REVIEW

The role of iron in obesity and diabetes

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Abstract : Iron is an essential trace metal for all life, but excess iron causes oxidative stress through catalyzing the toxic hydroxy-radical production via the Fenton reaction. The number of patients with obesity and diabetes has been increasing worldwide, and their onset and development are affected by diet. In both clinical and experimental studies, a high body iron content was associated with obesity and diabetes, and the reduction of body iron content to an appropriate level can ameliorate the status and development of obesity and diabetes. Macrophages play an essential role in the pathophysiology of obesity and diabetes, and in the metabolism and homeostasis of iron in the body. Recent studies demonstrated that macrophage polarization is related to adipocyte hypertrophy and insulin resistance through their capabilities of iron handling. Control of iron in macrophages is a potential therapeutic strategy for obesity and diabetes. J. Med. Invest. 69 : 1-7, February, 2022

Keywords **:** *iron, obesity, diabetes, macrophage*

INTRODUCTION

Nutrients can be divided into two categories : macronutrients, including carbohydrates, protein, and fat, and micronutrients such as vitamins and minerals. Minerals are also classified into macro-minerals and micro-minerals. Micro-minerals include nine minerals contained within the following seven metal elements : iron, zinc, copper, manganese, chrome, iodine, and selenium. Among the metal elements, iron is an essential trace metal for all life, and it is necessary for normal growth and development. On the other hand, excess iron is a well-known inducer of oxidative stress through the catalyzation of toxic hydroxy-radical production via the Fenton reaction. Indeed, hereditary iron overload disorders demonstrate a typical phenotype of cardiomyopathy, liver cirrhosis, and diabetes due to oxidative stress induced by ectopic tissue accumulation of excess iron (1). Therefore, cellular and whole-body iron homeostasis is strictly regulated to achieve sufficient iron uptake and inhibit toxic iron accumulation.

In the past 30 decades, increased body iron or iron intake have been reported be associated with a variety of diseases, including liver disease (2, 3), cardiovascular disease (4, 5), cancer (6, 7), Alzheimer's disease (8, 9), and kidney disease (10). Therefore, iron is considered to play an essential role in pathophysiology and its regulation maybe a therapeutic strategy for ameliorating the pathological conditions in the above diseases. Moreover, iron is related to obesity (11) and diabetes (12). In this review, we summarize recent findings and advances regarding the role of iron in obesity and diabetes, including those from our studies.

1. IRON METABOLISM IN THE BODY

The iron content is approximately 5 g in the body of an adult male. Of this, 65% is hemoglobin iron in red blood cells. The rest is stored iron in the liver, spleen, and bone marrow, and is also used as heme iron that activates enzymes in all cells. In humans, iron is generally ingested orally from the diet. Iron derived from food is mainly divided into heme iron and non-heme iron. Non-heme iron is mainly ferric iron and it is reduced to ferrous iron by duodenal cyctochrome b (Dcytb) present in the upper small intestine (13), and it is absorbed and moved into the small intestinal cells by divalent metal transporter (DMT1), an iron importer (14). On the other hand, heme iron is absorbed in the small intestine by the heme carrier protein (HCP) and degraded by heme oxigenase-1 (HO-1) (15). Absorbed iron moves into the cells from the lumen side of the small intestine, which is released to the blood vessel side via ferroportin (FPN) in basolateral enterocytes, an iron-exclusive exporter. The released ferrous iron is oxidized to ferric iron by hephaestin in basolateral enterocytes, ferric iron binds to transferrin, and it is then transported throughout the body (16).

The amount of iron absorbed from food is as low as a few mg per day and the amount excreted is also as low as 1 mg per day in the desquamation of the gastrointestinal mucosa, urine, sweat, and stool. Therefore, most iron in the body is recycled by processing hemoglobin iron from red blood cell waste in the reticuloendotheial system. Senescent erythrocytes are phagocytosed by macrophages, and hemoglobin in the red blood cells is degraded to heme and globin. Heme is also degraded to ferrous iron, carbon monoxide, and biliverdin by heme oxygenase-1 (HO-1) (17). Ferrous iron is spontaneously oxidized to ferric iron, which binds to ferritin and is stored in the reticuloendotheial system in the spleen and liver. The stored iron is mobilized and binds to plasma transferrin in the blood circulation (18). A large amount of released iron is reused for hemoglobin synthesis of erythroblasts in bone marrow. Transferrin receptor-1 (TfR1), a molecule that takes up iron bound to transferrin, is highly expressed in erythroblasts. Heme is synthesized by iron transported from TfR1 to the cytoplasm and combines with globin protein to form hemoglobin. The liver is the most important organ for iron storage, although body iron is mostly located in erythrocytes (>70%) as hemoglobin. Iron is transported into the liver through TfR-dependent (19) and -independent pathways via DMT1 (20). Iron transported into hepatocytes is mainly stored in ferritin and a part of it exists as free ionized iron, known as the labile iron pool

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(LIP) (21). Some of the ferritin synthesized in the cytoplasm is secreted into the blood. In clinical practice, the serum ferritin concentration is used as a marker of the amount of iron stored in the body (22). Figure 1 shows a concise schematic diagram of iron metabolism under normal condition.

Hepcidin and erythroferrone (ERFE) are important regulators of body iron homeostasis. Hepcidin is a hepatocyte-derived hormone to control FPN in the small intestine and macrophages (23). More specifically, hepcidin reduces iron efflux from intracellular iron by binding FPN, and induces the reduction of FPN expression, its internalization, and degradation (24). Increased hepcidin levels may impair the mobilization of stored iron and iron absorption due to the degradation of FPN, disturbing iron utilization, whereas decreased hepcidin levels may promote the mobilization of stored iron and iron absorption, altering iron utilization. Subsequently, erythroferrone (ERFE), derived from erythroblasts in the bone marrow and spleen, was recently identified as a new mediator that suppresses hepcidin production (25). The production and secretion of ERFE are regulated by erythropoietin, and an increase in circulating ERFE directly affects hepatocytes by inhibiting hepcidin production, leading to the increased availability of stored iron from macrophages and hepatocytes, in addition to the increased absorption of dietary iron from enterocytes. ERFE is a new factor in the relationship between erythropoiesis and iron metabolism ; however, further investigation is necessary to clarify how ERFE, hepcidin, and iron metabolism are linked.

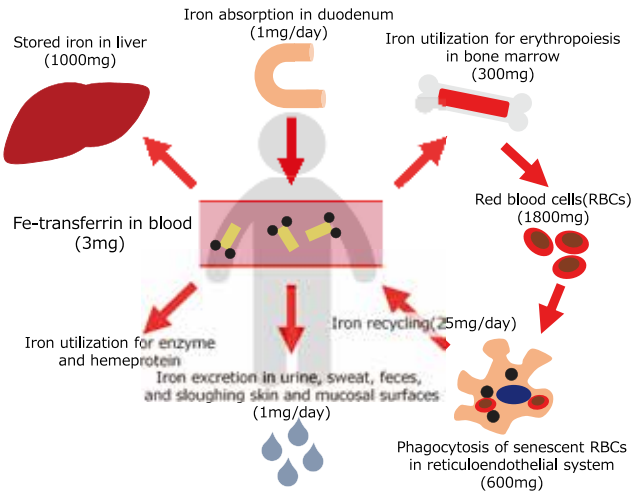

Figure 1. Iron metabolism in human under normal condition. A normal adult body contains about 5g of iron, of which 80% is in hemoglobin, myoglobin, and iron-containing enzymes and 1000 mg of iron is stored such as liver, spleen and so on. About 1 mg of iron is lost each day through urine, sweat, feces, sloughing of cells from skin and mucosal surfaces, including the lining of the gastrointestinal tract every day. About 1 mg of iron absorption also occurs predominantly in the duodenum and upper jejunum daily. Most of the iron in the body is recycled when old red blood cells are taken out of circulation and destroyed, with their iron phagocyted by macrophages in reticuloendothelial system, and returned to the storage pool for reuse.

2. IRON AND OXIDATIVE STRESS

Iron is essential for maintaining homeostasis in the body, but excess iron causes cytotoxicity and tissue injury through the production of reactive oxygen species (ROS) (26). The pathway of iron-induced ROS is mediated through hydroxyl radical production via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$) (Figure 2). Moreover, Fe3+ is reduced to Fe^{2+} by superoxide anion radicals ($O_2{}^{\bullet-} - + Fe^{3+} + \rightarrow Fe^{2+} + O_2$). Hydrogen peroxide reacts with superoxide to produce the most cytotoxic ROS. Hydroxyl radicals react in the presence of iron by the combination of this reaction and the Fenton reaction (Haber-Weiss reaction). Thus, iron induces ROS production through the Fenton/Haber-Weiss reaction, causing cell death, denaturation, and cancer due to the oxidation of cell components such as proteins, lipids, lipoproteins, and nucleic acids (27). Ferric iron is transported extracellularly via FPN and converted to ferric iron by ferroxidase of ceruloplasmin. Ferric iron normally exists to bind to transferrin outside the cell or is taken up by ferritin within the cell, inhibiting the above radical reaction by removing the free iron. Thus, the body iron metabolism is strictly regulated under normal conditions, which prevents the unnecessary production of ROS via the Fenton/Haber-Weiss reaction.

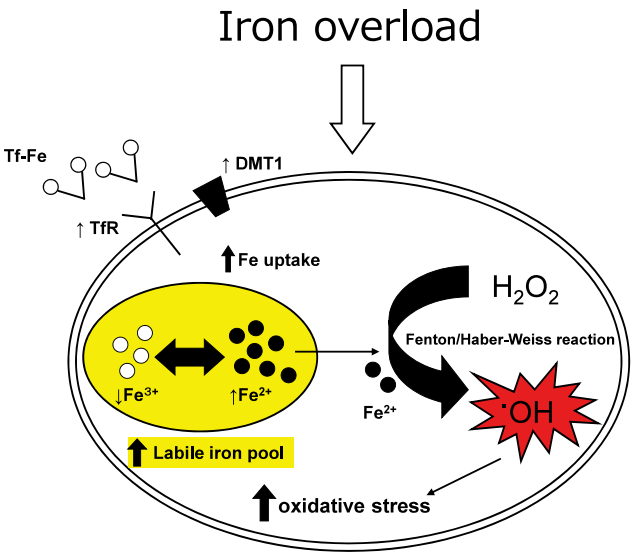

Figure 2. Oxidative stress production induced by alteration of intracellular iron dynamics. Iron overload increases the intracellular labile iron pool, promoting hydroxyl radical production via the Fenton reaction.

3. IRON AND DISEASE

In iron overload diseases, such as hereditary hemochromatosis, thalassemia, myelodysplasia syndrome, and regenerative anemia, transferrin becomes saturated and exceeds the iron-binding ability of transferrin, and the residual iron is taken into parenchymal organs, such as the liver and heart, as non-transferrin-bound iron (28). The excess (free) iron is thought to induce ROS via the Fenton/ Haber-Weiss reaction, causing liver damage/cirrhosis, cardiac damage, and pancreatic injury in general iron overload diseases. In recent years, iron-mediated oxidative stress was revealed to be involved in the pathophysiology of non-iron overload diseases. For example, viral hepatitis C causes iron to accumulate in hepatocytes and the degree of iron deposition correlates with hepatitis activity. Iron removal by phlebotomy ameliorated hepatitis activity (29, 30). Similarly, ROS induced by iron deposition cause hepatocyte damage in alcoholic liver disease (31, 32) and non-alcoholic fatty liver disease (33). In Alzheimer's disease, marked iron deposition was

observed in the brain compared with the control group (8), and an increased brain iron content accelerated cognitive deterioration in patients with Alzheimer's disease (9). Thus, as expected, iron removal suppresses neural damage and death in some neurodegenerative diseases, including Alzheimer's disease (34, 35). Iron is therefore thought to be a substrate of oxidative stress via the Fenton reaction in Alzheimer's disease. In addition, the amount of stored iron (6) or iron intake (7) is associated with the risk of cancer. Indeed, iron removal is an attractive therapeutic strategy for a new class of anti-cancer drugs (36). A new role of iron was demonstrated in the above non-iron overload diseases.

4. IRON, OBESITY, AND DIABETES

4-1. Clinical aspects

Obesity is a major risk factor associated with impaired glucose tolerance and insulin resistance, leading to metabolic syndrome, including type 2 diabetes mellitus (37). Regarding iron in obesity, several studies revealed the association between body iron storage and obesity. The level of serum ferritin is clinically used as a marker of body iron storage, although there is a limitation (38). There are few studies on the relationship between ferritin levels and obesity. The serum ferritin level is positively correlated with the waist-hip ratio (39, 40), body mass index (40, 41), and visceral fat accumulation (42). Serum ferritin also increases in the presence of metabolic syndrome (43) and high ferritin levels at baseline were associated with an increased prevalence of metabolic syndrome in both sexes at the end of a six-year follow-up period (44).

On the other hand, obesity is also affected by the state of iron deficiency. The first evidence of an association between hypoferremia and obesity was reported in 1962 (45) and subsequent studies confirmed this association (46). In a meta-analysis including 26 cross-sectional and case-control studies comprising 13,393 obese individuals and 26,621 non-overweight participants, the obese participants had lower serum iron concentrations and lower transferrin saturation than non-obese participants, resulting in a significantly higher risk of iron deficiency (odds ratio (OR) : 1.31) (47). In addition, a high level of soluble transferrin receptor, which indicates iron deficiency, increases the risk of developing diabetes in obese individuals (48). Several potential mechanisms of obesity-related iron deficiency have been proposed. Inflammation indicated by C-reactive protein is higher in obese subjects than in non-obese subjects, leading to inflammation-mediated functional iron deficiency (49). In addition, hepcidin is higher in obese children, causing iron deficiency by impaired iron absorption (50). However, the relationship between body iron content and obesity remains controversial and further studies are necessary.

Many reports suggested a link between iron content and diabetes. Serum ferritin levels are higher in patients with type 2 diabetes of both sexes. There is a positive association between type 2 diabetes and high plasma ferritin concentrations in newly diagnosed diabetes (OR is 4.94 for men and 3.61 for women) (41). In a population-based, cross-sectional study of 3289 middle-aged and elderly Chinese participates, high serum ferritin levels were associated with a higher prevalence of type 2 diabetes (51). A high ferritin concentration and a lower ratio of transferrin receptors to ferritin are potential markers for an increased risk of type 2 diabetes even in healthy women without known diabetes risk factors (52). Increased meat intake is associated with type 2 diabetes (53). The above association was confirmed in several studies (54), and is related to the high heme content of meat and high dietary heme intake (41, 55). In addition, body iron reduction by iron chelator (56) or phlebotomy (57, 58) improves glycemic control and insulin resistance. Frequent blood donation resulting in decreased iron stores is associated with a low prevalence of diabetes in healthy individuals (59). Thus, an increased body iron content is correlated with the onset and prevalence of type 2 diabetes. The above studies have limitations because serum ferritin levels are mainly used for estimating iron levels in the body without assessing tissue iron deposition. Iron deposition is generally evaluated by biopsy (60) as well as magnetic resonance imaging (61) in liver of patients with non-alcoholic fatty liver disease, however, there is no clinical study to demonstrate the detection of iron deposition in fat and pancreas of obese and diabetic subjects. Further studies are necessary for examine the occurrence of iron deposition in fat and pancreas in patients with obese and diabetes.

4-2. Experimental aspects

Mice have been widely used for iron study because iron metabolism system in mice is the same principles as human such as iron regulatory hormones and iron transporters (62). In experimental studies, iron is an essential regulator of adipogenesis through lipid handling (63), mitochondrial biogenesis (63), and insulin resistance (64), aiding in adipocyte expansion and dysfunction. Genetically obese mice with diabetes (*ob*/*ob*) exhibited increased iron levels in fat compared with lean mice (65). Similarly, high fat diet (HFD)-induced obesity and diabetes also led to an increased iron content in adipose tissue in mice, but not in the liver (66). Therefore, iron may be a therapeutic target for obesity and diabetes through ameliorating unhealthy adipocyte hypertrophy, and several basic studies demonstrated the ameliorative effects of body iron reduction on obesity and diabetes. An iron-restricted diet or phlebotomy improved the diabetic condition through the maintenance of pancreatic β-cell function and inhibition of oxidative stress in Otsuka Long-Evans Tokushima Fatty rats. Serum ferritin levels were higher in this rat model, suggesting body iron alteration under the condition of obesity and diabetes (67). Similarly, dietary iron restriction and iron chelation improved glucose tolerance through the protection of pancreatic β-cell function in *ob*/*ob* mice (68). The effects of iron reduction on insulin secretion in β-cell may be mediated through hypoxia-inducible factor signaling (69). In addition, iron reduction by deferoxamine increased the expression of GLUT1 and insulin receptor, and activated the Akt-FOXO1 pathway in hepatoma cell lines and the rat liver, thereby promoting glucose uptake and insulin signaling (70). Deferoxamine also inhibited the development of obesity in a diabetic state by ameliorating inflammatory cytokines and oxidative stress in white adipose tissue of KKAy mice (71) and lipid metabolism in *ob*/*ob* mice (72). Recently, adipocyte iron reduction by adipocyte-specific TfR deletion or FPN-overexpression was reported to mitigate HFD-induced obesity and diabetes by restricting lipid absorption from enterocytes, suggesting that adipocyte iron levels regulate adipocyte-enterocyte-dependent systemic lipid metabolism (73). Iron reduction in the body and adipose tissue may lead to the amelioration of metabolic disorders, including obesity and diabetes, whereas iron supplementation reduced HFD-induced obesity (74, 75). The reason for the different effects of iron on adipogenesis is unknown and further investigations are needed.

5. MACROPHAGE IRON IN OBESITY AND DIABETES

Chronic low-grade inflammation is associated with the development of insulin resistance, obesity, and diabetes (76). In adiposity, an increase of infiltrated macrophages is observed in visceral fat (77). The amounts of infiltrating macrophages in fat are positively correlated with adipocyte hypertrophy and recruited

macrophages are also responsible as a source of inflammatory cytokines (77, 78). Body weight loss leads to a reduction of infiltrated macrophages and inflammatory cytokines (79). Increased pro-inflammatory cytokines also cause insulin resistance in obesity. In particular, tumor necrosis factor-α (TNF-α) induces insulin resistance in adipocytes and the secreted TNF-α is mainly derived from macrophages (80, 81). This suggests that infiltrated macrophages play a central role in the development of a vicious cycle between adipocytes and macrophages in obese adipose tissue.

Macrophages are classified into two major populations that polarize either the proinflammatory phenotype (M1 : classically activated) or the anti-inflammatory phenotype (M2 : alternatively activated) (82). In healthy/lean subjects, alternatively activated M2 macrophages with CD206 and CD301 expression secrete anti-inflammatory cytokines, such as IL-10 and IL-1RA, to attenuate inflammation. On the other hand, in obese subjects, classically activated M1 macrophages expressing CD11c and F4/80 secrete pro-inflammatory cytokines, such as TNF-α and IL-1β, thereby inducing an inflammatory response in adipose tissue (83, 84).

Orr *et al*. investigated resident macrophages in adipose tissue using the ferromagnetic isolation technique (66). There were two populations of macrophages with either a low iron content or high iron content. In HFD-induced obese mice, the marked increase in macrophages with a low iron content resulted in a relative decrease in the number of macrophages with a high iron content. In addition, macrophages with a high iron content changed to the M1 phenotype with Ccr7 and TNF-α in HFD-induced obese mice, although they presented the M2 phenotype with IL-10 and Stab-1 expression in lean mice. The iron content of macrophages was reduced and the iron content in adipocytes increased in HFD-induced obese mice, suggesting that macrophages are a source of iron for adipocytes when obesity induces an abnormal iron distribution.

These two phenotypes are also characterized by the divergent expression of iron-related proteins and iron content (85, 86). M1 macrophages express high levels of the iron-storage protein H-ferritin (ferritin heavy chain ; FTH), and low levels of the iron import protein TfR and iron export protein FPN, thereby maintaining increased cellular iron content. In contrast, M2 macrophages express low levels of FTH and high levels of TfR and FPN, reducing the cellular iron content. This difference in polarization between M1 and M2 macrophages may involve intracellular iron content, affecting macrophage function, especially the proinflammatory response (86) (Figure 3). Indeed, increased iron content promotes the production of lipopolysaccharide (LPS)-induced inflammatory cytokines in hepatic macrophages (87), and an iron chelator suppressed cytokine production in mouse bone marrow macrophages (88). In our previous study, F4/80 expression (infiltrated M1 macrophages) colocalized with FTH protein expression in obese fat of KKAy mice (Figure 4) (71). Therefore, the coordination of FTH protein and intracellular iron content may be a determining factor in the polarization of macrophages with an inflammatory phenotype.

Regarding the above hypothesis, we recently investigated the role of macrophage FTH in HFD-induced obesity and diabetes using macrophage-specific FTH knockout (KO) mice (89). The iron content in macrophages of FTH KO mice was approximately two-thirds of that in wild-type (WT) mice. The macrophages highly expressed FPN and Hmox1, and weakly expressed TfR, suggesting M2-like polarization via the deletion of FTH. Anemia was not noted in FTH KO mice and there were no differences in red blood cell counts or hemoglobin between WT mice and KO mice. Body weight and white adipose tissue weight increased in WT mice during HFD feeding, but not in FTH KO mice.

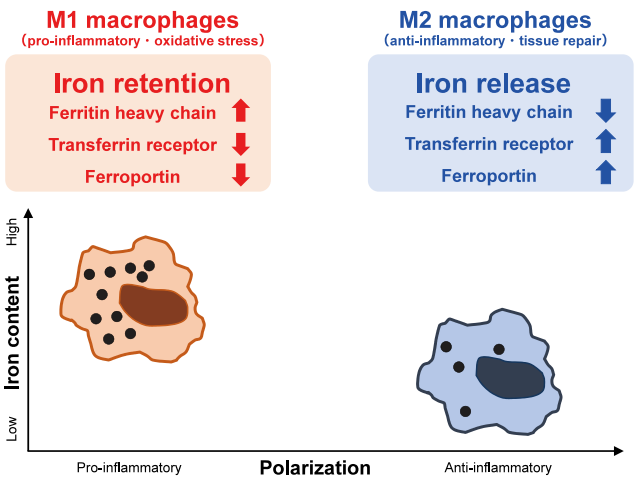

Figure 3. The difference in intracellular iron metabolism between M1 and M2 macrophages. M1 macrophages retain iron by increasing FTH, and reducing TfR and FPN. M1 macrophages release iron by reducing FTH, and increasing TfR and FPN. (Modified from (66))

Figure 4. Colocalization of macrophages and FTH expression in adipose tissue. The image of consecutive sections demonstrates that F4/80 expression colocalizes with FTH expression in hypertrophied fat of obese and diabetic KKAy mice. (Modified from Tajima and Ikeda *et al*. (71))

WT mice exhibited a HFD-induced increase in inflammatory cytokines, macrophage infiltration of adipose tissue, and oxidative stress, which were alleviated in FTH KO mice. WT mice fed HFD had a high iron content in white adipose tissue and spleen, which was not observed in FTH KO mice fed HFD. On the other hand, iron content in macrophages was not increased by HFD feeding, although the iron content was lower in FTH KO mice fed a normal diet or HFD than in WT mice. HFD-induced impairment of glucose tolerance and insulin sensitivity was alleviated in FTH KO mice. Furthermore, energy expenditure, mRNA expression of thermogenic genes, and body temperature were higher in FTH KO mice with HFD than in WT mice with HFD, leading to less body weight gain induced by HFD. This study demonstrated the roles of macrophage iron levels in the development of obesity and diabetes through regulating inflammatory responses and energy metabolism in adipose tissue. A scheme of our hypothesis is shown in Figure 5.

On the other hand, mice with myeloid-specific FPN deletion exhibited preserved insulin sensitivity, although they had mitochondrial defects, such as a reduction in mitochondrial reserve capacity, suggesting that macrophages resist iron excess without inducing metabolic disorders (90). Further studies are needed to elucidate the role of macrophage iron in obesity and diabetes.

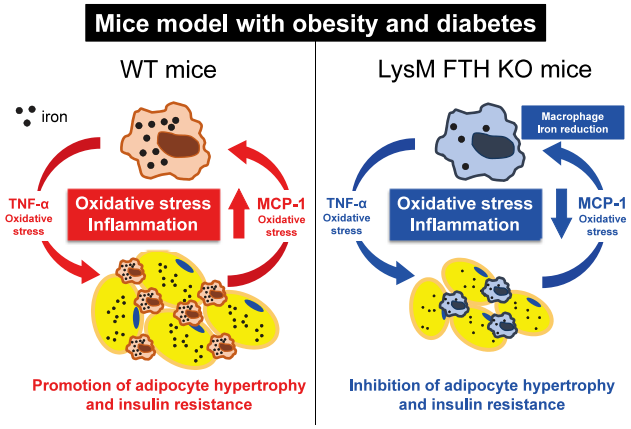

Figure 5. A schema of the hypothesized effects of macrophage iron in the cycle of inflammation and oxidative stress between macrophages and adipocytes in mice with HFD-induced obese and diabetes. Specific reduction of macrophage iron suppresses HFD-induced inflammation and oxidative stress in adipose tissue, preventing the development of obesity and diabetes.

CONCLUSION AND PERSPECTIVE

To summarize, this review suggests a close relationship between iron content and obesity and diabetes mellitus through controlling adipocyte expansion and insulin resistance. The current study supports that body iron content estimated by serum ferritin is positively correlated with fat accumulation and insulin resistance. Iron reduction leads to the amelioration of these conditions ; however, nonspecific iron depletion causes anemia and it is difficult to apply in a clinical setting. The interaction between macrophages and adipocytes creates a vicious cycle of inflammation and oxidative stress, further exacerbating obesity and diabetes, and iron is involved in this cycle by regulating macrophage polarization and supplying iron to adipocytes. Although further investigation of the role of macrophages in iron homeostasis in other tissues is needed, control of macrophage-specific iron content may be a new therapeutic target for regulating obesity and diabetes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this review.

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