における長期大量輸血後の高度鉄過剰症に関して報告されている．それに対してNAFLDを含めた慢性肝疾患の鉄過剰は軽度から中等度のものであり，その測定は検査系の感度の問題から不可能であった．今回我々が構築した測定系はNAFLD患者だけでなく，健常者の血清NTBI濃度を測定することが可能であった．その結果，NAFLD症例において細胞毒性の高い血清NTBIが正常の群と高値群が存在し，特に男性症例において高値症例が多く存在していることが示された．

血清NTBIと他の鉄関連マーカーとの関係では，健常者においては血清フェリチン，% Tfともに相関を認めなかった．しかし，軽度から中等度の鉄過剰のみられるNAFLD症例において血清

NTBI値は血清フェリチンとは相関を認めなかったが，% Tfとは正の相関を示していた．血清フェリチンは肝内貯蔵鉄のよいマーカーではあるが，肝細胞障害の強い場合にApo-ferritinが血中に逸脱するため，実際の貯蔵鉄量と解離することがある．今回，NAFLD症例において% TfとNTBIが相関していたことは，その測定値の信頼性の高さを示すものと考えられる．そして，% Tf高値NAFLD症例においては全身的な鉄毒性に暴露されていることが確認され，このような病態がNAFLDの肝外病変である心血管系疾患にも関与している可能性があると考えられる．

提示症例はNAFLDの鉄代謝異常を考察するうえで興味深い症例である．食事療法，運動療法によって体重減少がみられ，肝機能も改善しており，実際にフォローアップの肝生検では脂肪沈着が改善していた．しかし，血清フェリチン高値が持続しており，肝組織像も脂肪沈着以外の炎症，線維化および鉄沈着も改善していなかった．現在，NAFLDに対する除鉄治療の有効性は報告されているが[10]，本例ように血清ALT値が正常である肝内鉄過剰症例における除鉄治療の適応についてはその血清フェリチンのカットオフ値を含め議論が必要と考えられる。本例では血清NTBIの高値も持続していたことから，潜在的な全身的鉄毒性が持続していると考え，NAFLDの肝外病変である心血管系疾患のリスク軽減も考慮し，瀉血療法を試みている．今後，NAFLD症例が増えるとともに本例のようなケースも多く経験されると考えられるので，その除鉄治療の適応に関しては検討が必要である．

5．結　語

NAFLD症例において血清NTBIが高い群，つまり，全身的に鉄細胞毒性の強い群が存在し，特に男性で多い傾向があった．NAFLDにおいて血清NTBIは鉄毒性の評価に有用な血清マーカーと考えられた．

文　献

1) 高後　裕，生田克哉．Iron Overloadと鉄キレート療法．総論－生体鉄代謝の分子機構．25-35．メディカルレビュー社
2) Cabantchik ZI, Breuer W, Zanninelli G, et al. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol. 2005 Jun; 18(2): 277-87.
3) Angulo P, Keach JC, Batts KP, et al. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999; 30: 1356-62
4) Gosriwatana I, Loreal O, Lu S, Brissot P, et al. Quantification of non-transferrin-bound iron in the presence of unsaturated transferrin. Anal Biochem. 1999 Sep 10; 273(2): 212-20.
5) Kato J, Miyanishi K, Kobune M, et al. Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol. 2007 Oct; 42(10): 830-6.
6) Harrison-Findik DD, Schafer D, Klein E, et al. Alcohol metabolism-mediated oxidative stress down-regulates hepcidin transcription and leads to increased duodenal iron transporter expression. J Biol Chem. 281(32): 22974-82. 2006.
7) Ohtake T, Saito H, Hosoki Y, et al. Hepcidin is down-regulated in alcohol loading. Alcohol Clin Exp Res. 31(1 Suppl): S2-8. 2007.
8) Le Lan C, Loréal O, Cohen T, et al. Redox active plasma iron in C282Y/C282Y hemochromatosis. Blood. 2005 Jun 1; 105(11): 4527-31.
9) Piga A, Longo F, Duca L, Roggero S, et al. High nontransferrin bound iron levels and heart disease in thalassemia major. Am J Hematol. 2009 Jan; 84(1): 29-33.
10) Valenti L, Fracanzani AL, Dongiovanni P, et al. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case-control study. Am J Gastroenterol 2007; 102: 1251-8

2．生体内不安定鉄と鉄毒性と鉄キレート療法

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Summary　鉄は生体にとって必須の金属であるが，過剰に存在すると容易にラジカル産生を引き起こすため有害となる。そのため，血液中ではトランスフェリン，細胞内ではフェリチンといった鉄結合蛋白によって鉄はfreeであることを回避されている。しかし，これらのcapacityを超えると，血液中では非トランスフェリン結合鉄が，また，細胞内ではlabile iron poolと呼ばれる「不安定な鉄」が出現し，生体に障害をもたらす。鉄キレート療法はこれらの有害な鉄を除去する治療法であり，最近，新規経口鉄キレート剤が本邦でも使用可能になり注目されている。

1．生体内鉄代謝の概要

鉄は，ヘモグロビンの構成要素として赤血球造血に必須なだけではなく，DNA合成など全身の細胞の様々な代謝に必要であり，生体にとって必要不可欠な金属元素である[1)]。

生体内における鉄の動き，すなわち生体内鉄代謝の概要を生体に鉄が取り込まれるところからみてみると，食事に含まれる鉄は消化管にて吸収されて血液中に入り，トランスフェリン（transferrin：Tf）と呼ばれる蛋白に結合した形で運搬される[2)]。一部の鉄は肝臓での貯蔵や筋肉などの全身の組織での利用にまわされるが，大部分の鉄は骨髄での赤血球造血に利用されている。一方，生体は鉄を積極的に体外に放出する機構を有しておらず，通常では消化管粘膜上皮細胞の脱落などに伴うわずかな損失しかなく，それを補う分だけ消化管から吸収している。生体内のほとんどの鉄は各臓器を行き来しながら生体内にとどまり，半閉鎖的な回路を構築している。生体で利用される大部分の鉄は，網内系で寿命となった赤血球のヘモグロビンから鉄が取り出され，再びTfと結合して全身を循環し再利用されることによってまかなわれている（図1）[3)]。

Tf (transferrin；トランスフェリン)

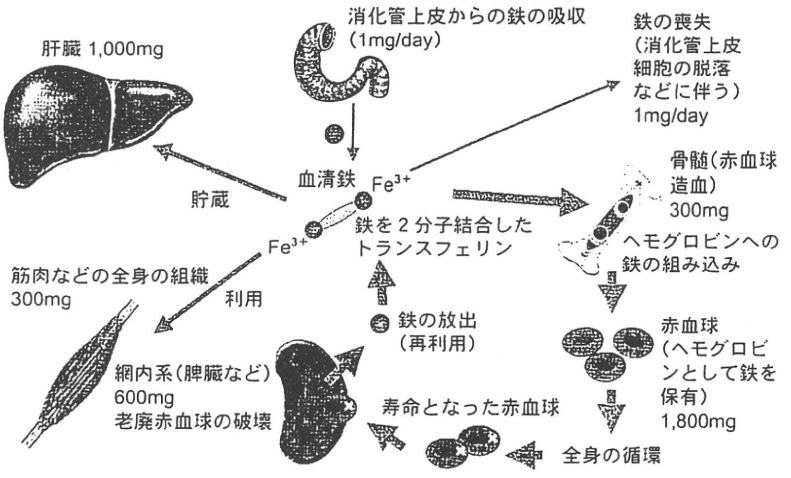

図1　生体内鉄代謝の概要

生体内で多くの鉄は赤血球造血に利用され，赤血球中のヘモグロビンに含まれ全身を循環しているが，一部は肝臓に貯蔵されたり，筋肉などの全身の細胞によって利用されている。生体は鉄を積極的に体外に排出する機構を有しておらず，通常はこれに見合った少量しか消化管から吸収しておらず，大部分の鉄は老廃赤血球を網内系で破壊して再利用してまかなっている。そのため，生体内鉄代謝は半閉鎖系を構築していると言える。

2．鉄の毒性

鉄代謝は多くの分子が複雑に関与して調節されていることが最近の研究で明らかになってきている[3]。これは，鉄が生体内に必要不可欠である一方で，過剰に存在するようになると逆に毒性を呈してくるためである。そこで次に，鉄が生体に対して及ぼす毒性について見ていく。

鉄は遷移金属であり，細胞内環境において，electron donorである2価鉄とelectron acceptorである3価鉄の間で容易に反応するため，生体内での様々な生化学的反応に利用されている。しかしながら，こうした特性は逆に有害なラジカルを産生するよう働き得るため，過剰状態では生体に対して毒性を示すこととなる。これは主にFenton反応によるもので，最終的にスーパーオキシド（O_2^-）や過酸化水素（H_2O_2）から，ヒドロキシラジカル（OHラジカル：$OH^\cdot$）が産生される（図2）。これらは活性酸素種reactive oxygen species（ROS）と呼ばれるが，OHラジカルはその中でも最も強力なもので，多糖類，蛋白質，核酸，脂質などを標的とし，細胞成分の酸化によって細胞に障害を与え細胞死を引き起こしたり，変性疾患の発症や発癌とも関連すると考えられている[4]。

また，鉄過剰状態ではOHラジカルのみではなく，peroxyl（$ROO^\cdot$），alkoxyl（$RO^\cdot$），thiyl（$RS^\cdot$），thiyl-peroxyl（$RSOO^\cdot$）などの各種のラジカルが産生される。

ROSにより生体内の様々な分子が修飾されるが，その一部は酸化ストレスのマーカーとして利用されている。主に脂質過酸化の指標としては

ROS (reactive oxygen species)

$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^{\cdot}$ (Fenton 反応) $Fe(III) + O_2^- \rightarrow Fe(II) + O_2$
(net reaction) $H_2O_2 + O_2^- \rightarrow OH^- + OH^{\cdot} + O_2$ (Haber - Weiss 反応)

図2　Fenton 反応および Harber-Weiss 反応
生体内に鉄が過剰になると，これらの反応を介してラジカルが産生され，生体に対して障害をもたらす。

4-hydroxy-2-nonenal (HNE) が，また，DNA 障害の指標としては 8-hydroxy-2'-deoxyguanosine (8-OHdG) が知られている。アルコール性肝障害における肝細胞での HNE と鉄の局在が一致するという報告[5]や，C 型慢性肝炎において瀉血と鉄制限食により 8-OHdG の低下が認められるという報告[6]は，ともに鉄が ROS を介して肝障害をもたらすことを示唆するものと考えられる。

こうした ROS 産生は，特に「free (自由)」な鉄が関与するため，生体内では鉄はヘム鉄（ヘモグロビンやミオグロビンなど），フェリチン，ヘモジデリン，トランスフェリンなどといった，鉄結合蛋白に結合する形となり，free な状態で存在することは回避されている[7]。しかしながら，生体が何らかの原因によって鉄過剰状態になると，鉄結合蛋白に結合しえなくなった free な鉄が出現してくることになる。Free な鉄は種々の形態をとっているが，その結合は弱いものであり，容易に ROS 産生などに関与することから，いわゆる「不安定鉄」と呼ばれる。これには，循環血液中における非トランスフェリン結合鉄 (non-transferrin bound iron：NTBI) や，細胞内における不安定鉄プール (labile iron pool：LIP) といったものがあげられる[8~11]。

3．循環血液中での不安定鉄：非トランスフェリン結合鉄

まず，循環血液中における不安定鉄について見てみる。血液中では鉄は主に Tf に結合した状態である。この Tf は肝臓で産生され全身の血液中を循環している分子量約8万の糖蛋白であり，Tf1 分子で2分子の鉄を結合することができる[2]。Tf の鉄に対する親和性は非常に高く，正常な状態においては消化管から流入した鉄や網内系から再利用に回された鉄はすぐに Tf に結合することができ，そのため，鉄は血液中で free の状態にならずにすむ[7]。鉄の中でも，特に free の状態で存在する鉄は，redox cycling を介した ROS を産生する反応に容易に関与して生体に毒性をもたらすが，Tf と結合することで，こうした鉄の毒性が回避されている。血液中において，Tf の鉄を結合する capacity は，total iron binding capacity (TIBC) という形で表わされるが，通常は大きくて十分な予備力を有しており，正常な場合では 30%程度の Tf しか鉄で飽和されていない。しかし，厳密に制御されているはずの生体内鉄代謝調節が，何らかの原因によって崩れてしまい鉄が体内に過剰に存在する方向に傾くと，まず血液中で Tf が過剰な鉄を結合していくが，次第に Tf の鉄を結合できる capacity が飽和されてくる。Tf 飽和度が 100%もしくはそれに近い状態になると，Tf に結合しきれなかった鉄が free の形で血液中に出現してくることになり，これを総称して NTBI と呼ぶ[8]。生体内鉄代謝が崩れ鉄過剰に傾き得る原因として代表的なものは，サラセミア，

HNE (4-hydroxy-2-nonenal)　8-OHdG (8-hydroxy-2'-deoxyguanosine)
NTBI (non-transferrin bound iron；非トランスフェリン結合鉄)　LIP (labile iron pool；不安定鉄プール)
TIBC (total iron binding capacity)

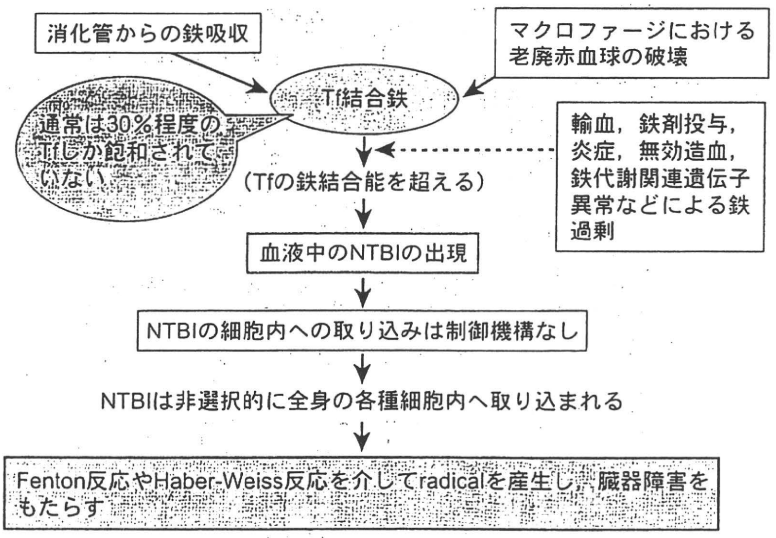

図3　NTBI の出現と臓器障害

消化管から吸収された鉄や，マクロファージにおける老廃赤血球の破壊により得られた鉄は，血液中ではトランスフェリン (transferrin：Tf) と結合して存在する。通常は 30%の Tf しか飽和されていないが，輸血，鉄剤投与，炎症などに伴う鉄過剰が加わると，次第に Tf の鉄の結合能力を超えてしまい，血液中に非トランスフェリン結合鉄 (non-transferrin bound iron：NTBI) が出現してくる。NTBI の細胞内への取り込みには制御機構がなく，全身の細胞に非選択的に取り込まれてしまい，最終的に Fenton 反応などを介してラジカル産生を引き起こし，細胞・臓器障害をもたらす。

骨髄異形成症候群，再生不良性貧血などの輸血依存性を呈する血液疾患，遺伝性ヘモクロマトーシス，鉄剤の投与を受けている透析患者などが知られている[12]。さらに最近では，全身感染症，C 型慢性肝炎，アルコール性肝障害[13]，各種の化学療法中[14]，cardiopulmonary bypass[15] などの際にも，NTBI が出現してくることが明らかとなっている。最近特に注目されているのは輸血による鉄過剰症で，輸血は短期間に急激に生体内に鉄を負荷する状態をつくるため，頻回で長期間にわたる輸血は容易に鉄過剰症をきたす。以前は，Tf に結合しきれない状態になって，すなわち Tf 飽和度が 100%になって初めて血中に出現すると考えられていたが，最近では必ずしもそうではなく，Tf の結合能がある程度残っていても血液中に free な鉄を認める場合が確認されてきており，Tf 飽和度が 80%程度から血液中で NTBI が検出されると考えられるようになってきた。

Tf に結合している Tf 結合鉄は，Tf と非常に高い親和性で結合する細胞膜表面の transferrin receptor 1 (TfR1) を介して，骨髄中の赤芽球のように鉄を大量に必要とする細胞に選択的に取り込まれ利用されている[16]。このように Tf 結合鉄に関しては行き先の方向性が明確であるが，NTBI にはこうした方向性がないと考えられており，そのため様々な細胞に無秩序に入り込み，最終的には細胞内鉄過剰状態をもたらし，ROS 産生などを介して細胞障害を引き起こす(図3)。例

TfR1 (transferrin receptor 1)

えば，無トランスフェリン血症と呼ばれる稀な疾患では，血清中のTf量が非常に少ないためTfと結合する鉄量は極めて少なく，その一方でNTBIが非常に増加し肝臓などに鉄の蓄積を引き起こす。

NTBIについては，1978年にHershkoらが提唱してからすでに30年ほど経過するが，詳しいことは実は未だによくわかっていない[17]。これまでの情報から，NTBIは単一の形態で存在するものではなく，heterogeneousなものであると考えられている。まず，Tfと結合できなかった鉄は，血液中で様々な物質と結合する形になるが，例えばcitrateやalbuminがその代表として考えられている。また，NTBIは様々なキレート剤でキレートしてみることで，heterogeneousなものであるということがわかる。Desferrioxamine (DFO)でキレートされる分画や，oxalateでmobilizeされなければdetectできない分画などがあり，疾患それぞれで認められるNTBIが異なっていると考えられている。

最近，labile plasma iron (LPI)という用語が使用されるようになってきているが，これはNTBIのうち，特にredox cyclingに影響を及ぼすactiveな分画で鉄キレート剤によってキレートされ，全身の細胞に対して極めて細胞障害をもたらしやすい分画を示している[18]。

臨床的にはNTBIが測定できると，特に鉄過剰状態における鉄代謝の状態を詳細に把握することができるため非常に有用と考えられるが，これが極めて難しく，現在まで様々な定量法の報告はあるが，まだ信頼性・普遍性・簡便性のどれをも満たすような方法はないのが実情である。現在までに報告されている方法には以下のようなものがある。① 最初にNTBIをshuttle molecule (ethylene diamine tetraacetic acid〔EDTA〕，nitrilotriacetic acid〔NTA〕など)でmobilizeさせ，その後血清蛋白をMW 30,000でcut-offされるmicrofilterで分離し，低分子量の分画をatomic absorptionもしくはcolorimetry，またはcolorimetryを組み合わせたHPLCで解析する方法は，現時点では最も信頼性はあるとされている。一方，ごく限られた施設でしか施行し得ない方法である[19]。② 上記方法に対して，NTBIのmobilizationと検出を血清蛋白の分離なしで行う方法がある。はじめに抗生物質であるbleomycinの鉄キレート能を利用してNTBIを捕捉し，次にbleomycinと鉄の複合体をascorbateなどの強力な還元剤の存在下でラジカル産生を誘導する。ここにDNAなどの酸化を受けうる物質を加えることで，酸化量とbleomycin-鉄複合体との関係が得られ，すなわちNTBIがどれだけ存在したか定量できる。ここで使用するascorbateは，還元剤としての作用とmobilizerとしての作用の両方を担うことになる。利点は比較的簡便に測定でき得ることであるが，原理的に間接的測定法であるため信頼性にやや劣ると考えられている[20]。③ 次に，蛍光標識したDFO (fluorescein tagged DFO：Fl-DFO)もしくはTf(fluorescein tagged Tf：Fl-Tf)を用いる方法があげられる。まず，血清に対しこれらをdirectに加えることで，NTBIの中でもDFO-chelatableなNTBI分画が結合し，その結果，蛍光が減弱するので，この変化を検出することでDFO-chelatable-NTBI量を定量することができる。加えて，oxalateやNTAでmobilize処理することでnon-DFO-chelatableなNTBI分画もFl-DFOに結合するため，これを検出できるというものである。Fl-Tfはoxalateと結合した鉄とは結合するが，Tfからの鉄を奪わないというものとして開発されており，特にoxalateでmobilizeされるNTBI分画を定量する[21]。利点としては感

DFO (Desferrioxamine)　LPI (labile plasma iron)　EDTA (ethylene diamine tetraacetic acid)
NTA (nitrilotriacetic acid)　Fl-DFO (fluorescein tagged DFO)　Fl-Tf (fluorescein tagged Tf)