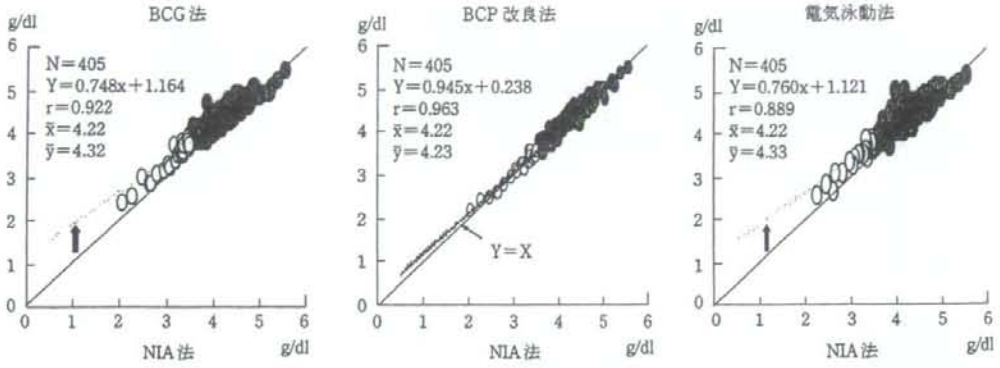

Figure 1 免疫法と各測定法との血清アルブミン値の相関

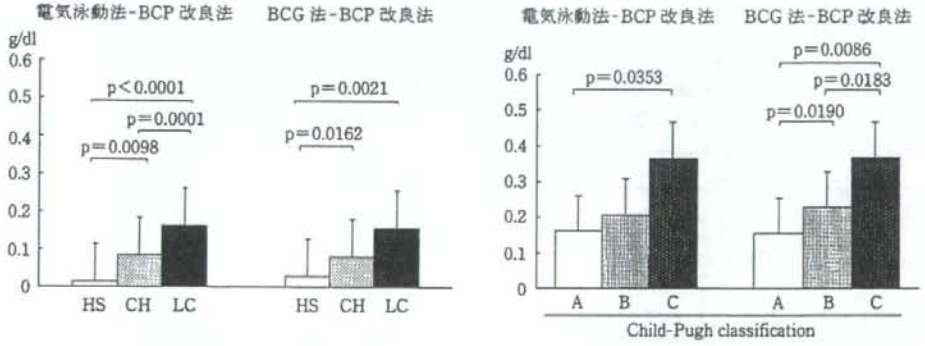

Figure 2 慢性肝疾患における血清アルブミン値と測定法による差
HS; healthy subject, CH; chronic hepatitis, LC; liver cirrhosis

法に比較して高値の傾向がみられたが，BCP 改良法は免疫法との低値例においても乖離することがなかった。

B. 慢性肝疾患における血清アルブミン値と測定法による差

慢性肝疾患における血清アルブミン値の各測定法による差について検討した(Fig. 2)。電気泳動法と BCP 改良法の測定値の差は健常者で−0.04±0.19g/dl，慢性肝炎で 0.07±0.19g/dl，肝硬変で 0.18±0.16g/dl であり，健常者に比較して慢性肝炎，肝硬変で電気泳動法での測定値は BCP 改良法の測定値に比較して有意に高値であった(p=0.0098，p<0.0001)。BCG 法と BCP 改良法の測定値の差は健常者で 0.06±0.12g/dl，慢性肝炎で 0.14±0.15g/dl，肝硬変で 0.16±0.13g/dl であり，健常者に比較して慢性肝炎，肝硬変で BCP 改良法の測定値に比較して BCG 法の測定値は有意に高値であった(p=0.0162，p=0.0021)。肝硬変を Child-Pugh 分類[4]で Grade A，Grade B，Grade C の 3 群に分け比較検討した。電気泳動法と BCP 改良法の測定値の差は Grade A で 0.16±0.16g/dl，Grade B で 0.21±0.14g/dl，Grade C で 0.36±0.10g/dl であり，Grade A に比較して Grade C で BCP 改良法の測定値に比較して電気泳動法の測定値は有意に高値であった(p=0.0353)。BCG 法と BCP 改良法の測定値の差はそれぞれ 0.16±0.13g/dl，0.23±0.09g/dl，0.37±0.08g/dl であり，Grade A に比較して Grade B，Grade C で BCG 法の測定値は BCP 改良法の測定値に比較して有意に高値であった(p=0.0190，p=0.0086)。

C. 浮腫の合併の有無による血清アルブミン値の測定差

血清アルブミンは血漿膠質浸透圧の保持に極めて

重要であり，血清アルブミン値の低下により血漿膠質浸透圧が低下し浮腫や腹水が出現する。そこで浮腫の合併の有無による血清アルブミン値の測定差について検討した(Fig. 3)。電気泳動法と BCP 改良法の差は浮腫非合併群では 0.12±0.20g/dl，浮腫を合併している群では 0.24±0.15g/dl であり，浮腫合併例では BCP 改良法に比較して電気泳動法の測定値は有意に(p=0.0001)高値であった。また BCG 法と BCP 改良法の差は浮腫非合併群では 0.16±0.14g/dl，浮腫合併群では 0.25±0.13g/dl であり浮腫合併例では BCP 改良法の測定値に比較して BCG 法の測定値は有意に(p<0.0001)高値であった。

D. SGA による血清アルブミン値の測定差

最近注目されている栄養評価法である自覚的包括的栄養指標(SGA)で血清アルブミン値の測定値の差を比較検討した(Fig. 4)。電気泳動法と BCP 改良法の差は SGA-A(栄養状態良好)では 0.14±0.20g/dl，SGA-B(中等度の栄養状態不良)で 0.24±0.15g/dl と SGA-A に比較して SGA-B で BCP 改良法の測定値に比較して電気泳動法の測定値は高値の傾向であった(p=0.0576)。また BCG 法と BCP 改良法の差も SGA-A では 0.18±0.14g/dl，SGA-B で 0.26±0.18g/dl と SGA-A に比較して SGA-B では BCP 改良法の測定値に比較して BCG 法の測定値は有意に(p=0.0176)高値であった。

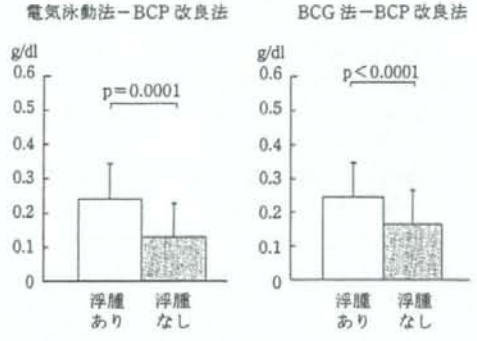

Figure 3 浮腫の合併の有無による血清アルブミン値の測定差

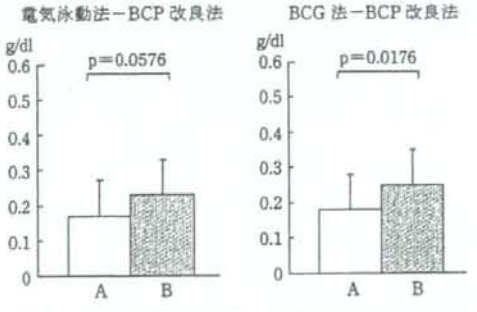

Figure 4 自覚的包括的栄養指標(SGA)による血清アルブミン値の測定差
SGA-A; 栄養状態良好，
SGA-B; 中等度の栄養状態不良

III. 考　　察

慢性肝疾患患者において蛋白栄養状態を的確に評価することは重要であることはいうまでもないが，臨床の現場において明らかな低アルブミン血症があるにも拘らず腹水や浮腫がみられない症例や逆に血清アルブミン値は正常であるにも拘らず浮腫や腹水がみられる症例も少なからず存在する。この原因として慢性肝疾患におけるアルブミンの質的な変化が推測される。そこで今回，血清アルブミン値の測定法として実用的基準法である免疫法と日常一般法である BCP 改良法，BCG 法，電気泳動法で測定し比較検討を行った。BCP 法はアルブミンに特異的な方法であるが酸化型アルブミンが増加するような病態下においてはその測定値は高値を呈することが知られている。BCP 改良法はその問題点を改良した方法である[1]。今回の我々の検討において BCP 改良法の測定値は免疫法での測定値と遜色はみられず免疫法に匹敵する方法[5][6]と考えられた。各測定法の差の原因として BCG 法ではアルブミンの特異性に問題[7]があり，肝硬変患者ではグロブリン分画の影響を受けている可能性や黄疸の存在などが影響している可能性が指摘され，電気泳動法が高値の原因は，染色に用いるポンソ 3R の染色性が γ-グロブリンを基準とすると，アルブミンはその 1.5 倍の呈色率を示すとされ各種の蛋白質を正確に検出していない[8]などが指摘されている。しかし，渡辺らは[9]血清アルブミン値低値例において酸化型アルブミンが増加し，肝硬変の病態が進行するに伴い酸化型アルブミンが増加することを報告しているが，今回の我々の検討でも広く臨床の場で用いられているアルブミンの測定法の BCG 法や電気泳動法での測定値は BCP 改良法での測定値に比較して高値であった。また慢性肝疾患の病態の進行に伴い BCP 改良法の測定値に比較して BCG 法，電気泳動法の測定値は明らかに高値

であった[10][11]。今回の検討でも浮腫合併例では浮腫非合併例に比較してBCG 法と電気泳動法の測定値はBCP 改良法の測定値に比較して高値であったことからBCP 改良法に比較して BCG 法と電気泳動法が高値であったことはアルブミンの質的変化の一端を反映している可能性も推測された。また SGA は，血清アルブミンや身体計測値などとよく相関し，栄養不良による感染症などの合併症を予測するのに有用といわれている。我々の SGA における検討でも栄養状態良好群に比較して栄養状態不良群で BCP 改良法の測定値に比較して BCG 法と電気泳動法の測定値は高値であった。したがって現在臨床の現場で用いられている BCG法，電気泳動法では必ずしも病態を的確に評価できるとは考えにくい[12]。しかし，アルブミン低値例においても BCP 改良法と免疫法の測定値はほぼ一致したことから慢性肝疾患患者における血清アルブミン値の測定法として BCP 改良法は免疫法に匹敵する測定法と考えられた。

IV. 結　　語

慢性肝疾患患者における血清アルブミン値は，測定法によりその測定値が大きく異なり，特に病態が進行するに伴い，また蛋白栄養状態が悪化するに伴い BCP 改良法に比較して BCG 法と電気泳動法による測定値は高値となる。しかし，BCP 改良法での測定値は実用的基準法の免疫法とアルブミン低値例においても優れた相関を示すことからアルブミンの測定は少なくとも BCP 改良法で行うことが重要であると考えられた。

本論文の要旨は，第 52 回日本臨床検査医学会（福岡）で発表した。

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公募のお知らせ

本誌では，シリーズ「病院検査部が行うコンサルティング業務の実際」について，
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基礎編 貧血の分子病態—総論—

鉄代謝と病態

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Iron metabolism and anemia

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Abstract

Iron is essential for all living organisms. Iron is taken up from the foods by enterocytes of the duodenum and proximal jejunum, and then released into the plasma and transported to whole body by binding to transferrin. Transferrin-bound iron is utilized mainly for erythropoiesis at the bone marrow, in which iron is essential for the formation of heme. Recently, a new anti-microbial peptide, named hepcidin, was identified, and hepcidin is found to function as the regulator of body iron metabolism by inhibiting iron uptake at enterocyte and iron release from reticuloendothelial macrophages. Hepcidin is produced by hepatocytes, and the expression is modulated by inflammation so that hepcidin is thought to be involved in the pathophysiology of anemia of chronic disease. Research in the iron metabolism field has been developing rapidly these years, and the new innovational therapies for the disease caused by the dysregulation of iron metabolism are expected.

Key words: anemia, iron metabolism, hepcidin, anemia of chronic disease (ACD), mitochondria, heme

はじめに

鉄は，生体内に存在する金属元素の中では最も多い．全身の細胞の分裂や増殖，様々な代謝などに必須であるが，ヘモグロビンの構成要素でもあり，赤血球における酸素の運搬になくてはならないものである．しかしながら，逆に鉄が過剰に存在してしまうと，細胞に対して毒性を示してしまうため，生体内において鉄代謝は巧妙に制御される必要がある[1]．

十数年前まではトランスフェリン(transferrin: Tf)，トランスフェリン受容体(現在ではトランスフェリン受容体1〔transferrin receptor 1: TfR1〕と呼ばれる)，フェリチンに関する理解が生体内鉄代謝に対する我々の理解のほとんどを占めていた．しかし，1996年に欧米で多い遺伝性ヘモクロマトーシスの原因遺伝子として同定されたHFEの発見以後[2]，数々の鉄代謝関連分子の発見が相次ぎ，生体における鉄動態にはこれら数多くの分子が関与して複雑に動いている

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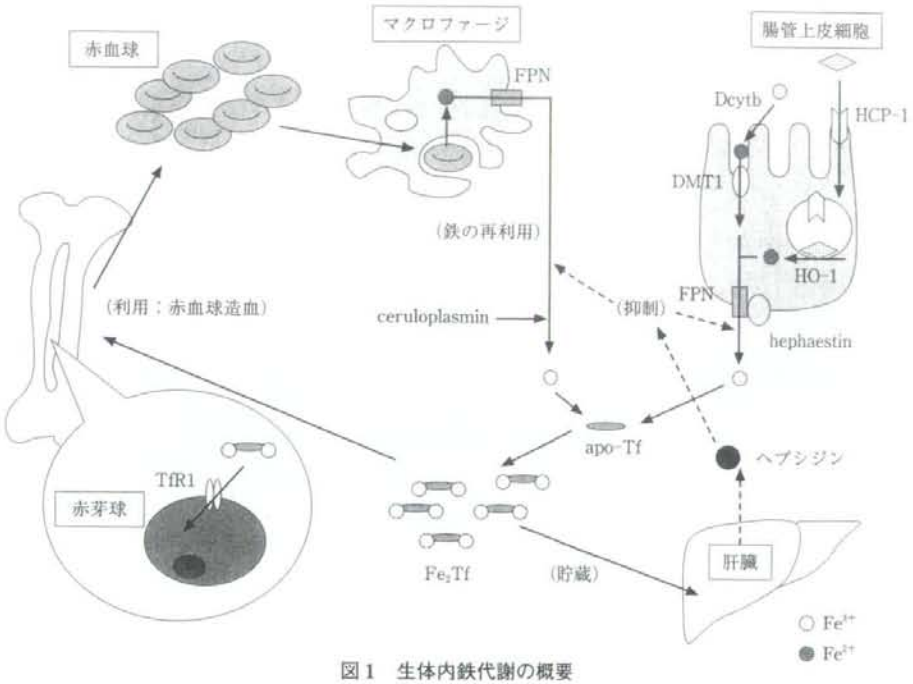

図1 生体内鉄代謝の概要

食事中に主に3価として含まれる非ヘム鉄は，上部小腸の腸管上皮細胞の腸管内腔側細胞膜上に存在するDcytbによって2価に還元され，DMT1によって腸管細胞内に運ばれ，その後血管内腔側に存在するferroportinによって血管内に放出され，hephaestinによって3価鉄に酸化され，Tfに結合し，全身に運搬される．多くの鉄は骨髄でほとんどTfR1を介して赤芽球内に取り込まれる．産生された赤血球は全身を循環するが，老廃赤血球は網内系のマクロファージにより捕捉され破壊される．そこで得られた鉄はferroportinを介して2価鉄として放出され，ceruloplasminにより3価鉄に酸化され，Tfと結合し再び体内を循環し再利用される．一部の鉄は肝細胞に貯蔵される．生体には鉄を積極的に体外に放出する機構がなく，こうした利用・再利用が大部分を占め，半閉鎖的回路となっている．

TfR1: transferrin receptor 1，FPN: ferroportin，HCP-1: heme carrier protein-1，HO-1: heme oxygenase-1，DMT1: divalent metal transporter 1，Dcytb: duodenal cytochrome b，Tf: transferrin（apo-Tf: Feと結合していないTf，Fe_2Tf: Fe分子を2分子結合したTf）．

ことが明らかとなってきた．現在理解されている概略を**図1**として示す．

1．食事からの鉄吸収

まず，食事からの鉄の吸収についてみていくが，食事中に含まれる鉄は，非ヘム鉄とヘム鉄に大別される．非ヘム鉄は主に3価鉄の形で存在しているが，上部小腸における腸管上皮細胞の腸管内腔側細胞膜上に存在しているduodenal cytochrome b（Dcytb）によって2価に還元され[3]，それから2価鉄トランスポーターであるdivalent metal transporter 1（DMT1）と呼ばれる分子によって腸管細胞内に運ばれる[4]．一方でヘム鉄は，最近同定されたheme carrier protein-1（HCP-1）によって細胞内へ取りこまれ，heme oxygenase-1によって分解される[5]．腸管細胞内に入った鉄は，その後血管内腔側に存在するferroportinによって2価鉄の形で血管内に放出されるが[6]，放出された2価鉄は，hephaestinと呼ばれる分子によって3価鉄に酸化される[7]．3価鉄の形となった鉄は，通常1分子のTfに対し2分子結合し，全身を運搬される

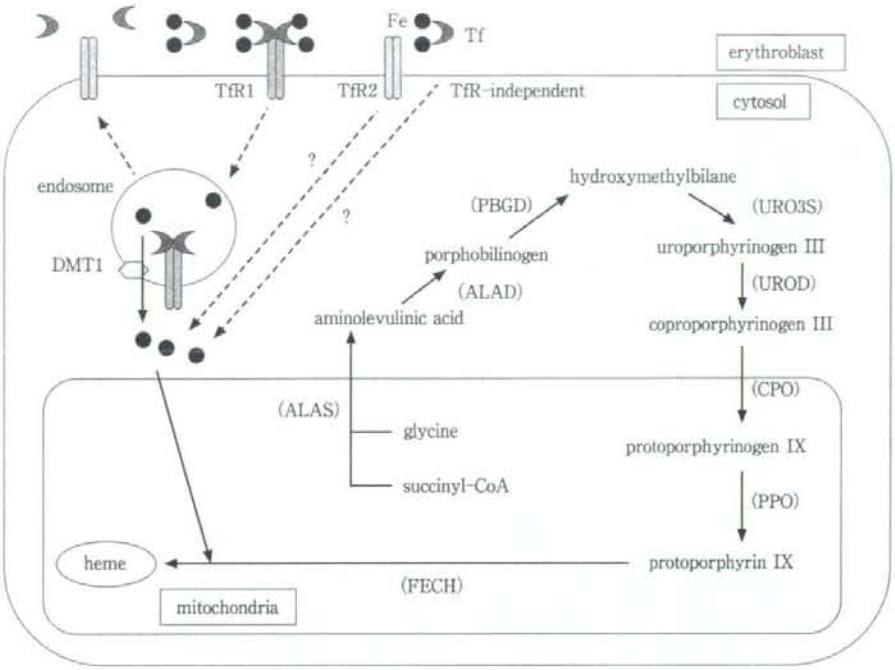

図2 骨髄赤芽球におけるヘムの合成経路と鉄の取り込み経路

肝細胞ではTfR1のほかにTfR2，およびTfR非依存性経路も想定されており，それらによってTf結合鉄が取り込まれ，その後，ヘムの合成経路の最終段階でprotoporphyrin IXに組み込まれる．

TfR1: transferrin receptor 1, TfR2: transferrin receptor 2, DMT1: divalent metal transporter 1, ALAS: aminolevulinic acid synthase, ALAD: aminolevulinic acid dehydratase, PBGD: porphobilinogen deaminase, UROS: uroporphyrinogen III synthase, UROD: uroporphyrinogen decarboxylase, CPO: coproporphyrinogen oxidase, PPO: protoporphyrinogen oxidase, FECH: ferrochelatase.

ことになる．

2. 鉄の利用

Tfに結合し全身を運搬されるTf結合鉄の大部分は骨髄の赤芽球においてTfR1を介して取り込まれ，赤血球造血に利用される．産生された赤血球はその後骨髄を出て全身を循環することになるが，約120日の寿命を終えた老廃赤血球は網内系のマクロファージにより捕捉され破壊される．この老廃赤血球の破壊によって鉄が得られるが，こうして得られた鉄はferroportinを介して2価鉄として再び血液中に放出される．その際にはceruloplasminのもつ鉄酸化作用によって3価鉄に酸化されることで血液中のTfに結合できるようになり，Tfと結合した鉄は再び体内を循環し再利用される．

造血などで利用されなかった一部のTf結合鉄は，全身の細胞において鉄を必要とする酵素に使用されたり，肝臓に貯蔵される．肝臓の肝細胞では，Tf結合鉄はTfR1を介した経路で取り込まれるが，このほかに肝細胞はTfR1のホモログ分子であるTfR2も発現しており，これを介した経路でもTf結合鉄を取り込む可能性もあり[8]，更にTfR非依存性経路も想定され[9]，複数の経路を利用していると考えられている．

一方で，生体は鉄を積極的に体外に放出する機構を備えていない．このため，生体内で動的に動いている鉄は，体内で利用・再利用されて

いる鉄が大部分を占めており，半閉鎖的な回路が構築されている．

3. 骨髄赤芽球における鉄代謝

骨髄の赤芽球では，ヘモグロビンが合成されるが，その構成成分はヘムとグロビンである．そのうち，**図2**に示すヘムの合成において，鉄は必須のものである．まず，骨髄赤芽球中のミトコンドリア内において，glycine と succinyl CoA からアミノレブリン酸合成酵素(aminolevulinic acid synthase: ALAS)によってアミノレブリン酸(aminolevulinic acid: ALA)が合成される．ALAS には様々な組織で発現している ALAS1 と，赤芽球系前駆細胞のみに発現している ALAS2 が存在しているが，この ALAS2 に変異が生じることで起こる疾患として X-linked sideroblastic anemia (XLSA)が知られている．これは小球性低色素性貧血を呈するが，鉄がたまったミトコンドリアが核の周囲に存在する鉄芽球を認めるものである．

合成された ALA は細胞質に移動するが，この ALA を前駆体として，その後 porphobilinogen, hydroxymethylbilane, uroporphyrinogen III, coproporphyrinogen III などの合成過程を経て，protoporphyrinogen IX が形成される過程で再びミトコンドリア内に入り，更に protoporphyrin IX が形成され，このピロール環の中心に ferrochelatase によって鉄が組み込まれ，ヘムが合成される[10]．ヘムは赤芽球の細胞質内で合成されたグロビン蛋白質と結合してヘモグロビンが形成され，分子状酸素を運搬することができる機能を獲得する．すなわち，正常な機能をもつヘモグロビンは，鉄，ポルフィリン環，グロビン蛋白質の3種から構成されている．鉄の赤芽球における欠乏，ポルフィリン環への鉄の組み込みの障害，グロビン合成障害などが生じると，赤血球の形成不全が起こり，いずれも小球性低色素性貧血を引き起こす．

4. 鉄代謝調節因子ヘプシジン

上述のように，生体には鉄を積極的に体外に排出する機構が存在しないため，生体内全体の鉄のバランスは，必然的に消化管での吸収と網内系での貯蔵・放出のレベルで調節を受けることになる．こうした調節は，骨髄での造血状態や，肝での鉄貯蔵状態に影響を受けることもわかっていたが，生体内での鉄の吸収・貯蔵・利用の部位が各々物理的に離れているため，鉄代謝全体を調節する何らかの液性因子の存在が想定されていた．想定はされながらも長い間同定されることのなかった鉄代謝調節因子であったが，2000年に入って状況が一変した．2000-01年にかけて2つのグループによって，新規の内因性抗菌ペプチドが発見され，ヘプシジンと名づけられた[11,12]．ヘプシジンは，活性型が25アミノ酸という短いペプチドである．遺伝子は第19番染色体上(19q13)に位置し，主に肝臓において産生される．当初は内因性抗菌ペプチドとして発見されたヘプシジンであったが，その後，鉄過剰状態のマウスの肝臓においてヘプシジン遺伝子の発現が誘導されていること，ヘプシジン遺伝子欠損マウスでは鉄過剰状態を引き起こすこと，ヘプシジン遺伝子トランスジェニックマウスでは極度の鉄欠乏性貧血のため生下直後に死亡することなどが次々と判明してきた．これらの知見の集積より，ヘプシジンは，消化管での鉄吸収およびマクロファージからの鉄放出を抑制することで生体内鉄量を負に調節する鉄代謝調節ホルモンとして機能すると考えられるようになり，鉄代謝の分野で大きな話題を呼ぶことになった[13]．

現在までに考えられているヘプシジンの分子生物学的な作用機序は，肝臓で産生された後，液性因子として全身を循環し，網内系マクロファージや消化管吸収上皮に発現している ferroportin に結合し，細胞膜表面の ferroportin を減少させるように働くとされている[14]．こうした作用によって，最終的には消化管においては鉄吸収を抑制する方向に作用し，網内系においては再利用されるべき鉄の放出を抑制する方向に作用することになる．

5. ヘプシジン発現亢進による ACD の発症

慢性炎症に伴う貧血(anemia of chronic

Nippon Rinsho Vol 66, No 3, 2008-3

disease: ACD)は，各種感染症，膠原病，悪性疾患などといった慢性的な炎症を伴う疾患をもつ患者において認められる貧血で，臨床的には頻度も高く重要な病態である[15]．ACDでは，血清鉄の低下と，網内系細胞への鉄沈着が認められるが[16]，その病態形成に関する詳細は長年不明であった．ところが，ヘプシジンの機能が判明し，更に炎症状態でヘプシジン発現が亢進することも発見されるようになると[17]，**図3**に示すように，生体内での様々な炎症状態においてヘプシジンが増加すると，ヘプシジンは消化管からの鉄吸収とマクロファージからの鉄放出を抑制する方向に作用し，最終的に造血に利用できる鉄は減少する方向に傾き，ACDが発症すると考えられるようになった．更に，炎症状態でヘプシジン発現が増加する機構についての研究が続けられ，interleukin-6(IL-6)[18]やinterleukin-1β(IL-1β)[19,20]の関与などが判明してきているが，まだ完全に詳細が明らかになっているわけではない．こうした研究の進展から，将来有望な治療法が開発されることが期待される．

図3 ヘプシジンとACD発症

生体に各種の感染，膠原病，悪性腫瘍などの存在によって慢性の炎症状態が存在すると，IL-6やIL-1βなどの炎症性サイトカインが増加し，それが肝細胞に作用してヘプシジンの産生を増加させる．

ヘプシジンは生体内鉄代謝のnegative regulatorとして機能し，消化管での鉄吸収およびマクロファージからの鉄の放出を抑制するため，最終的に造血に利用できる鉄に減少を来し，貧血(anemia of chronic disease: ACD)を引き起こす．

おわりに

生体内鉄代謝は多くの関連分子の関与により巧妙に制御されており，近年新規分子の発見など知見も多い．特に，ヘプシジンをはじめとしたそれらの分子に異常が生じると鉄代謝調節が破綻し，ACDなどの発症に結びつくことがわかり，これらの分子による病態の発症機構を分子生物学的に突き詰めることにより，新規治療法の開発が期待されて，今後も注目される領域である．

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METABOLISM, CANCER AND GENETICS

Dysregulation of systemic iron metabolism in alcoholic liver diseases

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Key words
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Abstract

Alcoholic liver diseases (ALD) are frequently associated with iron overload. Until recently, the effects of ethanol in hepatic iron uptake and intestinal iron absorption have not been clarified in detail. Two possible mechanisms for iron overload are the uptake of iron into hepatocytes in a specific manner through the increased expression of transferrin receptor (TfR) 1; and increased intestinal iron absorption by the lowering of hepcidin. It is worthwhile to examine whether a similar mechanism is present in the development of steatosis and non-alcoholic steatohepatitis (NASH). Hepatocytes have several iron uptake pathways. Ethanol increases transferrin (Tf)-mediated uptake via a receptor-dependent manner, but downregulates the non-Tf-bound iron uptake. According to immunohistochemical study, TfR1 was increased in hepatocytes in 80% of hepatic tissues of patients with ALD, but was not detected in normal hepatic tissues. In an experimental model, ethanol exposure to the primary cultured-hepatocytes in the presence of iron increased TfR1 expression and ^{59}Fe-labeled Tf uptake. In patients with ALD, intestinal iron absorption is increased by oral iron uptake assay. The regulatory hormone for iron homeostasis, hepcidin is downregulated in ethanol-loaded mice liver. As well as ALD, a similar mechanism was present in the mouse model fed with a high-fat diet, a model of the initial phenomenon of steatosis. The common mechanism for hepatic iron deposition and the triggering role of iron may be present in the development of ALD and non-alcoholic fatty liver disease/NASH.

Introduction

Body iron metabolism is strictly regulated in physiological conditions, but it is becoming clear that several factors including alcohol, hepatitis C virus (HCV) infection, steatohepatitis etc. affect iron metabolism and the outcomes of their own diseases.[1] Alcoholic liver diseases (ALD), which are characterized by fatty liver, fibrosis, hepatitis and cirrhosis, are frequently associated with mild to severe iron overload. In advanced cases, such as cirrhosis, the reticuloendothelial iron deposition is dominant, in which endotoxemia and hypercytokinemias are deeply involved. However, in ALD of earlier stages, such as fatty liver and fibrosis, iron deposition is very mild and iron is preferentially present in hepatocytes. These findings indicate that alcohol itself or its metabolites primarily affect and dysregulate overall body iron metabolism, including hepatocyte iron uptake and intestinal iron absorption in a specific manner such as via the newly discovered hormone, hepcidin. Concerning the fundamental pathogenesis of ALD, the production of reactive oxygen species (ROS) is considered to be responsible. During the oxidation process of ethanol, superoxide (O_2^-) is produced and is transformed to hydroxyl radical (OH^-), which is the most potent oxidant via the Fenton reaction in the presence of free iron.[2] Actually, in the intragastric infusion model of ALD, supplementation of carbonyl iron advances fibrosis and cirrhosis.[3] Two possible mechanisms of the role of alcohol in the early stage of disease can be seen in Fig. 1; one is increased uptake of iron into hepatocytes and the other is increased intestinal iron absorption.[4]

Iron accumulation in hepatocytes by ethanol

It is well known that Japanese patients with ALD have a phenotype that is rather mild compared with that of severe alcoholic siderosis seen in the USA.[5] In our study dealing with Japanese ALD,[6] as well as in the rat model,[7] there is a positive correlation between iron deposition and histological intensity of a lipid-peroxidation product, 4-hydroxy-2-nonenal (HNE)-protein adduct, suggesting that free iron responsible for the Fenton reaction may be present predominantly in hepatocytes, and that ROS-induced cell damage is increased.

Hepatocytes have several pathways for iron uptake: transferrin (Tf)-mediated and non-mediated pathways.[8] Plasma iron is usually bound to Tf and iron-bound Tf is taken up via its specific receptor. In addition, non-Tf-bound iron (NTBI) is thought to contribute iron uptake to hepatocytes through either a divalent metal transporter (DMT1)[9] or ZIP14.[10] As shown in Fig. 2, we have found that ethanol augmented ^{59}Fe-bound Tf, but inhibited ^{59}Fe-citrate

Journal of Gastroenterology and Hepatology 23 (2008) Suppl. 1; S78–S81

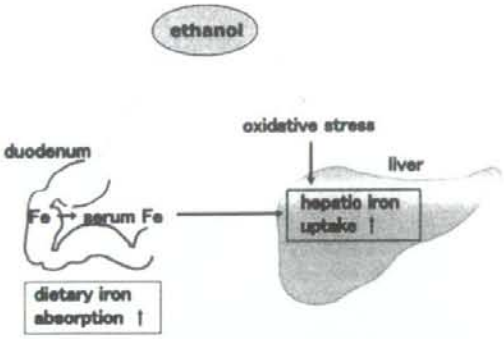

Figure 1 Two possible mechanisms of dysregulated hepatic iron accumulation in alcoholic liver disease (ALD) in the early stage of the disease. One is increased uptake of iron into hepatocytes and the other is increased intestinal iron absorption.

(NTBI), suggesting that Tf-bound iron may have an important role for hepatic iron uptake by ethanol. Although there are two molecules of Tf receptor, TfR1 and TfR2, TfR1 has a high affinity to serum Tf and is considered to be functional. However, in normal hepatocytes, TfR2 is constitutively expressed, but TfR1 is downregulated, suggesting that TfR1 does not contribute to the steady-state iron uptake. By immunohistochemical study of TfR1, the expression was increased in hepatocytes in 80% of hepatic tissues in Japanese patients with ALD, but was not detected in normal hepatic tissues.[11] It is noteworthy that the mean duration of abstinence of patients who demonstrated positive TfR1 expression in hepatocytes was significantly shorter than that of patients who demonstrated negative TfR1 expression. Taken together, it is possible that ethanol may augment TfR1. In the rat primary hepatocyte culture, the expression of TfR1 is upregulated in the presence of ethanol and iron by western blotting and ^{35}S-methionine metabolic labeling, suggesting that ethanol or its metabolite may affect the regulation of TfR1 and iron uptake. This increased TfR1 expression was regulated by increasing the activity of iron regulatory protein (IRP).[12]

Role of hepcidin in alcoholic iron overload

Body iron homeostasis is regulated strictly among processes such as dietary iron absorption, transport in circulation, and utilization or storage in bone marrow and liver. Increase in intestinal iron absorption is one of the mechanisms of the increase of body iron in alcoholics.[13] In patients with hereditary hemochromatosis, serum pro-hepcidin was lower than that in normal controls, suggesting that iron absorption is increased even with high iron storage.[14] It was also speculated that downregulation of hepcidin might be one of the important factors for the pathogenesis of iron overload in ALD.[15] In the ethanol-loaded mouse model which has a mild steatotic change, the hepcidin 1, 2 mRNA and protein expressions were significantly lower than in those of control.[16] In addition, alcohol-loading might disrupt the sensing signal of inflammatory cytokines and then downregulate hepcidin expression, following the increased iron absorption from the small intestine. Concerning the mechanism of hepcidin downregulation by alcohol, a decreased hepcidin expression in mouse liver is accompanied by increases of DMT1 and ferroportin 1, and a decrease of hepcidin promoter activity and DNA-binding activity of CCAAT/enhancer-binding protein (C/EBP).[17] In hemochromatotic Hfe(−/−) mice treated with ethanol, a further decrease in hepcidin mRNA expression was observed, in association with the decrease of C/EBP alpha, which may have implications for the liver injury observed in alcoholic liver disease and genetic hemochromatosis in combination with alcohol.[18]

Steatosis as an inducer of dysregulation of iron metabolism

Non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD) is a clinical entity characterized by the histopathological changes nearly identical to those induced by alcohol intake. In US population, approximately 25% are obese, and at least 20% of the obese individuals have hepatic steatosis, and it is suggested that obesity and steatosis affect liver disease progression.[19] A mild or moderate excess iron is frequently accumulated in liver tissue with NASH. Actually, the prevalence of the *HFE* gene mutation associated with hereditary hemochromatosis is increasing in patients with NASH, with the evidence strongly suggesting that iron is one of the important factors for the development of NASH.[20] It was also reported that phlebotomy is effective against NASH and the rise of oxidative stress markers related to the grade of iron overload in the liver.[21] However, the mechanism of iron overload in NASH is still unknown. Recently, our study using the high-fat diet mouse model suggested a strong link between the activation of peroxisome proliferator-activated receptor gamma (PPARγ) and the downregulation of cAMP response element-binding protein (CREB)[22] in addition to an increase of adipose differentiation-related protein (ADPR).[23] Among these molecules, the downregulation of CREB may be crucial, because CREB activation contributes to survival signals such as anti-apoptotic protein Bcl-2[24] and iron chelator desferrioxamine-increased CREB binding to the D-loop DNA of the mitochondrial genome in neurons.[25] The downregulation of CREB, which is associated with an activation of PPARγ by high-fat diet stimulation, may potentiate further dysregulation of iron metabolism. Actually, we found a significant increase in mRNAs of TfR1 and DMT1, and a decrease of hepcidin mRNA in association with bodyweight gain, mild steatosis and increased HNE immunostaining in this model (Miyoshi *et al.*, unpubl. data, 2007). As iron accumulation in the liver tissue after 16 weeks on a high-fat diet was not yet significant, our data strongly suggests that initial high-fat diet introduction upregulates TfR1 expression and downregulates hepcidin expression in the liver tissue, and upregulates DMT1 expression in the duodenum. Taken together, a high-fat diet itself has a capacity to accelerate intestinal iron absorption and hepatic iron uptake, as does ethanol. Therefore, it seems likely that iron is one of the important factors triggering NASH/NAFLD to develop, rather than a secondary factor.

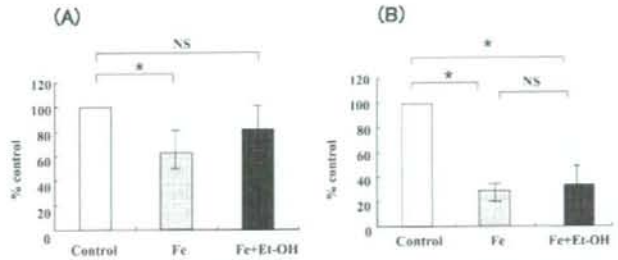

Figure 2 (a) Transferrin-bound iron uptake examined by incubation with ^{59}Fe-transferrin for 1 h after 24 h in the iron-deficient condition (control), with 20 μM iron (Fe), and with 20 μM iron and 25 mM ethanol (Fe + Et-OH). ^{59}Fe-transferrin uptake of iron-loaded hepatocytes was decreased to 63% compared with control hepatocytes. Additional ethanol exposure had a higher uptake at 82% of the control hepatocytes. There was no significant difference between control and iron- and ethanol-loaded hepatocytes. The experiment was repeated four times. (b) Non-transferrin-bound iron uptake examined by incubation with ^{59}Fe ferric chloride for 24 h in the iron-deficient condition (control), with 20 μM iron (Fe), and with 20 μM iron and 25 mM ethanol (Fe + Et-OH). Non-transferrin-bound ^{59}Fe uptake of iron-loaded hepatocytes was decreased to 29% compared with control hepatocytes. The additional ethanol exposure produced 34% of the iron uptake compared with the control hepatocytes. There was a significant difference between control and iron- and ethanol-loaded hepatocytes. The experiment was repeated four times. NS, not significant. $^*P < 0.05$. (From [12] with modifications with authors' permission.)

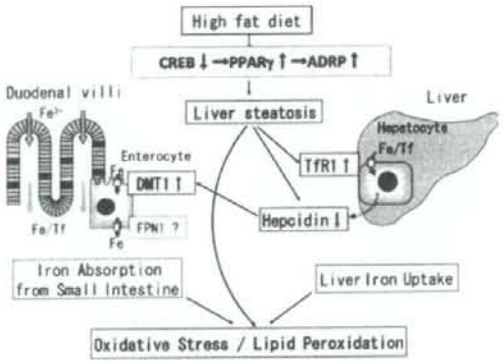

Figure 3 A high-fat diet itself has the capacity to accelerate intestinal iron absorption and hepatic iron uptake as well as ethanol. It seems likely that iron is one of the important factors triggering the development of non-alcoholic steatohepatitis/non-alcoholic fatty liver disease, rather than a secondary factor.

Conclusion

It is important to rationalize the finding of mild deposition of iron in earlier stages of ALD and to clarify the molecules involving the hepatic iron uptake in the presence of ethanol. In addition to the upregulation of TfR1 expression in hepatocytes, which is implicated in hepatic iron overload in alcoholic liver diseases, the decrease of hepcidin is also responsible for the increase of iron uptake. As shown in Fig. 3, a similar mechanism may be present in NASH or NAFLD through the production of ROS by a high-fat diet. A common pathway via steatosis/iron/oxidative stress should be considered for the development of liver fibrosis and carcinogenesis by iron as the initial progression stage.

Conflict of interest

No conflict of interest has been declared by the authors.

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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)

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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.

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.

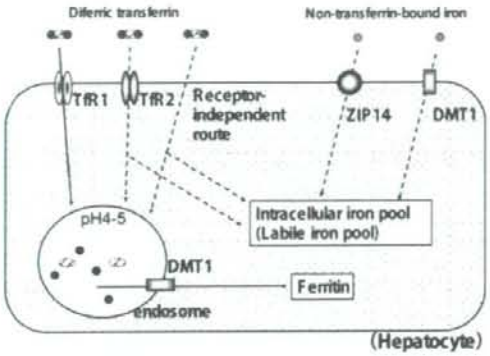

Fig. 1 Routes for iron uptake by hepatocytes. Hepatocytes have several pathways for iron uptake from the circulation. Concerning uptake of Tf-bound iron (Fe_2-Tf) at physiological concentrations, there are three pathways involving TfR1, TfR2, and TfR-independent mechanisms. The pathway via TfR1 is a 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).

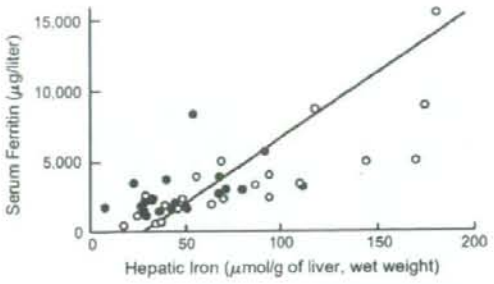

Fig. 2 Comparison of hepatic iron and serum ferritin concentrations. Indirect estimation is compared with the reference method, based on the direct measurement of hepatic iron levels by chemical analysis or magnetic-susceptibility studies. *Open circles* denote the values at the start of the trial (before 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