Review

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Iron homeostasis: a new job for macrophages in adipose tissue?

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Elevated serum ferritin and increased cellular iron concentrations are risk factors for diabetes; however, the etiology of this association is unclear. Metabolic tissues such as pancreas, liver, and adipose tissue (AT), as well as the immune cells resident in these tissues, may be involved. Recent studies demonstrate that the polarization status of macrophages has important relevance to their iron-handling capabilities. Furthermore, a subset of macrophages in AT have elevated iron concentrations and a gene expression profile indicative of iron handling, a capacity diminished in obesity. Because iron overload in adipocytes increases systemic insulin resistance, iron handling by AT macrophages may have relevance not only to adipocyte iron stores but also to local and systemic insulin sensitivity.

Obesity and metabolic syndrome are associated with increased iron

Elevated body iron stores have repeatedly been linked to factors comprising the metabolic syndrome, including obesity, dyslipidemia, hypertension, fasting hyperglycemia, and diabetes [1–7] ([8] for review). An analysis of four different studies on the link between iron and diabetes demonstrated that men and women in the highest quintile of serum ferritin (Ft) levels – the primary intracellular iron-storage protein that can be released in overload situations (see Glossary) – had a relative risk of greater than 3.5 for developing diabetes (reviewed in [9]). In fact, this condition is now known as dysmetabolic iron-overload syndrome (DIOS) [10] and it has even been suggested that serum Ft may serve as a surrogate marker for insulin resistance (IR) [11]. Although inflammatory cytokines can influence iron storage in various cell types, studies have shown that the link between elevated iron and obesity/diabetes is independent of inflammation [1,3]. It is also not associated with dietary iron intake or absorption [12] or with β cell function [13]. Further supporting the relationship between iron and metabolic disease, a recent 7 year prospective study found that serum Ft was positively associated with HOMA2 (Homeostasis Model Assessment 2)-IR, and both hepatic and adipocyte IR [14]. This aligns well with the finding that IR is associated with changes in iron-handling proteins in AT [15]. Furthermore, iron depletion by phlebotomy, even in healthy subjects, has been shown to improve insulin sensitivity [16]. Although extensive research efforts have been focused on understanding the association between obesity and the metabolic syndrome, mechanisms underlying the impact of iron on metabolic health have not been well elucidated.

AT secretes numerous cytokines, chemokines, adipokines, and other proteins that act locally and systemically

Glossary

Anemia of chronic disease: disease where chronic inflammatory states (chronic inflammation, malignancy, obesity, etc.) induce hepcidin expression via IL-6. Chronic elevation of hepcidin results in tissue iron storage, reduced plasma iron availability, and anemia due to reduced erythropoiesis.
Cluster of differentiation 91 (CD91): a scavenger receptor that binds heme–hemopexin complexes, among many other ligands. CD91 is involved in many key cellular processes, such as intracellular signaling, lipid homeostasis, and clearance of apoptotic cells.
Cluster of differentiation 163 (CD163): a scavenger receptor that binds to hemoglobin–haptoglobin complexes (see Box 1 for more information).
Divalent metal transporter 1 (DMT1): protein that belongs to a family of highly conserved metal ion transporters. DMT1 transports ferrous iron (Fe^{2+}) into enterocytes or across endosomal membranes. Body iron stores regulate its expression and it helps to maintain iron homeostasis.
Dysmetabolic iron-overload syndrome (DIOS): term used to describe a syndrome where elevated iron overload – indicated by increased serum ferritin – is associated with metabolic disease.
Ferroportin 1 (Fpn1): this protein is the only known iron-export protein. Hepcidin induces the internalization of ferroportin from the cell surface.
Ferritin (Ft): complex that consists of ferritin heavy chains and ferritin light chains, transcribed from two different genes. Iron is stored intracellularly complexed to ferritin. Serum ferritin concentrations are reflective of total body iron stores.
Heme oxygenase 1 (Hmox1): this enzyme is responsible for converting heme into CO, Fe^{2+}, and bilirubin.
Labile iron pool (LIP): this term refers to the chelatable intracellular iron, which is transitory and redox-active.
M1-like (classically activated) macrophages: *in vitro* polarization of macrophages with inflammatory mediators such as LPS and IFNγ results in M1 polarization and an inflammatory cytokine profile. *In vivo,* macrophages recruited to AT in obesity display evidence of M1-like polarization.
M2-like (alternatively activated) macrophages: *in vitro* polarization of macrophages with TH2 cytokines such as IL-4 and IL-13 results in M2 polarization and an anti-inflammatory cytokine profile. *In vivo,* resident AT macrophages display M2-like gene expression profiles.
MFe^{hi}: macrophages isolated from AT based on their natural ferromagnetic properties because of increased iron content. In both lean and obese mice, MFe^{hi} cells are more M2-like with anti-inflammatory properties, but these cells become more inflammatory after high fat diet-induced obesity.
MFe^{lo}: macrophages in AT that are not ferromagnetic. In lean mice, MFe^{lo} cells are primarily M2-like but differ from MFe^{hi} because of their decreased iron content. In obese mice the MFe^{lo} population still includes both non-ferromagnetic M2 cells, but consists predominantly of newly recruited M1-like ATMs.
Reactive oxygen species (ROS): chemically reactive molecules containing oxygen, such as OH·. Free iron readily induces ROS production.
Transferrin receptor 1 (TfR1): endocytic recycling receptor that binds to transferrin-bound iron.

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to control lipid, glucose, and energy homeostasis [17]. Furthermore, in obesity, a proinflammatory milieu within the AT alters its ability to maintain these homeostatic controls. Interestingly, almost all cells of the innate and adaptive immune system have been found in both lean and obese AT, with their proportions changing to promote inflammation and IR in obesity (reviewed in [18]). In addition to knowledge regarding the inflammatory potential of AT, it is now understood that iron overload of adipocytes also contributes to local and systemic IR [19]. The association between obesity, inflammation, and adipocyte iron overload is an area of new interest.

Despite the link between iron overload and obesity mentioned above, inflammation in obesity also leads to iron-deficient anemia [20]. Linking obesity with both tissue iron overload and iron-deficient anemia may seem counterintuitive; however, this can be understood by parsing out systemic and cell autonomous regulation of iron storage, as detailed below.

Regulation of systemic and cellular iron

Systemic iron handling

Iron is absorbed from the diet through apical transporters on duodenal enterocytes (Figure 1). It is released basally from enterocytes through the only known mammalian iron exporter, ferroportin-1 (Fpn1). In serum, iron is found in three main forms: Fe^{3+} bound to transferrin (Tf), heme bound to hemopexin, and hemoglobin bound to haptoglobin. In bone-marrow erythroblasts, iron is used to form hemoglobin for incorporation into nascent erythrocytes. Senescent erythrocytes are phagocytosed by splenic macrophages, and the iron is recycled back to the erythroblasts (Figure 1). In fact, 10-fold more iron is recycled by macrophages than is absorbed through the duodenum [21]. Resident macrophages are responsible for iron cycling in many tissues, including bone marrow, spleen [22], liver [23], and lung [24]. Macrophages also express receptors for all three types of iron that are present in serum (Figure 1); transferrin receptor (TfR1) binds to Fe^{3+}–Tf, cluster of differentiation 91 (CD91) binds to heme–hemopexin, and CD163 binds to haptoglobin–hemoglobin (Hp–Hb). Box 1 provides more information on CD163 and its relevance to tissue macrophage inflammation.

The systemic uptake and release of iron is regulated through the acute-phase reactant protein hepcidin, which is secreted by hepatocytes. Hepcidin induces Fpn1 endocytosis and degradation [25], and therefore regulates the primary control points of plasma iron: absorption of iron from the intestine, release of stored iron from hepatocytes, and recycling of iron by macrophages. This is also the primary pathway for regulated iron clearance whereby hepcidin reduces Fpn1 on enterocytes, leading to the

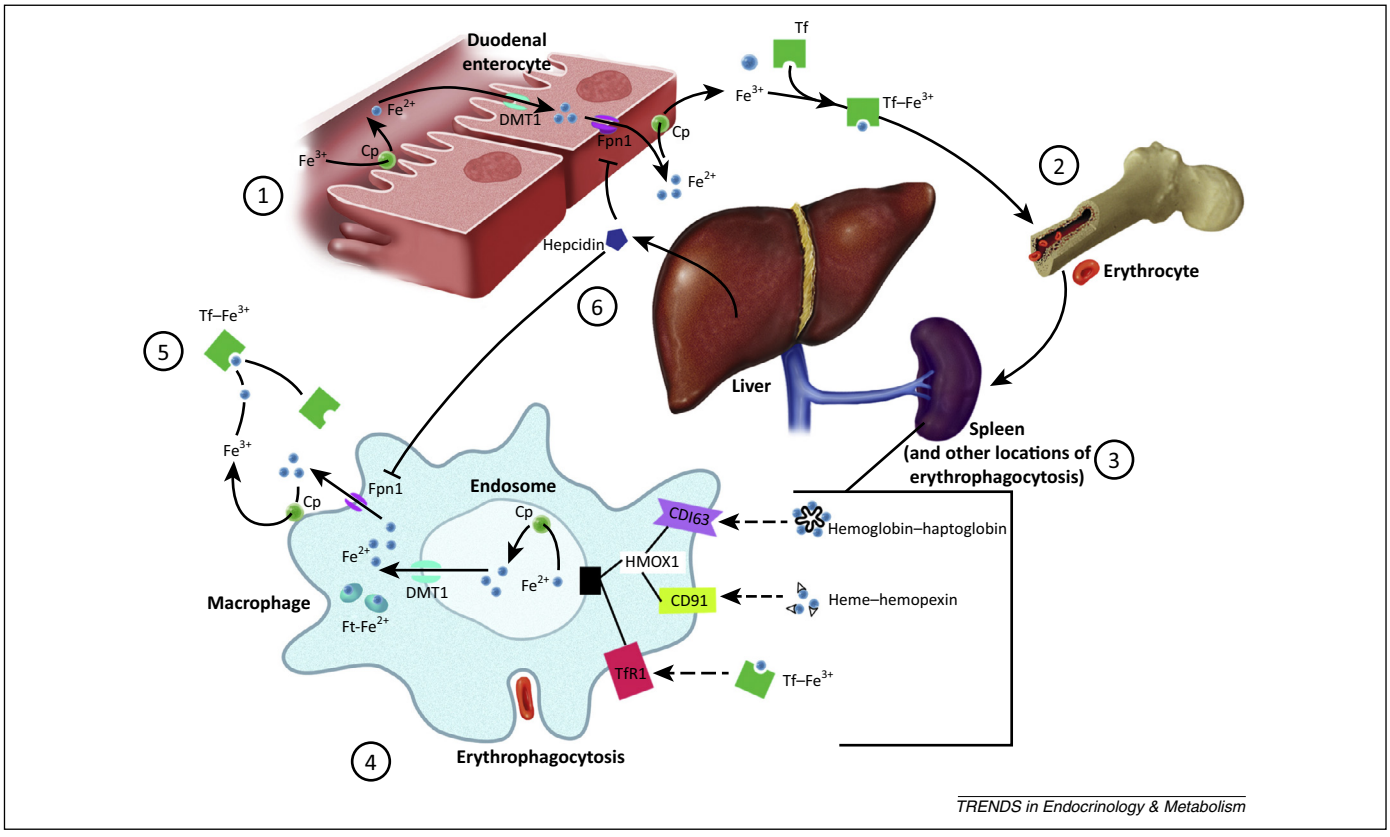

Figure 1. Schematic of systemic and cellular iron handling. Dietary iron is taken up via DMT-1 on enterocytes after reduction by Cp. It is transferred into the plasma through Fpn1, where it is again oxidized and binds Tf. Thereafter, it is primarily transported to bone marrow to produce heme for erythrocyte formation. In the spleen, most of the iron taken up by macrophages derives from phagocytosis of senescent red blood cells. However, systemic macrophages can also reabsorb iron through three primary receptors, depending on what form it is in: CD163 binds hemoglobin–haptoglobin complexes, CD91 binds heme–hemopexin complexes, and TfR1 binds Tf–Fe^{3+}. Once bound, the receptor is endocytosed and a change in pH induces the reduction of iron and its release into the cytoplasm. In the cytoplasm, iron exists in two forms; bound by Ft or incorporated into proteins. Iron is released from cells through Fpn1, again to be bound by Tf. Hepcidin is secreted from the liver in response to acute and chronic inflammatory stimuli and functions to degrade Fpn1, primarily on enterocytes and macrophages. Abbreviations: Cp, ceruloplasmin; DMT-1, divalent metal transporter 1; Fpn1, ferroportin 1; Ft, ferritin; Hmox1, heme oxygenase 1; Tf, transferrin; TfR1, transferrin receptor 1.