Pediatr Nephrol (2012) 27:33–40
DOI 10.1007/s00467-011-1832-y

EDUCATIONAL REVIEW

Hepcidin in anemia of chronic kidney disease: review for the pediatric nephrologist

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Received: 30 November 2010 / Revised: 16 February 2011 / Accepted: 17 February 2011 / Published online: 13 March 2011

Abstract Anemia coincident with hyporesponsiveness to erythropoiesis-stimulating agents is an ongoing and prevalent problem in children with chronic kidney disease (CKD). The recently identified iron-regulatory protein hepcidin appears likely to play a significant role in this problem. Hepcidin up-regulation in the setting of CKD, with subsequent increased serum levels, results in impaired iron absorption from the intestine and decreased iron release from body storage sites. Ultimately, in the setting of such elevated levels, a state of functional iron deficiency may develop and lead to anemia due to iron-restricted erythropoiesis. Elevated hepcidin levels are expected in the face of decreased glomerular filtration rate and inflammation. Based on current evidence, it seems likely that hepcidin represents a potentially modifiable mediator of anemia of CKD and is thus a potential target for future anemia therapy. Currently, increased removal via intensified dialysis and-/or blockade of the inflammatory pathway appear to be two viable generic strategies for reducing hepcidin levels. Goals of directly manipulating the hepcidin pathway should offer the pediatric clinician new options for treating the complex anemia associated with CKD.

Keywords Hepcidin · Anemia · Iron · Erythropoiesis-stimulating agent

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Introduction

Before treatment with erythropoietin alfa (EPO) became the standard of care for treating anemia in dialysis patients in the late 1980s, iron overload was of primary concern to practicing nephrologists [1]. Whereas most patients in the pre-EPO era received frequent packed red blood cell (RBC) transfusions and developed iron deposition, post-EPO, many of those same patients required supplemental iron to maintain the rapid pace of erythropoiesis seen while being treated with EPO [2]. Iron overload and organ damage caused by repeated transfusions was once the normal condition of an end-stage renal disease (ESRD) patient. This might cause some to question the justification for the current level of concern around the use and safety of iron agents given IV to treat anemia of chronic kidney disease (CKD). However, better understanding of the interactions of the iron and erythropoietic pathways and the risks and benefits of therapies targeting each will guide the clinician in safe and efficacious management of anemic CKD patients.

Most recently, hepcidin as an iron-regulatory protein has attracted attention in the nephrology community as a new, and potentially key, mediator of the chronic anemia pathway in CKD and ESRD populations. We briefly describe the key features of iron metabolism relevant to anemia of CKD. We then detail our understanding of the role and potential impact of hepcidin in anemia in general and as it relates to CKD and ESRD patients. We conclude the review with suggestions for clinical application of this molecule in pediatric anemia management in the CKD/ESRD population, with a brief overview of potential advances in understanding iron handling and manipulating iron metabolic pathways. Pediatric data, when available, are highlighted throughout the review.

Production of healthy red blood cells

RBCs, or erythrocytes, are produced in the bone marrow from a committed cell line, colony-forming unit – erythrocyte. The production and maturation of an RBC is a complex process involving many growth factors and differentiation inducers. In the renal failure population, erythropoietin, iron, vitamin B_{12}, folic acid, and perhaps Vitamin C are normally considered to play vital roles in the production of healthy mature RBCs. Carnitine, although not generally considered as a major component outside the setting of hemodialysis, may also contribute, as its lack increases RBC membrane fragility and leads to a shorter RBC lifespan. (A detailed review of erythropoiesis is beyond the scope of this paper, but the following two textbook chapters discuss the topic in relation to the normal physiology—Guyton and Hall, Textbook of Medical Physiology, 10th edn, Chap. 32, pp. 382–391—and that of the CKD patient—Greenbaum, in Comprehensive Pediatric Nephrology, by Geary and Schaefer. Chap. 49, 761–763) [3, 4].

Erythropoiesis-stimulating agent (ESA) hyporesponsiveness

Anemia coincident with hyporesponsiveness to erythropoiesis-stimulating agents (ESA) is a prevalent problem in children with CKD. More than 20% of children with stage 4 CKD can be shown to have this condition as defined by persistently low hemoglobin levels while on ESA treatment [5]. Current practice for managing ESA hyporesponsiveness in most centers is further escalation of ESA dose [6]. However, and despite the absence of direct evidence in the pediatric population, recent adult data in nondialysis CKD populations [Correction of Hemoglobin and Outcomes in Renal Insufficiency trial and Trial to Reduce Cardiovascular Events with Aranesp Therapy (CHOIR and TREAT)] caused concern among pediatric nephrologists as to the advisability of this strategy. In these two adult trials, the ongoing escalation of ESA dose in patients who were persistently anemic appeared to increase the risk of adverse cardiovascular events, stroke, and death [7, 8].

Although beyond the scope of this review, a number of studies in children demonstrated the value of iron supplementation in relation to ESA hyporesponsiveness. Gillepsie and Wolf, in their 2004 meta-analysis of four such trials, demonstrated that in terms of increases in hemoglobin, hematocrit, ferritin, and transferrin saturation (TSAT) values, and decrease in ESA requirements, there was a positive correlation with supplemental IV iron therapy with an effect size that varied from 0.62 [95% confidence interval (CI) 0.11–1.13) to 1.86 (95% CI 1.58–2.15) [9]. Further individual pediatric studies reporting similar findings are reviewed in detail in the 2006 Kidney Disease Outcomes Quality Initiative (KDOQI) Anemia Guidelines, pp. S101–102 [10]. Discussions around other less common causes of ESA hyporesponsiveness in both adults and children, e.g., hyperparathyroidism, bacteremia, pure red-cell aplasia, aluminum toxicity, etc., can likewise be found in the 2006 KDOQI Anemia Guidelines [10].

Iron-restricted erythropoiesis

Any condition that decreases iron availability for incorporation into RBC may lead to the condition of iron-restricted erythropoiesis. Absolute iron deficiency, due either to insufficient iron intake or chronic blood loss, is the most common cause of this condition and is well reviewed in a paper by Handelman and Levin [11]. Iron sequestration syndromes, including anemia of chronic disease or inflammation, on the other hand, result when sufficient iron is present in the body but not available to the marrow for effective or efficient erythropoiesis [12]. Anemia of inflammation, a complex topic well beyond the scope of this paper, has been well described in numerous chronic medical conditions, including rheumatologic disease, cancer, chronic renal failure, and possibly heart disease [13–15]. Finally, a "functional iron deficiency" may be defined as the condition in which the demand for iron by an ESA-stimulated bone marrow outpaces the available iron supply [12].

Iron metabolism

Iron storage and transport

Healthy RBC production in the bone marrow requires a reliable supply of iron. However, due to the potential free-radical generation and resulting cell damage associated with excess free iron, serum levels of this metal must be precisely controlled [16]. Sophisticated physiologic mechanisms for regulating serum and cellular iron stores have allowed humans to maintain iron in a soluble state suitable for circulation within the blood and for transfer into and out of cells [16]. Iron homeostasis in humans is regulated by two main mechanisms: iron absorption from the intestine, and the recycling of iron from senescent RBCs. The senescent cells are taken up by reticuloendothelial macrophages where their iron is recycled and stored in the form of ferritin, a cellular iron storage protein. This iron is ultimately released back into the circulation via ferroportin channels in cell membranes on an as needed basis and becomes available for erythropoiesis. Hepatocytes also serve as iron-storing cells [17, 18]. Transferrin, a plasma iron transport protein, binds tightly but reversibly to the iron molecule and transports it safely to active sites of

erythropoiesis in the bone marrow. When a transferrin protein loaded with iron encounters a transferrin receptor on the surface of an erythroid precursor cell in the bone marrow, it binds to and is transported into the cell, at which time it releases its iron [19]. Figure 1 depicts the process of iron cycling.

Assessment of iron status

Evaluating iron sufficiency for erythropoiesis is complex in patients with CKD. First, one must document the presence of sufficient iron stores. Then availability of those stores to the bone marrow compartment for incorporation into mature RBC must be assessed. In clinical practice, serum ferritin is considered a reflection of total body iron stores, and TSAT is an indicator of iron available for transport to the bone marrow. Normally, about 33% of transferrin is saturated with iron, but this may be reduced when iron supply to plasma from other absorptive and storage sites, such as the gut and macrophages, is reduced [16]. Serum ferritin is difficult to interpret as an independent marker of iron status in any setting of inflammation, such as CKD or ESRD, as it is an acute-phase reactant, the production of which is upregulated in inflammatory states. Whereas it is often presumed that serum ferritin concentration reflects a steady-state leakage of intracellular stored ferritin, serum levels may not accurately reflect total body stored iron [17], and the process by which intracellular ferritin enters the circulation, from which it is directly measured, is not well understood.

A number of other nontraditional markers of iron status in CKD have been promoted as more sensitive markers of functional iron deficiency. These include reticulocyte hemoglobin content (CHr), which measures the immediate incorporation of iron into reticulocytes; and percentage of hypochromic red cells (PHRC), which is defined as RBC with cellular hemoglobin concentration <28 g/dl. Both tests provide better estimates of iron availability for erythropoiesis in patients with elevated ferritin levels [17, 20]. However, clear cutoff values in these populations are unclear, and as few centers have the required equipment, neither test has had wide-spread adoption in the clinical setting.

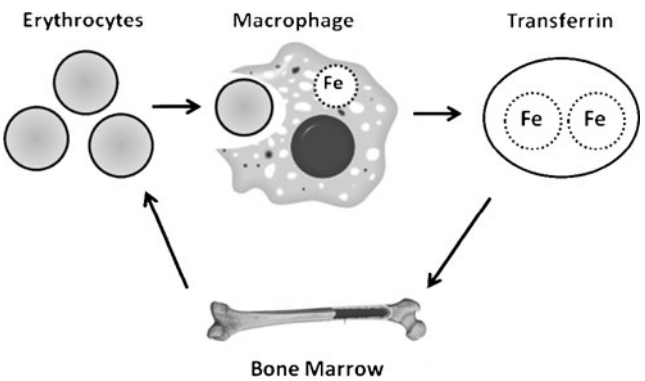

Fig. 1 The process of iron cycling from senescent erythrocytes to the bone marrow *Fe* iron

In otherwise healthy children and in the setting of absolute iron deficiency, serum ferritin values are <10 mg/ml and TSAT <10%. However, iron deficiency is defined differently by the pediatric KDOQI guidelines for children with CKD/ESRD who are on ESAs. Here, iron-replete or sufficient for effective erythropoiesis is defined as a serum ferritin>100 ng/ml and TSAT>20% [21]. Despite these robust iron targets, which are often exceeded in day-to-day practice, such children often have persistent anemia. In the setting of such anemia, despite serum ferritin and/or TSAT at or well above the recommended targets, one can presume the presence of a functional iron deficiency. In this scenario, despite iron sufficiency on a whole-body level, the iron available for erythropoiesis is suboptimal, and an iron-restricted anemia results.

Risks of excess iron

In CKD/ESRD patients chronically treated with iron supplementation, iron overload is a significant concern, with nephrologists often hesitating to administer additional iron to patients with serum ferritin levels above 500–800 ng/ml. Indeed, iron overload states are associated with multiple adverse clinical effects, including liver dysfunction, heart disease, and skin changes [22, 23]. Additionally, chronic intravenous administration of iron likely causes increased oxidative stress in CKD patients [22]. Thus, escalation of iron therapy, similar to that of ESA dose, in an attempt to overcome ESA hyporesponsiveness, has practical limitations.

Hepcidin

Hepcidin, an acute-phase protein produced in the liver, performs the function of a negative regulator of iron utilization and is crucial in the handling of iron availability for erythropoiesis [18]. Accumulating research suggests that in the setting of CKD, an excess of hepcidin results in impairment of both iron absorption from the intestine and release from body storage sites—a state of functional iron deficiency. In the setting of this form of iron-restricted erythropoiesis, even adequate to elevated serum ferritin levels and treatment with exogenous ESAs may not result in adequate correction of anemia.

Structure and function

The 84-amino-acid hepcidin precursor, preprohepcidin, is produced by hepatocytes and is encoded by the human

hepcidin gene (*HAMP*; OMIM 606464). Subsequent enzymatic cleavage of this molecule results in the production of the bioactive, 25-amino-acid hepcidin. This hepcidin has a physiologic role in preventing iron overload, and humans with mutations in the hepcidin gene develop clinical symptoms associated with iron overload, e.g., hemochromatosis [13, 18].

Regulation

Multiple inducers and suppressors of *HAMP* gene transcription, and thus hepcidin production, have been identified. Production is induced by inflammation, iron loading, and/or an iron replete state and is suppressed in the setting of anemia and in response to hypoxia. This induction of hepcidin by inflammation is thought to be a protective host-defense mechanism that limits iron availability during acute bacterial infection [13, 18, 24, 25]. In the case of iron loading, increasing hepcidin expression serves as defense against excess iron within the blood stream. Conversely, suppression of hepcidin expression during anemic or hypoxic states serves to increase the amount of iron released from cellular or tissue storage in the form of ferritin and makes it more readily available for erythropoiesis in the marrow. Erythropoietic activity itself, including increased levels of endogenous or exogenous erythropoietin, also appear to exert suppressive effects on *HAMP* gene transcription and levels of hepcidin [13, 24–26].

Mechanism of action

Hepcidin controls blood iron levels by down-regulating the absorption of iron via intestinal enterocytes and by inhibiting the release of iron from iron-storing reticuloendothelial cells. The mechanism for this is through a hepcidin-induced down-regulation of ferroportin, the major transmembrane iron transporter present on the surface of iron-storing cells and enterocytes [13, 16, 27]. Hepcidin binds directly to these ferroportin channels, inducing their internalization and degradation and effectively trapping iron within macrophages, hepatocytes, and enterocytes [13, 28]. This also contributes to reduced intestinal iron absorption (see Fig. 2) [16, 28].

The end result of the reduction in these channels is that iron absorption is decreased, and any current stores are sequestered outside the bone marrow and thus unavailable for erythropoiesis.

Laboratory measurement

Many early studies in humans measured the nonbiologically active 64-amino-acid hepcidin prohormone, prohepcidin, due to the early availability of a commercial prohepcidin immunoassay. However, it has now become apparent that prohepcidin levels do not correlate with levels of the active form of the protein or with urine hepcidin levels, and measurement of human prohepcidin is no longer considered clinically useful [27, 29]. In addition, whereas some studies have performed hepcidin assays using mass spectroscopic techniques, most of these assays are semiquantitative and require the use of equipment that is not widely available [29]. The bioactive form of hepcidin (25-hepcidin) can now be measured in human serum by a reliable and commercially available enzyme-linked immunosorbent assay (ELISA) developed by Ganz et al. in 2008 [29].

Normal ranges of hepcidin levels in healthy individuals have been explored and reported. In an adult series of 114 healthy volunteers, the 5–95% range of hepcidin concentrations was 29–254 ng/ml in men (median 112 ng/ml) and 17–286 ng/ml in women (median 65 ng/ml) [29]. This variation in hepcidin levels by sex has been noted in other studies of healthy individuals and in some cases has been explained by variation in ferritin levels by sex [26]. In another small series of healthy controls (20 children and 24 adults), median serum hepcidin levels have been reported as 25.3 ng/ml in children and 72.9 ng/ml in adults [25]. In healthy individuals, there does appear to be some diurnal variation in serum hepcidin levels, with peak levels noted in the afternoon [26, 30]. However, this diurnal pattern appears to be blunted in patients with CKD and on dialysis, possibly due to decreased renal clearance of the protein in the setting of decreased glomerular filtration rate (GFR) [26].

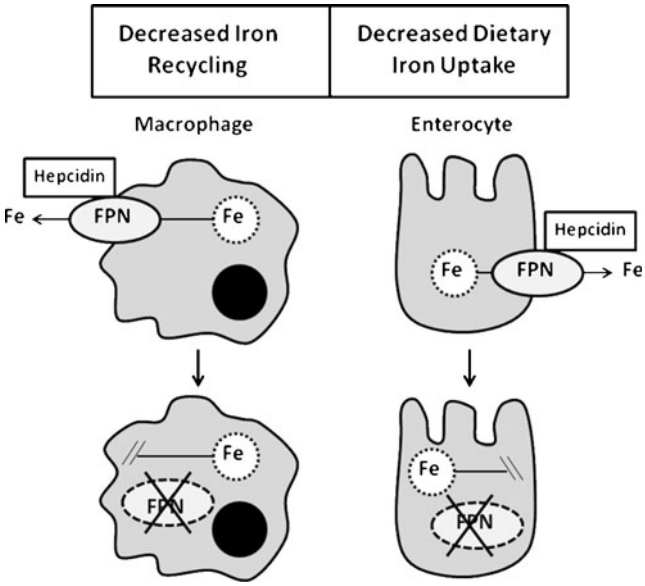

Fig. 2 Mechanism of action of hepcidin via direct binding to, internalization, and degradation of ferroportin, *Fe* iron, *FPN* ferroportin channel

Hepcidin in chronic kidney disease

In the setting of CKD, increased circulating levels of hepcidin mediate a state of functional iron deficiency, resulting in insufficient iron being available for erythropoiesis despite adequate or even increased body iron stores as reflected by serum ferritin levels. Higher hepcidin levels likely play a significant role in anemia and ESA resistance in CKD patients. This has been demonstrated in a mouse model of hepcidin overexpression in which typical features of the anemia of inflammation, including decreased bone marrow response to erythropoietin and iron accumulation within macrophages, was observed [31]. Multiple mechanisms by which serum hepcidin levels may be elevated in the CKD and ESRD populations have been proposed. First, hepcidin is freely filtered at the glomerulus, excreted by the kidney, and detectable in the urine; its serum concentration is known to increase in the setting of decreased excretion in patients with decreased GFR [16]. Ashby et al. noted an inverse correlation between serum hepcidin and estimated GFR (eGFR) in adults with mild to moderate CKD [26], and this correlation has been borne out in other studies [32]. Supporting this role of decreased renal elimination as a potential mechanism for increased hepcidin levels, residual renal function (as measured by urine output) has been associated with lower serum hepcidin levels in hemodialyzed and peritoneally dialyzed patients compared with their anuric counterparts [33].

In addition, as an acute-phase reactant, hepcidin production is directly induced by inflammation, particularly by the proinflammatory cytokine interleukin-6 (IL-6) [18, 34]. When infused into healthy volunteers, IL-6 causes an abrupt decrease in serum iron levels and increase in urinary hepcidin concentration within hours [35]. Dialysis patients demonstrate elevated plasma levels of proinflammatory cytokines [36] due, perhaps, to increased incidence of infections, the uremic milieu, and, in adults, the presence of comorbidities such as atherosclerosis. Decreased renal function in the CKD and ESRD populations may intensify these inflammatory responses due to decreased clearance and hence prolonged half-life of these molecules [37, 38].

In children specifically, studies have confirmed the finding of elevated proinflammatory cytokines [IL-6 and tumor necrosis factor alpha (TNF-α)] among dialysis patients compared with age-matched controls [39, 40]. It should also be noted that increased levels of inflammatory markers have been detected in both adults and children at earlier stages of CKD, well before the initiation of renal replacement therapy [40–42]. IL-6 levels in particular are elevated among adults with CKD stages 3–5 when compared with healthy individuals [43] and in adult hemodialysis patients appear predictive of ESA hyporesponsiveness [44].

Finally, iron loading or supplementation, commonly used in the CKD and ESRD population, has also been shown to induce hepcidin gene expression [18, 45]. Consistent positive correlations between hepcidin and serum ferritin levels have been demonstrated in adult and pediatric CKD patients as well as in healthy controls [18, 25, 27, 34, 46].

Hepcidin in pediatrics

Zaritsky et al. performed studies of hepcidin levels in children and adults with CKD. In a study of 48 children with stages 2–4 CKD, 26 children on dialysis, and 32 adults with stages 2–4 CKD, serum hepcidin levels were compared with those of healthy pediatric and adult controls. The highest hepcidin levels were noted in children on dialysis (median 652.4 ng/ml), followed by adults with stages 2–4 CKD (median 269.9 ng/ml) and children with stages 2–4 CKD (median 72.9 ng/ml) [25]. Hepcidin levels among children with both ESRD and earlier-stage CKD were significantly higher than in healthy pediatric controls. Among the pediatric CKD patients, in multivariate modeling, hepcidin was positively correlated with ferritin and high-sensitivity C-reactive protein [25].

Clinical implications

As the role of hepcidin in anemia of CKD has been illuminated in the literature, nephrologists have been hopeful that it could be used to more accurately depict/predict iron status of CKD patients than ferritin and hemoglobin values alone. In particular, there has been speculation that measuring hepcidin might serve to identify which patients will benefit from intravenous iron therapy despite apparent sufficiency of body stores. As discussed above, interpreting elevated serum ferritin levels in CKD is challenging, and the most common markers of iron status used in current clinical practice, ferritin and TSAT, do not accurately predict response to either ESA or iron treatment. In fact, elevated serum ferritin levels in patients with CKD may lead nephrologists to discontinue supplemental iron therapy out of concern for iron overload, but in the presence of elevated hepcidin, these ferritin levels are unlikely to represent the iron available for erythropoiesis but, rather, would be a marker for functional iron deficiency on the basis of a reticuloendothelial blockade.

To address this subject, a recent study performed by Tessitore et al. in 56 adult hemodialysis patients receiving

maintenance ESA therapy examined whether serum hepcidin (measured by mass spectrometer) was useful as a predictor of hemoglobin response to intravenous iron infusion compared with conventional markers of iron status (ferritin and TSAT). They found that neither hepcidin, ferritin, nor TSAT values were predictive of response to iron therapy; the only significant predictor identified was percentage of hypochromic RBC at a threshold of >6% [46]. This result suggests that, although a consistent correlation between hepcidin and ferritin is seen in CKD patients, its utility as a predictor of responsiveness to ESA escalation is limited at this time. More studies are needed in this area to further define the diagnostic utility of serum hepcidin levels, particularly in children.

Despite the absence of methods targeted to reduce the upregulation of hepcidin directly, there is emerging evidence that dialysis decreases interdialytic hepcidin levels [47]. A recent study in adult and pediatric hemodialysis patients demonstrated a nearly 50% decrease in hepcidin levels during a standard-length dialysis treatment [48]. Taken together, these results suggest that hemodialysis patients in whom ESA hyporesponsiveness/ iron-restricted erythropoiesis is a chronic problem might benefit from more frequent and longer duration hemodialysis sessions to lower serum hepcidin levels. Similarly, although limited data exists, hepcidin would appear to be removed by peritoneal dialysis [33].

Finally, a recent study demonstrated that ESA therapy itself is associated with decreasing levels of hepcidin in ESRD patients [26]. This suggests that perhaps ESA therapy should be initiated earlier in the face of anemia of CKD, even when reduction of GFR might not be sufficient to produce significant reduction in erythropoietin levels.

Future considerations

Hepcidin is likely a modifiable mediator of the anemia of CKD and a potential (and attractive) target for future therapies in this population. Although there are no available hepcidin antagonists for use in humans, in a mouse model, the neutralization of hepcidin by a monoclonal antibody has been shown to restore responsiveness to erythropoietin [30, 49]. Similarly, an anti-IL-6 antibody has been demonstrated to decrease hepcidin production and improve anemia in a non-CKD setting, suggesting that anticytokine therapies in general might be useful in decreasing hepcidin production [30, 50]. Given that hepcidin is in large part eliminated by renal clearance, potential interventions to significantly decrease hepcidin levels in patients with progressive CKD will likely need to be directed toward decreasing hepcidin gene transcription and production.

Summary

Elevated serum hepcidin levels have emerged as a key mediator of the anemia of CKD. It appears hepcidin acts by decreasing iron absorption from the gut and by decreasing the number of ferroportin channels on the surface of iron-storing cells, leading to a form of iron-restricted erythropoiesis. Similarly, as a result of this inability to mobilize iron stores for RBC production, hepcidin seems likely to play a key role in the development of ESA resistance as related to iron availability.

Although hepcidin production can be directly upregulated by proinflammatory cytokines, and C-reactive protein and hepcidin have been shown to correlate in several inflammatory non-CKD disease states, associations between hepcidin and IL-6/ C-reactive protein have not been consistent in human studies of CKD [18, 26, 30, 46]. Among some CKD patients, it may be that decreased GFR plays a relatively more significant role in decreasing hepcidin clearance than inflammation does in inducing its production. A number of exposures have been identified that may modify hepcidin levels and/or production in patients with CKD, including iron and ESA therapy, dialysis, and potentially direct hepcidin antagonists. Thus far, the diagnostic utility of hepcidin as a predictor of response to ESA or iron therapies has not been well established.

Finally, while ESA therapies will likely always be required for effective management of the majority of anemic CKD patients, our improved understanding of the role of hepcidin should allow for more intelligent, effective, and safe use of iron therapy in concert with this. Hepcidin reduction could also serve to improve dietary iron absorption and decrease the requirement for intravenous iron administration. Future goals of directly manipulating the hepcidin pathway seem promising and should offer the adult and pediatric clinician new options for treating the complex anemia associated with the CKD and ESRD populations.

Acknowledgment The authors thank Dr. Cindy Roy for valuable assistance in designing the figures.

Questions: (answers will appear following the reference list)

1. Elevated serum ferritin levels can be observed in the setting of:
   a. Total body iron overload
   b. Acute inflammation
   c. Reticuloendothelial blockade
   d. All of the above

2. Hepcidin gene transcription is down-regulated by:
   a. Inflammatory conditions
   b. Enteral iron ingestion
   c. Tissue hypoxia
   d. Intravenous iron administration
3. Ferroportin channels are found in the cell membranes of which cell types?
   a. Macrophages and enterocytes
   b. Macrophages and RBC
   c. Enterocytes and RBC
   d. Lymphocytes and macrophages
4. Probable mechanisms for increased hepcidin levels in CKD include:
   a. Decreased renal clearance
   b. Induction by inflammatory cytokines
   c. Chronic iron therapy
   d. All of the above
5. Serum hepcidin levels have been shown to be modified by:
   a. Nonsteroidal anti-inflammatory drugs
   b. Dialysis
   c. Hemapheresis
   d. ACE inhibitors

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Answers:

1. d. All of the above
2. c. Tissue hypoxia
3. a. Macrophages and enterocytes
4. d. All of the above
5. b. Dialysis