Obesity and iron deficiency in chronic kidney disease: the putative role of hepcidin

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Hepcidin is a 25-amino acid peptide with a defensin-like structure that is primarily synthesized in hepatocytes [1, 2]. It was independently isolated ~10 years ago by two groups seeking peptides with antimicrobial activity in urine [3] and plasma [4]. Hepcidin was originally shown to have a weak selective antimicrobial activity against certain bacteria; thus, its name reflected the site of major tissue expression (‘hep’ for hepatocyte) and its antimicrobial properties (‘cidin’) [1, 2]. However, soon after its isolation, independent studies suggested an important role of hepcidin in iron regulation [5, 6]; currently, a large body of evidence strongly supports the role of hepcidin as the ‘master regulator’ of iron homeostasis [2, 7].

Hepcidin reduces the efflux of recycled iron from both splenic and hepatic macrophages and also inhibits iron absorption from the gut [1, 2] (Figure 1). The cellular mechanisms of hepcidin action seem to be tissue specific. In reticuloendothelial macrophages, hepcidin was previously shown to bind to the cellular iron export channel ferroportin, inducing its internalization and subsequent degradation [8, 9]. Recent data suggest that in intestinal...
epithelial cells, hepcidin mainly acts by decreasing the protein levels of the apical divalent metal transporter 1, another iron channel, through proteasome internalization and degradation [10–13]. A number of in vitro studies suggest that hepatic hepcidin transcription is upregulated by inflammatory cytokines, bone morphogenetic proteins (BMPs) and iron, and is downregulated by iron deficiency, hypoxia and ineffective erythropoiesis [14, 15]. Thus, under physiological conditions, hepcidin acts by maintaining stable extracellular plasma iron concentrations and iron stores and also by providing adequate iron for erythropoiesis.

A role of hepcidin in innate immunity is consistent with its upregulation by inflammatory cytokines, such as interleukin (IL)-6, IL-1α and IL-1β, and acute inflammatory stimuli, such as lipopolysaccharide (LPS) [2, 16, 17]. However, this increase in hepcidin production during inflammation has important consequences for iron status. The inhibitory effect of hepcidin on iron release from macrophages, hepatocytes and enterocytes, seen from an evolutionary perspective, was clearly beneficial to the host by preventing bacteria from accessing tissue iron during an ‘acute’ infectious attack; however, it is also a major contributor to hypoferraemia and eventually anaemia of chronic inflammatory disease, where pro-inflammatory cytokines are chronically raised [1, 2, 18, 19]. Elevated hepcidin levels have been recorded in patients with end-stage renal disease undergoing haemodialysis [20, 21]. It is now believed that hepcidin elevation is largely due to the inflammatory milieu accompanying uraemia, and the resulting functional iron deficiency is another major contributor towards the anaemia associated with chronic kidney disease [7], compounding the problem of inadequate erythropoietin production.

An association between obesity and iron deficiency has been recognized for many years [22, 23]. Although several different factors have been previously proposed to explain this association, a sole major mechanism could not be established. However, in recent years, adipose tissue has been increasingly recognized as an active endocrine organ, secreting various hormones (i.e. leptin, resistin, adiponectin and visfatin) and cytokines [23, 24]. These substances have important systemic effects, e.g. by contributing towards the development of insulin resistance, low-grade inflammation, endothelial dysfunction and other disturbances that cluster within the context of the ‘metabolic syndrome’ and may mediate the relationship between obesity and atherosclerotic cardiovascular disease [25–27]. This recent view of obesity as a state of low-grade inflammation, along with the evolution of hepcidin as a key player in inflammation-induced functional iron deficiency and anaemia, has led to studies aiming to investigate a potential role of hepcidin in obesity-related hypoferraemia. This article summarizes the recent evidence from both in vitro data and human studies in this field, strongly supporting a causal role of inflammation and hepcidin elevation in the development of iron deficiency in obese individuals, which may exacerbate the anaemia associated with chronic kidney disease.

**Obesity and iron deficiency**

Obesity is one of the most critical public health problems worldwide since excess body fat is associated with increased all-cause mortality and increased risk of several morbidities (including type 2 diabetes, dyslipidaemia and hypertension), and obesity rates are increasing worldwide, particularly among children and adolescents [28, 29]. On the
other hand, dietary iron deficiency is the most common global mineral deficiency, affecting an estimated 1.5 billion people worldwide. In the USA alone, >6% of reproductive-aged women and 10% of children and adolescents were reported to be iron deficient [29, 30].

The first data suggesting an association between obesity and iron deficiency were published in the early 1960s [31, 32]. Subsequently, several population studies confirmed a relationship between excess body weight and low iron levels in children and adolescents [33, 34], adult men and women [35–37] and post-menopausal women [38]. It has also been shown that the increase in obesity observed in transition countries is associated with a worsening in iron status [39]. Of note, the association between body mass index (BMI) and ferritin levels, which is considered as the main indicator of iron stores in healthy individuals, does not follow the same pattern; several studies have shown that ferritin is elevated in obese subjects [40–42]. Although theoretically that could be due to iron excess in obese subjects, it is generally believed that this elevation is due to low-grade inflammation accompanying obesity rather than to iron overload or repletion since serum ferritin is also an acute-phase reactant, affected by several pro-inflammatory cytokines [23, 43].

A number of mechanisms that can explain the relationship between obesity and iron deficiency have previously been proposed [23]. A diet rich in carbohydrates and fat but low in essential nutrients has been long considered the most important cause for this relation [32] and reports suggested that overweight individuals had lower iron intakes [44]. However, other investigators failed to confirm this finding; a recent study did not show differences in intake of haeme and non-haeme iron or other dietary factors that can affect iron absorption between obese and non-obese people; however, fat mass per se remained a significant negative predictor of serum iron level [45]. An alternative explanation for the above relationship is that iron depletion could be a result of greater iron requirements of obese subjects due to their larger blood volume, as supported by animal data [46]. Furthermore, it has also been suggested that the causality of this association is reverse, i.e. that perinatal iron deficiency might lead to increased visceral adiposity, accelerated by sedentary lifestyle and lack of physical activity [47, 48].

A causal mechanism for the obesity–hypoferraemia association involving the upregulation of hepcidin has evolved only recently, after the conception of the visceral adipose tissue as an endocrine and paracrine organ, releasing, among other active peptides, pro-inflammatory cytokines such as IL-6 and tumour necrosis factor-alpha (TNF-α) [23]. As previously discussed, low-grade inflammation induced by such cytokines has been proposed to contribute to the development of insulin resistance and associated morbidities in obese individuals [25]. Anaemia of chronic inflammation is a condition characterized by hypoferraemia and high to normal serum ferritin levels, currently attributed to a large extent to inflammatory cytokines (especially IL-6), inducing increased production of hepcidin [18, 19]. To this end, obesity has been recently viewed as a chronic inflammatory state which could induce iron deficiency through the same pathway [37].

**The role of adipose tissue in hepcidin production: in vitro data**

Expression of hepcidin in hepatocytes (its main production site) has been previously shown to be downregulated by iron deficiency, hypoxia and ineffective erythropoiesis [14, 15]. On the other hand, iron overload, but also inflammatory cytokines such as IL-6 [2, 16, 17] and BMPs [49], can induce hepatic hepcidin production. Indeed, IL-6 seems to be the major mediator in increasing hepatic hepcidin expression in response to inflammation (Figure 2). Acute inflammatory stimuli such as LPS were shown to enhance hepcidin expression in cultured cell lines and in a murine model, mainly through the mediation of IL-6, as this increase in hepcidin was abolished in the presence of anti-IL-6 antibodies [2, 16, 17]. At the subcellular level, IL-6 stimulates hepcidin messenger RNA (mRNA) expression by inducing the binding of the signal transducer and activator of transcription (STAT) factor 3 to the hepcidin promoter [50].

Accumulating data also suggest that adipocytes also possess the molecular machinery to produce hepcidin, and this production is mainly regulated by inflammatory mediators (Figure 2). In a pivotal study, Bekri et al. [51] examined the expression of hepcidin in liver and adipose tissue cells from three groups of severely obese patients (with diabetes, with non-alcoholic steatohepatitis and without either of these conditions). They found that hepcidin was expressed both at the mRNA and the protein level not only in hepatocytes but also in adipocytes. In addition, the hepcidin mRNA expression in hepatocytes was not different between obese people and non-obese controls, but the adipose tissue expression was much higher in obese individuals; this increased expression was not affected by the presence of diabetes or non-alcoholic steatohepatitis. To further explore the association between hepcidin production and inflammation, the authors examined the expression of IL-6 and C-reactive protein (CRP) in the tissues studied. Of note, hepcidin mRNA levels were closely related to the expression of IL-6 and CRP in the adipocytes, but not in the hepatocytes. Moreover, incubation of adipose tissue cells with inflammatory stimuli such as LPS or IL-6 significantly increased hepcidin mRNA [51]. Taken together, these results showed for the first time that adipose tissue was another source of hepcidin production and that the induction of hepcidin expression by inflammatory stimuli was not restricted to hepatic tissue.

Interestingly, a feedback control mechanism of iron deficiency on hepcidin expression could be relevant only to hepatic and not to adipose tissue hepcidin production. In the aforementioned study of Bekri et al. [51], 17 of the 25 patients presented with low transferrin saturation (TSAT) (<25%) and 6 of them were anaemic. A significant correlation was found between the hepcidin mRNA expression in the liver and the serum TSAT levels (higher hepcidin mRNA levels in patients with higher TSAT), suggesting the existence of a negative feedback regulation system. In contrast, hepcidin mRNA expression in adipose tissue was similar in iron-replete and iron-deplete groups and there was no correlation seen between adipose hepcidin mRNA and TSAT. Thus, the iron status regulation of hepcidin...
expression may be tissue specific and, in obese individuals, hepcidin production from adipose tissue could be mainly driven by inflammation.

An association between inflammatory status and hepcidin production from adipose tissue was also suggested by a recent study from Vokurka et al. [52]. The authors aimed to investigate changes in adipose hepcidin production during an acute-phase reaction, by measuring hepcidin mRNA expression in isolated subcutaneous and epicardial adipose tissue collected from 12 subjects at the beginning and at the end of cardiac surgery (aortocoronary bypass or valvular surgery). Hepcidin mRNA expression was significantly increased at the end of the surgery in subcutaneous but not in epicardial adipocytes. With regard to changes in expression of other proteins involved in iron metabolism, transferrin receptor 1 mRNA expression increased and ferroportin mRNA expression decreased in both adipose tissue depots after surgery. In addition, the serum levels of IL-6 and TNF-α were markedly increased after surgery, whereas serum ferritin increased, serum iron and transferrin decreased and serum transferrin saturation showed a non-significant downward trend [52]. These data suggest that such acute inflammatory changes could increase hepcidin expression in subcutaneous adipose tissue and this could, in turn, lead to changes in serum biomarkers characteristic of functional iron deficiency.

Adipocyte hypoxia is also present in adipose tissue of obese individuals and was recently shown to influence hepcidin expression. Adipocyte hypoxia has been previously reported to produce marked differences in gene expression, not only in genes expected to be regulated by hypoxia (such as vascular endothelial growth factor and haeme oxygenase) but also in those leading to increased expression of the inflammatory cytokines, such as TNF-α, IL-1 and IL-6 [53]. An in vitro study [54] examined whether this adipocyte hypoxia could affect hepatocyte hepcidin production. To this end, adipocytes were cultured at either standard conditions (19% O₂) or hypoxic conditions (1% O₂); hypoxic cells had significantly higher IL-6 and leptin expression than control cells. Treatment of hepatic cells with media from hypoxic adipocytes significantly increased hepcidin promoter activity and hepcidin mRNA levels compared to cells treated with normoxic adipocyte media. Similarly, treatment of hepatic cells with media from adipocytes previously treated with LPS resulted in increased hepcidin mRNA levels [54]. Therefore, apart from producing hepcidin, hypoxic adipocytes in obese individuals may also increase hepatic hepcidin expression.

Leptin is a 16-kDa non-glycosylated peptide hormone, which is considered as the prototype of adipokines. It is mainly produced by adipocytes, and circulating leptin levels are directly correlated with white adipocyte mass; its physiological role is to decrease food intake and increase energy consumption through action on specific hypothalamic receptors [24]. Structurally, leptin belongs to the class I cytokine superfamily, sharing a number of common biological features with IL-6. For this reason, Chung et al. [55] examined whether leptin could affect hepatic production and release of hepcidin by exposing human hepatoma cells to leptin. Indeed, hepcidin mRNA expression was significantly elevated in leptin-treated cells, a response partially inhibited by pre-incubation with a janus kinase (JAK) 2 inhibitor. Furthermore, leptin also increased the hepcidin promoter activity, an effect blocked by mutations of the transcription factor STAT3-binding pathway [55]. These results indicate that leptin may also induce hepatic hepcidin production, via pathways similar to IL-6.
Haemojuvelin is a glycosylphosphatidylinositol-anchored membrane protein mainly expressed in the liver, skeletal muscle and heart cells, which acts as a co-receptor of BMP to enhance hepcidin expression in liver cells [56]. Although several aspects of the role of haemojuvelin in hepcidin regulation are still unclear, it currently seems that in conditions of iron deficiency or hypoxia, haemojuvelin is cleaved by furin (which is negatively regulated by iron concentration) at the C-terminus, producing soluble extracellular domain fragments [57, 58]. This soluble haemojuvelin could downregulate hepcidin expression by competing with hepatocyte membrane-bound haemojuvelin for BMP binding. Luciani et al. [59] recently showed that haemojuvelin mRNA and protein were expressed in adipose tissue; this haemojuvelin mRNA expression was highly increased in adipose tissue from obese patients and correlated with mRNA hepcidin expression levels. On the other hand, blood concentrations of soluble haemojuvelin were significantly increased in obese patients, in relation to normal controls [59]. With currently available data, a strict cause-and-effect relationship between the different forms of haemojuvelin and hepcidin (either expressed in tissue samples or measured in peripheral blood) is difficult to ascertain, and further studies in the field are warranted; however, haemojuvelin may play a regulatory role in hepcidin production, not only in hepatocytes but also in adipose tissue.

**Hepcidin elevation in obesity: human studies**

Recent human studies provide further support for a possible role of obesity in upregulating hepcidin levels and causing iron deficiency (Table 1). Del Giudice et al. [60] performed a study in Italian children (average age of 11 years) comparing 60 obese (69.2 ± 17.1 kg) and 50 children of normal weight (average 36.5 ± 15.7 kg). Obese children had significantly lower serum iron and transferrin saturation compared to controls, despite having marginally higher ferritin levels (37.8 ± 16 versus 31.4 ± 19 ng/mL, P = 0.06). The levels of leptin, IL-6 and hepcidin were also significantly higher in obese compared to normal weight children. Significant positive correlations between hepcidin and the degree of obesity, hepcidin and leptin levels, as well as inverse correlations between hepcidin and serum iron, and hepcidin and transferrin saturation were observed, suggesting a role for increased hepcidin production in the link between obesity and disrupted iron metabolism [60].

Another study compared iron status, dietary iron intake and bioavailability as well as circulating levels of hepcidin, leptin and IL-6 in 118 children between 6 and 14 years of age living in Switzerland [61]. Among these, 85 were overweight (53.0 ± 14.1 kg) and 33 of normal weight (34.3 ± 9.2 kg). There were no significant differences between the two groups in total and non-haeme dietary iron intake and total iron bioavailability, measured with appropriate equations; if anything, haeme iron was higher in overweight children following a higher consumption of meat. However, the levels of serum transferrin receptor and the

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of design/comparison</th>
<th>Number of subjects</th>
<th>Duration of follow-up</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies comparing obese and non-obese individuals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Del Giudice et al. [60]</td>
<td>Cross-sectional two-group comparison</td>
<td>110</td>
<td>—</td>
<td>Lower iron and TSAT; higher leptin, IL-6, and hepcidin levels in obese children</td>
</tr>
<tr>
<td>Aeberli et al. [61]</td>
<td>Cross-sectional two-group comparison</td>
<td>118</td>
<td>—</td>
<td>Higher sTfR, leptin, IL-6, CRP and hepcidin levels in obese children</td>
</tr>
<tr>
<td>Tussing-Humphreys et al. [62]</td>
<td>Case–control (matched for Hb levels)</td>
<td>40</td>
<td>—</td>
<td>Higher sTfR, CRP and hepcidin levels in obese subjects</td>
</tr>
<tr>
<td><strong>Studies on weight loss</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amato et al. [63]</td>
<td>Prospective cohort (single group)</td>
<td>15</td>
<td>6 months</td>
<td>Decreases in BMI, leptin and hepcidin levels; increases in serum iron and TSAT levels with weight loss</td>
</tr>
<tr>
<td>Tussing-Humphreys et al. [64]</td>
<td>Prospective cohort (single group)</td>
<td>20</td>
<td>6 months</td>
<td>Decreases in BMI, CRP, hepcidin and serum transferrin receptor levels; increase in Hb with weight loss</td>
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*aTfR, serum transferrin receptor.*
prevalence of iron-deficient erythropoiesis (suggested by an increased serum transferrin receptor concentration) were significantly higher in the overweight children. Furthermore, leptin, IL-6, CRP and hepcidin were all significantly higher in overweight children. The authors concluded that the reduced iron availability for erythropoiesis in overweight children is unlikely to be due to low dietary iron intake but rather due to reduced iron absorption and/or increased iron sequestration mediated by hepcidin [61].

Tussing-Humphreys et al. [62] also investigated the association between obesity and hepcidin in a case–control study of 20 obese (BMI 50.1 ± 11.0 kg/m²) pre-menopausal females and 20 non-obese (BMI 21.9 ± 4.9 kg/m²), free of inflammation controls matched for haemoglobin levels. Despite similar haemoglobin concentrations, being in the low-normal range in the two groups (12.2 ± 1.4 versus 12.1 ± 0.9 g/dL), and similar dietary iron intake, obese women had significantly higher serum transferrin receptor levels compared to controls. Iron and TSAT levels were lower and serum ferritin was higher in obese women; however, different differences did not reach statistical significance. In addition, obese subjects had 10-fold higher serum hepcidin levels, as well as higher CRP and erythropoietin levels and a trend towards higher IL-6 levels [62], findings also indicating a role for hepcidin-mediated inhibition of iron absorption and utilization.

**Human studies on the effect of weight loss on hepcidin levels**

Further support for the role of elevated hepcidin in obesity-related iron deficiency is provided from two recent pilot studies examining the effect of intentional weight loss on hepcidin levels (Table 1). The first study included 15 children aged between 9 and 16 years and a BMI exceeding the 95th percentile for age and sex according to reference values [63]. Subjects underwent a 6-month weight loss programme that included a self-selected diet of 60% of the recommended dietary energy allowances for age and sex, physical exercise and behavioural therapy. At the end of 6 months, a significant decrease in BMI from 29.9 ± 4.1 to 26.5 ± 4.5 was accompanied by significant decreases in leptin and hepcidin levels and a marginally significant reduction in IL-6. Furthermore, there was a significant improvement in iron absorption and significant increases in serum iron and TSAT levels with weight loss, whereas ferritin levels remained unchanged [63].

Another recent study reported on the iron status and hepcidin levels of 20 severely obese pre-menopausal females before and after 6 months of restrictive bariatric surgery [64]. At baseline, the prevalence of iron depletion, as evidenced by increased serum transferrin receptor concentration, included 45% of the population. At 6 months after surgery, there were significant reductions in weight (from 130.0 ± 23.5 to 106.7 ± 26.3 kg) and BMI (from 47.6 ± 7.9 to 39.5 ± 7.5 kg/m²) coupled with significant reductions in both CRP and serum hepcidin. With regard to iron status, a significant decrease in serum transferrin receptor concentration, along with non-significant increases in serum iron and TSAT and decrease in serum ferritin were observed. Of note, there was a significant and clinically meaningful increase in haemoglobin, from 12.1 ± 1.3 to 13.3 ± 1.2 [64]. In another report from the same group, the mechanism of the above changes in hepcidin and iron status was further investigated by studying the *ex vivo* stimulated production of several cytokines in whole-blood cultures of 17 obese women undergoing bariatric surgery and 19 controls [65]. Interestingly, *ex vivo*-stimulated production of IL-6 and TNF-α was normalized after surgery, but interferon-gamma (IFN-γ) was not. Clearly, additional studies are required to fully elucidate the role of various inflammatory substances on hepcidin regulation in obesity.

**Conclusions**

The discovery of hepcidin in recent years was a breakthrough in our understanding of iron regulation in physiological and pathophysiological conditions. Several lines of evidence point to inflammation-induced hepcidin production as the mainstay of functional iron deficiency and anaemia of chronic disease. This association could be of particular importance for individuals with chronic kidney disease; indeed, the inflammatory state accompanying uraemia seems to be also related to increased hepcidin production, hence providing another pathway for anaemia and an explanation for resistance to erythropoietin treatment in these patients. A recent expansion of *in vitro* and *in vivo* data suggests that adipose tissue is an additional site of hepcidin production, and the low-grade inflammation of obesity can also trigger hepcidin increase, whereas weight loss may improve both hepcidin levels and deranged iron balance. These data provide a novel and increasingly solid explanation for the repeatedly observed association between obesity and poor iron status. The fact that an increasingly large proportion of patients with kidney disease are obese, coupled with associated problems of anaemia, iron deficiency and resistance to erythropoietin agents, provides a strong rationale for further studies designed to elucidate the role of hepcidin in the link between obesity and iron deficiency in chronic kidney disease.

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