Published in final edited form as:

Trends Endocrinol Metab. 2015 February ; 26(2): 101–109. doi:10.1016/j.tem.2014.12.005.

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

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Abstract

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.

Keywords

iron; adipose tissue; inflammation; macrophage

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] (See [8] for a review of this topic). 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 reference [9]). In fact, this condition is now called

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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 to beta 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-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 for 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 to control lipid, glucose, and energy homeostasis [17]. Furthermore, in obesity, a pro-inflammatory 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, or hemoglobin bound to haptoglobin. In bone marrow erythroblasts, iron is used to form hemoglobin for incorporation into nascent erythrocytes. Senescent erythrocytes are phagocytized by splenic macrophages and the iron is recycled back to the erythroblasts (Figure 1). In fact, ten times 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 Fe^{3+} -Tf, cluster of Differentiation 91 (CD91) binds heme-hemopexin, and CD163 binds haptoglobin-hemoglobin (Hp-Hb). BOX 1 provides more information on CD163 and its relevance to tissue macrophage inflammation.

BOX 1**CD163 – more than the hemoglobin-haptoglobin receptor?**

CD163 is a scavenger receptor that binds and endocytoses haptoglobin-hemoglobin (Hp-Hb) complexes to prevent hemoglobin-induced toxicity. In fact, endocytosis and lysosomal degradation of Hp-Hb complexes followed by the conversion of heme into CO, biliverdin, and iron by Hmox1 is highly anti-inflammatory. CD163 is only expressed on monocytes and macrophages. In fact, CD163 is primarily expressed on M2-like macrophages [76] and its expression is often used to identify M2-polarization of macrophages. It is generally upregulated by anti-inflammatory cytokines and suppressed by inflammatory cytokines. CD163 can be cleaved at the cell surface by the TNF α Converting Enzyme (TACE; ADAM17) [77] resulting in the release of soluble CD163 (sCD163). During acute inflammation, TACE is activated and plasma levels of sCD163 rise; sCD163 is therefore sometimes used as a biomarker of tissue macrophage activation. CD163 expression on tissue macrophages is increased in autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, Crohn's disease, and celiac disease and it is also highly expressed on tumor-associated macrophages.

sCD163 is elevated in obesity, diabetes, and gestational diabetes [78] and is also a known risk factor for preterm birth in obese women [79]. Recent work has shown that AT and placenta from women with gestational diabetes release more sCD163 and have more macrophages expressing CD163 [80]. These data suggest that tissue macrophages in AT and placenta may contribute to systemic sCD163 levels. Furthermore, they raise the possibility that AT and placental macrophage iron handling may be relevant to the progression of gestational diabetes.

Obesity is associated with both increased sCD163 and reduced adiponectin expression. This could be coincidental; however, recent evidence points to a direct role of adiponectin in regulating macrophage CD163 expression [78] and M2 polarization [81].

Given the various diseases associated with CD163 and sCD163, and the fact that CD163 is a receptor for Hp-Hb, it is curious that little work has been done to determine the role of resident macrophage iron handling in tissue homeostasis and in disease progression. Furthermore, it is not clear whether sCD163 is simply a biomarker of tissue macrophage inflammation, or whether it plays a role in the pathophysiology of metabolic diseases associated with chronic inflammation. We may be just at the tip of the iceberg in understanding the contribution of macrophages to local and systemic iron homeostasis. For an excellent review on CD163, please see [82].

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 sequestration of iron in cells that will be sloughed off and excreted [26]. In acute inflammation, hepcidin-induced iron

sequestration results in dramatic reductions in plasma iron as a bacteriostatic mechanism. Hepcidin is also synthesized by adipocytes [27] and macrophages [28]. Under homeostatic conditions hepcidin production is induced through signaling of the bone morphogenetic protein 6 (BMP6)/Smad pathway with a co-receptor for BMP, hemojuvelin. During inflammation, this is accomplished via the interleukin 6 (IL6)/STAT3 pathway (See [29] for a review on regulation of hepcidin).

Effects of obesity on systemic iron concentrations

Inflammatory signaling, often associated with chronic diseases such as obesity, is associated with increased expression of hepcidin via IL-6 induction and can lead to anemia of chronic disease – a disease in which tissue iron overload and anemia occur concomitantly. Increased circulating hepcidin can explain this apparent conundrum. Cells such as macrophages, enterocytes, and hepatocytes have high iron uptake and depend on Fpn1 for iron release [30]. Therefore, when hepcidin is up-regulated, Fpn1 is down-regulated and iron is sequestered, reducing its availability in plasma. A consequence of decreased plasma iron levels is reduced availability for erythroblastosis, thereby leading to anemia. Thus, in the case of chronic inflammation-induced hepcidin expression, increased serum Ft reflective of tissue iron overload occurs concomitantly with anemia.

Obesity is not only associated with AT inflammation, but also hypoxia [31]. Hepcidin expression is down-regulated in the presence of hypoxia through the hemojuvelin/BMP6 pathway via hypoxia-inducible factors [32]. Interestingly, this indicates an opposite effect of inflammation and hypoxia on hepcidin expression. Therefore, the role for hypoxia in regulating AT hepcidin warrants further investigation.

Obesity has also been directly linked to changes in iron storage. For example, in the liver, high-fat/high-sucrose diet induces hepatic iron storage [33]. However, there are still some conflicting findings concerning the impact of diet-induced obesity of liver iron storage, as some studies have shown a decrease in liver iron in obese mice [34, 35]. It is thought that iron accumulation in certain cells not only alters iron-handling genes but also changes inflammatory cytokine expression. Taken together, there is still much to be learned regarding the reciprocal regulation of iron and inflammation in obesity.

Systemic iron regulation in genetic iron overload disorders

The relationship between obesity and cellular iron overload contrasts with findings in genetic forms of iron overload, such as hereditary hemochromatosis. Genetic causes of iron overload are most commonly caused by defects in hepcidin leading to inappropriately high Fpn1 on the cell surface [36]. With constitutively elevated Fpn1, enterocytes continually import dietary iron into serum, and excess iron is stored in tissues such as the liver, heart, and endocrine organs that have naturally lower Fpn1 levels. Iron is initially not deposited in macrophages and enterocytes because of their maintained Fpn1 levels; however, in later stages of this disease macrophages can become iron-loaded. These disorders are associated with an increased prevalence of diabetes (likely due to iron-mediated toxicity to the pancreas [37]) and DIOS. It has also been shown that genetic variants in iron-related genes, such as *HFE*, *Slc40a1*, and even beta-globin genes, can impact the pathogenesis of DIOS [38, 39].

Cellular regulation of iron

Following endocytosis via one of the iron receptors, iron is released by acidification of the endosome, and transported across the endosomal membrane by divalent metal transporter 1 (DMT-1) to be bound by Ft, or incorporated into iron-containing proteins. The proteins involved in cellular iron handling are translationally controlled in response to intracellular iron concentrations [40]. This occurs through iron-response proteins (IRPs) that can bind iron-response elements (IREs) to either stabilize or de-stabilize mRNAs for iron-related transcripts. In iron-deplete cells, IRP1 and IRP2 bind IREs in the 3' UTR of *TfR1* and *Slc11a2* (gene for DMT-1) and in the 5' region of *Fth1* and *Ftl1* (genes for Ft heavy and light chains, respectively) and *Slc40a1* (gene for Fpn1). Binding in the 3' UTR stabilizes the mRNA for *Tfrc* (gene for TfR1) and *Slc11a2*, whereas binding in the 5' UTR inhibits translation of *Fth1* and *Slc40a1*. Ultimately, protein levels of TfR1 and DMT-1 are elevated, while levels of Ft and Fpn1 are reduced. The overall effect is that more iron is imported into the cell, less is bound by Ft, and less is exported. Effectively, active IRPs increase the cell's labile iron pool (LIP) the transitory, chelatable, and redox-active iron pool in a cell. When the cell becomes iron-replete, IRP1 is bound by an iron-sulfur cluster to form c-aconitase, and IRP2 is ubiquitinated and degraded (See [40] for a review of cellular iron handling). Ft levels increase and Ft is released at concentrations proportional to its expression in the cells. Due to this fine-tuned regulation, Ft is used as a surrogate measure of body iron stores in humans [11].

ADIPOCYTES AND IRON: BALANCE IS KEY

Based on extensive data correlating obesity and metabolic syndrome to iron overload, it is of no surprise that scientific attention has recently turned to iron in AT. *In vitro* and *in vivo* studies by several different groups have begun to elucidate the role of iron in lipid handling, mitochondrial biogenesis, and other adipocyte functions.

Iron in the balance of adipocyte lipid handling

Iron can impact adipocyte lipid handling in several ways: lipid accumulation during adipogenesis, lipid release during lipolysis, and lipid peroxidation. As shown by *in vitro* 3T3-L1 differentiation models, adipocytes require iron for adipogenesis [41]. Conversely, knockdown of Tf or Ft light chain, as well as iron chelation, result in iron depletion and antiadipogenic effects in human AT explants [41]. Iron also alters adipocyte lipolysis and lipid peroxidation. For example, iron treatment of primary adipocytes is sufficient to increase lipolysis [42] and reduce insulin-mediated glucose uptake [43]. The mechanism underlying this iron-induced lipolysis is not through canonical pathways such as cAMP, MAP kinase, protein kinase C, or phosphatidylinositol 3-kinase. Rather, it can be inhibited by a free-radical scavenger, and can be mimicked by 4-hydroxy-2-nonenal (4-HNE), a by-product of lipid peroxidation [42] (reviewed in [44]). Lipid peroxidation is an end product of excess reactive oxygen species (ROS) in adipocytes, and increases with increased iron. Therefore, iron likely induces lipolysis through a pro-oxidant mechanism. Taken together, regulation of adipocyte iron exposure in a temporal and concentration dependent manner is a priority for maintaining healthy AT expansion.

Iron requirement for mitochondrial function and biogenesis

Mitochondria depend on iron for many functions, including regulation of cellular redox potential, regulation of apoptotic cell death, and production of iron-sulfur clusters, steroids, and heme [45]. Adipocyte iron depletion decreases expression of the mitochondrial transcription factor, *Tfam*, indicating decreased mitochondrial transcription and replication. Conversely, mitochondrial biogenesis is upregulated during adipogenesis. Iron and IRPs have been shown to reversibly stimulate mitochondrial biogenesis and activity [41], which is also a primary mechanism for the efficacy of pharmaceutical agonists of peroxisome proliferator-activated receptor gamma commonly used to treat diabetic patients [46]. Therefore, adipocyte iron concentrations must be maintained within a proper range to allow for proper mitochondrial biogenesis and function, without being so high that oxidative stress occurs.

Impact of iron overload on adipocytes

Several recent studies have attempted to illuminate the effect of iron overload on adipocyte function. Feeding mice a high iron diet causes IR in visceral AT [10], while a low iron diet is associated with improved insulin sensitivity in genetically obese leptin deficient ob/ob mice [47]. Furthermore, in mice, high dietary iron is associated with a decrease in the insulin-sensitizing adipokine, adiponectin [19], as well as an increase in the insulin-resistance inducing adipokine, resistin [10], despite a decrease in fat mass. An adipocyte-specific *Fpn1* knockout mouse model was developed to induce adipocyte iron accumulation [19]. These mice demonstrated decreased systemic glucose tolerance in a mechanism driven by reduced serum adiponectin. This parallels studies showing that serum Ft is the strongest predictor of low serum adiponectin in humans [19, 48, 49]. High serum Ft is also associated with adipocyte-specific IR, even after controlling for a host of covariates [48]. Therefore, high serum Ft could be an indicator of adipocyte dysfunction, via changes in adipokine secretion and adipocyte IR.

Mechanisms underlying adipocyte dysfunction with high iron

There is no known central mechanism for the impact of iron on adipocytes; however, iron is known to impact adipocytes' mitochondrial function and adiponectin production, as mentioned above. Excess free intracellular iron leads to amplified production of ROS. These increased ROS alter mitochondrial function in adipocytes, contributing to IR (see [50] for review of mitochondrial dysfunction in AT). In fact, oxidative stress from ROS impairs mitochondrial function in adipocytes through FOXO1 [51], and FOXO1 activation is also thought to inhibit adipocyte differentiation [52]. Interestingly, altered FOXO1 activation also underlies the association between adipocyte iron overload and reduced adiponectin expression – but by FOXO1 deacetylation, rather than dephosphorylation [19]. These findings suggest that multiple mechanisms for altering adipogenesis and adipocyte function converge on the transcription factor FOXO1, and that iron-induced oxidative stress and adiponectin can be added to the list. Despite this extensive background outlining the impact of iron on adipocyte function, little is understood about how iron availability is regulated in AT.

IRON HANDLING BY RESIDENT AT MACROPHAGES

As mentioned above, AT contains many different immune cells – the most abundant of which are macrophages (Figure 2). In lean AT, these macrophages have a gene expression profile most similar to an alternatively activated, M2-like phenotype [53]. Their localization in interstitial spaces, whereby each adipocyte is touching several macrophages, suggests communication between these two cells types and a role for macrophages in AT homeostasis. Some potential roles of resident AT macrophages (ATMs) include AT development and expansion [54, 55], antigen presentation [56], and catecholamine synthesis [57] (reviewed in [58]). In obesity, there is a large influx of macrophages, such that up to 40% of cells in AT are macrophages [59]. The macrophages in obese AT accumulate in crown-like structures around dead adipocytes [60, 61] and have a gene expression profile reflective of a more classically activated, M1-like phenotype [53]. These M1 like macrophages are thought to contribute to IR in AT via their secretion of inflammatory cytokines.

MFe^{hi} macrophages in lean and obese AT

Because macrophages play a prominent role in iron metabolism in other tissues (see BOX 2), ATMs might play a role in iron handling and contribute to the local control of iron metabolism in AT. A recent report showed that 25% of macrophages in lean AT have a 2-fold increase in intracellular iron stores, allowing them to be isolated based upon their ferromagnetic properties [35]. The authors referred to this population of ATMs as “MFe^{hi}” and the remaining ATMs as “MFe^{lo}” (Figure 2 & Figure 3A). Although it has been thought that all ATMs in lean mice are M2-like, compared to MFe^{lo} cells, MFe^{hi} cells have even greater expression of M2 genes, and a reduction in M1 genes. Furthermore, MFe^{hi} ATMs have a 2-fold elevation in all iron metabolism-related genes quantified, including *Cd163*, *Tfr1*, *Hmox1*, *Fth*, *Ftl*, and *Slc40a1*. The increase in all iron-related genes suggests a role for these macrophages in iron-recycling rather than iron-storage. In obesity, newly recruited macrophages do not take on a role in iron handling, and the MFe^{hi} cells become more inflammatory and appear to lose their ability to handle iron properly (Figure 2). Importantly, this change in MFe^{hi} cells with regards to iron handling in obesity occurs concomitantly with adipocyte iron overload and reduced adipocyte adiponectin expression (Figure 2). Taken together, these studies suggest the possibility that MFe^{hi} cells are a unique population of macrophages that regulate iron homeostasis in AT. These MFe^{hi} ATMs have several characteristics to call attention to: (1) they are M2 polarized in the context of increased intracellular iron, (2) their polarization and phenotype change with obesity, and (3) they express elevated levels of *Hmox1*.

BOX 2

Iron handling by macrophages in disease states

Macrophages are responsible for iron handling in most tissues, especially during resolution of inflammatory processes. In some cases, iron uptake by macrophages appears to promote restoration of tissue homeostasis, but macrophage iron overload can also contribute to disease processes.

Hemorrhagic plaques. Alternatively activated macrophages have increased iron content and atheroprotective effects during atherosclerotic plaque rupture. These M2-like macrophages, termed “Mhem”, and “M(Hb)” [83–85] take up iron from the local milieu in hemorrhagic plaques. Concomitantly, Mhem/M(Hb) macrophages upregulate lipid export genes. Functionally, this protects the cells from lipid peroxidation that occurs if elevations in iron and lipid are present simultaneously.

Wound healing. Although M1-like macrophages fight infection acutely, anti-inflammatory M2-like macrophages contribute to resolution of inflammation during wound healing. Interestingly, in chronic venous leg ulcers, iron-overloaded macrophages show an unrestrained M1-like, pro-inflammatory phenotype, while still expressing high levels of the M2 markers, CD163 and CD206 [86].

Cancer. Alternatively activated macrophages in the tumor microenvironment are associated with increased iron release, which correlates with tumor cell proliferation. In this system, M2-like macrophages promote angiogenesis, while M1-like cells tend to sequester iron and decrease tumor development [87].

Multiple Sclerosis lesions. M1-like macrophages on the edges of demyelinated lesions have increased depositions of iron. Interestingly, these macrophages tend to be myelin-deplete, while myelin-laden “foamy” macrophages do not contain iron and resemble an M2 phenotype [88]. The presence of M2-like iron-laden macrophages is higher in patients with active relapsing multiple sclerosis.

These examples demonstrate that the polarization of macrophages, their iron content, and their ability to recycle iron, contribute to the overall outcome of disease processes and resolution of inflammation. There remains much to be learned about mechanisms by which macrophages respond to environmental cues unique to these tissues and alter their iron handling status.

M2-like macrophages have an iron-recycling phenotype

A long-standing dogma in the field of immunology is the association between M1-polarized macrophages and increased intracellular iron. This view is grounded in the observation that iron is sequestered by M1-polarized macrophages in serum following spikes in hepcidin during acute inflammation [62]. In addition, this view holds that patterns of gene expression in macrophages either favor iron storage (increased import and storage genes) or iron release (decreased storage and increased export genes). The study of MFe^{hi} cells discussed above contradicts these dogma showing that MFe^{hi} ATMs are highly M2-polarized, have increased iron content, and also have high expression of all iron-related genes, *i.e.* iron uptake, storage, and release [35] (Figure 3A). This could be a phenomenon unique to MFe^{hi} cells in AT; however, other studies support the observation that M2-polarized macrophages have an iron-cycling phenotype [63, 64] (Figure 3B). When comparing M2-polarized to M1-polarized macrophages *in vitro*, TfR1 and Fpn1 are both up-regulated in M2 macrophages, while Ft is down-regulated. M2 macrophages also have enhanced binding of IRPs to mRNA, corresponding to an increased LIP and a more flexible iron-cycling phenotype. Characterization of *in vitro* polarized macrophages versus those found naturally responding

to various environmental cues *in vivo* leaves many questions open with regards to mechanisms by which macrophage polarization and iron handling are linked.

While it is apparent that polarization plays a role in determining macrophages' iron content, secondary factors may further influence their iron status. For example, in M1-polarized macrophages, secondary treatment with nitric oxide (NO) activates IRP1 and IRP2, causing iron retention [65]. In fact, NO synthase inhibitors are able to completely inhibit IFN γ /LPS induced IRP activation [66]. This type of secondary effector may explain why both M1-polarized during infection, and M2-polarized macrophages in lean AT, have increased iron. A difference in NO production (or other potential effectors unique to AT), and the impact on tissue-specific iron handling has not been studied and is a ripe area for future studies (see BOX 3).

Box 3

OUTSTANDING QUESTIONS

- What is the primary mode of iron uptake by ATMs and adipocytes?
- Do MFe^{hi} AT macrophages have a large LIP and how does this change in obesity?
- Are there secondary signals that modulate IRPs and the LIP in MFe^{hi} cells?
- To what extent do resident macrophages, such as MFe^{hi} ATMs, control tissue iron concentrations?
- What is the importance of Hmx1 in resident macrophages of various tissues, and does this depend on the macrophage's iron burden?

Mechanisms by which obesity may alter the MFe^{hi} phenotype

In obese mice, MFe^{hi} ATMs have decreased iron content as well as decreased expression of iron uptake and release genes [35]. Cytokines such as TNF α , IL-6, IL-1 β , and saturated fatty acids (SFAs), are important modulators of inflammation in obese AT (Figure 4). Exposure of primary peritoneal macrophages to SFAs induces inflammation as expected, while at the same time reducing expression of iron metabolism genes [35]. Other inflammatory molecules have not been studied extensively in the context of iron handling in ATMs; however, in one recent report, high-iron macrophages in the spinal cord were shown to be uniquely susceptible to M1 polarization by TNF α [67]. Thus, the inflammatory milieu of obese AT may explain why MFe^{hi} ATMs take on a more M1-like phenotype (Figure 4). The susceptibility of MFe^{hi} ATMs to inflammatory polarization relative to the other ATMs has not been explored, but may partially explain increased adipocyte dysfunction in iron-overloaded humans.

Obesity is associated with an increase in the cytokine IL-6. In fact, 30% of serum IL-6 is thought to come from visceral AT, and this is attributed to macrophages rather than adipocytes [68]. IL-6 directly induces the production of hepcidin [69]. Interestingly, in obesity, hepcidin can be secreted by visceral AT, and like IL-6, it is produced mainly from

macrophages [70]. Whether the hepcidin can be expressed in MFe^{hi} cells is not known. The production of hepcidin by ATMs indicates that they may be responsible for sensing and responding to changes in local iron concentrations, causing reduced iron cycling by macrophages and iron retention in adipocytes. Differences in IL-6 expression in obesity could also explain changes in hepcidin and iron content between tissues in obesity. For example, hepcidin does not increase in the liver in obesity, which may explain why increases in iron content are seen in AT more than in liver in obesity [35].

IL-4 is a potent T helper type 2 cytokine whose expression is reduced in AT in obesity. IL-4 has been shown to induce M2-like macrophage polarization; it also inhibits NO-induced IRP activation by reducing binding of IRP1 and IRP2 to RNA [71], thereby decreasing the LIP (Figure 4). Furthermore, IL-4 enhances production of *TfR1* mRNA through a non-IRP dependent pathway. Thus, IL-4 may serve as a node of control for both macrophage polarization and iron handling.

Heme oxygenase 1 and macrophage iron handling

Heme oxygenase 1 (Hmox1, gene *Hmox1*) is an enzyme that metabolizes heme into iron, bilirubin and CO. It is part of the cellular defense against oxidative stress and has largely been considered protective against cellular dysfunction. For example, peritoneal macrophages from Hmox1 knockout mice have increased ROS and the inflammatory cytokine MCP-1 [72]. However, the cellular role of Hmox1 may be much more context-dependent than previously appreciated. In a recent study, macrophage-specific *Hmox1* knockout mice on a high fat diet had decreased AT inflammation and a dramatic improvement in nearly every metabolic parameter compared to controls [73]. These findings not only contradict the dogmatic “anti-inflammatory” categorization of Hmox1, but also obfuscate the finding that *Hmox1* is one of the genes most highly up-regulated in MFe^{hi} ATMs, which are very polarized to the anti-inflammatory M2 phenotype. However, iron content of macrophages could be very important in determining the impact of Hmox1 on cellular function. For example, splenic macrophages are absent in *Hmox1* deficient mice and humans [74]. Splenic macrophages have high iron content due to persistent heme recycling, and their absence in *Hmox1* deficient mice indicates that Hmox1 may be required for the viability of macrophages with high heme or iron content. In the context of previous studies, it would be interesting to determine whether the increased *Hmox1* expression in MFe^{hi} ATMs is a by-product of their change in iron content, or if *Hmox1* is beneficial to MFe^{hi} ATMs in the context of high iron. Interestingly, *Hmox1* expression in MFe^{hi} ATMs is reduced in obese mice (Figure 3A and 4). Paralleling this, *Hmox1* in human M2-polarized macrophages is decreased by the inflammatory cytokine IFN γ and increased by IL-4 [75].

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Taken together, the published literature suggests a strong association between iron storage and metabolic regulation. Based upon available technologies and publications, the current view holds that serum Ft is an indication of systemic iron-overload. However, this may be an oversimplification, as the site of iron-overload cannot be determined simply from measuring serum Ft. It is clear that many different metabolic tissues, including pancreas, liver, and AT, may be relevant to the metabolic perturbations associated with iron-overload

[6]. In fact, new data suggests that iron metabolism may be regulated at the tissue level. This is especially apparent in AT, where iron levels must be sufficiently high to facilitate adipogenesis, while not so high that they induce IR. In addition, the gene expression profile of MFe^{hi} ATMs suggests M2 polarization and an iron recycling phenotype, a phenotype substantiated by *in vitro* studies published by other groups. Together, these data suggest a tight regulation of macrophage phenotype and iron handling, an observation that is important for understanding the role of macrophages in multiple different tissues. Key priorities for future studies will be to determine the role of resident macrophages in tissue iron homeostasis. With regards to AT, one of the major outstanding questions is whether MFe^{hi} ATMs contribute to the fine-tuned and localized control of AT iron concentrations, perhaps acting as “ferrostats”. Furthermore, it is not known whether MFe^{hi} cells and adipocytes can exchange iron. Finally, it is yet to be determined how MFe^{hi} cells become inflammatory in obesity and what effect this has on their function in AT. The concept that resident macrophages could contribute to tissue iron homeostasis may have broader implications beyond AT. For example, elevated sCD163 is related to several different diseases (BOX 1) and macrophage iron handling is known to be relevant in other tissue (BOX 2).

Acknowledgments

MJH was supported by a T32 GM07347 from the National Institute of General Medical Studies for the Vanderbilt Medical-Scientist Training Program, and is currently supported by an individual NIH NRSA (F30 DK103438). KRP is supported by a T32, Training in Pharmacological Sciences grant (T32 GM007628). AHH is supported by VA Merit award I01 BX002195, NIH grant DK095456 and an AHA Established Investigator Award (12EIA827). We would like to thank W. Reid Bolus for creating Figure 2.

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 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 by 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	<i>in vitro</i> polarization of macrophages with inflammatory mediators such as LPS and IFN γ results in M1 polarization and an inflammatory cytokine profile. <i>In vivo</i> , macrophages recruited to AT in obesity display evidence of M1-like polarization
M2-like (alternatively activated) macrophages	<i>in vitro</i> polarization of macrophages with T _H 2 cytokines such as IL-4 and IL-13 results in M2 polarization and an anti-inflammatory cytokine profile. <i>In vivo</i> , 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 $\cdot$. Free iron readily induces ROS production
Transferrin receptor 1 (TfR1)	endocytic recycling receptor that binds transferrin-bound iron

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Highlights

- Studies have linked elevated body iron stores to obesity and metabolic syndrome in humans.
- Iron levels in adipose tissue are important for adipogenesis and adipocyte function.
- Iron-enriched macrophages in adipose tissue have an iron-cycling profile, increased Hmox1 expression and their phenotype is altered in obesity.
- In obesity, adipocytes become iron-overloaded concomitant with reduced macrophage iron content.

Figure 1. Schematic of systemic and cellular iron handling

Dietary iron is taken up via DMT1 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-Fe³⁺. 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 three forms; bound by Ft, incorporated into proteins, or retained as part of the unbound LIP. 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. [Cp: ceruloplasmin; DMT-1: divalent metal transporter 1; Fpn1: ferroportin 1; Tf: transferrin; TfR1: transferrin receptor 1; Hmox1: Heme oxygenase 1; Ft: ferritin].

Figure 2. Alterations of macrophage and adipocyte iron handling in obese mice

In lean AT, resident all macrophages are M2-like (possibly because of IL-4 secretion by other immune cells) and are interstitially space. A subpopulation of macrophages has a 2-fold increase in iron concentration and is referred to as MFe^{hi}, while the remaining ATMs are referred to as MFe^{lo}. In obesity, newly recruited ATMs are pro-inflammatory, M1-like, and localized to crown like structures around dead adipocytes and contribute to an inflammatory milieu of elevated TNFα, IL-1β, and IL-6. In addition, the remaining MFe^{hi} ATMs sequester less iron, concomitant with adipocyte iron-overload. The MFe^{hi} macrophages also become less M2-like and have reduced expression of iron handling genes such as *Hmox1*. Adipocyte iron overload in other models has been shown to result in increased lipolysis and lipid peroxidation, decreased adiponectin and increased ROS production. Thus, changes in MFe^{hi} iron handling are hypothesized to impact AT homeostasis by increasing adipocyte iron concentrations. [IL-4: interleukin-4; ROS: reactive oxygen species; SFA: saturated fatty acid; TNFα: tumor necrosis factor α; IL-1β: interleukin 1β].

Figure 3. Inflammatory nature and iron content of macrophages

(A) Adipose tissue (AT) macrophages can be magnetically isolated based upon natural paramagnetic properties due to increased iron stores. MFe^{hi} macrophages have elevated iron concentrations compared to MFe^{lo} macrophages. In both lean and obese mice, MFe^{hi} macrophages have increased expression of M2-like anti-inflammatory genes and decreased expression of M1-like pro-inflammatory genes, as compared to MFe^{lo} macrophages from the same animals. It is known that obesity induces a pro-inflammatory phenotype in AT macrophages. In high fat diet-induced obesity, MFe^{hi} macrophages become more inflammatory compared to MFe^{hi} macrophages from lean mice, but do not exhibit the extent of M1-like inflammatory properties observed in MFe^{lo} macrophages from obese animals. The MFe^{lo} cells in obese mice consist of resident macrophages that are lower in iron, but also, the newly recruited M1-like macrophages. (B) *In vitro* polarized M1 and M2 macrophages have unique properties regarding iron handling. M1 macrophages have decreased iron uptake and export, and a smaller LIP while M2 macrophages have an iron recycling phenotype. [LIP: labile iron pool; *Ft*: gene for ferritin; *Hmox1*: gene for heme oxygenase 1; IRP: iron response protein]. The height along the y-axis in panels A and B is arbitrary.

Figure 4. Proposed mechanisms by which obesity could impact MFe^{hi} cells

In lean AT, MFe^{hi} ATMs are highly M2-polarized and express elevated levels of Hmox1 and thereby could breakdown heme into CO, bilirubin, and iron in an anti-inflammatory process. In addition, they are exposed to elevated levels of IL-4, which could increase TfR1 and NO. An increase in NO has been shown to increase IRP binding and therefore the labile iron pool in other settings. In obesity, the MFe^{hi} ATMs become more M1-like, likely due to the milieu of SFA and inflammatory cytokines such as TNF α and IL-1 β . A decrease in Hmox1 could result in increased ROS. In vitro studies with cultured macrophages have shown similar mechanisms to occur; however, these have yet to be tested in MFe^{hi} ATMs. [AT: adipose tissue; Hmox: heme oxygenase 1; TfR1: transferrin receptor; NO: nitric oxide; ROS: reactive oxygen species; SFA: saturated fatty acid; TNF α : tumor necrosis factor α ; IL-1 β : interleukin 1 β].