Role of Iron Deficiency and Overload in the Pathogenesis of Diabetes and Diabetic Complications

Qiuju Liu[†,1,3,4], Liang Sun[†,2], Yi Tan[1], Guanjun Wang[3], Xu Lin*[,2] and Lu Cai*[,1,3]

[1]*Departments of Medicine, Radiation Oncology, and Pharmacology and Toxicology, the University of Louisville, Louisville, USA;* [2]*Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Graduate University of the Chinese Academy of Sciences, Shanghai, China;* [3]*The First and* [4]*the Second Hospitals of Jilin University, Changchun, China*

Abstract: Iron is one of the essential minerals that are required for a variety of molecules to maintain their normal structures and functions and for cells to live, grow, and proliferate. The homeostasis of iron results from a tightly coordinated regulation by different proteins involved in uptake, excretion and intracellular storage/trafficking. Although it is essential, iron can also be toxic once in excess amounts. Through Fenton reaction, iron as a transit mineral can generate various reactive oxygen or nitrogen species; therefore, abnormal metabolism of iron can lead to several chronic pathogenesis. Oxidative stress is one of the major causative factors for diabetes and diabetic complications. Increasing evidence has indicated that iron overload not only increases risks of insulin resistance and diabetes, but also causes cardiovascular diseases in non-diabetic and diabetic subjects. Temporal iron deficiency was found to sensitize insulin action, but chronic iron deficiency with anemia can accelerate the development of cardiovascular diseases in non-diabetic and diabetic patients. In this review, therefore, we will first outline iron homeostasis, function, and toxicity, and then mainly summarize the data regarding the roles of iron deficiency and overload in the pathogenesis of diabetes and diabetic complications, as well as the possible links of iron to diabetes and diabetic complications. In the end, the possible therapy using iron chelators for diabetes and diabetic complications will also be discussed.

Keywords: Diabetes, diabetic complications, insulin resistance, iron deficiency, iron overload, iron, ferritin, anemia.

1. INTRODUCTION

Prevalence of diabetes, a disorder of metabolism, is globally increasing. Most of the food we eat is broken down into glucose, which is the main source of fuel for the body. After digestion, glucose passes into the bloodstream, where it is used by cells for growth and energy. For glucose to get into cells, insulin must be present. Insulin is a hormone produced by the pancreas. When we eat, the pancreas automatically produces the right amount of insulin to move glucose from blood into our cells. In people with diabetes, however, the pancreas either produces little or no insulin (Type 1 diabetes), or the cells do not respond appropriately to the insulin that is produced (Type 2 diabetes). Glucose builds up in the blood, overflows into the urine, and passes out of the body in the urine. Thus, the body loses its main source of fuel even though the blood contains large amounts of glucose.

Once we have crossed the reversible stage of pre-diabetes and enter diabetic stage, certain changes start developing in our body. These changes occur due to high blood glucose level with instability in the hormones as well as vessels and nerves. When these changes become permanent in body serious diabetic complications develop and the body indicates these changes by steady symptoms. Therefore, both type 1 and type 2 diabetes have the same long-term complications, including cardiomyopathy, retinopathy, nephropathy, and neuropathy [1,2]. Several mechanisms for the onset of diabetes and the development of diabetic complications have been proposed, one of which may be the abnormal homeostasis of trace elements such as iron [3-5].

Iron is one of the important essential minerals, and its abnormal homeostasis such as deficiency or overload is associated with the pathogenesis of various chronic diseases, including diabetes [3,6]. Although recent two reviews have discussed the role of iron in diabetes [7,8], the focuses in these reviews are the associations of iron overload with the risk of insulin resistance and type 2 diabetes. Therefore, this review will briefly outline iron homeostasis, biological function and toxicity, and then will mainly update the epidemiological studies showing the association of iron deficiency or overload with the impaired glucose tolerance, diabetes and diabetic complications. New insights into understanding the possible mechanisms by which iron deficiency or overload may cause the onset of diabetes and diabetic complications are provided. In the last part, the potential use of iron chelators as well as its combination with other components for the therapy of diabetic patients will be also discussed.

2. IRON HOMEOSTASIS, BIOLOGICAL FUNCTION AND TOXICITY

Iron is one of the essential minerals that are required for a variety of molecules to maintain their normal structures and functions and for cells to live, grow, and proliferate. Several reviews are available regarding the chemical, physiological and toxicological features of iron [3,6,9,10]; therefore, we only outline these pieces of information incorporated with the update knowledge regarding its homeostatic regulation, biological function, and toxicity in this section in order to help understanding the contents in other sections.

2.1. Iron Homeostatic Regulation

Under most conditions, dietary iron is a critical determinant of body's iron status since once absorbed it is not ac-

*Address correspondence to these authors at the 511 South Floyd Street, MDR 533, Louisville, KY 40202, USA; Tel: (502) 852-5215; Fax: (502) 852-6904; E-mail: L0cai001@louisville.edu

294 Tai-Yuan Rd., Shanghai, 200031, China. Tel: + 86 (21) 5492-0249; Fax: + 86 (21) 54920291; E-mail: xlin@sibs.ac.cn

[†]These Authors are Equally contribution.

0929-8673/09 $55.00+.00

tively excreted. Iron absorption is influenced by a number of factors, but feedback regulation and bioavailability of iron-containing foods are the two keys. An overview of iron metabolism is provided in Fig. (**1A**). Iron in foods is absorbed as heme and non-heme forms in the proximal small intestine into blood. In the blood, the iron is bound to the plasma protein transferrin (Tf, see the summary of abbreviations in Table **1**) and distributed to the body's tissues and organs [6,10]. Quantitatively, most iron is incorporated into erythrocytes for heme synthesis, but all body cells require some iron to meet their metabolic requirements. During adulthood, iron stores gradually increase in men, and start to increase after menopause in woman.

Table 1. Abbreviations

AGEs	advanced glycated endproducets
Ang II	angiotensin II
CHD	chronic hypertension disease
CO	carbon monoxide
DCYTB	duodenal cytochrome b
DFO	Deferoxamine
DMT1	divalent metal transporter
Fe2+-O	Ferryl
Fe2+-O2	Perferryl
FPT-1	ferroportin 1
HbA1c	hemoglobin A1c
HCP1	heme carrier protein
HH	Hemochromatosis
HIF	hypoxia-inducible factor
HO	heme oxygenase
LIP	labile iron pool
LPS	Lipopolysaccharide
Tf	Transferring
TfR-1	Tf receptor-1
TGF-ß1	transforming growth factor beta1
TZD	Thiazolidinedione
RBP	retinol-binding protein
ROS	reactive oxygen species
RNS	reactive nitrogen species
STZ	Streptozotocin

Fig. (**1B**) illustrates the absorption of dietary iron in intestinal cells and exportation of iron into blood. Both heme iron and inorganic iron have their specific absorption pathways [10,11]. Through the putative intestinal heme iron transporter (HCP1), heme iron is transported into cells and ferrous iron (Fe2+) is liberated within the endosome or lysosome. Transport of non-heme iron from the intestinal lumen into the enterocytes is mediated by the divalent metal ion transporter 1 (DMT1). DMT1 transports only Fe2+, but most dietary iron that enters the duodenum is in the ferric form (Fe3+). Therefore, Fe3+ must be first reduced to Fe2+, possibly by the brush border ferric reductase, duodenal cytochrome b (DCYTB) or by other reducing agents, such as ascorbic acid. Once inside the enterocyte, iron that is not directly transferred to the circulation is stored as ferritin and ultimately is lost when the cell is sloughed at the villus tip. Efflux of iron across the basolateral membrane into the blood is mediated by the transport protein ferroportin 1 (FPT-1). FPT-1 also mediates export of iron for other cells, including macrophages.

Hepcidin is a peptide hormone secreted by the liver that plays a central role in the regulation of iron homeostasis. Hepcidin regulates the entry of iron into blood by directly binding to the FPT-1 on basolateral membranes of enterocytes, the plasma membranes of macrophages and other type cells [12-14]. The binding of hepcidin leads to the internalization and degradation of FPT-1, thereby blocking cellular iron export, and reducing plasma iron (Fig. **1B**). Hepcidin thus acts as a suppressor of cellular iron release. In iron deficiency (see Table **2** for the definition), hepcidin release from the liver is decreased, thereby increasing iron absorption to the maximum. Iron deficiency also stimulates duodenal expression of DMT1, DCYTB, and FPT-1, and thereby increases iron absorption [6,10,11]. In contrast, iron excess (see Table **2**) stimulates liver to secrete more hepcidin that decreases the export of iron from enterocytes and iron release from reticuloendothelial systems [13,14].

Fig. (**1C**) shows the metabolism of iron in the erythrocyte. The TF-bound iron in the blood is distributed to the cell in the tissues or organs *via* its Tf receptor-1 (TfR-1). The Tf-TfR1 complexes are internalized into the cell *via* receptor-mediated endocytosis. After a reduction in endosomal pH, Tf-bound iron is released and then transported across the endosomal membrane *via* the DMT1 [14]. After release from Tf and entry into the cytosol, iron becomes a part of the poorly characterized compartment known as intracellular labile iron pool (LIP). The capacity to storing iron in ferritin is essential for life in mammals, but the mechanism by which cytosolic iron is delivered to ferritin is unknown [10,14] until a recent study [15]. Shi *et al.* found that human ferritins expressed in yeast contained little iron, but human poly (rC)-binding protein 1 (PCBP1) increased the amount of iron loaded into ferritin when expressed in yeast [15]. PCBP1 was found able to bind to ferritin and iron that facilitated iron loading into ferritin. Depletion of PCBP1 in human cells inhibited ferritin iron loading and increased cytosolic iron pools. Therefore, PCBP1 can function as a cytosolic iron chaperone in the delivery of iron to ferritin [15].

In addition, an outer mitochondrial membrane protein mitoNEET was recently discovered as an iron-containing protein to play an important role in the control of maximal mitochondrial respiratory rate [16]. Based on the biophysical data and domain fusion analysis, mitoNEET was considered probably to function in Fe-S cluster shuttling and/or in redox reactions [17]. The storage of iron in proteins with Fe-S clusters, such as ferritin and mitoNEET, makes it unavailable for Fenton reaction (see the below), providing a protection for cells against the damaging effects of "free iron". Therefore, whether mitNEET plays a critical role in iron homeo-

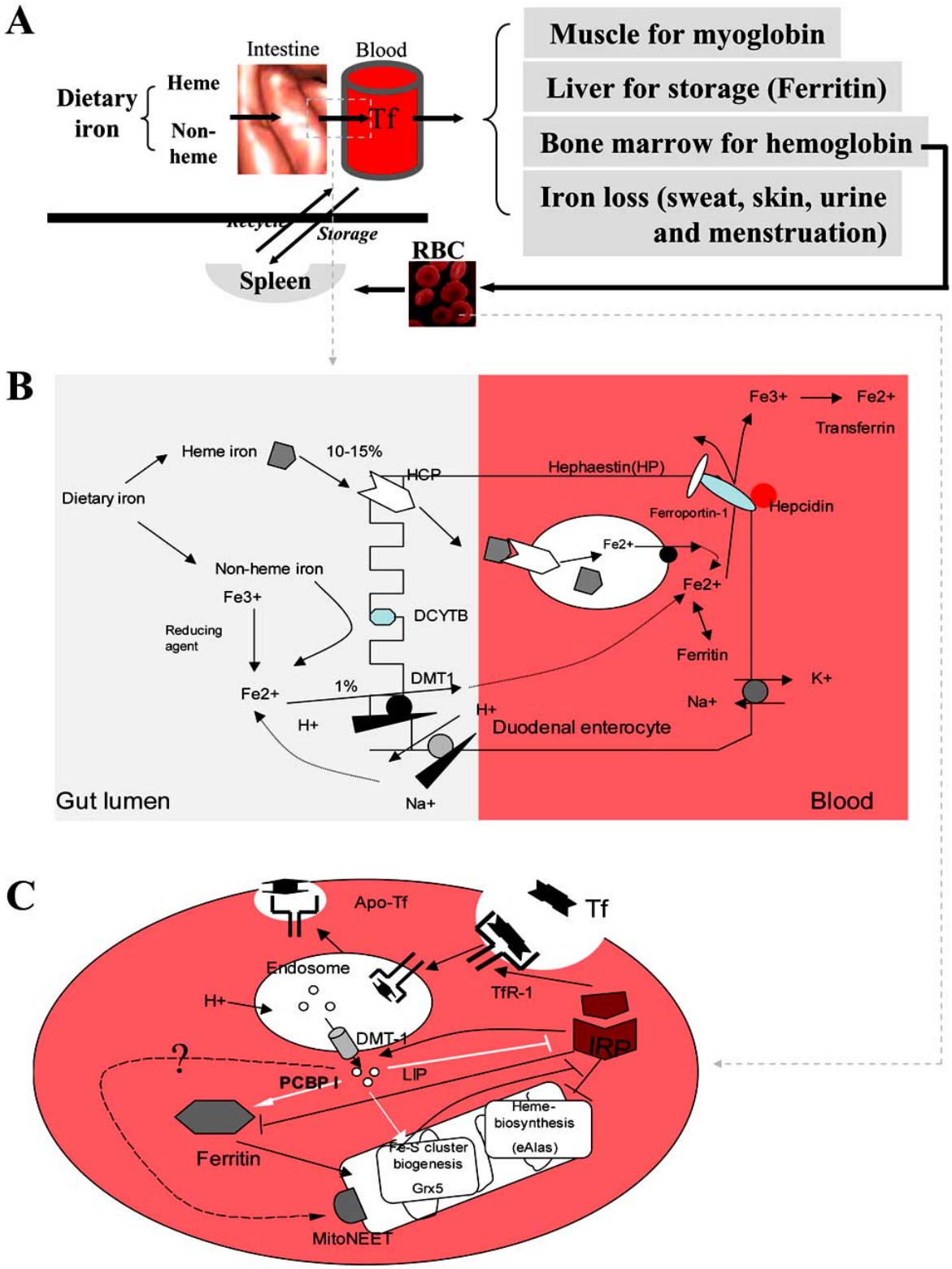

Fig. (1). Schematic illustration of iron metabolism. Panel **A** is to indicate the overview of iron homeostasis. Panel **B** is to indicate how heme iron and inorganic iron are absorbed into intestinal cell and exported into blood. Panel **C** is to illustrate how iron bound Tf is taken into cell and used for heme protein synthesis. Tf: transferrin; RBC, red blood cells; HCP, heme iron transporter; DMT1, divalent metal-ion transporter; DCYTB, duodenal cytochrome b; TfR-1, transferrin receptor 1; IRP, iron-regulatory protein; LIP, labile iron pool; PCBP1, poly (rC)-binding protein 1; mitNEET, a recently identified an outer mitochondrial membrane protein that is an iron-containing protein and plays an important role in the control of maximal mitochondrial respiratory rate [86]. Grx5, glutaredoxin 5. In the panel **C**, IRPs control the expression of erythroid 5-aminolevlinate synthase (eALAS), the first and rate-limiting enzyme in the heme biosynthetic pathway. IRP activity is modulated by the LIP (IRP1 + IRP2) and by Fe-S-cluster biosynthesis. This figure was made based on the published materials [8,11,13].

Table 2. Definition and Common Reasons of Iron Deficiency and Overload

	Main criteria	Common reasons
Iron deficiency	1. Bone marrow staining: storage iron reduced; sideroblast ≤ 20%. 2. Serum ferritin ≤ 12 ng/ml 3. Transferrin saturation < 10%	1. Increased demand of iron. 2. Increased lose of iron. 3. Less iron uptake 4. Others: inflammation, chronic disease.
Iron overload	1. Serum Ferritin > 300ng/ml (men) > 150ng/ml (women) 2. Transferrin saturation > 50%	Primary: Hemachromatosis Secondary: (1) Transfusional iron overload (2) Long term hemodialysis. (3) Dietary iron overload. (4) Chronic liver disease.

Notes: (1) These values were obtained based on a few publications [6,8,11,152]; (2) The numbers are provided just for references of serum ferritin levels. In fact, clinical diagnosis of iron deficiency needs comprehensively analysis of several measurements and case situation. That is because iron deficiency may exist in patients with inflammation or malignancy even with a normal serum ferritin.

stasis as ferritin and how the LIP iron is incorporated into mitNEET remain to be further investigated (Fig. **1C**).

2.2. Biological Functions of Iron

Iron plays a crucial role in oxygen sensing and transport, electron transfer, and catalysis [10,18]. A significant fraction of cellular iron is associated with proteins in the form of heme, a common prosthetic group composed of protoporphyrin IX and a Fe2+ ion. The insertion of Fe2+ into protoporphyrin IX, catalyzed by ferrochelatase in the mitochondria, defines the terminal step of the heme biosynthetic pathway (Fig. **1C**). Heme is then exported to the cytosol for incorporation into hemoproteins. The most abundant mammalian hemoproteins, hemoglobin and myoglobin (Fig. **2**), serve as oxygen carriers in the erythroid tissue and in the muscle, respectively. Oxygen binding is mediated by the heme moieties. Other hemoproteins include various cytochromes and enzymes, such as oxygenase, peroxidase, nitric oxide (NO) synthase, or guanylate cyclase. The heme moiety may also function in electron transfer reactions (e.g., in cytochromes a, b, and c), as a substrate activator (e.g., in cytochrome oxidase, cytochrome P450, catalase) or as an NO sensor (in guanylate cyclase) [10,18,19].

Heme degradation is catalyzed by the microsomal heme oxygenase 1 (HO-1) and its homologues HO-2 and HO-3 [10,19]. As shown in Fig. (**2**), heme degeneration produces a liberated Fe2+ that can be reutilized, a carbon monoxide (CO) that may be involved in signaling pathways, and a molecule biliverdin that is further enzymatically converted to the bilirubin. Bilirubin, when oxidized, reverts to become biliverdin once again. Therefore, bilirubin has been assumed to play an important antioxidant role against oxidative stress [19,20].

2.3. Chemical Features and Toxicity of Iron

Iron is chemically active in forming a variety of coordination complexes with organic ligands in a dynamic and flexible mode, and potential to switch between Fe2+ and

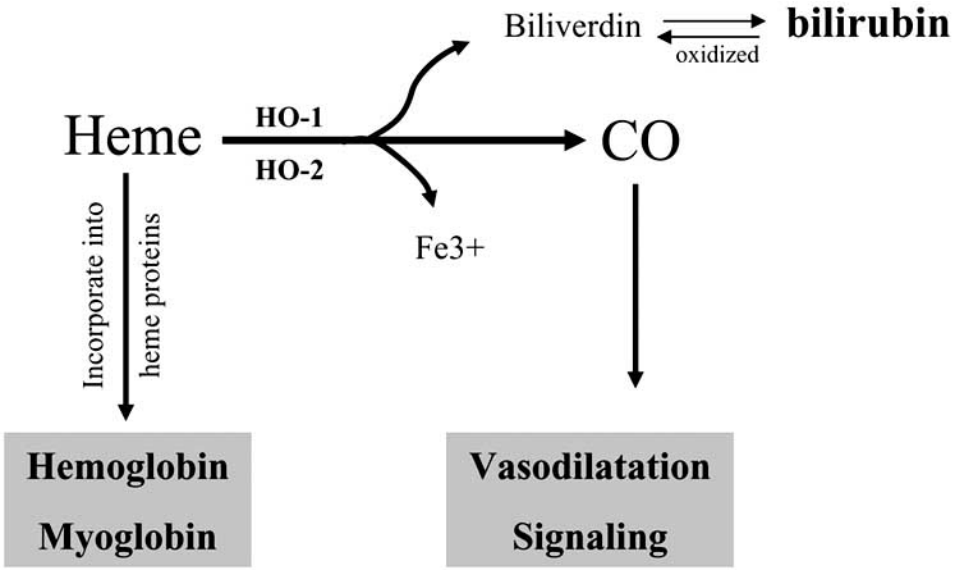

Fig. (2). Schematic illustration for the fate of heme. Heme exported into the cytosol is transported to mitochondria for synthesizing hemoproteins, such as the two most abundant heme proteins: hemoglobin and myoglobin. In addition, heme is degraded by canalization of microsomal heme oxygenase 1 (HO-1) or HO-2 to produce a liberated Fe2+, a carbon monoxide (CO), and a molecule biliverdin that is further enzymatically converted to the bilirubin.

Fe3+. The efficiency of Fe2+ as an electron donor and of Fe3+ as an electron acceptor is a fundamental feature for many biochemical reactions and renders iron to an essential mineral and nutrient. However, the ability of iron to cycle between its two stable oxidation states is also potentially to generate reactive oxygen or nitrogen species (ROS or RNS) such as hydroxyl radical ($OH^{\bullet}$) *via* Fenton and Haber–Weiss reactions, as indicated in Fig. (**3A**) [9,21,22]. Heme iron may catalyze the formation of radicals, mainly *via* formation of oxoferryl intermediates (Fig. **3B**). Finally, Fe2+ can also contribute as a reactant, rather than as a catalyst, to free radical generation by a direct interaction with oxygen, *via* ferryl (Fe2+–O) or perferryl ($Fe2+–O_2$) iron intermediates (Fig. **3C**) (see reviews [9,22]).

ROS and RNS, including superoxide, nitric oxide, peroxynitrite, hydrogen peroxide and hydroxyl radical, are highly reactive and potentially damaging to cells and tissues [9,22], although at low concentrations these species may also act as second messengers, gene regulators, and/or mediators of cellular activation [23]. To control and balance the production of ROSs and RNSs, the cell builds up a set of antioxidants and detoxifying enzymes such as superoxide dismutase, catalase, and glutathione peroxidase that can scavenge excessive ROSs or RNSs. An increase in the steady state levels of ROS and/or RNS beyond the antioxidant capacity of the organism, called oxidative (or nitrosative) stress, is encountered in many pathological conditions, such as diabetes and diabetic complications [2,3,10,23].

3. IRON DEFICIENCY

3.1. Iron Deficiency, Glycated Hemoglobin Levels, and Insulin Resistance

Iron deficiency is the reduction of storage iron, shown by reduced plasma ferritin concentration and decreased bone marrow iron staining (Table **1**). The major form of glycated hemoglobin in diabetic patients is hemoglobin A1c (HbA1c). In non-diabetic subjects, the HbA1c reaches a steady state with 3.0% to 6.5% of the hemoglobin while the HbA1c fraction is abnormally elevated in patients with chronic hyperglycemia and correlates positively with glycemic control. An early study by Davis *et al*. demonstrated that iron-deficiency in a 68-year-old female patient with diabetes was associated with a marked rise in HbA1c (from 10.7% to 15.4%), and if the iron-deficiency anemia was corrected, the HbA1c level fell to 11.0% [24]. Tarim *et al*. [25] also performed a prospective study including 37 patients with type 1 diabetes (11 patients were iron-deficient and the remaining 26 were iron-sufficient). Patients with iron deficiency had higher levels of HbA1c than patients without iron deficiency. After iron supplementation for three months, these patients showed a significant decrease in HbA1c levels.

The HbA1c-enhancing effect of iron deficiency was observed not only in diabetic individuals, but also in non-diabetic individuals [25-27]. For instance, a survey was performed based on 730 university students. Eighty-one stu-

A

$$Fe2^{+} + H_2O_2 \rightarrow Fe3^{+} + OH^{-} + OH^{\bullet} \quad \text{(Fenton reaction)}$$

$$Fe3^{+} + O_2 \rightarrow Fe2^{+} + O_2^{\bullet-}$$

Net reaction

$$H_2O_2 + O_2^{\bullet-} \xrightarrow{Fe} OH^{-} + OH^{\bullet} + O_2 \quad \text{(Haber-Weiss reaction)}$$

B

$$Heme\text{-}Fe2^{+}\text{-}O_2 + H_2O_2 \rightarrow Heme\text{-}Fe4^{+}\text{-}OH^{\bullet} + O_2 + OH^{-}$$

$$Heme\text{-}Fe4^{+}\text{-}OH + ROOH \rightarrow Heme\text{-}Fe3^{+} + ROO^{\bullet} + O_2H_2^{-}$$

C

$$Fe2^{+} + H_2O_2 \longrightarrow Fe2^{+}\text{-}O + H_2O$$

$$Fe2^{+} + O_2 \longrightarrow [Fe2^{+}\text{-}O_2 \rightarrow Fe3^{+}\text{-}O_2^{\bullet-}] \longrightarrow Fe3^{+} + O_2^{\bullet-}$$

Fig. (3). Illustration of the generation of ROS by iron *via* Fenton-reaction. Panel **A** illustrates the generation of the hydroxyl radical by iron *via* the Fenton reaction; the net Haber–Weiss reaction is also indicated. Panel **B** illustrates how heme iron catalyzes generation of oxygen radicals *via* oxoferryl intermediates. Panel **C** outlines the direct interaction of iron with oxygen. This figure was modified based on a published materials [22].

dents were anemia, of which 47 were iron deficient with an average of HbA1c being 6.2 ± 0.6%. When they were treated with oral iron and followed up for 20 weeks, HbA1c fell significantly to 5.3 ± 0.5 % [26]. Hashimoto *et al.* [28] recently also demonstrated that the HbA1c , but not serum glycated albumin, is elevated in late pregnancy due to iron deficiency, and correction of iron deficiency resulted in a correction of HbA1c . These studies thus suggest that among non-diabetic or diabetic individuals with similar level of glycemia, iron deficiency anemia is associated with higher concentrations of HbA1c. Iron replacement therapy drops HbA1c in both diabetic and non-diabetic individuals [26,27]. This implies that the iron states must be considered during the interpretation of HbA1c concentrations in diabetic or non-diabetic patients.

An interpretation is that lipid peroxidation can cause hemoglobin glycation [29]. Human erythrocytes in phosphate buffered saline (pH 7.4) were incubated with 5 or 50 mmol/l glucose. Level of HbA1c was higher in erythrocytes incubated with 50 mmol/l glucose than that in erythrocytes incubated with 5 mmol/l glucose. Selvaraj *et al.* [29] further showed that the increase in HbA1c levels was blocked significantly when erythrocytes were pretreated with either lipoic acid or taurine, both which markedly reduced the lipid peroxide levels. The level of HbA1c in erythrocytes incubated with glucose along with the presence of lipid peroxides was significantly increased compared to erythrocytes incubated with glucose alone. Sundaram *et al.* [30] also reported the increase in the plasma lipid peroxides of the patients with iron deficient anemia, and the correction of the increased lipid peroxides in the patients with iron deficient anemia by iron supplementation therapy. In addition, low levels of antioxidant levels stimulate the formation of hemoglobin glycation [31]. For instance, in eighteen patients with nephropathic syndrome, both HbA1c and lipid peroxide levels were significantly increased, along with a significant decrease in glutathione levels compared with fifteen healthy controls. If erythrocytes were depleted of glutathione by pretreatment with 1-chloro-2, 4-dinitrobenezene, these cells become more sensitive to high glucose-induced hemoglobin glycation [31]. Therefore, lipid peroxides play a role in hemoglobin glycation and antioxidants can inhibit the formation of glycated hemoglobin by lowering the levels of lipid peroxides[30,31].

In summary, iron deficiency anemia in diabetic and non-diabetic individuals was associated with the increased HbA1c, which can be corrected after iron deficiency was corrected. The association of increased HbA1c with iron deficiency anemia may be due to the increased lipid peroxidation derived from anemia.

3.2. Blood Donation, Insulin Sensitivity and Cardiovascular Diseases

It is known that blood donation can cause a transient anemia or iron-deficiency; therefore, whether iron-deficient anemia in the blood donors affects the prevalence of insulin resistance and metabolic syndrome has been questioned [32]. Male blood donators (n = 21) who had donated blood between 6 months and 5 years were carefully matched with non-donators (n = 66) for age, body mass index (BMI), waist-to-hip ratio, and cardiovascular risk profile, including blood lipids, blood pressure, and smoking states. Although these two groups had similar blood hematocrits and blood hemoglobin concentrations, frequent blood donators (2-10 donations) significantly increased insulin sensitivity, decreased insulin secretion, and significantly reduced iron stores (serum ferritin, 101.5 ± 74) than nondonators (162 ± 100 ng/mL; P = 0.017) [32]. Similarly, in type 2 diabetic patients [33], Fernandez-Real *et al*. also found that serum ferritin and transferrin saturation index decreased significantly at 4 and 12 months in patients who had blood letting (500 ml of blood for three times at 2-week intervals of each blood letting), along with significant decreases in blood HbA1c levels and insulin resistance as compared with those observed in a matched no-blood-letting group of diabetic patients with high-ferritin.

However, the above findings were not supported by all studies. In a study conducted by Jiang *et al.* [34], 389 healthy blood donators and 239 non-donators have been questioned for the times of blood donation within the past 30 years. These peoples were divided into never, 1-5, 6-9, 10-19, 20-29, and ≥30 times blood donations, with an average of serum ferritin concentrations of 187, 186, 187, 160, 93, and 64 ng/mL for the first seven groups, respectively. There was no appreciable association between blood donation times and the risk of type 2 diabetes.

Consistent with the above study showing the correlation of frequent blood donation with reduced risks of insulin resistance and type 2 diabetes [32], an early study found that blood donation decreased cardiac incidence for non-diabetic subjects [35]. In this study, Myers *et al*. have enrolled patients for age ≥ 40 years without clinically apparent vascular diseases (1985-1987) [35]. The occurrence of cardiovascular events (myocardial infarction, angina, and stroke), procedures (angioplasty, bypass surgery, claudication, and endarterectomy), nitroglycerin use or death (all cause mortality) was analyzed against levels of blood donation in men and women. It was found that blood donation in men, but not in women, was associated with reduced risk of cardiovascular events. In another retrospective cohort study with a total of 1508 adults who donated more than 1 unit of whole blood each year between 1988 and 1990 (frequent donators) and 1508 age- and sex-matched adults who donated only a single unit in that 3-year period (casual donators) [36], cardiovascular events were found to be 6.3% in frequent donators and 10.5% in casual donators, suggesting that frequent and long-term whole blood donation is associated with a lower risk of cardiovascular events. Furthermore, frequent blood donations were also found to decrease the body iron stores and oxidative stress, and enhance vascular function as compared to the less frequent donators [37].

However, there were also a few studies with different results [38-40]. Zacharski *et al*. have tested whether reducing body iron stores through phlebotomy would influence clinical outcomes in a cohort of patients with symptomatic peripheral arterial disease (PAD). They recruited 641 PAD patients as control group and 638 PAD patients as bloodletting group who have donated defined volumes of blood at 6-month intervals (avoiding iron deficiency). No significant difference between groups for the all-cause mortality or

death plus nonfatal myocardial infarction and stroke was found [38]. Zheng *et al*. [39] have conducted a prospective cross-sectional study in twenty-one high-frequency blood donators (donations ≥ 8 times in last 2 years) and twenty-one low-frequency blood donators (1-2 donations in the last 2 years) aged 50-75 years. Serum ferritin was significantly decreased (23 vs. 36 ng/ml, $P < 0.05$) in high-frequency blood donation compared with low-frequency donation, but did not improve insulin sensitivity or protect the vascular endothelium from the adverse effects of acute hyperglycemia after oral glucose loading. Engberink *et al*. collected data on blood donation history and intima-media thickness of the common carotid artery in 819 subjects (50-70 years) from the blood bank registries in The Netherlands. Serum ferritin was lower in current donators (n=443; 44 ng/mL) than in ex-donators (n=120; 114 ng/mL) and never-donators (n=256; 124 ng/mL). They found that the intima-media thickness of the common carotid artery was only slightly reduced in frequent donators (i.e., ≥ 49 times during life or ≥ 2 times per year), but it was not statistically significant. They concluded that frequent blood donation, resulting in lowered body iron, might give some protection against accelerated atherosclerosis [40].

These discrepancies for the outcomes among the above studies may be due to the following reasons: (a) Different fractions of populations selected from healthy and unhealthy groups may have different responses to frequent blood donations [32]; (b) In the study of Zacharski *et al*. [38] the blood donators were selected from those without lowering ferritin although they have had blood donations; (c) The ferritin levels in the control group may be important. Although this endpoint was not available from the early studies conducted by Meyers *et al*. [36], the two studies from Zheng *et al*. [39] can indicate the importance of ferritin levels in the control group. For instance, in the latest study by Zheng *et al*. [39] that showed no correlation of blood donations with the risk of cardiovascular events, ferritin levels in frequent and unfrequent blood donation groups were only 23 and 36 ng/ml, respectively, which was not a remarkably different between two groups even though it was statistical difference ($P < 0.05$). In contrast, in another study that showed a positive correlation of blood donation with the risk of cardiovascular events [37], the difference for the serum ferritin between high-frequency blood donators (17 ng/mL) and low-frequency blood donators (52 ng/mL; $P<0.001$) was remarkable; (d) The case number is also a factor to be considered. There were only 21 cases in high-frequent blood donators and 21 low-frequent blood donators in the study with no correlation [39] while there were 40 and 42 cases in these two groups, respectively in the study with correlation [37]. Accordingly, systemic studies with uniformed measurements remain to clarify the risks or benefits of frequent blood donations for cardiovascular diseases. Therefore, although a few studies indicated the improvement of insulin sensitivity by blood donating, it would not be encouraged in the clinics. In addition, the safety of the repeated and frequent blood donations is also a concern [41,42]. What is the importance of the occasional improvement of insulin resistance and cardiovascular events by blood letting found in certain studies for our practice has to be further investigated.

3.3. Cautions for the Diabetic Patients in Terms of Blood Donation

Anemia is a frequent complication of diabetic patients mainly due to the decreased renal function [43,44]. Anemia with iron deficiency will also increase the lipid peroxidation and HbA1c levels, suggesting the increase in oxidative stress, as discussed above. We know that diabetic complications (retinopathy, neuropathy, heart disease, peripheral arterial disease) are almost, if not all, related to the oxidative stress and damage [1,2] and may be also related to the ischemic damage if the diabetic patients with high blood pressure and atherosclerosis [1]; therefore, if diabetic patients are suffering from anemia, the risk of these late-stage complications will be significantly increased [45-47].

It has been reported that chronic anemia with a severe iron deficiency due to long-term bloodletting increased cardiomyopathy [48]. Correcting anemia with and without iron deficiency seems to slow, in certain extents, the progression of microvascular and macrovascular complications, including postural hypotension from autonomic neuropathy, retinopathy, and loss of renal function from diabetic nephropathy [49-51]. Therefore, regardless whether blood donation in healthy donators is associated with a decrease in blood iron stores along with increase in insulin sensitivity, it may not be suitable for diabetic patients, and remains to be defined in diabetic populations.

4. IRON OVERLOAD

4.1. Iron Overload and Insulin Resistance or Type 2 Diabetes

Iron overload means an increase of storage iron, regardless of the presence or absence of tissue damage. It involves primarily hepatocytes in case of digestive hyperabsorption (hemochromatosis and dyserythropoiesis) and macrophages in case of transfusional excess (Table **1**). Fernandez-Real *et al*. have performed a relative early study in 36 healthy subjects to indicate a significant correlation of serum ferritin with insulin resistance [52]. This correlation was confirmed by subsequently epidemiological studies, i.e.: increased heme iron (derived from animal products) was significantly associated with an increased risk of insulin resistance and type 2 diabetes among a variety of populations, including Asians, Americans and Europeans [53-61]. This association between iron overload and insulin resistance or type 2 diabetes as well as controversial reports was discussed in detail in a recent review [8]. Therefore, we just outline several features regarding this association in this section.

Forouhi *et al*. demonstrated that serum ferritin is an important and independent predictor of the development of diabetes [59]. The baseline serum ferritin in 360 type 2 diabetic patients was significantly higher than that in 758 control participants after adjustment for other known risk factors (age, body mass index, sex, family history, physical activity, smoking habit) and dietary factors [59].

It is known that increased serum ferritin is frequently observed in nonalcoholic fatty liver disease (NAFLD) that is often accompanied with hepatic insulin resistance. Valenti *et al*. recently demonstrated that iron depletion by phlebotomy

in 64 NAFLD patients significantly decreased insulin resistance as compared to 64 non-phlebotomy NAFLD patients who were matched for age, sex, ferritin, obesity, and alanine aminotransferase levels and underwent lifestyle modifications [62]. Based on this study, iron depletion seemed to improve insulin resistance more effectively than lifestyle changes alone.

Dietary consumption of red meat has been considered to increase heme iron intake, therefore whether risks of insulin resistance and type 2 diabetes are associated with dietary consumption of various types of meat had been extensively investigated (see the review [8]). Schulze *et al.* found that diets containing high processed meat were related to increase risk of type 2 diabetes after eliminating the impact of dietary fatty acids and cholesterol on this effect [63]. In their study, however, serum iron level was not available. In contrast, vegetarians who had lower body iron stores, as indicated by a serum ferritin concentration compared with meat-eaters, were more insulin sensitive than meat eaters [54]. Since dietary cholesterol was not directly measured in this study, it was unclear whether dietary cholesterol may be involved in the development of insulin resistance or type 2 diabetes. In the same study [54], however, 6 male meat-eaters were phlebotomized to reduce body iron stores to a level similar to that of vegetarians. After this intervention serum ferritin concentration in the 6 meat-eaters significantly fell from a mean of 85.3 mg/l before phlebotomy to a mean of 27 mg/l after phlebotomy. The effect of iron lowering on insulin resistance was significantly evident, showing a significant enhancement of insulin-stimulated glucose disposal after iron depletion [54].

The above study [54] seems to indicate the importance of serum iron derived from dietary meat in the development of insulin resistance. However, there was another study showing a contrast finding. Jiang *et al*. have further subdivided heme-iron intake to two groups based on red meat and sources other than red meat, and then examined each source to the risk of diabetes [34]. They found that diabetes risk increased with increasing heme-iron intakes from red meat, but not with increasing heme-iron intakes from the groups with sources other than red meat, such as chicken and fish. These contradictory results suggest that heme iron may not be a causative factor for metabolic syndrome or type 2 diabetes, rather than be one of several metabolic syndrome-related influencing factors. This concept was supported by the following facts.

There was a significant difference between man and woman, and between different racial populations for the association of heme iron levels with the insulin sensitivity or risk of type 2 diabetes [64-66]. Acton *et al.* found that the mean serum ferritin concentration was significantly greater in women with diabetes in all racial/ethnic groups and in Native-American men with diabetes than in those without diabetes [65]. One previous study [64] observed that a relationship between serum ferritin levels and insulin resistance exists in woman but not in men, which is consistent with another study conducted in Jiangsu Province [66] that serum ferritin was also significantly associated with the risk of diabetes in Chinese women, but not significantly in men. In contrast to the above two studies, a recent study for the population of North China indicated that associations among higher serum ferritin level, higher heme iron intake, and elevated risk of diabetes were found without gender difference [67].

Even in Chinese women, body mass index also affects the risk of meat consumption for type 2 diabetes [68]. High consumption of total unprocessed meat was related to a modest reduction in the risk of type 2 diabetes among normal weight women, but was associated with a modest increase in risk of type 2 diabetes among obese women. Furthermore, poultry consumption was not associated with a high risk of type 2 diabetes among obese participants. Processed meat consumption was associated with an increased risk of developing type 2 diabetes, particularly for obese participants, suggesting the important contribution of components of processing to the risk of diabetes [68].

In summary, these studies imply that heme-iron levels derived from different diets, including red meat, was positively associated with the increased risk of insulin resistance and diabetes under most conditions; however, whether it is a direct cause remains a concern since the correlation of increased heme iron with insulin resistance or diabetes did not exist under certain other conditions. For instance, iron-overload significantly increases the risk of insulin resistance or type 2 diabetes in Asian women (but not Asian men), and also in certain racial men like Native-American men with diabetes, probably because certain unrecognized confounding factors exist in these populations.

4.2. Iron Overload and Type 1 or Gestational Diabetes

Pancreatic toxicity of iron overload that leads to diabetes has been indicated in hereditary hemochromatosis (HH) (see review [69]). HH is a highly prevalent iron overload disorder that is, unless recognized and treated, fatal, and as the disease progresses, patients develop iron-induced tissue damage, resulting in serious illnesses such as diabetes. The discovery in 1996 that mutations in a gene coding for a novel protein (HFE) were responsible for the majority of cases of HH has made a great advance for us to understand the etiology of HH, and also possible to early diagnose it at a much earlier stage than previously. As a result, the earlier diagnosis of HH has significantly decreased the prevalence of diabetes in HH patients since it is possible for us to commence phlebotomy prior to the development of complications of iron overload such as diabetes [70].

The nature of the development of diabetes in HH is complex. Generally two mechanisms may contribute to the development of hyperglycemia and diabetes in hemochromatotic patients; liver iron overload, leading to insulin resistance, and pancreatic β-cell iron accumulation, resulting in cell damage and diminished insulin secretion (see review: [69]). Recent studies from thalassemic patients [71] and experimental animal models [72,73] support that β-cell iron deposits-induced decrease in insulin secretion capacity may play an important trigger for diabetes in hemochromatotic patients.

In order to investigate the influence of dietary heme iron intake on the gestational diabetes, Zhang *et al.* have carried out a study with a large population, including 13,110 women

who were free of cardiovascular disease, cancer, type 2 diabetes and history of gestational diabetes [74]. Subjects were taken for a validated semi-quantitative food frequency questionnaire in 1991, and followed up until 1998 in the Nurses' Health Study II. Total 758 incident cases of gestational diabetes were recorded [74]. Two major dietary patterns (i.e. "prudent" and "Western") were identified through factor analysis. The prudent pattern was characterized by a high intake of fruit, green leafy vegetables, poultry and fish, whereas the Western pattern was characterized by high intake of red meat, processed meat, refined grain products, sweets, French fries and pizza. They found that intakes of red meat and processed meat were both significantly associated with a higher risk of gestational diabetes even after adjusting other possible confounding factors [74]. This is supported by other studies [75,76]. These findings are very interesting since the likelihood of gestational diabetes is significantly reduced with maternal iron deficiency anemia, which probably acts also as a surrogate for general nutritional deficiency [77]. Based on these two studies, overall nutritional improvement and correction of anemia might be the contributing factors to the increasing prevalence of diabetes and gestational diabetes in the developing world [78,79]. Apparently, further studies on this issue are warranted, especially in developing countries.

4.3. Iron Overload and Diabetic Complications

Increasing evidence indicates that iron overload played a pathologic role in diabetic complications since iron overload is able to damage vascular and heart in non-diabetic subjects (see the review [6]). It has been recognized that diabetic patients have the most compensative mechanisms impaired by hyperglycemia or hyperlipidemia, which make the organs or tissues more susceptible to oxidative stress [1,2,80]. Therefore, the complications, mainly including microvascular and macrovascular disorders (neuropathy, retinopathy, nephropathy, cardiomyopathy) and vascular dysfunction (hypertension and arteriosclerosis), may be enhanced in the diabetic patients with increased serum and organ iron.

Fernandez-Real *et al.* [33] recruited a total of 28 type 2 diabetic male patients with serum ferritin levels >200 ng/ml and randomized them to iron depletion by blood letting (three times of 500 ml blood at 2-week intervals) or to control. Vascular reactivity (high-resolution external ultrasound) was evaluated at baseline and at 4 and 12 months thereafter. The two groups of patients were matched for age, body mass index, pharmacological treatment, and chronic diabetic complications. They found that iron depletion improved vascular dysfunction in type 2 diabetic patients with high ferritin concentrations, suggesting the contributing role of increased serum iron in ferritin form to diabetic vascular complications. Gross *et al*. [81] and de Mello *et al*. [82] have assessed the effects of replacing red meat in the usual diet with chicken or vegetarian low-protein diet on renal function of type 2 diabetes patients. They found that in macroalbuminuric type 2 diabetic patients, withdrawing red meat from the diet improved the renal function, shown by reducing the urinary albumin excretion rate. The above three studies indirectly indicated that reduction of serum ferritin levels by blood letting or reduction of red meat intake all have beneficial outcomes for delaying or preventing the cardiovascular complications in diabetic patients.

Recently, Qi *et al*. further provided evidence to support the correlation of iron overload to the risk of chronic cardiac diseases in the diabetic patients [83]. In this study, they prospectively assessed the associations of long-term intakes of dietary iron and red meat with the risk of chronic heart diseases among 6,161 women who reported a diagnosis of type 2 diabetes. They found that from 1980 through 2000 there were 550 incident cases of chronic heart diseases. After adjustment for age and body mass index, high intakes of both heme iron and red meat were significantly associated with the increased risk of fatal chronic heart diseases, coronary revascularization, and total chronic heart diseases. Furthermore, the positive association between heme iron and red meat intakes and chronic heart diseases was found to be more evident among postmenopausal women compared with premenopausal women [83].

These studies seem to support the notion that increase in serum iron levels is associated with an increase of cardiovascular complications in diabetic patients; however, further studies to confirm these findings remain required since there are several limitations in these studies: (a) small case number (28 patients) in the study by Fernandez-Real *et al*. [33]; (b) no direct measurement of serum iron or ferritin levels after the red meat was removed so that the prevention could not be directly related to reduction of serum iron levels [82,84].

5. SEVERAL HINTS FOR THE INVOLVEMENT OF IRON IN THE PATHOGENESIS OF DIABETES AND DIABETIC COMPLICATIONS

As aforementioned that iron is a transitional metal and a strong pro-oxidant, it can catalyses several cellular reactions that result in the production of ROS and/or RNS, leading to oxidative stress [9,21,22]. The oxidative stress can cause pancreatitis and β-cell death that results in type 1 diabetes and the chronic oxidative stress in the liver, muscle and adipose tissue causes inflammatory response and insulin resistance in these tissues (see reviews [55,69]). In addition, Rajpathak *et al.* [8] has briefly summarized the potential mechanisms for link of iron with type 2 diabetes. Therefore, this review will mainly discuss a few specific possibilities for iron to be linked to diabetes and diabetic complications in addition to oxidative stress derived from iron's Fenton reaction.

5.1. mitoNEF4

Mitochondrial dysfunction is associated with insulin resistance and the development of type 2 diabetes [85], and diabetic complications [23]. Pharmacological agents extensively used to treat insulin resistance such as the thiazolidinedione (TZD) pioglitazone are known to enhance oxidative capacity and normalize lipid metabolism. Although TZDs are conventionally thought to operate through binding to peroxisome proliferator-activated receptors (PPAR), a recent study by Colca and colleagues discovered mitoNEET as a binding target of pioglitazone [86]. As shown in Fig. (**1C**), mitoNEET as an integral protein of the outer mitochondrial membrane is an iron-containing protein to play an important

role in the control of maximal mitochondrial respiratory rate [87]. In fact, mitoNEET contained a redox-active pH-labile Fe-S cluster and lost 2Fe and 2S upon cofactor extrusion; therefore, mitoNEET probably functions as a Fe-S cluster shuttling in redox reactions [17].

In the inflammation-induced Parkinson's disease model, where microglia activation leads to oxidative stress, mitochondrial dysfunction, and dopaminergic neurodegeneration, pioglitazone prevented dopaminergic neurons through the protection of mitochondrial damage [88]. Deficiency of mitoNEET protein in mice results in a compromise in the respiratory capacity of heart mitochondria [17]. Therefore, whether iron dyshomeostasis affects mitoNEET function remains unclear, but if so, it may result in mitochondrial dysfunction to cause metabolic syndrome and cardiovascular diseases in diabetic patients.

5.2. Advanced Glycated Endproducts

Hyperglycemia modifies the amino groups of proteins by a process of non-enzymatic glycation to form advanced glycated endproducts (AGEs), leading to potentially deleterious effects on the protein structures and functions. Since AGEs play an important role in the pathogenesis of diabetic cardiovascular complications [1,3,9,23,89], iron overload may contribute to diabetic complications by accelerating AGE generation *via* Fenton reaction to form ROSs and RNSs [1,3,9,23].

Cussimanio *et al.* [90] found that hemoglobin and myoglobin are extremely susceptible to damage by high levels of glucose *in vitro* through a process that leads to complete destruction of the essential heme group. In addition to the expected formation of AGEs on lysine and other side-chains, this process also induces a release of the iron from these two proteins during the non-enzymatic glycation process [90-92]. Therefore, diabetic hyperglycemia glycates iron-binding proteins that release more iron into circulation, while free iron will further accelerate AGE process *via* Fenton reaction. This circle reaction will promote oxidative stress and damage, leading to the development of diabetic complications.

Several studies have shown the increase in iron levels in the heart or the kidney of diabetic patients and animals. We have investigated the iron content in the diabetic kidney [93]. Male Sprague Dawley rats were treated with streptozotocin (STZ) for induction of the hyperglycemia, and 6 month after diabetes, renal iron contents were significantly increased as compared to control kidney. Study by Singh *et al.* [94] also showed the increase in cardiac iron in diabetic rats. Six to 8 weeks after Wister rats were induced to be hyperglycemic with STZ, iron levels was significantly increased in the right ventricle of the diabetic heart compared to control. This increased organ iron levels will stimulate AGE formation; therefore, the organ iron overload is most likely a cause for the development of complications.

5.3. Iron Enhancement of the Pathological Effects of Angiotensin II

Angiotensin II (Ang II), acting *via* its AT_1 and AT_2 receptor-mediated NADPH oxidase activation, has been shown to be involved in a wide range of pathogenic processes in the heart, including the induction of apoptosis and fibrosis under diabetic conditions [1,2,89,95,96]. The pathogenic effect of Ang II on the hearts and kidneys was also found to be closely associated with the increased iron or up-regulation of iron metabolism-related genes [97-101].

Ishizaka [97,98,100,101] gave rats infusion of Ang II for continuously 7 days, which significantly increased the depositions of iron in the proximal tubular epithelial cells and in the hearts of these rats. Treatment of Ang II-infused rats with an iron chelator, deferoxamine (DFO), blocked the abnormal iron deposition both in the kidneys and the heart, resulting in a significant suppression of Ang II-induced increase renal and cardiac fibrosis and renal dysfunction. These results suggest that Ang II causes renal and cardiac injury, in part, by inducing the deposition of iron in the organs. Iron chelation and free radical scavenger can prevent Ang II-induced renal and cardiac pathogenesis, by suppression of oxidative damage caused by deposited iron in these organs.

A more recent study from Ishizaka *et al*. [97] has revealed the effect of Ang II on the localization and expression of TfR, DMT1, FPN-1, and hepcidin mRNA in the rat kidney. Weak expression of TfR, DMT1, FPN-1, and hepcidin mRNA was observed in the kidneys of control rats. In contrast, after 7 days of Ang II infusion the expression of these mRNAs was more widely distributed. Staining of serial sections revealed that some, but not all, of the renal tubular cells positive for these genes in the kidney of Ang II-infused animals contained deposited iron. Real-time polymerase chain reaction showed that the mRNA expression of TfR, DMT1, FPN, and hepcidin increased about 1.9-fold, 1.7-fold, 2.3-fold, and 4.7-fold, respectively, after Ang II infusion as compared to that of controls, and that these increases could be suppressed by the concomitant administration of Ang II receptor antagonist losartan. These results implicated that Ang II can regulate iron metabolism-related gene expression and the up-regulated iron-metabolism related genes may accelerate the iron deposition in the kidney and cause oxidative damage. Therefore, iron overload should be considered as causative factor for diabetic complications.

5.4. HO-1

HO is an enzyme that catalyzes the degradation of heme, as shown in Fig. (**2**). This produces biliverdin, iron, and CO [19]. There are three known HO isoforms: HO-1 is an inducible isoform in response to stress such as oxidative stress, hypoxia, heavy metals, and cytokines; HO-2 is a constitutive isoform which is expressed under homeostatic conditions. Both HO-1 and HO-2 are ubiquitously expressed and catalytically active; HO-3 is not catalytically active, but is thought to work in oxygen sensing.

In diabetes, the interaction of AGEs with their AGE receptor leads to oxidative stress and induction of the HO-1 [102-104]. Given that CO is a product of HO activity, an early study [105] has tried to measure the elevation of exhaled CO levels as evaluation of diabetic HO up-regulation. Diabetes increases tissue levels of HO-1 as an antioxidant (see Fig. **2**, *via* direct production of bilirubin) at the early stage in animal models and also in patients [102-104], but significantly decreased its expression at the late stage as a dyscompensative effect [106,107]. Considering that HO-1 is

assumed to prevent abnormal intracellular iron accumulation by degradation of heme (Fig. **1** & **2**) and diabetic up-regulation of Ang II leads to iron deposition to tissues as discussed above, up-expression of HO-1 at the early stage of diabetes may be a mechanism to meliorate iron-induced renal toxicity.

With genetic or pharmacological approaches, it has been clearly indicated that lowing or increasing HO-1 significantly enhanced or prevented diabetes and diabetic complications [108-112]. Therefore, HO-1 not only determines iron homeostasis, but also plays an important role in preventing diabetes and diabetic complications. Although at the early stage of diabetes, overexpression of HO-1 in the tissues as a compensative mechanism can efficiently remove the deposit iron and protect from oxidative damage, but diabetes at the late stage often impairs tissue defense systems, including HO-1 expression which will lead to iron deposition and induce oxidative damage. To support the above notion, diabetes was found not only to impair HO-1 expression and activity, but also to reduce antioxidant capacity [113-115], which will increase the susceptibility of the tissues in diabetic subjects to oxidative damage caused by diabetes, and even to impair iron-binding capacity by glycation [80].

It should be mentioned, in addition to antioxidant action of HO-1, a few studies also found the pro-oxidant role of HO-1 in certain conditions, including diabetes. Farhangkhoee *et al*. treated STZ-induced diabetic rats with a potent inhibitor of HO system, SnPPIX, at the onset of diabetes. In no-treated diabetic rats, no significant alterations of the HO system following 1 week of diabetes. However, 1 month of diabetes caused increased oxidative stress as demonstrated by higher levels of 8-OHdG-positive cardiomyocytes, in association with increased HO isozyme mRNA and protein expression, and augmented HO activity as well as increased number of cardiomyocytes with stainable iron. SnPPIX treatment resulted in reduced number of 8-OHdG-positive cardiomyocytes in parallel with reduced HO activity. This study demonstrated that diabetes-induced oxidative stress in the heart is, in part, due to up-regulated HO expression and activity. These results also provide evidence of pro-oxidant activity of HO in the diabetic hearts, which could be mediated by increased redox-active iron [116]. In a following study from the same group, they further demonstrated that high levels of glucose can stimulate up-regulation of HO mRNA and activity in the endothelial cells. The increased HO activity was found to associate with oxidative DNA damage [117]. Therefore, the above two studies suggest that HO by itself and *via* elaboration of other vasoactive factors may cause cardiac and endothelial injury and functional alteration. In line with this notion, a recent study showed that overexpression of HO-1 exacerbated early brain injury after intracerebral haemorrhage [118]. Therefore, to understand these features of HO is very important in the context of chronic diabetic complications.

5.5. An Interaction Between Iron and Retinol-Binding Protein

Retinol-binding protein (RBP) is an important, specific transport protein for retinol (vitamin A) in the circulation, and has been considered as secreted only from hepatocytes and only functioning as delivering retinol to tissues [119]. Emerging evidence has indicated that RBP4 can be produced by adipocytes as a new adipokine, and also be elevated in insulin-resistant mice and humans with obesity and type 2 diabetes [120]. Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance [120]. In subsequent human studies, this association was supported by the finding that RBP4 levels and the level of insulin resistance were positively correlated in people with obesity and impaired glucose tolerance, and in patients with type 2 diabetes [121-123]. Therefore, plasma RBP4 concentration has been considered as a possible biomarker of nephropathy and cardiovascular diseases in type 2 diabetic subjects [124].

The mechanisms by which RBP4 affects insulin sensitivity and diabetic complications are largely unknown. Because vitamin A is required for effective utilization of iron and maintenance of normal hemoglobin concentration [125,126], Fernández-Real *et al*. [127] hypothesized that iron-associated insulin resistance may be associated with the impaired insulin action caused by RBP4. To define this notion, serum ferritin and RBP4 concentrations and insulin resistance were evaluated in 132 healthy middle-aged men, in a replication independent study (20 healthy men and 13 healthy women), and also in 13 patients with type 2 diabetes before and after iron depletion. They found a positive correlation between circulating RBP4 and serum ferritin in both independent studies. Serum RBP4 concentration was higher in men than women in parallel to increased ferritin levels. On multiple regression analyses to predict serum RBP4, serum ferritin contributed significantly to RBP4 variance after controlling for body mass index and age. Serum RBP4 concentration significantly decreased after iron depletion in type 2 diabetic patients. They further evaluated the effect of iron on RBP4 release *in vitro* in adipose tissue, in which the iron donor lactoferrin led to increased dose-dependent adipose tissue release of RBP4 and increased RBP4 expression, while iron chelators apotransferrin and DFO led to decreased RBP4 release. Therefore, these observations suggest that iron could play a role in the correlation of RBP4 with insulin resistance. The mechanisms by which iron depletion leads to improved insulin sensitivity may include both decrease of iron oxidative stress and decrease of RBP4 levels in the body system.

6. POSSIBLE THERAPY FOR DIABETES AND DIABETIC COMPLICATIONS USING IRON CHELATORS

Excess iron appears associated with insulin resistance and type 2 diabetes as well as increased risk of diabetic complications. Whether reducing serum iron by chelation can prevent or delay the development of diabetes and its complications has been questioned [3]. A few early studies showed contradictory outcomes. A study to use DFO for type 2 diabetic patients disclosed a well correlation of lowering the elevated serum ferritin levels with improvement of fasting glucose, triglyceride, and HbA1c levels (see review [3]). However, other two studies, in which five and nine type 2 diabetic patients with an elevated serum ferritin, respectively, were treated with DFO, did not show the improvement of glucose control although DFO treatment led to nor-

mal or near-normal serum ferritin values in the most patients (see review [3]). Analyzing these three early studies, Wilson *et al*. found that all these studies were short-term, from 5 to 13 weeks, and levels of serum ferritin were reduced at best to the high normal range, with most remaining above 250 ng/mL [3]. Thus, all the above therapeutic trials might be inadequate. For diabetic complications, Hattori *et al*. found that myocardial blood flow response to sympathetic stimulation is significantly impaired in long-term type 1 diabetic patients, and single infusion of DFO could significantly, but partially, revise the impairment of myocardial blood flow in these patients [128,129]. However, effect of long-term use of DFO on diabetic complications remains not available.

Several animal studies supported the beneficial effects of iron chelators on the onset of diabetes and diabetic complications. Treatment of diabetic animals with DFO restored the lipid peroxide levels to its control value [130], decreased the incidence and severity of STZ-induced or spontaneously developed type 1 diabetes [131], and reduced the risk of developing many diabetic complications [132-134]. These beneficial effects of DFO might be related to its antioxidant action [135] and inhibitory capacity of AGE generation [130,133]. A recent study [5] demonstrated that iron depletion by DFO up-regulated glucose uptake and insulin signaling in hepatoma cells and in rat liver. Up-regulation of insulin receptor by DFO was mimicked by the intracellular iron chelator deferasirox. Iron depletion increased insulin receptor activity, whereas iron supplementation had the opposite effect. DFO consistently increased insulin signaling key components to enhance glucose utilization. Iron depletion of Sprague-Dawley improved glucose clearance, and was associated with up-regulation of insulin receptor and downstream signaling action. In rats with fatty liver because of a high-calorie and high-fat diet, glucose clearance was increased by iron depletion and decreased by iron supplementation. Therefore, iron depletion by DFO up-regulates glucose uptake, and increases insulin receptor activity and signaling in hepatocytes *in vitro* and *in vivo* [5].

Although DFO had been extensively used in the above clinical and experimental studies, its unsatisfactory and toxic effects were gradually recognized [136]. Several other orally effective chelators thus have been developed to reduce toxic effects [136-139]. Among the new chelators, deferiprone (L1) was accepted to use alone or with others for the therapy of various chronic diseases such as thalassemia with less side-toxic effects [138,139].

There is high prevalence of insulin resistance and diabetes in iron loaded thalassemic patients. The combined use of DFO and L1 (DFO/L1) for 24-36 months resulted in an improvement of diabetes and impaired glucose tolerance in some patients [140]. More convincing evidence was available from a few recent studies [141-143], to confirm that DFO/L1 provided a greater efficacy in removing cardiac and hepatic iron accumulation than either chelator alone, along with a reversal of cardiac and hepatic dysfunction in patients with β-thalassemia major. The DFO/L1 therapy also markedly decreased ferritin levels and significantly improved the glucose responses at all times during an oral glucose tolerance test [141-143].

It should be clarified that although the application of L1/DFO showed beneficial effect for thalassemic patients under certain conditions, whether these iron chelators can also be used for diabetic patients without β-thalassemia major to prevent or delay the developments of cardiovascular diseases remain unclear. In addition, for diabetic patients the following precautions should also be taken. First, as aforementioned, iron deficiency with anemia, though might enhance insulin sensitivity, is most likely to enhance the risk of cardiovascular diseases [44,49,50,144,145]; therefore, for the diabetic patients, application of iron chelators may be considered only when the patients show significant elevation of iron overload. Second, it should be mentioned that even though L1 seems relatively safe, disturbance of zinc homeostasis (zinc deficiency) [146] and agranulocytosis as well as arthropathy in children [147] have been documented by chronic iron chelation with L1. It is well-known that zinc deficiency often occurs in diabetic patients [148] and increases the risk for diabetic complications [149]. Zinc is able to inhibit iron bioavailability [150], and supplementation of zinc to diabetic patients has been explored for its potential prevention of diabetic complications [23,148,151]. Therefore, the nutritional measurement of zinc levels may be taken

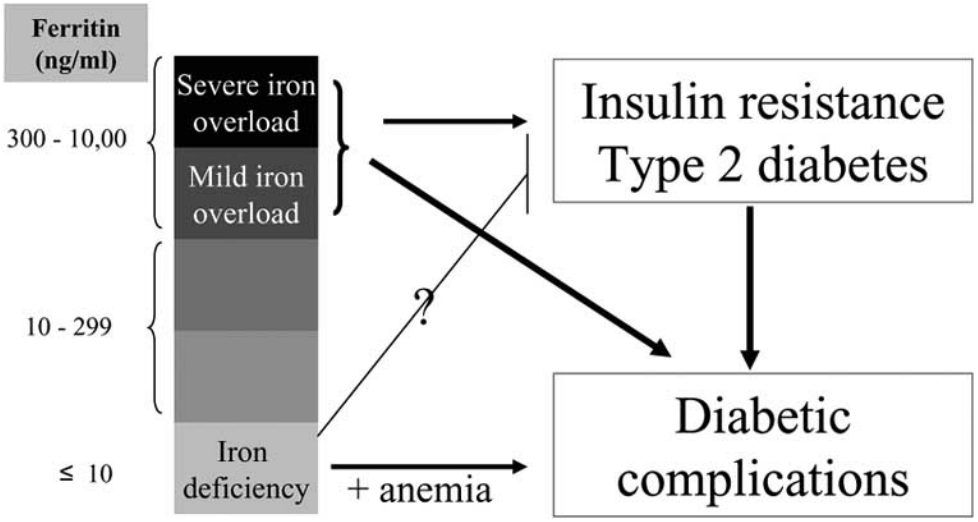

Fig. (4). Illustration of the relationships of iron deficiency or overload with diabetes, insulin resistance and diabetic complications.

before determining whether iron chelation is applied. In addition, iron chelation with iron chelators in preventing diabetes and diabetic complications may also be considered to use together with zinc or other antioxidants.

7. SUMMARY

Iron is a very important essential mineral for the cells in the body. Either deficiency or overload will contribute certain pathogenic outcomes. As summarized in Fig. (**4**), temporal mild iron deficiency seems to prevent the development of insulin resistance in normal population and to sensitive insulin response in the type 2 diabetes, but on the other hand, chronic iron deficiency with anemia enhances the risk of diabetic complications. Iron overload increases the prevalence of insulin resistance and even type 2 diabetes, and also increases the risk of cardiovascular disease in diabetic patients (Fig. **4**). Although confounding pathogenic factors such as cholesterol may exist, iron overload by dietary intake of red meat is likely to increase cardiovascular complications of diabetic patients. All these clinical observation strongly suggest the roles of iron in the pathogenesis of diabetes and diabetic complications. Studies using animal models have indicated that iron-derived ROS and RNS may be the major mediators for iron overload to cause insulin resistance, diabetes and diabetic cardiovascular complications. Several potential mechanisms for the link of iron to diabetes and diabetic complications remains to be further defined.

Although more systemic clinic observation remains needed for clarifying the outcomes of various iron chelators for diabetes and its complications, it is clear at least that dietary control to reduce iron intake, particular heme iron (animal products), will be beneficial for the prevention of insulin resistance, type 2 diabetes and diabetic complications.

ACKNOWLEDGMENTS

The work in the authors' laboratories was supported, in part, by research grants from American Diabetes Association (02-07-JF-02; 05-07-CD-02, to L. Cai) and from the CAS/SAFEA International Partnership Program for Creative Research Team (to X. Lin & L. Cai). Q.J. Liu is a recipient of Scholarship under State Scholarship Fund from China Scholarship Council.

REFERENCES

[1] Cai, L.; Kang, Y.J. Oxidative stress and diabetic cardiomyopathy: a brief review. *Cardiovasc. Toxicol.*, **2001**, *1*, 181-93.

[2] Chyun, D.A.; Young, L.H. Diabetes mellitus and cardiovascular disease. *Nurs. Clin. North Am.*, **2006**, *41*, 681-95, viii-ix.

[3] Wilson, J.G.; Lindquist, J.H.; Grambow, S.C.; Crook, E.D.; Maher, J.F. Potential role of increased iron stores in diabetes. *Am. J. Med. Sci.*, **2003**, *325*, 332-9.

[4] Kahn, S.E.; Hull, R.L.; Utzschneider, K.M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, **2006**, *444*, 840-6.

[5] Dongiovanni, P.; Valenti, L.; Ludovica Fracanzani, A.; Gatti, S.; Cairo, G.; Fargion, S. Iron depletion by deferoxamine up-regulates glucose uptake and insulin signaling in hepatoma cells and in rat liver. *Am. J. Pathol.*, **2008**, *172*, 738-47.

[6] Beutler, E.; Hoffbrand, A.V.; Cook, J.D. Iron deficiency and overload. *Hematology. Am. Soc. Hematol. Educ. Program*, **2003**, 40-61.

[7] Swaminathan, S.; Fonseca, V.A.; Alam, M.G.; Shah, S.V. The role of iron in diabetes and its complications. *Diabetes Care*, **2007**, *30*, 1926-33.

[8] Rajpathak, S.N.; Crandall, J.P.; Wylie-Rosett, J.; Kabat, G.C.; Rohan, T.E.; Hu, F.B. The role of iron in type 2 diabetes in humans. *Biochim. Biophys. Acta*, **2008**. [Epub ahead of print].

[9] Eaton, J.W.; Qian, M. Molecular bases of cellular iron toxicity. *Free Radic. Biol. Med.*, **2002**, *32*, 833-40.

[10] Rouault, T.A. The role of iron regulatory proteins in mammalian iron homeostasis and disease. *Nat. Chem. Biol.*, **2006**, *2*, 406-14.

[11] Zimmermann, M.B.; Hurrell, R.F. Nutritional iron deficiency. *Lancet*, **2007**, *370*, 511-20.

[12] Farnaud, S.; Rapisarda, C.; Bui, T.; Drake, A.; Cammack, R.; Evans, R.W. Identification of an iron-hepcidin complex. *Biochem. J.*, **2008**, *413*, 553-7.

[13] De Domenico, I.; Ward, D.M.; Kaplan, J. Hepcidin regulation: ironing out the details. *J. Clin. Invest.*, **2007**, *117*, 1755-8.

[14] Oates, P.S. The role of hepcidin and ferroportin in iron absorption. *Histol. Histopathol.*, **2007**, *22*, 791-804.

[15] Shi, H.; Bencze, K.Z.; Stemmler, T.L.; Philpott, C.C. A cytosolic iron chaperone that delivers iron to ferritin. *Science*, **2008**, *320*, 1207-10.

[16] Chen, S.; Khan, Z.A.; Karmazyn, M.; Chakrabarti, S. Role of endothelin-1, sodium hydrogen exchanger-1 and mitogen activated protein kinase (MAPK) activation in glucose-induced cardiomyocyte hypertrophy. *Diabetes Metab. Res. Rev.*, **2007**, *23*, 356-67.

[17] Wiley, S.E.; Paddock, M.L.; Abresch, E.C.; Gross, L.; van der Geer, P.; Nechushtai, R.; Murphy, A.N.; Jennings, P.A.; Dixon, J.E. The Outer Mitochondrial Membrane Protein mitoNEET Contains a Novel Redox-active 2Fe-2S Cluster. *J. Biol. Chem.*, **2007**, *282*, 23745-9.

[18] Bruick, R.K. Oxygen sensing in the hypoxic response pathway: regulation of the hypoxia-inducible transcription factor. *Genes Dev.*, **2003**, *17*, 2614-23.

[19] Kikuchi, G.; Yoshida, T.; Noguchi, M. Heme oxygenase and heme degradation. *Biochem. Biophys. Res. Commun.*, **2005**, *338*, 558-67.

[20] Baranano, D.E.; Rao, M.; Ferris, C.D.; Snyder, S.H. Biliverdin reductase: a major physiologic cytoprotectant. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 16093-8.

[21] Cai, L.; Tsiapalis, G.; Cherian, M.G. Protective role of zinc-metallothionein on DNA damage *in vitro* by ferric nitrilotriacetate (Fe-NTA) and ferric salts. *Chem. Biol. Interact.*, **1998**, *115*, 141-51.

[22] Papanikolaou, G.; Pantopoulos, K. Iron metabolism and toxicity. *Toxicol. Appl. Pharmacol.*, **2005**, *202*, 199-211.

[23] Cai, L. Suppression of nitrative damage by metallothionein in diabetic heart contributes to the prevention of cardiomyopathy. *Free Radic. Biol. Med.*, **2006**, *41*, 851-61.

[24] Davis, R.E.; McCann, V.J.; Nicol, D.J. Influence of iron-deficiency anaemia on the glycosylated haemoglobin level in a patient with diabetes mellitus. *Med. J. Aust.*, **1983**, *1*, 40-1.

[25] Tarim, O.; Kucukerdogan, A.; Gunay, U.; Eralp, O.; Ercan, I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. *Pediatr. Int.*, **1999**, *41*, 357-62.

[26] El-Agouza, I.; Abu Shahla, A.; Sirdah, M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. *Clin. Lab. Haematol.*, **2002**, *24*, 285-9.

[27] Coban, E.; Ozdogan, M.; Timuragaoglu, A. Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. *Acta Haematol.*, **2004**, *112*, 126-8.

[28] Hashimoto, K.; Noguchi, S.; Morimoto, Y.; Hamada, S.; Wasada, K.; Imai, S.; Murata, Y.; Kasayama, S.; Koga, M. HbA1C, but not serum glycated albumin, is elevated in late pregnancy due to iron deficiency. *Diabetes Care*, **2008**, *31*, 1945-8.

[29] Sathiyapriya, V.; Bobby, Z.; Vinod Kumar, S.; Selvaraj, N.; Parthibane, V.; Gupta, S. Evidence for the role of lipid peroxides on glycation of hemoglobin and plasma proteins in non-diabetic asthma patients. *Clin. Chim. Acta*, **2006**, *366*, 299-303.

[30] Sundaram, R.C.; Selvaraj, N.; Vijayan, G.; Bobby, Z.; Hamide, A.; Rattina Dasse, N. Increased plasma malondialdehyde and fructo-

samine in iron deficiency anemia: Effect of treatment. *Biomed. Pharmacother.*, **2007,** *61*, 682-5.

[31] Balamurugan, R.; Selvaraj, N.; Bobby, Z.; Sathiyapriya, V. Increased glycated hemoglobin level in non-diabetic nephrotic children is associated with oxidative stress. *Indian J. Physiol. Pharmacol.*, **2007**, *51*, 153-9.

[32] Fernandez-Real, J.M.; Lopez-Bermejo, A.; Ricart, W. Iron stores, blood donation, and insulin sensitivity and secretion. *Clin. Chem.*, **2005**, *51*, 1201-5.

[33] Fernandez-Real, J.M.; Penarroja, G.; Castro, A.; Garcia-Bragado, F.; Hernandez-Aguado, I.; Ricart, W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes.*, **2002**, *51*, 1000-4.

[34] Jiang, R.; Ma, J.; Ascherio, A.; Stampfer, M.J.; Willett, W.C.; Hu, F.B. Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. *Am. J. Clin. Nutr.*, **2004**, *79*, 70-5.

[35] Meyers, D.G.; Strickland, D.; Maloley, P.A.; Seburg, J.K.; Wilson, J.E.; McManus, B.F. Possible association of a reduction in cardiovascular events with blood donation. *Heart*, **1997**, *78*, 188-93.

[36] Meyers, D.G.; Jensen, K.C.; Menitove, J.E. A historical cohort study of the effect of lowering body iron through blood donation on incident cardiac events. *Transfusion*, **2002**, *42*, 1135-9.

[37] Zheng, H.; Cable, R.; Spencer, B.; Votto, N.; Katz, S.D. Iron stores and vascular function in voluntary blood donors. *Arterioscler. Thromb. Vasc. Biol.*, **2005**, *25*, 1577-83.

[38] Zacharski, L.R.; Chow, B.K.; Howes, P.S.; Shamayeva, G.; Baron, J.A.; Dalman, R.L.; Malenka, D.J.; Ozaki, C.K.; Lavori, P.W. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. *JAMA*, **2007**, *297*, 603-10.

[39] Zheng, H.; Patel, M.; Cable, R.; Young, L.; Katz, S.D. Insulin Sensitivity, Vascular Function, and Iron Stores in Voluntary Blood Donors. *Diabetes Care*, **2007**, *30*, 2685-93.

[40] Engberink, M.F.; Geleijnse, J.M.; Durga, J.; Swinkels, D.W.; de Kort, W.L.; Schouten, E.G.; Verhoef, P. Blood donation, body iron status and carotid intima-media thickness. *Atherosclerosis*, **2008**, *196*, 856-62.

[41] Newman, B.H.; Pichette, S.; Pichette, D.; Dzaka, E. Adverse effects in blood donors after whole-blood donation: a study of 1000 blood donors interviewed 3 weeks after whole-blood donation. *Transfusion*, **2003**, *43*, 598-603.

[42] Farrugia, A. Iron and blood donation- an under-recognised safety issue. *Dev. Biol. (Basel)*, **2007**, *127*, 137-46.

[43] Thomas, M.C. The high prevalence of anemia in diabetes is linked to functional erythropoietin deficiency. *Semin. Nephrol.*, **2006**, *26*, 275-82.

[44] Thomas, M.C. Anemia in diabetes: marker or mediator of microvascular disease? *Nat. Clin. Pract. Nephrol.*, **2007**, *3*, 20-30.

[45] Vlagopoulos, P.T.; Tighiouart, H.; Weiner, D.E.; Griffith, J.; Pettitt, D.; Salem, D.N.; Levey, A.S.; Sarnak, M.J. Anemia as a risk factor for cardiovascular disease and all-cause mortality in diabetes: the impact of chronic kidney disease. *J. Am. Soc. Nephrol.*, **2005**, *16*, 3403-10.

[46] Shu, D.H.; Ransom, T.P.; O'Connell, C.M.; Cox, J.L.; Kaiser, S.M.; Gee, S.A.; Rowe, R.C.; Ur, E.; Imran, S.A. Anemia is an independent risk for mortality after acute myocardial infarction in patients with and without diabetes. *Cardiovasc. Diabetol.*, **2006**, *5*, 8.

[47] Iseki, K.; Kohagura, K. Anemia as a risk factor for chronic kidney disease. *Kidney. Int. Suppl.*, **2007**, S4-9.

[48] Sohn, I.S.; Jin, E.S.; Jang, S.T.; Cho, J.M.; Kim, C.J.; Bae, J.H.; Moon, J.Y.; Lee, S.H.; Kim, M.J. Bloodletting-induced cardiomyopathy: Reversible cardiac hypertrophy in severe chronic anemia from long-term bloodletting with cupping. *Eur. J. Echocardiogr.*, **2007**. *9*, 585-6.

[49] Ascic-Buturovic, B.; Heljic, B. Effects of hyperglycemia and iron deficiency on kidney and heart function in type 2 diabetes disease. *Bosn. J. Basic. Med. Sci.*, **2006**, *6*, 78-81.

[50] Ritz, E.; Laville, M.; Bilous, R.W.; O'Donoghue, D.; Scherhag, A.; Burger, U.; de Alvaro, F. Target level for hemoglobin correction in patients with diabetes and CKD: primary results of the Anemia Correction in Diabetes (ACORD) Study. *Am. J. Kidney Dis.*, **2007**, *49*, 194-207.

[51] Silverberg, D.S.; Wexler, D.; Iaina, A.; Schwartz, D. The correction of anemia in patients with the combination of chronic kidney disease and congestive heart failure may prevent progression of both conditions. *Clin. Exp. Nephrol.*, **2008**. [Epub ahead of print].

[52] Fernandez-Real, J.M.; Ricart-Engel, W.; Arroyo, E.; Balanca, R.; Casamitjana-Abella, R.; Cabrero, D.; Fernandez-Castaner, M.; Soler, J. Serum ferritin as a component of the insulin resistance syndrome. *Diabetes Care*, **1998**, *21*, 62-8.

[53] Kim, N.H.; Oh, J.H.; Choi, K.M.; Kim, Y.H.; Baik, S.H.; Choi, D.S.; Kim, S.J. Serum ferritin in healthy subjects and type 2 diabetic patients. *Yonsei. Med. J.*, **2000**, *41*, 387-92.

[54] Hua, N.W.; Stoohs, R.A.; Facchini, F.S. Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. *Br. J. Nutr.*, **2001**, *86*, 515-9.

[55] Jehn, M.; Clark, J.M.; Guallar, E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care*, **2004**, *27*, 2422-8.

[56] D'Souza, R.F.; Feakins, R.; Mears, L.; Sabin, C.A.; Foster, G.R. Relationship between serum ferritin, hepatic iron staining, diabetes mellitus and fibrosis progression in patients with chronic hepatitis C. *Aliment. Pharmacol. Ther.*, **2005**, *21*, 519-24.

[57] Rajpathak, S.; Ma, J.; Manson, J.; Willett, W.C.; Hu, F.B. Iron intake and the risk of type 2 diabetes in women: a prospective cohort study. *Diabetes Care*, **2006**, *29*, 1370-6.

[58] Wrede, C.E.; Buettner, R.; Bollheimer, L.C.; Scholmerich, J.; Palitzsch, K.D.; Hellerbrand, C. Association between serum ferritin and the insulin resistance syndrome in a representative population. *Eur. J. Endocrinol.*, **2006**, *154*, 333-40.

[59] Forouhi, N.G.; Harding, A.H.; Allison, M.; Sandhu, M.S.; Welch, A.; Luben, R.; Bingham, S.; Khaw, K.T.; Wareham, N.J. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. *Diabetologia*, **2007**, *50*, 949-56.

[60] Jehn, M.L.; Guallar, E.; Clark, J.M.; Couper, D.; Duncan, B.B.; Ballantyne, C.M.; Hoogeveen, R.C.; Harris, Z.L.; Pankow, J.S. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Am. J. Epidemiol.*, **2007**, *165*, 1047-54.

[61] Vari, I.S.; Balkau, B.; Kettaneh, A.; Andre, P.; Tichet, J.; Fumeron, F.; Caces, E.; Marre, M.; Grandchamp, B.; Ducimetiere, P. Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care*, **2007**, *30*, 1795-801.

[62] Valenti, L.; Fracanzani, A.L.; Dongiovanni, P.; Bugianesi, E.; Marchesini, G.; Manzini, P.; Vanni, E.; Fargion, S. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case-control study. *Am. J. Gastroenterol.*, **2007**, *102*, 1251-8.

[63] Schulze, M.B.; Manson, J.E.; Willett, W.C.; Hu, F.B. Processed meat intake and incidence of Type 2 diabetes in younger and middle-aged women. *Diabetologia*, **2003**, *46*, 1465-73.

[64] Sheu, W.H.; Chen, Y.T.; Lee, W.J.; Wang, C.W.; Lin, L.Y. A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. *Clin. Endocrinol (Oxf).*, **2003**, *58*, 380-5.

[65] Acton, R.T.; Barton, J.C.; Passmore, L.V.; Adams, P.C.; Speechley, M.R.; Dawkins, F.W.; Sholinsky, P.; Reboussin, D.M.; McLaren, G.D.; Harris, E.L.; Bent, T.C.; Vogt, T.M.; Castro, O. Relationships of serum ferritin, transferrin saturation, and HFE mutations and self-reported diabetes in the Hemochromatosis and Iron Overload Screening (HEIRS) study. *Diabetes Care*, **2006**, *29*, 2084-9.

[66] Shi, Z.; Hu, X.; Yuan, B.; Pan, X.; Meyer, H.E.; Holmboe-Ottesen, G. Association between serum ferritin, hemoglobin, iron intake, and diabetes in adults in Jiangsu, China. *Diabetes Care*, **2006**, *29*, 1878-83.

[67] Luan de, C.; Li, H.; Li, S.J.; Zhao, Z.; Li, X.; Liu, Z.M. Body iron stores and dietary iron intake in relation to diabetes in adults in North China. *Diabetes Care*, **2008**, *31*, 285-6.

[68] Villegas, R.; Shu, X.O.; Gao, Y.T.; Yang, G.; Cai, H.; Li, H.; Zheng, W. The association of meat intake and the risk of type 2 diabetes may be modified by body weight. *Int. J. Med. Sci.*, **2006**, *3*, 152-9.

[69] Pietrangelo, A. Hereditary hemochromatosis. *Annu. Rev. Nutr.*, **2006**, *26*, 251-70.
[70] O'Sullivan, E.P.; McDermott, J.H.; Murphy, M.S.; Sen, S.; Walsh, C.H. Declining prevalence of diabetes mellitus in hereditary haemochromatosis-The result of earlier diagnosis. *Diabetes Res. Clin. Pract.*, **2008**, *81*, 316-20.
[71] Angelopoulos, N.G.; Zervas, A.; Livadas, S.; Adamopoulos, I.; Giannopoulos, D.; Goula, A.; Tolis, G. Reduced insulin secretion in normoglycaemic patients with beta-thalassaemia major. *Diabet. Med.*, **2006**, *23*, 1327-31.
[72] Shirasuga, N.; Hayashi, K.; Awai, M. Pancreatic islets after repeated injection of Fe3+-NTA. An ultrastructural study of diabetic rats. *Acta. Pathol. Jpn.*, **1989**, *39*, 159-68.
[73] Ramey, G.; Faye, A.; Durel, B.; Viollet, B.; Vaulont, S. Iron overload in Hepc1(-/-) mice is not impairing glucose homeostasis. *FEBS Lett.*, **2007**, *581*, 1053-7.
[74] Zhang, C.; Schulze, M.B.; Solomon, C.G.; Hu, F.B. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. *Diabetologia.*, **2006**, *49*, 2604-13.
[75] Chen, L.; Lei, L.; Jin, T.; Nordberg, M.; Nordberg, G.F. Plasma metallothionein antibody, urinary cadmium, and renal dysfunction in a Chinese type 2 diabetic population. *Diabetes Care*, **2006**, *29*, 2682-7.
[76] Afkhami-Ardekani, M.; Rashidi, M. Iron status in women with and without gestational diabetes mellitus. *J. Diabetes Complications*, **2008**, [Epub ahead of print].
[77] Lao, T.T.; Ho, L.F. Impact of iron deficiency anemia on prevalence of gestational diabetes mellitus. *Diabetes Care*, **2004**, *27*, 650-6.
[78] King, H. Epidemiology of glucose intolerance and gestational diabetes in women of childbearing age. *Diabetes Care*, **1998**, *21* (Suppl 2), B9-13.
[79] Janghorbani, M.; Stenhouse, E.; Jones, R.B.; Millward, A. Gestational diabetes mellitus in Plymouth, U.K.: prevalence, seasonal variation and associated factors. *J. Reprod. Med.*, **2006**, *51*, 128-34.
[80] Van Campenhout, A.; Van Campenhout, C.; Lagrou, A.R.; Moorkens, G.; De Block, C.; Manuel-y-Keenoy, B. Iron-binding antioxidant capacity is impaired in diabetes mellitus. *Free Radic. Biol. Med.*, **2006**, *40*, 1749-55.
[81] Finck, B.N.; Lehman, J.J.; Leone, T.C.; Welch, M.J.; Bennett, M.J.; Kovacs, A.; Han, X.; Gross, R.W.; Kozak, R.; Lopaschuk, G.D.; Kelly, D.P. The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus. *J. Clin. Invest.*, **2002**, *109*, 121-30.
[82] de Mello, V.D.; Zelmanovitz, T.; Perassolo, M.S.; Azevedo, M.J.; Gross, J.L. Withdrawal of red meat from the usual diet reduces albuminuria and improves serum fatty acid profile in type 2 diabetes patients with macroalbuminuria. *Am. J. Clin. Nutr.*, **2006**, *83*, 1032-8.
[83] Qi, L.; van Dam, R.M.; Rexrode, K.; Hu, F.B. Heme iron from diet as a risk factor for coronary heart disease in women with type 2 diabetes. *Diabetes Care*, **2007**, *30*, 101-6.
[84] Gross, J.L.; Zelmanovitz, T.; Moulin, C.C.; De Mello, V.; Perassolo, M.; Leitao, C.; Hoefel, A.; Paggi, A.; Azevedo, M.J. Effect of a chicken-based diet on renal function and lipid profile in patients with type 2 diabetes: a randomized crossover trial. *Diabetes Care*, **2002**, *25*, 645-51.
[85] Stark, R.; Roden, M. ESCI Award 2006. Mitochondrial function and endocrine diseases. *Eur. J. Clin. Invest.*, **2007**, *37*, 236-48.
[86] Colca, J.R.; McDonald, W.G.; Waldon, D.J.; Leone, J.W.; Lull, J.M.; Bannow, C.A.; Lund, E.T.; Mathews, W.R. Identification of a novel mitochondrial protein ("mitoNEET") cross-linked specifically by a thiazolidinedione photoprobe. *Am. J .Physiol. Endocrinol. Metab.*, **2004**, *286*, E252-60.
[87] Wiley, S.E.; Murphy, A.N.; Ross, S.A.; van der Geer, P.; Dixon, J.E. MitoNEET is an iron-containing outer mitochondrial membrane protein that regulates oxidative capacity. *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 5318-23.
[88] Hunter, R.L.; Choi, D.Y.; Ross, S.A.; Bing, G. Protective properties afforded by pioglitazone against intrastriatal LPS in Sprague-Dawley rats. *Neurosci. Lett.*, **2008**, *432*, 198-201.
[89] Zhou, G.; Li, C.; Cai, L. Advanced glycation end-products induce connective tissue growth factor-mediated renal fibrosis predominantly through transforming growth factor beta-independent pathway. *Am. J. Pathol.*, **2004**, *165*, 2033-43.
[90] Cussimanio, B.L.; Booth, A.A.; Todd, P.; Hudson, B.G.; Khalifah, R.G. Unusual susceptibility of heme proteins to damage by glucose during non-enzymatic glycation. *Biophys. Chem.*, **2003**, *105*, 743-55.
[91] Kang, J.H. Oxidative damage of DNA by the reaction of amino acid with methylglyoxal in the presence of Fe(III). *Int. J. Biol. Macromol.*, **2003**, *33*, 43-8.
[92] Xiao, H.; Cai, G.; Liu, M. Fe2+-catalyzed non-enzymatic glycosylation alters collagen conformation during AGE-collagen formation *in vitro*. *Arch. Biochem. Biophys.*, **2007**, *468*, 183-92.
[93] Cai, L.; Li, W.; Wang, G.; Guo, L.; Jiang, Y.; Kang, Y.J. Hyperglycemia-induced apoptosis in mouse myocardium: mitochondrial cytochrome C-mediated caspase-3 activation pathway. *Diabetes*, **2002**, *51*, 1938-48.
[94] Singh, J.; Chonkar, A.; Bracken, N.; Adeghate, E.; Latt, Z.; Hussain, M. Effect of streptozotocin-induced type 1 diabetes mellitus on contraction, calcium transient, and cation contents in the isolated rat heart. *Ann. N Y. Acad. Sci.*, **2006**, *1084*, 178-90.
[95] Li, C.; Cao, L.; Zeng, Q.; Liu, X.; Zhang, Y.; Dai, T.; Hu, D.; Huang, K.; Wang, Y.; Wang, X.; Li, D.; Chen, Z.; Zhang, J.; Li, Y.; Sharma, R. Taurine may prevent diabetic rats from developing cardiomyopathy also by downregulating angiotensin II type2 receptor expression. *Cardiovasc. Drugs Ther.*, **2005**, *19*, 105-12.
[96] Yaras, N.; Bilginoglu, A.; Vassort, G.; Turan, B. Restoration of diabetes-induced abnormal local Ca2+ release in cardiomyocytes by angiotensin II receptor blockade. *Am. J. Physiol. Heart Circ. Physiol.*, **2007**, *292*, H912-20.
[97] Ishizaka, N.; Saito, K.; Furuta, K.; Matsuzaki, G.; Koike, K.; Noiri, E.; Nagai, R. Angiotensin II-induced regulation of the expression and localization of iron metabolism-related genes in the rat kidney. *Hypertens Res.*, **2007**, *30*, 195-202.
[98] Ishizaka, N.; Saito, K.; Mitani, H.; Yamazaki, I.; Sata, M.; Usui, S.; Mori, I.; Ohno, M.; Nagai, R. Iron overload augments angiotensin II-induced cardiac fibrosis and promotes neointima formation. *Circulation*, **2002**, *106*, 1840-6.
[99] Saito, K.; Ishizaka, N.; Aizawa, T.; Sata, M.; Iso-o, N.; Noiri, E.; Mori, I.; Ohno, M.; Nagai, R. Iron chelation and a free radical scavenger suppress angiotensin II-induced upregulation of TGF-beta1 in the heart. *Am. J. Physiol. Heart. Circ. Physiol.*, **2005**, *288*, H1836-43.
[100] Saito, K.; Ishizaka, N.; Aizawa, T.; Sata, M.; Iso, O.N.; Noiri, E.; Ohno, M.; Nagai, R. Role of aberrant iron homeostasis in the upregulation of transforming growth factor-beta1 in the kidney of angiotensin II-induced hypertensive rats. *Hypertens Res.*, **2004**, *27*, 599-607.
[101] Ishizaka, N.; Saito, K.; Mori, I.; Matsuzaki, G.; Ohno, M.; Nagai, R. Iron chelation suppresses ferritin upregulation and attenuates vascular dysfunction in the aorta of angiotensin II-infused rats. *Arterioscler. Thromb. Vasc. Biol.*, **2005**, *25*, 2282-8.
[102] Cosso, L.; Maineri, E.P.; Traverso, N.; Rosatto, N.; Pronzato, M.A.; Cottalasso, D.; Marinari, U.M.; Odetti, P. Induction of heme oxygenase 1 in liver of spontaneously diabetic rats. *Free Radic. Res.*, **2001**, *34*, 189-91.
[103] Hayashi, K.; Haneda, M.; Koya, D.; Maeda, S.; Isshiki, K.; Kikkawa, R. Enhancement of glomerular heme oxygenase-1 expression in diabetic rats. *Diabetes Res. Clin. Pract.*, **2001**, *52*, 85-96.
[104] Song, J.; Sumiyoshi, S.; Nakashima, Y.; Doi, Y.; Iida, M.; Kiyohara, Y.; Sueishi, K. Overexpression of heme oxygenase-1 in coronary atherosclerosis of Japanese autopsies with diabetes mellitus: Hisayama study. *Atherosclerosis*, **2008**, [Epub ahead of print].
[105] Paredi, P.; Biernacki, W.; Invernizzi, G.; Kharitonov, S.A.; Barnes, P.J. Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease? *Chest*, **1999**, *116*, 1007-11.
[106] Bruce, C.R.; Carey, A.L.; Hawley, J.A.; Febbraio, M.A. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. *Diabetes*, **2003**, *52*, 2338-45.
[107] Adaikalakoteswari, A.; Balasubramanyam, M.; Rema, M.; Mohan, V. Differential gene expression of NADPH oxidase (p22phox) and hemoxygenase-1 in patients with Type 2 diabetes and microangiopathy. *Diabet. Med.*, **2006**, *23*, 666-74.

[108] Goodman, A.I.; Chander, P.N.; Rezzani, R.; Schwartzman, M.L.; Regan, R.F.; Rodella, L.; Turkseven, S.; Lianos, E.A.; Dennery, P.A.; Abraham, N.G. Heme oxygenase-2 deficiency contributes to diabetes-mediated increase in superoxide anion and renal dysfunction. *J. Am. Soc. Nephrol.*, **2006**, *17*, 1073-81.

[109] Ma, M.M.; Chen, J.L.; Wang, G.G.; Wang, H.; Lu, Y.; Li, J.F.; Yi, J.; Yuan, Y.J.; Zhang, Q.W.; Mi, J.; Wang, L.S.; Duan, H.F.; Wu, C.T. Sphingosine kinase 1 participates in insulin signalling and regulates glucose metabolism and homeostasis in KK/Ay diabetic mice. *Diabetologia*, **2007**, *50*, 891-900.

[110] Kruger, A.L.; Peterson, S.J.; Schwartzman, M.L.; Fusco, H.; McClung, J.A.; Weiss, M.; Shenouda, S.; Goodman, A.I.; Goligorsky, M.S.; Kappas, A.; Abraham, N.G. Up-regulation of heme oxygenase provides vascular protection in an animal model of diabetes through its antioxidant and antiapoptotic effects. *J. Pharmacol. Exp. Ther.*, **2006**, *319*, 1144-52.

[111] Li, M.; Kim, D.H.; Tsenovoy, P.L.; Peterson, S.J.; Rezzani, R.; Rodella, L.F.; Aronow, W.S.; Ikehara, S.; Abraham, N.G. Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. *Diabetes*, **2008**, *57*, 1526-35.

[112] Liu, X.; Wei, J.; Peng, D.H.; Layne, M.D.; Yet, S.F. Absence of heme oxygenase-1 exacerbates myocardial ischemia/reperfusion injury in diabetic mice. *Diabetes*, **2005**, *54*, 778-84.

[113] Merzouk, S.; Hichami, A.; Sari, A.; Madani, S.; Merzouk, H.; Yahia Berrouiguet, A.; Lenoir-Rousseaux, J.J.; Chabane-Sari, N.; Khan, N.A. Impaired oxidant/antioxidant status and LDL-fatty acid composition are associated with increased susceptibility to peroxidation of LDL in diabetic patients. *Gen. Physiol. Biophys.*, **2004**, *23*, 387-99.

[114] Duzguner, V.; Kaya, S. Effect of zinc on the lipid peroxidation and the antioxidant defense systems of the alloxan-induced diabetic rabbits. *Free Radic. Biol. Med.*, **2007**, *42*, 1481-6.

[115] Peerapatdit, T.; Patchanans, N.; Likidlilid, A.; Poldee, S.; Sriratanasathavorn, C. Plasma lipid peroxidation and antioxidiant nutrients in type 2 diabetic patients. *J. Med. Assoc. Thai.*, **2006**, *89* (Suppl 5), S147-55.

[116] Farhangkhoee, H.; Khan, Z.A.; Mukherjee, S.; Cukiernik, M.; Barbin, Y.P.; Karmazyn, M.; Chakrabarti, S. Heme oxygenase in diabetes-induced oxidative stress in the heart. *J. Mol. Cell Cardiol.*, **2003**, *35*, 1439-48.

[117] Chen, S.; Khan, Z.A.; Barbin, Y.; Chakrabarti, S. Pro-oxidant role of heme oxygenase in mediating glucose-induced endothelial cell damage. *Free Radic. Res.*, **2004**, *38*, 1301-10.

[118] Wang, J.; Dore, S. Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. *Brain*, **2007**, *130*, 1643-52.

[119] Quadro, L.; Blaner, W.S.; Salchow, D.J.; Vogel, S.; Piantedosi, R.; Gouras, P.; Freeman, S.; Cosma, M.P.; Colantuoni, V.; Gottesman, M.E. Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO J.*, **1999**, *18*, 4633-44.

[120] Yang, Q.; Graham, T.E.; Mody, N.; Preitner, F.; Peroni, O.D.; Zabolotny, J.M.; Kotani, K.; Quadro, L.; Kahn, B.B. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*, **2005**, *436*, 356-62.

[121] Kovacs, P.; Geyer, M.; Berndt, J.; Kloting, N.; Graham, T.E.; Bottcher, Y.; Enigk, B.; Tonjes, A.; Schleinitz, D.; Schon, M.R.; Kahn, B.B.; Bluher, M.; Stumvoll, M. Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. *Diabetes*, **2007**, *56*, 3095-100.

[122] Kowalska, I.; Straczkowski, M.; Adamska, A.; Nikolajuk, A.; Karczewska-Kupczewska, M.; Otziomek, E.; Gorska, M. Serum retinol binding protein 4 is related to insulin resistance and nonoxidative glucose metabolism in lean and obese women with normal glucose tolerance. *J. Clin. Endocrinol. Metab.*, **2008**, *93*, 2786-9.

[123] van Hoek, M.; Dehghan, A.; Zillikens, M.C.; Hofman, A.; Witteman, J.C.; Sijbrands, E.J. An RBP4 promoter polymorphism increases risk of type 2 diabetes. *Diabetologia*, **2008**, *51*, 1423-8.

[124] Cabre, A.; Lazaro, I.; Girona, J.; Manzanares, J.; Marimon, F.; Plana, N.; Heras, M.; Masana, L. Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease in type 2 diabetes. *J. Intern. Med.*, **2007**, *262*, 496-503.

[125] Mejia, L.A.; Chew, F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. *Am. J. Clin. Nutr.*, **1988**, *48*, 595-600.

[126] Roodenburg, A.J.; West, C.E.; Hovenier, R.; Beynen, A.C. Supplemental vitamin A enhances the recovery from iron deficiency in rats with chronic vitamin A deficiency. *Br. J. Nutr.*, **1996**, *75*, 623-36.

[127] Fernandez-Real, J.M.; Moreno, J.M.; Ricart, W. Circulating retinol-binding protein-4 concentration might reflect insulin resistance-associated iron overload. *Diabetes*, **2008**, *57*, 1918-25.

[128] Hattori, N.; Rihl, J.; Bengel, F.M.; Nekolla, S.G.; Standl, E.; Schwaiger, M.; Schnell, O. Cardiac autonomic dysinnervation and myocardial blood flow in long-term Type 1 diabetic patients. *Diabet. Med.*, **2003**, *20*, 375-81.

[129] Hattori, N.; Schnell, O.; Bengel, F.M.; Rihl, J.; Nekolla, S.G.; Drzezga, A.E.; Standl, E.; Schwaiger, M. Deferoxamine improves coronary vascular responses to sympathetic stimulation in patients with type 1 diabetes mellitus. *Eur. J. Nucl. Med. Mol. Imaging*, **2002**, *29*, 891-8.

[130] Young, I.S.; Tate, S.; Lightbody, J.H.; McMaster, D.; Trimble, E.R. The effects of desferrioxamine and ascorbate on oxidative stress in the streptozotocin diabetic rat. *Free Radic. Biol. Med.*, **1995**, *18*, 833-40.

[131] Roza, A.M.; Slakey, D.P.; Pieper, G.M.; Van Ye, T.M.; Moore-Hilton, G.; Komorowski, R.A.; Johnson, C.P.; Hedlund, B.E.; Adams, M.B. Hydroxyethyl starch deferoxamine, a novel iron chelator, delays diabetes in BB rats. *J. Lab. Clin. Med.*, **1994**, *123*, 556-60.

[132] Cameron, N.E.; Cotter, M.A. Neurovascular dysfunction in diabetic rats. Potential contribution of autoxidation and free radicals examined using transition metal chelating agents. *J. Clin. Invest.*, **1995**, *96*, 1159-63.

[133] Pieper, G.M.; Siebeneich, W. Diabetes-induced endothelial dysfunction is prevented by long-term treatment with the modified iron chelator, hydroxyethyl starch conjugated-deferoxamine. *J. Cardiovasc. Pharmacol.*, **1997**, *30*, 734-8.

[134] Cameron, N.E.; Cotter, M.A. Effects of an extracellular metal chelator on neurovascular function in diabetic rats. *Diabetologia*, **2001**, *44*, 621-8.

[135] El-Khatib, A.S.; Moustafa, A.M.; Abdel-Aziz, A.A.; Al-Shabanah, O.A.; El-Kashef, H.A. Effects of aminoguanidine and desferrioxamine on some vascular and biochemical changes associated with streptozotocin-induced hyperglycaemia in rats. *Pharmacol. Res.*, **2001**, *43*, 233-40.

[136] Lai, T.Y.; Lee, G.K.; Chan, W.M.; Lam, D.S. Rapid development of severe toxic retinopathy associated with continuous intravenous deferoxamine infusion. *Br. J. Ophthalmol.*, **2006**, *90*, 243-4.

[137] Kontoghiorghes, G.J.; Nasseri-Sina, P.; Goddard, J.G.; Barr, J.M.; Nortey, P.; Sheppard, L.N. Safety of oral iron chelator L1. *Lancet*, **1989**, *2*, 457-8.

[138] Kontoghiorghes, G.J. Future chelation monotherapy and combination therapy strategies in thalassemia and other conditions. comparison of deferiprone, deferoxamine, ICL670, GT56-252, L1NAll and starch deferoxamine polymers. *Hemoglobin*, **2006**, *30*, 329-47.

[139] Kontoghiorghes, G.J.; Eracleous, E.; Economides, C.; Kolnagou, A. Advances in iron overload therapies. prospects for effective use of deferiprone (L1), deferoxamine, the new experimental chelators ICL670, GT56-252, L1NA11 and their combinations. *Curr. Med. Chem.*, **2005**, *12*, 2663-81.

[140] Platis, O.; Anagnostopoulos, G.; Farmaki, K.; Posantzis, M.; Gotsis, E.; Tolis, G. Glucose metabolism disorders improvement in patients with thalassaemia major after 24-36 months of intensive chelation therapy. *Pediatr. Endocrinol. Rev.*, **2004**, *2* (Suppl 2), 279-81.

[141] Christoforidis, A.; Perifanis, V.; Athanassiou-Metaxa, M. Combined chelation therapy improves glucose metabolism in patients with beta-thalassaemia major. *Br. J. Haematol.*, **2006**, *135*, 271-2.

[142] Christoforidis, A.; Perifanis, V.; Tsatra, I.; Vlachaki, E.; Athanassiou-Metaxa, M. Evolution of OGTT in patients with beta-thalassaemia major in relation to chelation therapy. *Diabetes Res. Clin. Pract.*, **2007**, *76*, 6-11.

[143] Farmaki, K.; Angelopoulos, N.; Anagnostopoulos, G.; Gotsis, E.; Rombopoulos, G.; Tolis, G. Effect of enhanced iron chelation ther-

apy on glucose metabolism in patients with beta-thalassaemia major. *Br. J .Haematol.*, **2006**, *134*, 438-44.

[144] Thomas, G.N.; Chan, P.; Tomlinson, B. The role of angiotensin II type 1 receptor antagonists in elderly patients with hypertension. *Drugs Aging*, **2006**, *23*, 131-55.

[145] Ritz, E. Anemia and diabetic nephropathy. *Curr. Diab. Rep.*, **2006**, *6*, 469-72.

[146] al-Refaie, F.N.; Wonke, B.; Wickens, D.G.; Aydinok, Y.; Fielding, A.; Hoffbrand, A.V. Zinc concentration in patients with iron overload receiving oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one or desferrioxamine. *J. Clin. Pathol.*, **1994**, *47*, 657-60.

[147] Lucas, G.N.; Perera, B.J.; Fonseka, E.A.; De Silva, D.D.; Fernandopulle, M.; Karunatilaka, D.H.; Weerasinghe, I. Experience with the oral iron chelator deferiprone in transfusion-dependent children. *Ceylon. Med. J.*, **2002**, *47*, 119-21.

Received: September 30, 2008 Revised: November 24, 2008 Accepted: November 28, 2008

[148] Al-Maroof, R.A.; Al-Sharbatti, S.S. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics. *Saudi. Med. J.*, **2006**, *27*, 344-50.

[149] Soinio, M.; Marniemi, J.; Laakso, M.; Pyorala, K.; Lehto, S.; Ronnemaa, T. Serum zinc level and coronary heart disease events in patients with type 2 diabetes. *Diabetes. Care*, **2007**, *30*, 523-8.

[150] Olivares, M.; Pizarro, F.; Ruz, M. New insights about iron bioavailability inhibition by zinc. *Nutrition.*, **2007**, *23*, 292-5.

[151] Faure, P. Protective effects of antioxidant micronutrients (vitamin E, zinc and selenium) in type 2 diabetes mellitus. *Clin. Chem. Lab. Med.*, **2003**, *41*, 995-8.

[152] Umbreit, J. Iron deficiency: a concise review. *Am. J. Hematol.*, **2005**, *78*, 225-31.