度が高いとされ，かなり簡便であるが，蛍光強度のquentingで検出する方法のため，HPLC測定法に比較すると正確性に欠ける恐れがある。

4．細胞内での鉄代謝と細胞内不安定鉄

循環血液中に認められ得る不安定鉄としてNTBIがあげられたが，細胞内においても不安定な鉄は存在する。

血液中に存在する鉄は，ヘモグロビン合成やDNA合成をはじめとした各種の代謝に必須であるため全身の細胞に取り込まれるが，取り込まれた後の細胞内においても鉄は厳密に制御される必要がある。各種代謝に利用されず，細胞にとって余分となった鉄は，主に細胞質内のフェリチンと呼ばれる蛋白に格納される。フェリチンは，H-subunitとL-subunitという異なる2種類の分子が合計24個集合して構成されており，卵の殻のような形状で，内部に最大で4,500分子の鉄を貯蔵することができる[22]。このフェリチンのおかげで，細胞内では多くの鉄はfreeの形では存在しないですみ，過剰鉄が細胞に毒性を示すことが防がれている。しかし，細胞内の一部の鉄はLIPと呼ばれるfreeな形で存在すると考えられている。LIPは，細胞内各小器官と細胞質内での鉄の受け渡しなどを，種々の不安定な形態で速やかに行っていると考えられている鉄の「プール」である。フェリチンは十分な鉄貯蔵能力を有している上，細胞内鉄濃度が上昇すると，細胞はフェリチン蛋白の合成を亢進させてさらに格納能力を増やすように機能している。しかしながら，これらを超えるほど細胞内に多量の鉄が流入してくるようになると，フェリチンに格納しきれない分がLIPの増加につながる。LIPはfreeの鉄であるため容易にROS産生に働き，細胞障害をもたらすことになる。

LIPの存在形態に関しては詳細は不明な部分も多いが，Fe^{2+}とFe^{3+}両方の鉄イオン形態で存在し，鉄との親和性がそれほど強くない低分子物質と結合している。そのような低分子のキレート物質としては，アデニンやグアニンのような核酸，システインやチロシンのようなアミノ酸，あるいはクエン酸，アスコルビン酸，リン酸，リン脂質，ポリペプチドといった低分子化合物などが知られている。LIPは細胞内鉄総量の3～5%程度とその割合は極めて少ないとされるが，理論的には細胞内に取り込まれた鉄は，必ず一度はLIPを通過して目的となる鉄結合蛋白に至ることとなる。

LIPの動態には細胞内の種々の小器官が関与しており[23]，平衡状態でのLIPレベルは，細胞内への鉄の供給，細胞内における鉄の需要，細胞外への排出のバランスによって調整されている。組織によってその様式は大きく異なるものの，細胞外から細胞内への鉄の取り込みに関しては，例えばTfR1を介したTf結合鉄の取り込み経路や，Fe^{2+}のトランスポーターであるdivalent metal transporter 1（DMT1）[24, 25]やZIP14[26]などを介したNTBIの取り込み経路が知られている（図4）。TfR1を介した鉄取り込みであっても，細胞内エンドソーム内ではTfからはずれたFe^{3+}がFe^{2+}に還元された後，エンドソーム上のDMT1によって細胞内に移動するため，いずれの経路によっても，細胞質に移動した直後の鉄はFe^{2+}と考えられている。細胞にTf結合鉄やNTBIを負荷することにより細胞内のLIPレベルが増加することが知られており，細胞内への鉄供給がLIPレベルを規定する因子であると言えるが，これに加えて，細胞内からのLIPへの鉄供給も認められる。例えばフェリチンは，鉄を貯蔵し有害な鉄を隔離する機

DMT1 (divalent metal transporter 1)

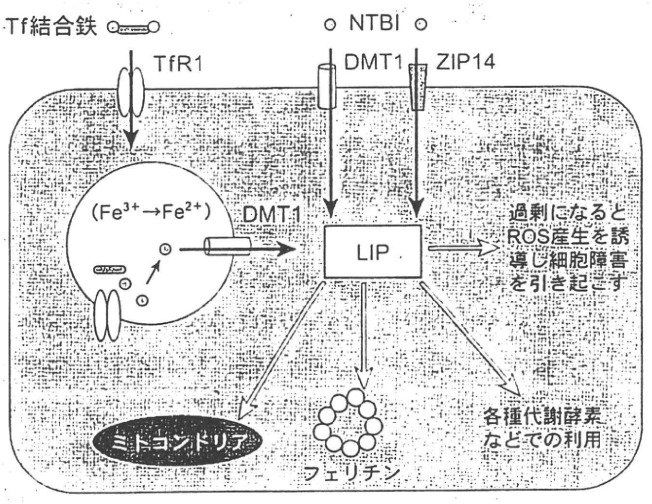

図4　細胞内のLIP

細胞は，トランスフェリン結合鉄(Tf結合鉄)をトランスフェリン受容体1 (transferrin receptor 1：TfR1)で取り込んだり，NTBIをDMT1やZIP14などのtransporterによって取り込む。これらはいずれにしても一度細胞質内においてLIPとなり，それからミトコンドリアで利用されたり，フェリチンに格納されたり，その他の各種代謝に利用されたりする。細胞内でのバランスが崩れLIPが過剰になると，細胞に対して障害をもたらす。

能を有しLIPレベルを減少させる蛋白であるが，逆にLIPレベルが減少した際には蓄えた鉄分子を放出しそのレベルを維持している。また，主たる赤血球の処理細胞である網内系細胞においては，ヘム蛋白がheme oxygenase-1により分解されて生じる鉄は，LIP形成に重要な因子となる。

LIPレベルは臓器の種類によって異なっている[27]。例えば，赤血球産生を行う赤芽球では，Tfからの鉄はミトコンドリアのferrochelataseに非常に効率的に渡されヘム合成に使用されるため，細胞質にはほとんどLIPは検出できないと報告されている[28]。一方，マクロファージなどの網内系細胞では，赤血球を貪食しヘモグロビンを分解し，heme oxygenase-1によるヘム鉄の放出が行われているため，LIPの増加が予測されている。また，鉄の貯蔵を主に担う肝細胞においてもLIPの存在が確認されている[11]。

各細胞内小器官におけるLIPレベルも，細胞の種類によって異なると考えられている。ミトコンドリアは細胞内の主要な鉄消費器官であるが，同時にスーパーオキシドの産生部位でもある。ミトコンドリアでは，ヘムや鉄-硫黄クラスター蛋白へ取り込まれる鉄と，ミトコンドリアに入る鉄量は厳密にリンクしていることから，ミトコンドリア内のLIPレベルは非常に少ないと考えられている。しかし，キレート可能な鉄の存在が培養肝細胞や心筋細胞のミトコンドリア内に認められ，キレート物質がミトコンドリア内でのROS産生を抑制することも報告されている[29]。また，鉄-硫黄クラスター蛋白合成に必須のfrataxin蛋白異常に起因するFriedreich's ataxiaや，ミトコンドリアの鉄トランスポーターであるABC7遺伝子の異常であるX連鎖性鉄芽球性貧血などのようなミトコンドリア内に鉄蓄積を来す疾患においては，ミトコンドリア内のLIPが増加し，病態形成に寄与している可能性も考えられている[30]。

5．鉄キレート療法

これまで見てきたように，循環血液中のNTBIや細胞内におけるLIPは，フリーの形で存在する「不安定鉄」であり，細胞障害を引き起こし，各種の臓器障害にも強く関連していることが明らかとなってきた。こうした「不安定鉄」に対する治療として，単純ではあるがそれらの不安定鉄を除去する方法がまず考えられ，鉄キレート剤を用いた鉄キレート療法が期待される（表1）。

わが国では鉄過剰症に対して使用可能な唯一の

表1　鉄キレート剤の比較

	Desferrioxamine	Deferiprone	Deferasirox
投与経路	静注，皮下注	経口	経口
血漿中半減期	短い（5～20分程度）	やや短い（< 2 hours）	長い（8～16 hours）
投与方法	反復投与や持続投与が望ましい	1日2～3回の分割投与	1日1回投与
Molar chelating efficiency	High (hexadentate)	Low (bidentate)	Moderate (tridentate)
副作用	聴覚障害，骨や成長への影響，注射部位への局所皮膚反応など	重篤な顆粒球減少など	消化器症状，軽度の発疹，軽度の血清クレアチニン上昇（腎障害）など

現在まで臨床応用されている3つの鉄キレート剤の比較を示す。

治療薬がDFOであった。DFOの登場はサラセミア・メジャー患者の生存率を向上させたが，血中半減期は約5～20分と短く，経口バイオアベイラビリティが低いのが欠点で，静脈内または皮下投与が行われるが，さらに十分な血中濃度を保つために反復投与や持続ポンプを使用しての皮下注射などの工夫が必要であった。そのため，コンプライアンスも当然のことながら不良であり，十分な臨床効果をあげられる症例は多くなかった。

欧州で使用されているdeferiproneは低分子量で2座配位の経口鉄キレート剤であり，心臓に蓄積した鉄を効果的に除去するとされるが，血中半減期が約1.5時間程度と短いため1日2～3回の分割投与が必要であり，さらに顆粒球減少症の副作用が出やすいという問題があった。

一方，最近本邦でも認可になったdeferasiroxは新規トリデント鉄キレート剤であり，鉄に高い選択性を示す3座キレート剤である。血中半減期が8～16時間と長いため，1日1回水に懸濁して服用することで，定期的なDFO投与と同等の有効性が認められる。有害事象も他剤にくらべ少なく，コンプライアンスを高めることができ，鉄過剰症の予防および治療に非常に期待が持たれている。Deferasiroxの投与で，サラセミアの患者においてLPIが実際に低下するという報告もある。さらに，deferasiroxは細胞内に入ることから細胞内LIPもキレートする可能性が考えられ，鉄キレート療法が生体内不安定鉄を取り除くことで，将来的に鉄過剰によってもたらされ得る臓器障害を軽減，もしくは回避する可能性が示唆されている。

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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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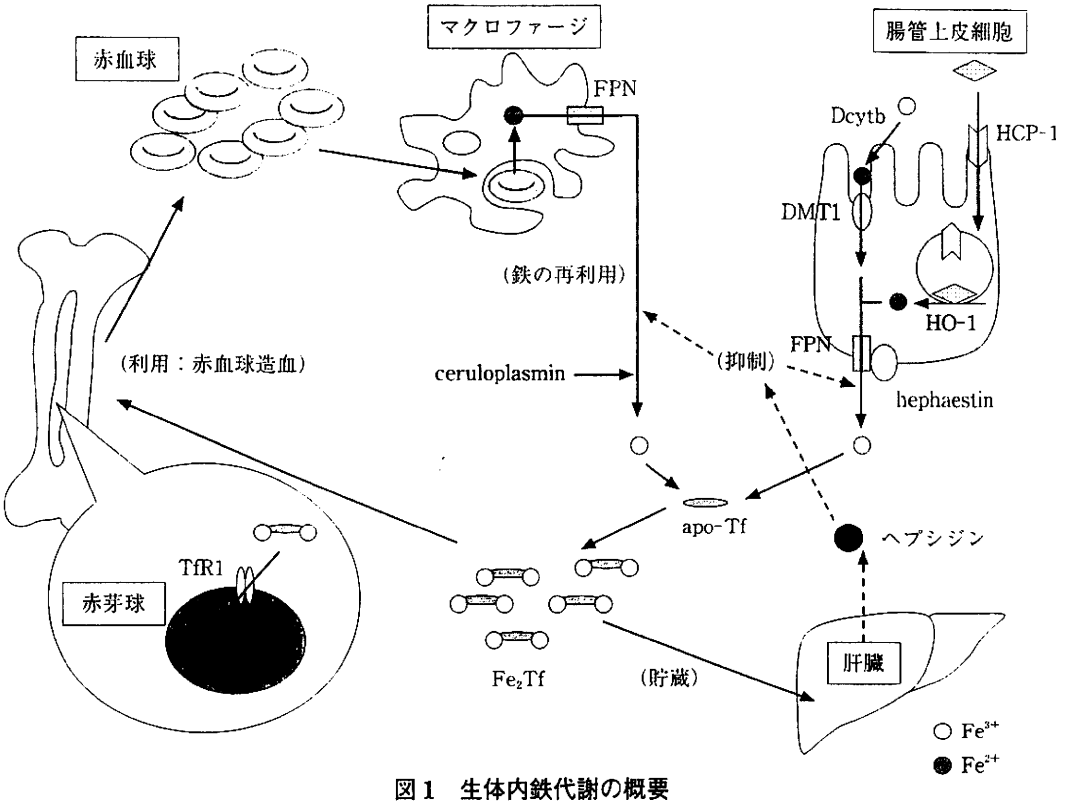

図1 生体内鉄代謝の概要

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

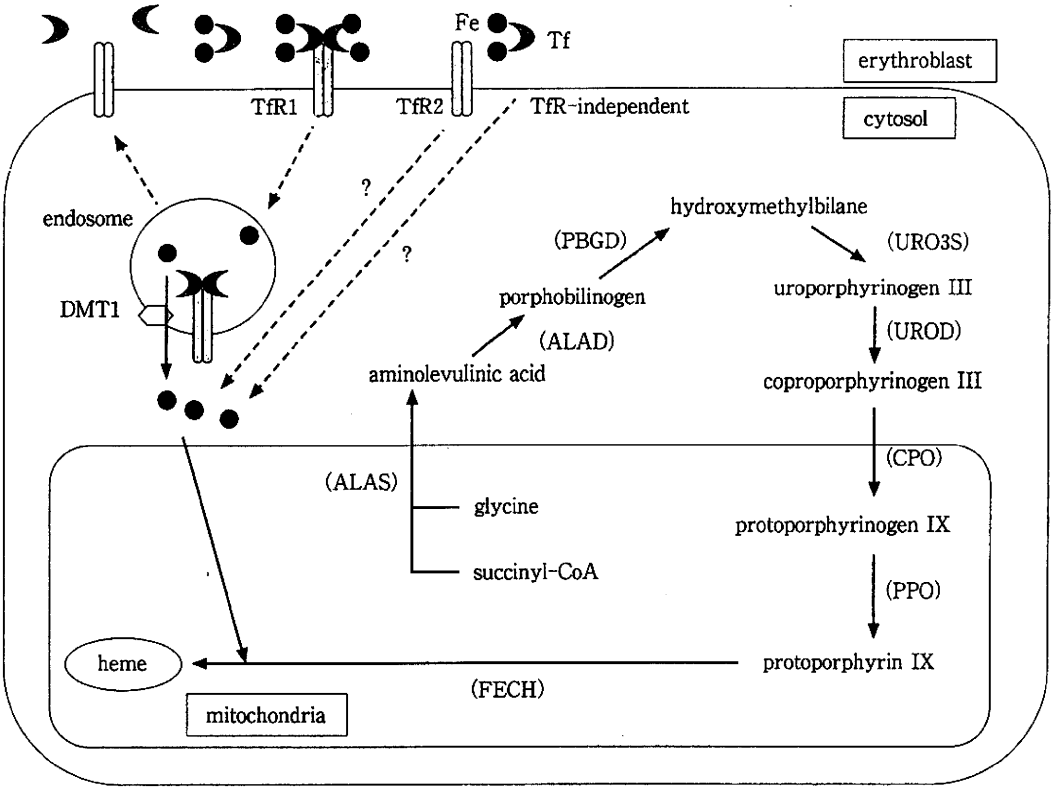

図2 骨髄赤芽球におけるヘムの合成経路と鉄の取り込み経路

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

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．

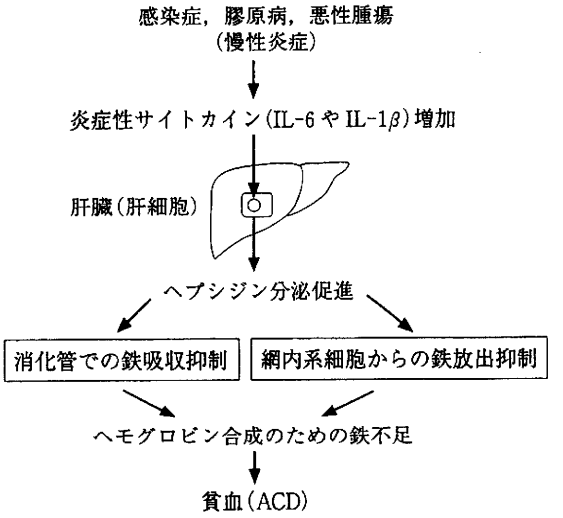

図3　ヘプシジンとACD発症

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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＜速　報＞

検査前食のエネルギー代謝に及ぼす影響—血清遊離脂肪酸による検討—

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瀧沢　義教[2)]　奥住　裕二[2)]　川村　憲弥[2)]　春木　宏介[2)]

緒言：肝硬変（LC）患者は早朝空腹時に飢餓状態にあるため検査前の絶食時間の延長によりエネルギー代謝状態が悪化することが報告[1)]されている．そのため飢餓状態の改善に検査前食が用いられている[2)]．遊離脂肪酸（NEFA）はLCで早朝飢餓状態で高値を呈することが知られている[3)]．そこで今回ウイルス性慢性肝疾患を対象にNEFAを空腹時に測定し，さらに腹部超音波検査（US）前後にも測定した．

対象と方法：検討対象は慢性肝炎（CH）123例（HBV 26例，HCV 97例，男性62例，女性61例，平均63歳）とLC 54例（HBV 3例，HCV 51例，男性19例，女性35例，平均68歳）である．食事群（16例：Child-Pugh Grade A 6例，B 10例）はUS終了まで絶食とし，US終了後とUS後の食事（600 kcal，蛋白20 g）摂取3時間後にNEFAを測定した．検査前食群（5例：Child-Pugh Grade B5例）はUS前にNEFAを測定し，カロリーメイトゼリー（大塚製薬株式会社）摂取3時間後にUSを施行し，NEFAも測定した．NEFAの測定は酵素法にて測定した．

結果：CHのNEFAは571±240 μEq/Lでありは780±240 μEq/LとLCで有意に高値（$p<0.0001$）であった．食事摂取およびカロリーメイト摂取後にNEFAは有意に低下した（Table 1）．

考察：検査前食群においても食事群と同様に検査前食によりNEFAは有意に低下した．飢餓状態により上昇したNEFAは食事摂取によりグルコースとNEFAから中性脂肪を再合成する．したがって食事群と同様にカロリーメイトゼリーでもNEFAが低下したことは200 kcalのカロリーメイトゼリーでもエネルギー代謝状態を改善でき，US検査前食として有用である．

索引用語：遊離脂肪酸，肝硬変，補食

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英文要旨

Effect of a nutritional supplement before abdominal ultrasonography on energy metabolism

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The serum free fatty acid levels were 571±240 μEq/L in patients with chronic hepatitis and 780±240 μEq/L; the serum free fatty acid levels were significantly higher in chronic hepatitis than in chronic hepatitis than in cirrhosis. The elevation of serum free fatty acid in cirrhosis is caused by starvation.

The serum free fatty acid levels were significantly decreased after the meal. 200 kcal of a nutritional supplement (Calorie Mate JERRY; Otsuka Pharmaceutical, Japan) was given to the patients before abdominal ultrasonography.

The free fatty acids levels were significantly decreased after a nutritional supplement.

Calorie Mate JERRY as a nutritional supplement improve metabolic disorder caused by fasting before abdominal ultrasonograhy.

Key words: non-esterified fatty acid, liver cirrhosis, free fatty acid, supplement

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Table 1　Change of serum NEFA level

meal	At fasting NEFA (μEq/L)	3rd hr after meal NEFA (μEq/L)	p
Diet	555.9 ± 328.3	73.3 ± 58.2	< 0.0001
Calorie Mate JERRY	997.8 ± 454.6	353.8 ± 321.0	0.0054

US: Ultrasonography
NEFA: non esterified fatty acid
NEFA (normal range: 140 ～ 850 μEq/L)

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Original Article

Effects of late evening snack on diurnal plasma glucose profile in patients with chronic viral liver disease

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Aim: Glycemic control is important to improve the prognosis in cirrhotic patients with complications from diabetes. A late evening snack (LES) has been recommended for cirrhotic patients. We investigated the effects of LES on diurnal plasma glucose levels.

Methods: Subjects comprised 47 patients with chronic viral liver disease (chronic hepatitis, $n = 11$; cirrhosis, $n = 36$) treated in the Department of Gastroenterology & Hepatology, Dokkyo Medical University Koshigaya Hospital. Diurnal variations in plasma glucose were first investigated with three meals/day, in accordance with the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines. Starting the next day, patients were given four meals including a LES, without changing meal content. Diurnal variations in plasma glucose were examined on day 7, and urine C-peptide immunoreactivity (CPR), and homeostasis model assessment insulin resistance (HOMA-IR) were investigated.

Results: With a LES, plasma glucose levels in patients with chronic hepatitis were significantly lower 2 hours before and 2 hours after dinner. In cirrhotic patients, significant decreases in plasma glucose levels were seen 2 hours after breakfast, before lunch, and before dinner. Significant decreases were noted in average plasma glucose levels and highest plasma glucose levels with four meals including a LES in patients with liver cirrhosis. This decrease was greater when maximum plasma glucose levels were higher on the three-meal regimen.

Conclusions: Improvements in plasma glucose levels were seen with four meals per day, including a LES, in viral chronic liver disease, particularly cirrhosis.

Key words: chronic liver disease, diabetes, late evening snack, plasma glucose

INTRODUCTION

A HIGH RATE OF complications have long been known to occur with diabetes against a background of chronic liver disease, particularly hepatitis C virus (HCV)-related chronic liver disease. Patients with non-alcoholic chronic liver disease and diabetes are also known to show a high rate of liver cancer,[1,2] and cirrhotic patients with complications of diabetes show an increased frequency of liver disease-related death and worsened prognosis.[3] However, prognosis can be improved with strict glycemic control in HCV-related cirrhosis patients with diabetes.[4] Glycemic control in patients with chronic liver disease with diabetes is therefore crucial for preventing the onset of liver cancer and improving prognosis. Patients with liver cirrhosis are in a hypermetabolic state and can thus reach a state of extreme hunger during morning fasting, and the non-protein respiratory quotient (npRQ) decreases because of an increased fat-burning rate. A late evening snack (LES) to improve the morning starving state[5] is recommended for patients with liver cirrhosis.[6] However, dietary management of diabetes in patients with method of diet intake in patients with diabetes is not clear. We therefore investigated the effects of LES on diurnal fluctuations in plasma glucose in patients with chronic viral liver disease.

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METHODS

Subjects

SUBJECTS COMPRISED 11 patients with chronic viral hepatitis (hepatitis B virus (HBV), $n = 2$; HCV, $n = 9$) and 36 patients with viral cirrhosis (HBV, $n = 8$;

Table 1 Data are expressed mean standard deviation. Turkey's method was used for statistical analysis

	n	Age (years) §	Male	Female	HBV	HCV	BMI (kg/m²)§
Chonic hepatitis	11	56 ± 14	8	3	2	9	23.5 ± 3.7
Liver cirrhosis	36	66 ± 8†	21	15	8	28	22.9 ± 3.7
Grade A‡	10	69 ± 4	6	4	1	9	23.5 ± 4.0
Grade B,C‡	26	65 ± 5	4	11	7	19	22.6 ± 3.6

†Chronic hepatitis vs liver cirrhosis, $P = 0.003$. ‡Child–Pugh classification. §Mean±standard deviation. BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus.

HCV, $n = 28$) hospitalized in the Department of Gastroenterology & Hepatology, Dokkyo Medical University Koshigaya Hospital between June 2002 and May 2004 (Table 1). Diagnoses were made comprehensively by liver specialists using blood biochemistry and abdominal ultrasonography. HCV-related hepatitis was diagnosed based on positive results for HCV-RNA from RT-PCR (real time polymerase chain reaction), and HBV-related hepatitis was diagnosed based on Hepatitis B surface antigen (HBsAg) positivity from enzyme immunoassay (EIA). This study was conducted in accordance with the Helsinki Declaration of the World Medical Association in 1975. Diurnal fluctuations in plasma glucose were monitored only in patients who had provided consent after receiving full explanations regarding chronic liver disease and glucose metabolism disorders. Patients complicated with liver cancer, patients with a history of taking branched-chain amino acid preparations and gastrointestinal tract surgery, and patients currently taking diabetes medications were excluded from this investigation.

Methods

In accordance with The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines,[7] diet therapy was conducted with 25–35 kcal/day of non-protein and 1.0–1.2 g/day of protein per 1 kg of standard weight in three meals/day. Diurnal fluctuations in plasma glucose were measured on day 7 after starting diet treatment with three meals (early morning fasting (0600), 2 h after breakfast (0900), before lunch (1200), 2 h after lunch (1400), before dinner (1800), 2 h after dinner (2000), and 4 h after dinner (2200). Diet treatment with four meals including a late evening snack (LES) was started on the day after diurnal fluctuations in plasma glucose were measured in all subjects. We served LES at 2200. To maintain the same daily energy intake with four meals, 70 kcal was taken from each of the three meals and that reduction of approximately 200 kcal was provided in the LES.[5] Seven days after starting diet treatment with four meals including LES, diurnal fluctuations in plasma glucose were again measured, and the effects of four meals including a LES on these fluctuations were investigated. Average plasma glucose levels were taken as the mean levels of the seven measurements within a day, and the highest level within the daily fluctuations was used as maximum plasma glucose level. Changes in average and highest plasma glucose levels before and after starting LES were investigated. In addition, plasma insulin levels were measured by chemiluminescent enzyme immunoassay during the morning fast, and insulin resistance was investigated before and after LES. Insulin resistance was calculated as fasting glucose level (mg/dL) × fasting insulin level (μU/mL) using homeostasis model assessment insulin resistance (HOMA-IR).[8] To investigate effects on insulin secretion of four meals including LES, 24-h urine collection was conducted on the day diurnal fluctuations in plasma glucose were measured, and urinary C-peptide (CPR) was measured by EIA.

Statistical analysis

All statistical tests were performed using SPSS software (SPSS, Chicago, IL). Data are expressed as mean ± standard deviation. Analysis was performed using the paired *t*-test and Tukey's test. The level of statistical significance was taken as $P < 0.05$.

RESULTS

Intakes of energy and three main nutrients

IN PATIENTS WITH chronic hepatitis, non-nitrogen energy intake was 31.7 ± 3.6 kcal/kg/day per standard body weight, protein intake was 1.1 ± 0.1 g/kg/day, fat intake was 50.9 ± 5.4 g/day, and carbohydrate intake was 278.2 ± 16.6 g/day. In patients with liver cirrhosis, non-nitrogen energy intake was 32.3 ± 3.5 kcal/kg/day per standard body weight, protein intake was 1.1 ± 0.2 g/kg/day, fat intake was 49.2 ± 6.5 g/day, and carbohydrate intake was 276.9 ± 15.8 g/day. No

significant difference in intake of either energy or these three main nutrients was seen between groups.

Influence of four meals including LES on diurnal plasma glucose profile for patients with chronic liver disease

In patients with chronic hepatitis, significant decreases in plasma glucose levels were seen before the evening meal and 2 h after the evening meal ($P = 0.0281$, $P = 0.0406$) with four meals including LES. In patients with liver cirrhosis, plasma glucose levels were significantly decreased 2 h after breakfast, before lunch, and before dinner ($P = 0.0160$, $P = 0.0004$ and $P = 0.0440$, respectively) with four meals including LES. However, no significant differences in minimum plasma glucose levels during diurnal fluctuations were apparent between the 3- and 4-meal regimens. In patients with liver cirrhosis (Child–Pugh grade A), no significant decreases in plasma glucose levels were seen after the evening meal with four meals including LES. However, in patients with liver cirrhosis (Child–Pugh grades B and C) plasma glucose levels were significantly decreased before lunch and 2 h after lunch ($P = 0.0268$ and $P = 0.0006$, respectively) with four meals including LES (Table 2).

Changes of average plasma glucose levels and highest plasma glucose levels in patients with liver cirrhosis

In patients with chronic hepatitis, average plasma glucose levels were 204 ± 75 mg/dL in three meals/day and 167 ± 46 mg/dL after LES. Highest plasma glucose levels were 256 ± 100 mg/dL on three meals/day and 212 ± 56 mg/dL after LES. No significant differences were seen in patients with chronic hepatitis. In patients with liver cirrhosis, highest plasma glucose levels were 232 ± 97 mg/dL on three meals/day and 222 ± 98 mg/dL after LES. No significant differences were seen. However, in patients with liver cirrhosis, average plasma glucose levels were decreased significantly after LES (182 ± 82 vs. 168 ± 70) ($P = 0.0099$) (Fig. 1). Besides, in patients with chronic liver disease (HCV), average plasma glucose levels were 181 ± 80 mg/dL in three meals/day and 1163 ± 65 mg/dL after LES. Highest plasma glucose levels were 231 ± 99 mg/dL on three meals/day and 212 ± 90 mg/dL after LES ($P = 0.0215$). In patients with chronic liver disease (HBV), average plasma glucose levels were 209 ± 82 mg/dL for three meals/day and 186 ± 64 mg/dL after LES. Highest plasma glucose levels were 261 ± 93 mg/dL for three meals/day and 249 ± 84 mg/dL after LES. No significant

Table 2 Trends in diurnal changes of plasma glucose before and after LES in patients with chronic liver disease. Data are expressed mean standard deviation. A paired *t*-test was used. Before LES, 3 meals/day; After LES, 4 meals/day including LES

	Chronic hepatitis			Liver cirrhosis			Child–Pugh grade A			Child–Pugh grade B,C		
	Before LES	After LES	*P*-value	Before LES	After LES	*P*-value	Before LES	After LES	*P*-value	Before LES	After LES	*P*-value
Cases (*n*)	11	11		36	36		10	10		26	36	
Fasting glucose (mg/dL)	142 ± 37	138 ± 51	0.7681	123 ± 47	120 ± 45	0.4766	117 ± 37	106 ± 21	0.2589	125 ± 50	125 ± 50	0.0875
2 h after breakfast (mg/dL)	239 ± 93	201 ± 64	0.1985	221 ± 102	193 ± 98	0.0160	205 ± 112	176 ± 119	0.3029	227 ± 100	200 ± 91	0.9935
Before lunch (mg/dL)	197 ± 101	163 ± 86	0.3838	181 ± 94	158 ± 81	0.0004	163 ± 107	149 ± 84	0.2781	187 ± 91	161 ± 82	0.0268
2 h after lunch (mg/dL)	228 ± 98	183 ± 52	0.1147	199 ± 101	187 ± 93	0.0928	190 ± 116	179 ± 97	0.2565	203 ± 96	190 ± 93	0.0006
Before dinner (mg/dL)	180 ± 65	136 ± 35	0.0281	158 ± 81	139 ± 68	0.0440	144 ± 81	129 ± 47	0.3602	163 ± 82	143 ± 74	0.1824
2 h after dinner (mg/dL)	230 ± 78	177 ± 58	0.0406	203 ± 93	194 ± 78	0.3522	203 ± 99	178 ± 66	0.1487	202 ± 92	201 ± 83	0.0800
4 h after dinner (mg/dL)	216 ± 78	173 ± 49	0.1682	190 ± 95	184 ± 81	0.5280	182 ± 103	173 ± 70	0.5198	193 ± 94	189 ± 86	0.8622
Minimum plasma glucose (mg/dL)	132 ± 32	113 ± 23	0.1055	117 ± 49	108 ± 41	0.0661	108 ± 40	101 ± 24	0.4849	120 ± 52	110 ± 46	0.6963

LES, late evening snack.

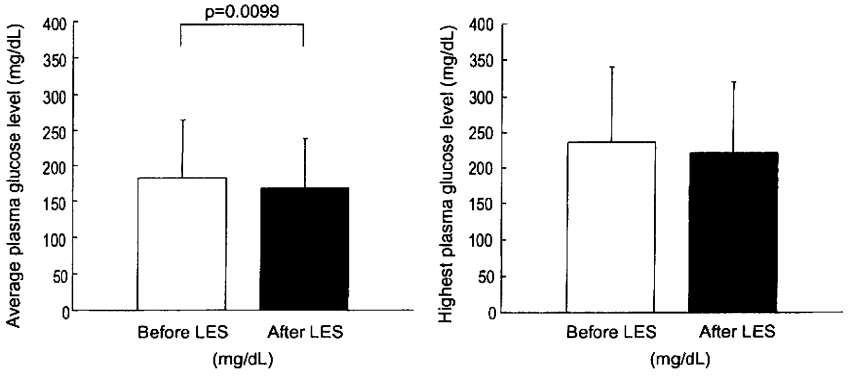

Figure 1 Changes of average plasma glucose levels and highest plasma glucose levels before and after LES in patients with liver cirrhosis. A paired *t*-test was used. Before LES, 3 meals/day; After LES, 4 meals/day including LES. LES, late evening snack.

differences were seen in patients with chronic liver disease (HBV).

Relationship between fasting plasma glucose levels before LES and changes of average plasma glucose levels after LES in patients with liver cirrhosis

Relationship between fasting plasma glucose levels before LES and changes of average plasma glucose levels after LES were investigated in patients with liver cirrhosis. Average plasma glucose levels were significantly decreased ($P = 0.0034$) after starting LES, when fasting plasma glucose level was higher before LES (Fig. 2), but no significant change was seen in patients with chronic hepatitis.

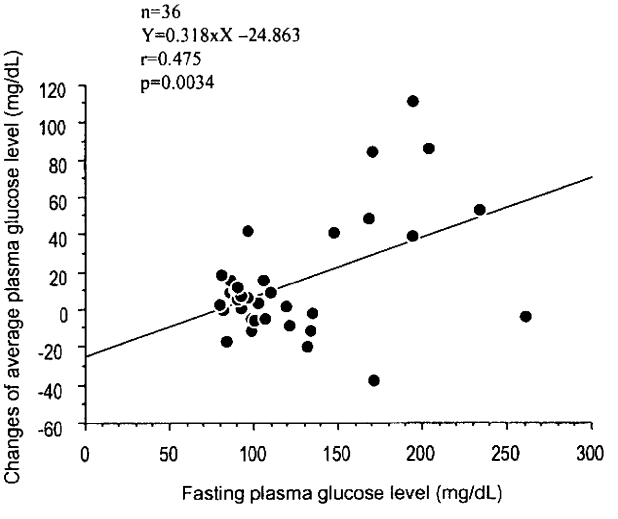

Figure 2 Relationship between fasting plasma glucose levels in 3 meals/day and changes of average plasma glucose levels after LES in patients with liver cirrhosis. The investigation was performed using Pearson's correlation coefficient.

Relationship between highest plasma glucose levels in three meals/day and changes of average plasma glucose levels and highest plasma glucose levels after LES

The highest plasma glucose levels in diurnal fluctuations before LES and changes in average plasma glucose levels and highest plasma glucose levels before and after LES were investigated (Fig. 3). In patients with chronic hepatitis, there were no significant changes in average plasma glucose levels and highest plasma glucose levels before and after LES in patients with chronic hepatitis. However, decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater ($r = 0.830$, $P = 0.0016$, $r = 0.826$, $P = 0.0017$) in proportion to the degree of highest plasma glucose levels.

The highest plasma glucose levels in diurnal fluctuations before LES and changes in average plasma glucose levels and highest plasma glucose levels before and after LES were investigated in patients with liver cirrhosis (Fig. 4). Decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater ($r = 0.558$, $P = 0.0004$, $r = 0.373$, $P = 0.0250$) in proportion to the degree of highest plasma glucose levels.

Effects of four meals with LES on insulin resistance

In patients with chronic hepatitis patients, insulin resistance (HOMA-IR) was 3.28 ± 1.37 before LES and 4.37 ± 1.55 with four meals including LES, showing no significant change. HOMA-IR in patients with liver cirrhosis was 5.75 ± 8.69 before LES and 3.77 ± 2.41 with four meals including LES, again showing no significant change.

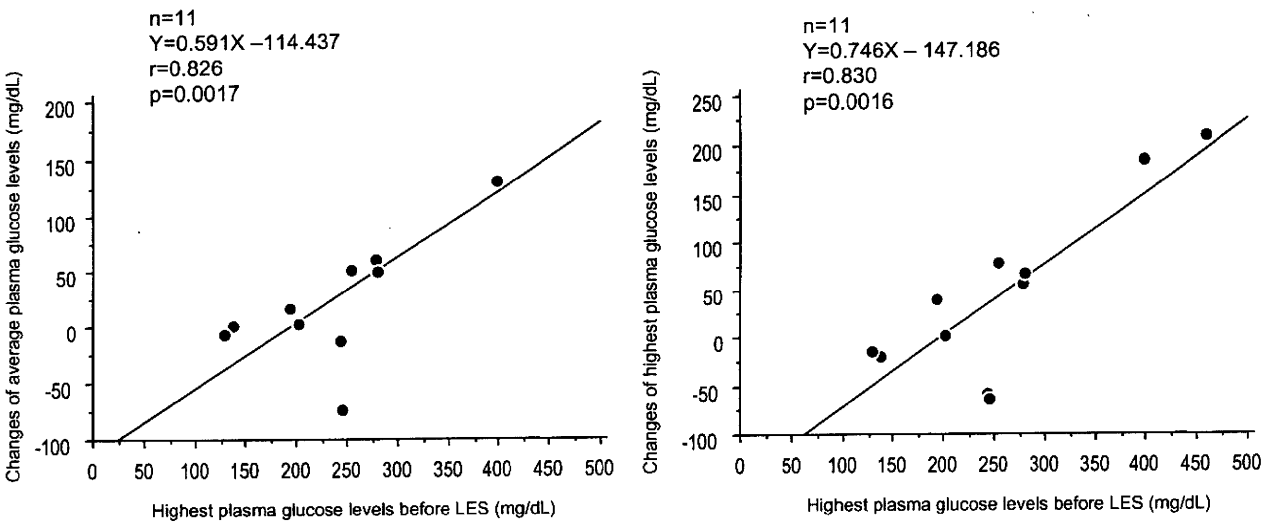

Figure 3 Relationship between highest plasma glucose levels in 3 meals/day and changes of average plasma glucose levels and highest plasma glucose levels after LES in patients with chronic hepatitis. The investigation was performed using Pearson's correlation coefficient.

Effects on insulin secretion of four meals including LES

In patients with chronic hepatitis urine C-peptide was 96.6 ± 17.2 μg/day before LES and 92.3 ± 34.1 μg/day with four meals including LES. Similarly, in patients with liver cirrhosis urine C-peptide was 97.6 ± 104.1 μg/day before LES and 90.4 ± 93.5 μg/day with four meals including LES. Neither change was significant. In addition, no significant differences were seen after stratifying the patients according to average plasma glucose levels and highest plasma glucose levels.

DISCUSSION

A HIGH RATE OF complications is seen with diabetes in patients with liver cirrhosis, and diabetes in patients with liver cirrhosis reduces long-term survival

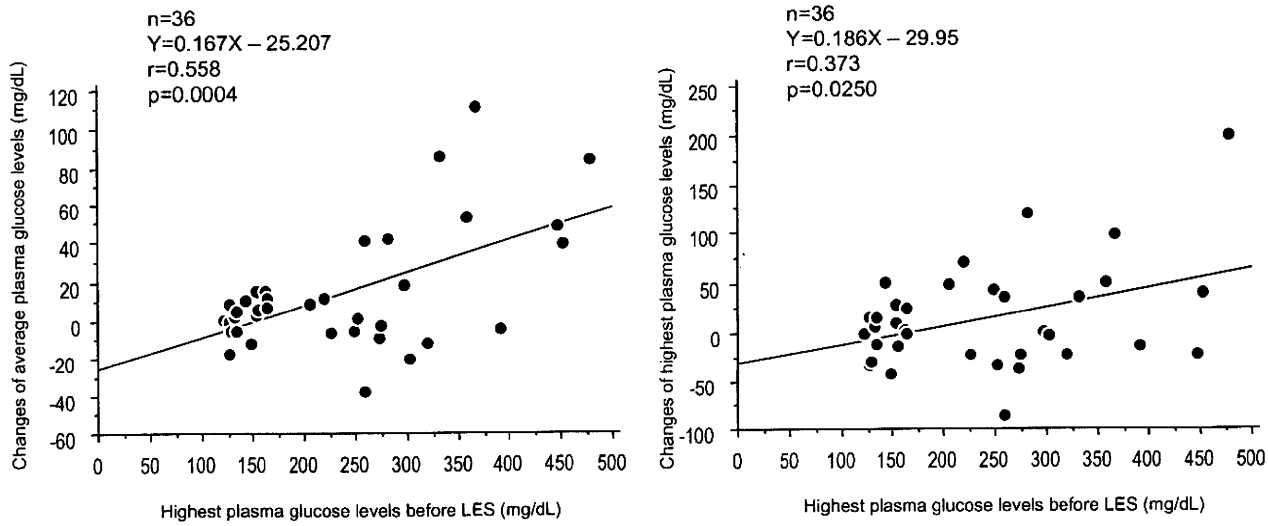

Figure 4 Relationship between highest plasma glucose levels in 3 meals/day and changes of average plasma glucose levels and highest plasma glucose levels after LES in patients with liver cirrhosis. The investigation was performed using Pearson's correlation coefficient.

in liver disease patients. Cause of death in these patients is often hepatic failure.[3] Variables that were found to have a significantly increased hazard ratio of liver cancer without the interaction term were sex, baseline serum albumin level, concurrent diabetes, baseline BMI, and baseline AFP level.[9] In hepatitis C, the virus itself is related to insulin resistance, and this insulin resistance has also been indicated to influence the progression of liver fibrosis.[10] In addition, HCV-related chronic hepatitis shows a high rate of liver cancer from complications with diabetes,[2,11] and hyperinsulinemia in liver cancer patients accelerates the rate of tumor growth for the liver cancer. If insulin secretion is suppressed by administration of octreotide, the rate of tumor growth is slowed,[12] indicating a relationship between insulin secretion and the rate of liver cancer growth. A relationship has also been indicated between insulin therapy and postoperative recurrence of liver cancer complicated with cirrhosis when the patient has diabetes.[13] Therefore, controlling plasma glucose to treat diabetes in patients with liver cirrhosis may reduce mortality rates related to liver disease. Moreover, suppressing insulin secretion through dietary measures may delay liver cancer recurrences and tumor growth rate.

Dietary measures for diabetes in patients with liver cirrhosis are important in consideration of the hepatic usage of glucose. In an investigation of glucose usage using an indirect calorimeter in liver cirrhosis, glucose usage was found to be slowed in liver cirrhosis, but increased in peripheral skeletal muscle and increased overall.[14] Compared with three meals/day, four meals/day including LES decreases the glucose intake per meal, so glucose can be supplied gradually with four meals including LES. Considering the state of glucose metabolism in cirrhotic patients, frequent meals may be effective in controlling plasma glucose in patients with liver cirrhosis.

We therefore investigated the effect of four meals including LES in diurnal fluctuations of plasma glucose in hospitalized patients. In patients with chronic hepatitis, the glucose contained in a single meal can be adequately processed in the liver, even with three meals/day, so we assumed that no major changes would be seen in the diurnal profile of plasma glucose with four meals including LES. In cirrhosis, however, uptake of glucose in the liver is slower than that in patients with chronic hepatitis, so with three meals the glucose contained in a single meal cannot be completely processed in the liver, and high plasma glucose is seen. With four meals including LES, the glucose supplied in meals can be processed in the liver because the amount of glucose per meal is decreased, so elevations in plasma glucose can be kept down. Improvements in the diurnal profile of plasma glucose were thus attributed to decreases in overall plasma glucose with four meals including LES. Therefore, average plasma glucose levels and highest plasma glucose levels were significantly decreased after four meals/day in patients with liver cirrhosis. However, even with four meals including LES, no significant decrease was seen in highest plasma glucose levels and highest plasma glucose levels in patients with chronic hepatitis, but a decreasing trend was seen.

No change in insulin secretion was seen with four meals including LES. In patients with liver cirrhosis, hepatic parenchymal cells decrease, tissue fibrosis occurs, and the glucose absorbed to rebuild lobular structures is not taken up by hepatic cells. As a result, glucose not taken up in the liver is thought to be oxidized in peripheral skeletal muscle and brain by the action of insulin and used as an energy source, so hyperinsulinemia is expressed.[15] Thus, in patients with liver cirrhosis eating three meals/day, the glucose that is not processed in the liver is used in peripheral tissues through the actions of insulin, and this increases insulin secretory capacity. With four meals including LES, plasma glucose is conjectured to decrease because glucose is processed adequately in the liver, so the amount of glucose transported to peripheral tissues is decreases, thereby decreasing insulin secretory capacity. In our investigation, however, no significant difference was seen in insulin secretion. This may have been because glucose was processed in peripheral tissues via insulin, since the amount of glucose supplied in 1 meal with a 4-meal regimen exceeded the glucose-processing capacity of the liver, and as a result no significant difference in insulin secretion was seen. Investigation of increasing the number of meals to more than 4 and decreasing the amount of glucose provided per meal may be warranted.

We investigated the kinds of cases in which four meals a day is effective, but in patients with chronic hepatitis, average and highest plasma glucose levels were not significantly decreased. However, decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater in proportion to the degree of highest plasma glucose levels. In patients with liver cirrhosis, average plasma glucose levels and highest plasma glucose levels were decreased significantly after LES. In addition, decreasing degrees of average plasma glucose levels and highest plasma glucose levels were significantly greater in proportion to the degree of highest plasma glucose levels. A regimen of four meals including LES should thus be actively used

with chronic liver disease patients showing high plasma glucose especially in patients with liver cirrhosis.

The present investigation examined only a small number of subjects, but was useful as an investigation of dietary measures aimed at lowering plasma glucose in cases of liver cirrhosis with diabetic complications. However, no change in insulin secretion was seen with four meals including LES. The present findings suggest that frequent meals of >four meals/day will need to be investigated in terms of dietary measures that also aim to suppress insulin secretion.

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Original Article

Evaluation of the effects of combination therapy with branched-chain amino acid and zinc supplements on nitrogen metabolism in liver cirrhosis

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Aim: Disorders of protein metabolism in liver cirrhosis can affect prognosis or cause complications. Treatment with branched-chain amino acid (BCAA) and zinc supplements has been shown to be effective against abnormal nitrogen metabolism in liver cirrhosis. There are, however, few studies on the effects of combining these supplements. In this study, the effect of combining BCAA and zinc treatment in cirrhosis was investigated.

Methods: Forty patients with liver cirrhosis who had blood albumin levels of 3.5 g/dL or less and blood zinc levels of 70 μg/dL or less were randomized to receive either BCAA alone or a combination of BCAA and zinc supplements. Blood albumin, the Fischer ratio, and ammonia levels were compared over 5–6 months of treatment.

Results: In the combination group, the post/pre treatment change ratio in blood ammonia levels decreased significantly (0.87 ± 0.26 vs. 1.22 ± 0.38, $P = 0.0033$), and the change ratio in the Fischer ratio increased significantly (1.22 ± 0.29 vs. 1.08 ± 0.16, $P = 0.0165$) in comparison with the BCAA monotherapy group. The change ratio in blood albumin levels showed no significant difference between the groups (1.01 ± 0.07 vs. 1.03 ± 0.08, $P = 0.4646$).

Conclusions: More improvement in disorders of nitrogen metabolism in liver cirrhosis occurred after administration of BCAA with zinc than after BCAA alone over 5–6 months of treatment. Further investigation is necessary to determine mechanisms of the action and longer-term clinical efficacy.

Key words: albumin, ammonia, branched-chain amino acid, Fischer ratio, urea-cycle

INTRODUCTION

LIVER CIRRHOSIS IS associated with various metabolic disorders, among which protein and energy malnutrition plays a role in the reduction of prognosis and the occurrence of complications such as hepatic encephalopathy.[1-5] Treatment using branched-chain amino acid (BCAA) has been demonstrated to contribute to improvement of the nitrogen metabolism, decrease in the incidence of complications, and improvement of prognosis.[6-8] In liver cirrhosis, BCAA is consumed in the skeletal muscle when ammonia that is no longer processed by the liver is detoxified via glutamine synthesis, resulting in BCAA deficiency.[9] Administration of BCAA corrects the amino acid imbalance and improves the protein metabolism, but BCAA itself imposes a nitrogen load and does not alleviate hyperammonemia.[6]

Liver cirrhosis is also associated with a high incidence of zinc deficiency, which contributes to nitrogen metabolism disorder. In particular, the urea-cycle enzyme ornithine-transcarbamylase, which plays a major role in ammonia metabolism in the liver, is a zinc enzyme. The activity of the urea-cycle enzymes is reduced by zinc deficiency. Several factors such as poor dietary intake, impaired intestinal absorption, and excessive urinary losses may be responsible for reduced whole-body zinc content.[10-13] Supplementation of zinc mainly improves nitrogen and ammonia metabolism in the liver.[10-14]

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