Table 1 Molecules involved in body iron metabolism

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

2 Molecular mechanisms of body iron metabolism

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

2.1 Intestinal iron absorption

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

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

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

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

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

2.3 Iron uptake and utilization in liver

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

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

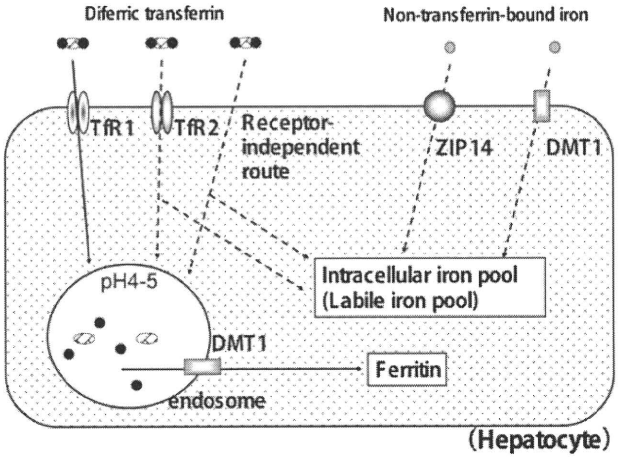

Fig. 1 Routes for iron uptake by hepatocytes. Hepatocytes have several pathways for iron uptake from the circulation. Concerning uptake of Tf-bound iron (Fe_2-Tf) at physiological concentrations, there are three pathways involving TfR1, TfR2, and TfR-independent mechanisms. The pathway via TfR1 is a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

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

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

2.4 Bone marrow iron metabolism and erythropoiesis

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

2.5 Systemic regulation of body iron metabolism

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

3 Forms of iron in serum and tissue

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

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

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

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

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

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

3.3 Serum ferritin

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

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

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

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

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

4 Measurement of body iron stores: comparison with serum ferritin

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

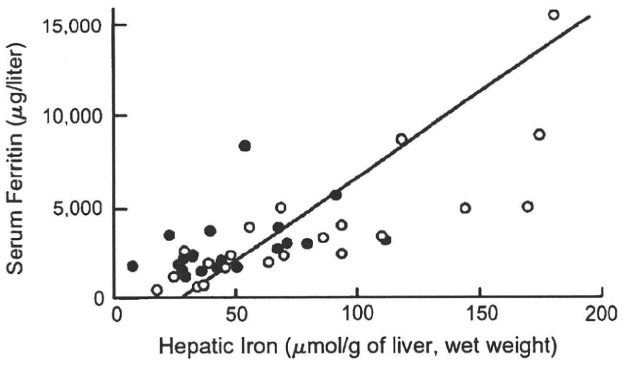

Fig. 2 Comparison of hepatic iron and serum ferritin concentrations. Indirect estimation is compared with the reference method, based on the direct measurement of hepatic iron levels by chemical analysis or magnetic-susceptibility studies. *Open circles* denote the values at the start of the trial (before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

4.1 Direct measurement

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

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

4.2 Physical measurement of body iron

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

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

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

5 Toxic effect of iron overload on organ function

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

5.1 Mechanism of iron toxicity

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

5.2 Iron overload syndrome

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

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

Table 3 Classification of iron overload

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

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

5.3 Organ dysfunction by excess iron

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

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

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

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

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

6 Conclusion

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

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

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doi:10.1111/j.1440-1746.2007.05290.x

METABOLISM, CANCER AND GENETICS

Dysregulation of systemic iron metabolism in alcoholic liver diseases

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Key words
alcohol, hepcidin, iron, steatohepatitis, transferrin receptor 1.

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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

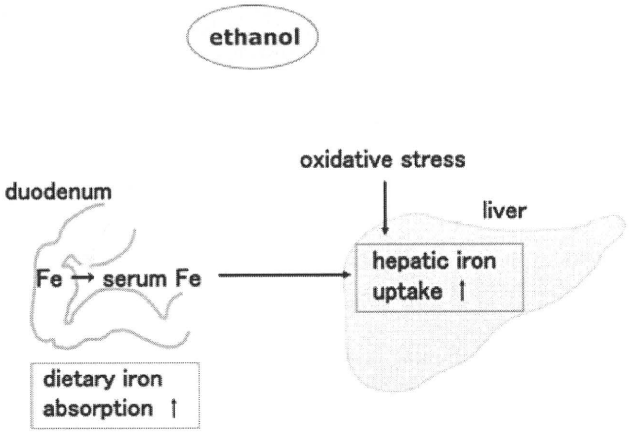

Figure 1 Two possible mechanisms of dysregulated hepatic iron accumulation in alcoholic liver disease (ALD) in the early stage of the disease. One is increased uptake of iron into hepatocytes and the other is increased intestinal iron absorption.

(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.

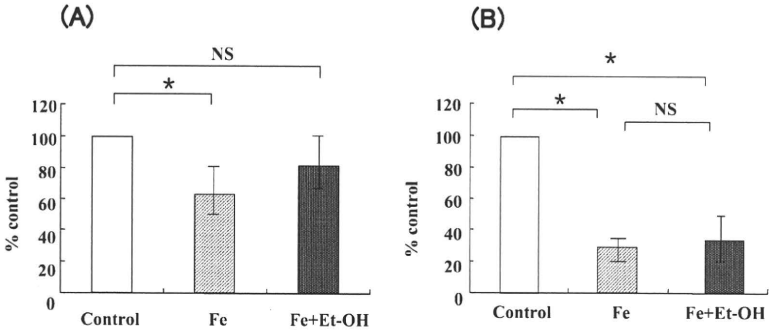

Figure 2 (a) Transferrin-bound iron uptake examined by incubation with ^{59}Fe-transferrin for 1 h after 24 h in the iron-deficient condition (control), with 20 μM iron (Fe), and with 20 μM iron and 25 mM ethanol (Fe + Et-OH). ^{59}Fe-transferrin uptake of iron-loaded hepatocytes was decreased to 63% 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.)

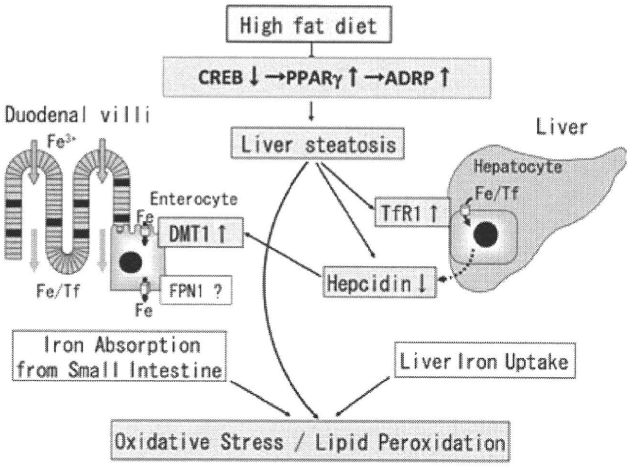

Figure 3 A high-fat diet itself has the capacity to accelerate intestinal iron absorption and hepatic iron uptake as well as ethanol. It seems likely that iron is one of the important factors triggering the development of non-alcoholic steatohepatitis/non-alcoholic fatty liver disease, rather than a 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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Relationship between Alcohol Consumption and Serum Adiponectin Levels: The Takahata Study—A Cross-Sectional Study of a Healthy Japanese Population

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Context: The relationship between alcohol consumption and serum adiponectin levels has not been fully explored in an Asian population.

Objective: Our goal was to determine whether alcohol consumption is associated with a change in adiponectin levels in a healthy Japanese population.

Design: This was a cross-sectional study.

Setting: Subjects were recruited from participants in a health check-up program.

Participants: This study included 2932 subjects (1306 men and 1626 women).

Main Outcome Measures: The effects of total weekly or daily volume of ethanol intake on serum adiponectin levels were evaluated. In addition, the correlation of clinical traits with serum adiponectin levels was examined. A multivariate regression model was used to control for possible confounding factors.

Results: Alcohol consumption was weakly correlated with decreased serum adiponectin levels in men [Spearman's ordered correlation coefficient (r_s) = −0.141; $P < 0.001$]; an even weaker correlation was seen in women (r_s = −0.055; P = 0.025). Multivariate analysis demonstrated that alcohol consumption was independently associated with hypoadiponectinemia.

Conclusion: In contrast to reports from the United States and Europe among White and Black subjects, our study demonstrated an inverse association between alcohol intake and serum adiponectin levels in Asian subjects, suggesting ethnic differences in the effects of alcohol consumption on serum adiponectin levels. (***J Clin Endocrinol Metab* 95: 3828–3835, 2010**)

Adiponectin, predominantly synthesized in adipose tissue, is a major modulator of insulin action and resistance (1). It is also related to lipid metabolism, particularly higher levels of high-density lipoprotein cholesterol (HDL-C) and lower levels of triglycerides (2). Higher adiponectin levels are associated with a lower risk of coronary heart disease (3, 4) and type 2 diabetes (5).

Light to moderate alcohol intake is associated with lower risk for coronary heart disease, potentially by increasing HDL-C levels (6) or enhancing fibrinolysis (7).

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-1862 Received September 1, 2009. Accepted April 19, 2010.
First Published Online May 5, 2010

Abbreviations: ADH, Alcohol dehydrogenase; ALDH2, acetaldehyde dehydrogenase type 2; ALT, alanine aminotransferase; BMI, body mass index; FBG, fasting blood glucose; γ-GTP, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HMW, high molecular weight; LDL-C, low-density lipoprotein cholesterol; r_s, Spearman's ordered correlation coefficient.

Several previous studies performed in White and Black populations investigated the association between adiponectin concentrations and the risk of developing cardiovascular disease or type 2 diabetes and showed that alcohol intake was associated with elevated serum adiponectin levels (3). In contrast, recent studies in mice and rats have demonstrated that chronic ethanol feeding decreases circulating adiponectin concentrations (8, 9).

As previously described, there are ethnic differences both in serum adiponectin levels (10) and in the risk of type 2 diabetes and cardiovascular disease between Asian and White individuals that are not explained by conventional risk factors (11). In light of these findings, we hypothesized that alcohol consumption may have a different effect on modulation of adiponectin levels in individuals of Asian descent. This relationship has not been fully elucidated on a large scale because of the limited number of subjects. Given the sample size available to us, we chose to evaluate the relationship between alcohol consumption and serum adiponectin levels among a Japanese general population while adjusting for potential confounding factors.

Subjects and Methods

Study population

This study is a part of the Japanese prospective, population-based study held in an agricultural area located about 350 km north of Tokyo. The design and methods of these studies have been reported elsewhere (12–14). Briefly, the study was designed to evaluate the role of lifestyle, diet, and genetic factors in the subsequent development of many common diseases. The study cohort consists of subjects recruited from participants in the regular health check-up program for residents. Since 2004, the baseline survey and subsequent follow-up surveys have been conducted annually. The survey collects information on lifestyle and anthropometric measurements and collects blood and urine specimens from participants on the morning of the survey. The study protocols were approved by the ethics committee at Yamagata University.

Of 3826 participants in the health check-up program from June 1, 2004, through November 30, 2005, the present study population started with 3166 subjects aged 40 yr or older who agreed to participate (83%). Written informed consent was obtained from all subjects. For this analysis, we restricted subjects to those with available information on drinking status and adiponectin levels (n = 3130). We also excluded those who ate breakfast before blood was drawn or those with missing information regarding biomedical variables, anthropometrical variables, or blood pressure. Thus, data from 2932 subjects (1306 men and 1626 women) who met all eligibility criteria were analyzed.

Data collection and measurements

Height, weight, and blood pressure were measured with the subject in light clothes and without shoes, and the body mass index (BMI) (kilograms per square meter) was calculated. After blood samples were drawn, they were frozen in aliquots at −70 C within 4 h and stored frozen until measurements. Biochemical variables evaluated in this study included levels of total adiponectin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides, fasting blood glucose (FBG), fasting serum insulin, alanine aminotransferase (ALT), and γ-glutamyltransferase (γ-GTP). Plasma glucose, serum lipids, and liver enzymes were assayed by routine automated laboratory methods in a single laboratory (BML Inc., Tokyo, Japan). Serum insulin concentrations were measured using a chemiluminescent immunoassay kit (Kyowa Medics, Tokyo, Japan), with intra- and interassay coefficients of variation of 2.0–3.0 and 0.9–4.7%, respectively. Plasma total adiponectin levels were determined by a human adiponectin ELISA (Otsuka Pharmaceutical Co., Tokyo, Japan). Intra- and inter-assay coefficients of variation were 3.3–3.6 and 3.2–7.3%, respectively. All biochemical measurements were performed using plasma samples collected after an overnight fast. The estimate of insulin resistance was done using the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated from FBG and fasting insulin levels using the following formula: FBG (milligrams per deciliter) × fasting plasma insulin (microunits per milliliter)/405.

Assessment of alcohol consumption and smoking history

Information on alcohol consumption and smoking habits of each individual was obtained in face-to-face interviews. Alcohol consumption was calculated on the basis of ethanol volume, and each drinker's status was defined according to the total weekly volume of ethanol intake. The amounts of alcoholic beverages, including beer, wine, and whisky, were converted to an equivalent amount of sake (rice wine). One hundred eighty milliliters of sake contains 20 g ethanol; 180 ml sake equals 500 ml beer, 180 ml wine, or 60 ml whisky in alcohol content. Information on smoking habits was categorized as current use, past use, or never. To assess the reliability of the amount of alcohol consumption, we compared the volume of ethanol intake in the present study with the information on similar items in the survey conducted using a self-administered questionnaire during May 16 through May 29, 2005. Among 1457 subjects who completed the lifestyle questionnaire, Spearman's ordered correlation coefficient (r_s) between the two variables was 0.71.

Statistical analysis

Because alcohol habits are gender related (15), the analysis was conducted according to gender. Variables are given as means ± SD for variables with a normal distribution, median (25th–75th percentile) for skewed variables or n (percent) for numerical or categorized variables. The skewed variables (adiponectin, glucose, insulin, and triglyceride levels) were log transformed before statistical analysis.

Alcohol consumption was treated both as a continuous variable and as a categorical variable: abstainer, less than 120 g/wk, 120–239 g/wk, and 240 g/wk or more. BMI (<22.0, 22.0–24.9, and ≥25.0) and HOMA-IR (<2.0, 2.0–3.9, and ≥4.0) were categorized before statistical analysis. One-way ANOVA was used for testing between multiple groups, and Dunnett's test was used for subsequent comparison of abstainers with other groups. An unpaired *t* test was used to compare continuous data, and the χ^2 test was used for the analysis of proportions between groups. Pearson's correlation coefficient or r_s was calculated to evaluate

TABLE 1. Characteristics of study participants

	Men (n = 1306)	Women (n = 1626)	*P* value[a]
Age (yr)			
40–49	142 (10.9)	188 (11.6)	0.351
50–59	312 (23.9)	426 (26.2)	
60–69	447 (34.2)	546 (33.6)	
≥70	405 (31.0)	466 (28.7)	
Adiponectin (μg/ml)	7.0 (5.1–9.9)	10.4 (7.4–14.9)	<0.001
BMI (kg/m^2)			
<22.0	424 (32.5)	550 (33.8)	0.731
22.0–24.9	485 (37.1)	588 (36.2)	
≥25.0	397 (30.4)	488 (30.0)	
Blood pressure (mm Hg)			
Systolic	136.1 ± 15.7	133.1 ± 16.1	<0.001
Diastolic	81.9 ± 9.9	77.5 ± 9.8	<0.001
Serum lipids (mg/dl)			
Total cholesterol	193.4 ± 31.0	207.3 ± 0.9	<0.001
HDL-C	56.3 ± 14.4	61.6 ± 14.2	<0.001
LDL-C	119.1 ± 28.9	128.9 ± 29.6	<0.001
Triglycerides	95 (69–136)	88 (65–118)	<0.001
Glucose tolerance			
Glucose (mg/dl)	96.9 ± 19.5	92.3 ± 13.3	<0.001
Insulin (μU/ml)	4.2 (3.0–7.0)	5.0 (3.9–8.0)	<0.001
HOMA-IR			
<2.0	1084 (83.0)	1292 (79.5)	0.001
2.0–3.9	184 (14.1)	303 (18.6)	
≥4.0	38 (2.9)	31 (1.9)	
Liver enzymes			
ALT (IU)	21 (17–29)	18 (15–24)	<0.001
γ-GTP (IU)	32 (21–52)	19 (14–26)	<0.001
Alcohol consumption (g/wk)			
None	351 (26.9)	1384 (85.1)	<0.001
<120	366 (28.0)	207 (12.7)	
120–239	285 (21.8)	28 (1.7)	
≥240	304 (23.3)	7 (0.4)	
Smoking habit			
Never	506 (38.7)	1495 (91.9)	<0.001
Current	445 (34.1)	88 (5.4)	
Former	355 (27.2)	43 (2.6)	

χ^2 test, unpaired *t* test, or Mann-Whitney *U* test was used for analyses. Data are n (%) unless otherwise indicated: mean ± SD for blood pressure, total cholesterol, HDL-C, LDL-C, and glucose; median (25th–75th percentile) for adiponectin, triglycerides, insulin, ALT, and γ-GTP.

[a] Men *vs.* women.

the relationship between two continuous or ordered variables. Multiple regression analysis was used with covariance analyses, and log-transformed adiponectin was used as the independent variable. In multivariable analyses, the impact of the effect of 10 g/d alcohol consumption was assessed. The SPSS 15.0 program for Windows (SPSS Inc., Chicago, IL) was used for the statistical analyses. $P < 0.05$ (two sided) was considered statistically significant.

Results

Characteristics of the 2136 subjects are shown in Table 1. There were significant differences in adiponectin levels, lipid levels, glucose, insulin, HOMA-IR, and both systolic and diastolic blood pressure between men and women. Levels of all these variables, except for HDL-C and triglycerides, were significantly higher in women than in men. Only 15% of female subjects were drinkers compared with 73% of men ($P < 0.001$).

The relationship between adiponectin concentrations and potentially confounding factors and alcohol intake are shown in Table 2. Using correlation analysis, we found a small and significant negative correlation for adiponectin concentrations and alcohol consumption in men (r_s) = −0.141; $P < 0.001$) and a weaker negative correlation in women ($r_s = -0.055$; $P = 0.025$). Significant negative correlations with adiponectin concentrations were observed in total cholesterol, LDL-C, triglyceride, BMI, blood glucose, insulin, HOMA-IR, ALT, γ-GTP, systolic and diastolic blood pressure, and smoking habits in both in men and women. A positive correlation was observed in HDL-C levels in both genders.

In the next analysis, we used categorized data on alcohol consumption to investigate the relationship between alcohol intake and serum adiponectin levels. As shown in Fig. 1, adiponectin levels significantly decreased in a dose-

TABLE 2. Relationship between serum adiponectin concentrations and other factors studied

	Men (n = 1306)		Women (n = 1626)	
	Adiponectin levels or correlation coefficient[a]	*P* value	Adiponectin levels or correlation coefficient[a]	*P* value
BMI (kg/m^2)				
<22.0	8.4 (6.2–12.1)	<0.001	12.9 (9.2–17.6)	<0.001
22.0–24.9	6.9 (5.1–9.4)		10.0 (7.3–14.4)	
≥25.0	6.0 (4.4–8.1)		9.0 (6.4–12.7)	
Blood pressure (mm Hg)				
Systolic	−0.009	0.749	−0.029	0.242
Diastolic	−0.100	<0.001	−0.027	0.275
Serum lipids (mg/dl)				
Total cholesterol	−0.113	<0.001	−0.029	0.245
HDL-C	0.329	<0.001	0.355	<0.001
LDL-C	−0.103	<0.001	−0.097	<0.001
Triglyceride	−0.390	<0.001	−0.307	<0.001
Glucose tolerance				
Glucose (mg/dl)	−0.091	0.001	−0.183	<0.001
Insulin (μU/ml)	−0.341	<0.001	−0.441	<0.001
HOMA-IR				
<2.0	7.6 (5.4–10.3)	<0.001	11.4 (8.3–15.9)	<0.001
2.0–3.9	5.3 (3.8–6.7)		7.5 (5.7–10.7)	
≥4.0	4.9 (3.4–7.0)		5.6 (4.3–7.7)	
Liver enzymes				
ALT (IU)	−0.264	<0.001	−0.185	<0.001
γ-GTP (IU)	−0.300	<0.001	−0.223	<0.001
Alcohol consumption (g/wk)	−0.141	<0.001	−0.055	0.025
Smoking habit				
Never	7.5 (5.4–10.4)	<0.001	10.5 (7.5–15.0)	0.002
Current	6.7 (4.7–9.3)		9.1 (5.9–13.9)	
Former	7.2 (5.0–10.0)		9.8 (6.6–14.7)	

ANOVA, Pearson's correlation coefficient, or Spearman's correlation coefficient was used for analyses.

[a] Data are median (25th–75th percentile) of serum adiponectin levels, Pearson's correlation coefficient, or Spearman's correlation coefficient.

dependent manner in men ($P < 0.001$). A similar trend was noted in women ($P = 0.029$), although the relationship was not as clear as that seen in men. In women, a borderline significant decrease of serum adiponectin levels was observed among drinkers who consumed less than 120 g/wk of ethanol compared with abstainers ($P = 0.053$). A decrease in serum adiponectin levels was not noted in those who consumed 120 g/wk or more of ethanol compared with abstainers.

We also examined the established relationship between alcohol consumption and HDL-C levels. Significant positive correlations were demonstrated ($r_s = 0.165$, $P < 0.001$ for men; and $r_s = 0.118$, $P < 0.001$ for women), indicating that these relationships were consistent with previous studies.

Subsequently, we conducted a multiple regression analysis to assess the effect of 10 g/d alcohol intake on adiponectin concentrations, controlling for potential confounding factors. We included age, sex, BMI, systolic blood pressure, LDL-C, HDL-C, triglycerides, glucose, HOMA-IR, ALT, and smoking habits as covariates. Alcohol consumption was independently associated with hypoadiponectinemia: 10 g/d ethanol intake was associated with a 0.028 (95% confidence interval = −0.040 to −0.016; $P < 0.001$) μg/ml decrease of log-transformed adiponectin concentrations (Table 3).

Discussion

In this population-based cross-sectional study, we found that alcohol intake and serum adiponectin levels were significantly inversely associated in men. A suggested inverse association was demonstrated in women who consumed less than 120 g/wk alcohol. The weak inverse association between alcohol consumption and serum adiponectin concentrations was found even after adjustment for possible confounding factors. These are contradictory observations when compared with several previous epidemiological and experimental reports performed in White and Black populations (4, 16), but they are consistent with experimental studies in animal models (8, 9). Recently, Kawamoto *et al.* (17) reported an inverse relationship between high molecular weight (HMW) adiponectin and alcohol consumption among healthy Japanese men in a cross-sectional study. HMW complex is the most active

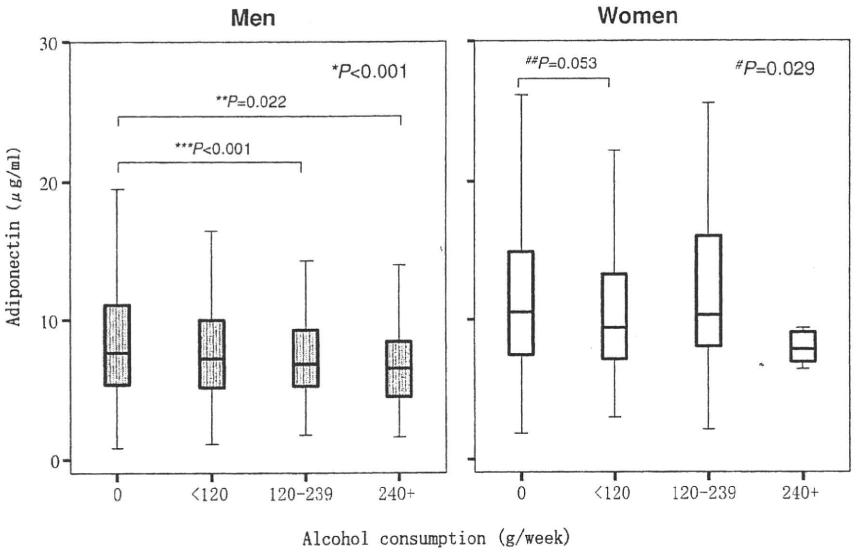

FIG. 1. Box plots illustrating serum plasma adiponectin concentrations for each level of alcohol consumption by gender. *Horizontal lines inside each box* represent medians, and the *top and bottom of the boxes* are the 25th and 75th quartiles, respectively. The *error bars* indicate 95% confidence intervals. *, $P < 0.001$ in men, and #, $P = 0.029$ in women for comparisons by ANOVA; **, $P = 0.022$, and ***, $P < 0.001$ in men, and ##, $P = 0.053$ in women for comparisons with abstainers in each group (Dunnett's test).

form of adiponectin and was closely associated with the type 2 diabetes when compared with total adiponectin (18). Moreover, it was shown that moderate alcohol consumption had different effects on HMW adiponectin, medium molecular weight adiponectin, and low molecular weight adiponectin (19). Further study is necessary to evaluate the effect of HMW on the association between serum adiponectin levels and alcohol consumption in a Japanese population.

Multiple regression analysis demonstrated that serum adiponectin levels were significantly related to sex, age, BMI, HDL-C, triglyceride, HOMA-IR, and ALT. All of the results are in good agreement with previous reports (3, 4, 10, 20, 21). Schulze *et al.* (4) observed an inverse relationship between plasma adiponectin levels and BMI and triglyceride but a positive relationship between plasma adiponectin levels and HDL-C and age in diabetic men. Ferris *et al.* (10) reported that serum adiponectin levels inversely correlated HOMA-IR in White subjects. A sex-based difference in plasma adiponectin levels was supported by previous studies (21, 22) and could be partly explained by differences in body fat distributions (22).

The consistent findings regarding the relationship between serum adiponectin levels and BMI, serum lipids, and insulin resistance and between alcohol consumption and HDL-C levels imply that factors related to ethnic differences, alcohol metabolism, and dietary intake may explain the discrepancies between our results and those of previous studies conducted in humans.

Alcohol is initially oxidized to acetaldehyde, mainly by the alcohol dehydrogenase (ADH) enzyme, and acetaldehyde is subsequently oxidized into acetate by the acetaldehyde dehydrogenase type 2 (ALDH2) enzyme (23). The gene that encodes these two representative alcohol-metabolizing enzymes displays polymorphisms that modulate individual differences in alcohol- and acetaldehyde-oxidizing capacity. Several ethnic differences in distribution of the ADH and ALDH2 genotypes, and in subsequent ethanol metabolism, have been demonstrated. First, the ADH class IV isozyme (σ-ADH), which is present predominantly in the upper gastrointestinal tract but not in the liver and which contributes to gastric ethanol oxidation, is absent or markedly decreased in 80% of Japanese people (24, 25). Second, about 85% of Japanese subjects are carriers of the ADH2*2 allele compared with only 5% or less of European and White American subjects (26). The ADH2*2 encodes an active enzyme and may be expected to generate more acetaldehyde because of this higher activity. Third, the ADH3*1 allele, coding for the rapidly acting ADH3, is more predominant (~95%) in Japanese subjects, whereas it is present in only 40–50% of White subjects (27). Finally, the ALDH2*2 allele, which encodes a catalytically inactive subunit, is present in about 45% of Japanese subjects, although it is extremely rare in White subjects (26). The latter three features indicate a failure to rapidly metabolize acetaldehyde, leading to excessive accumulation of acetaldehyde and higher susceptibility to acetaldehyde among a considerable number of Japanese subjects compared with White subjects. Ethanol and its metabolites, especially acetaldehyde, have been shown to have a toxic influence (23). Acetaldehyde is not only a highly toxic metabolite with extraordinary reactivity but was also shown to induce proinflammatory cytokines, TNF-α, and IL-1β in HepG2 cells (28), whereas TNF-α decreased the levels of adiponectin in human differentiated adipocytes (29). We assume that acetaldehyde and/or acetaldehyde adducts produced through oxidation of ethanol potentially modulate, in part, the association between alcohol intake and serum adiponectin concentrations in the Japanese population. Adjustments for polymorphisms in alcohol-metabolizing genes may explain the differences noted in ethnic groups.

Dietary factors play an important role in the development of type 2 diabetes and ischemic heart disease, because excess caloric intake contributes to the development of obesity, a major risk factor for both diseases. Studies on

TABLE 3. Multivariate-adjusted associations between serum adiponectin concentrations and alcohol consumption in 2932 subjects

Variables	Partial correlation coefficient	SE	Standardized partial correlation coefficient	95% confidence interval		*P* value
				Lower limit	Upper limit	
Sex (men,[a] women)	0.267	0.022	0.244	0.223	0.310	<0.001
Age (yr)	0.106	0.009	0.192	0.089	0.124	<0.001
BMI (<22,[a] 22–24.9, ≥25) (mm Hg)	−0.068	0.012	−0.099	−0.090	−0.045	<0.001
Systolic blood pressure (mm Hg)	0.000	0.001	−0.002	−0.001	0.001	0.902
LDL-C (mg/dl)	−0.001	0.000	−0.029	−0.001	0.000	0.058
HDL-C (mg/dl)	0.008	0.001	0.222	0.007	0.010	<0.001
Triglyceride (mg/dl)	−0.001	0.000	−0.081	−0.001	0.000	<0.001
Glucose (mg/dl)	−0.001	0.001	−0.025	−0.002	0.000	0.144
HOMA-IR (<2.0,[a] 2.1–3.9, ≥4.0)	−0.200	0.021	−0.170	−0.241	−0.158	<0.001
ALT (IU/liter)	−0.002	0.001	−0.060	−0.004	−0.001	<0.001
Smoking status (never,[a] current/former)	−0.031	0.022	−0.027	−0.074	0.011	0.147
Alcohol consumptions (10 g/d)	−0.028	0.006	−0.083	−0.040	−0.016	<0.001

Multiple regression analysis was used in covariance analyses for serum adiponectin concentrations after log transformation as independent variable.

[a] Reference category.

the dietary predictor of plasma adiponectin concentrations in animal models demonstrated that a high-fat diet is related to decreased serum adiponectin levels, just as it related to an increase in insulin resistance (30). Several controversial observations regarding fat intake have been reported when alcohol consumption accompanied this intake. High-fat, ethanol-containing food decreased serum adiponectin concentrations in mice (8) and rats (31). Decreases in serum adiponectin concentrations after ethanol feeding were dependent on the type of fat in the diet. Ethanol-containing diets high in unsaturated fats contributed to ethanol-induced decreases in adiponectin levels, whereas inclusion of saturated fats in the ethanol-feeding protocol prevented decreased adiponectin levels (9). A diet enriched in saturated fatty acids effectively reversed alcohol-induced necrosis, inflammation, and fibrosis despite continued alcohol consumption (32). The precise mechanism through which dietary fatty acids plus ethanol affect adiponectin expression and its secretion has yet to be determined. The protective action of saturated fatty acids is suggested to be partly caused by down-regulation of TNF-α (30, 33), which suppresses an adiponectin expression (29). In the Japanese population, both intake of total fat and that of saturated fats are lower than in the U.S. population (16, 34). The lower intake of saturated fat in the Japanese population may contribute to the different influence of alcohol consumption on adiponectin concentrations between Japanese and White subjects. However, it was not helpful to compare the effect of the intake of saturated fats with that of unsaturated fats in our study, because intake of these two fats was highly correlated ($r_s = 0.87$) among 1457 subjects who had completed the nutritional survey conducted in the same district using a self-administered questionnaire (unpublished data).

Carbohydrate intake may also be a factor that modulates the relationship between alcohol intake and adiponectin concentrations. In epidemiological studies, high glycemic loads, which were calculated by multiplying the carbohydrate content of each food by its glycemic index, were significantly associated with lower adiponectin concentrations in healthy men (16). For Japanese people, rice is the primary food that contributes to total carbohydrate and energy intake, which is seldom the case in Western populations. Data from the nutritional survey conducted in the same district (unpublished data) have shown that carbohydrate intake accounted for about 59% of total energy intake, and the mean glycemic load was about 206 among subjects aged 40 yr or over. Both parameters were higher than those of White adults (16). Although the effect of the dietary glycemic intake on the relationship between alcohol intake and adiponectin concentrations has not been fully elucidated, the higher intake of carbohydrate in the Japanese population may contribute to the different influence of alcohol consumption on adiponectin concentrations between Japanese and White subjects.

Our study demonstrated an inverse association between alcohol intake with serum adiponectin levels in men, with less clear findings in women. This discrepancy might be explained, in part, by the gender difference in ethanol metabolism. Women differ from men in several factors associated with alcohol metabolism (35), including 1) a lower gastric σ-ADH activity, which mediates the first-pass mechanism of ethanol in women, and 2) a decreased volume of ethanol distribution (body size and distribution space for alcohol, with water space being smaller

in women). However, these properties are not sufficient to explain the gender difference of the effect of alcohol intake on serum adiponectin concentrations. The small number of drinkers among our female subjects (15%) might cause difficulty in evaluating this result. Further study, including increasing the number female drinkers enrolled, is necessary to examine this inference.

There are potential limitations to this study. Because of its cross-sectional nature, this study did not provide a causal inference regarding the association between alcohol intake and serum adiponectin levels. However, information on the drinking habits of subjects was determined before the measurement of adiponectin concentrations; thus, an incorrect finding of an inverse association is unlikely. Data on drinking habits was based on face-to-face interviews, which leads to the possibility of misclassification of exposure (*e.g.* underreporting). However, it is also unlikely that this type of misclassification is directly dependent on adiponectin levels, which could be a nondifferential misclassification. Because our study subjects were recruited from participants in a health screening program, any generalization of these results to the normal population should be made with caution.

In conclusion, alcohol consumption was weakly associated with decreased serum adiponectin concentrations in apparently healthy Japanese subjects. Further investigations in Japanese subjects on alcohol metabolism and nutrition intake are necessary to clarify the factors that modulate this inverse effect, which differs from that seen in White subjects.

Acknowledgments

We thank Mr. Kazuo Goto, the Center for Disease Control and Prevention in Takahata, for his assistance with data collection.

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This study was partly funded by a Grant-in-Aid from the Global COE program of the Japan Society for the Promotion of Science.

The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Disclosure Summary: The authors have nothing to disclose.

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