Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 31 (3-4)
Sofia ● 2024

Review Articles

Renal Iron Metabolism and Its Role in the Kidney

Yavor Tabakov, Yordanka Gluhcheva*

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Corresponding author e-mail: yavor.tabakov@iempam.bas.bg

For a long time iron transport and utilization in the kidney have been underestimated. The aim of the present review is to discuss the available research data about kidney iron and its role in some renal pathologies resulting from disturbed iron metabolism. The complex dynamics between systemic and local cellular iron regulation reveal kidney's key role in iron homeostasis. This has been shown by the presence of several major iron transports in the nephrons and by the fact that the kidney synthesises erythropoietin (EPO), master regulator of erythropoiesis. Furthermore, renal pathologies have a wide-reaching negative effect on iron homeostasis and like any of the canonical organs involved in iron metabolism, the kidney is very susceptible to iron disorders. Iron metabolism has become a focus for novel therapeutic strategies for several renal pathological conditions.

Key words: iron metabolism, regulation, kidney, kidney pathologies

Introduction

Iron (Fe) is the fourth most abundant element on Earth; therefore, it is not surprising that almost all organisms have evolved to include this unique element and its properties in various cellular process. Iron primarily exists in either ferrous (Fe^{2+}) or ferric (Fe^{3+}) oxidation state in biological systems. In a healthy, non-malnourished human there are approximately 3-5 g of iron; 60-75% of which is bound to haem, forming haemoglobin and around 10% is incorporated in myoglobin. The remaining amount forms complexes with various other enzymes and proteins. Most of the inorganic iron (non-haem) is stored as ferritin (Ft) or haemosiderin in macrophages and hepatocytes and only a very small amount is bound to the circulating serum protein transferrin (Tf). Approximately

1-2 mg of iron is lost daily through sweat, blood loss, sloughing of intestinal epithelial cells, and desquamation. To compensate, the body absorbs an equal amount in the same time period. Additionally, iron must be recycled and tightly regulated within the system to support haemoglobin synthesis and other metabolic processes. In summary, iron is an essential trace element involved in oxidation-reduction reactions, oxygen transport and storage, and energy metabolism. In excess it can have a pathological effect on cells, since it is involved in reactive oxygen species (ROS) production and pathogenic microbes require it for survival [7].

The kidneys are paired bean-shaped organs, located retroperitoneally in the posterior abdomen at the level of the 12th thoracic rib. The main function of the kidneys is to maintain homeostasis by reabsorbing essential nutrients and elements from the blood, whilst excreting metabolic waste and xenobiotics via urine. For a long time, researchers overlooked the kidneys as a major contributor to systemic iron homeostasis. This was due to the widely accepted fact that any "free" iron in the blood is bound to transferrin and that the complex is too big to be filtered by the glomerulus. Nevertheless, several articles published in the past few years have brought up the presence of various metal transporters in the kidneys and have established their involvement in systemic iron homeostasis [21, 24].

The aim of the present review is to discuss the research data regarding iron homeostasis in the kidney and some renal pathologies resulting from disturbed iron metabolism.

Iron Metabolism

Inorganic dietary iron is absorbed at the brush border of the duodenal enterocytes via divalent metal transporter 1 (DMT1). Dietary iron is usually in the oxidized ferric state (Fe^{3+}), and it must be reduced before entry into the cell. This is done by the membrane-associated ferrireductase – duodenal cytochrome b (Dcytb) [26]. Haem iron is absorbed through a different manner that so far remains unconfirmed, but animal models have identified haem carrier protein 1 (HCP1) and/or haem transporter HRG1 (SLC48A1) as potential transporters. Haem iron is released intracellularly by the inducible haemoxygenase 1 (HOX1/HO-1) [11, 22]. Intracellular iron is used for multiple processes and most of it is transferred to the mitochondria for haem and Fe-S clusters production by mitoferrin [19]. Haem is indispensable for haemoglobin, cytochromes, and enzyme activity. Fe-S clusters are essential to proteins involved in genome maintenance, energy conversion, iron regulation and protein translation [3, 6]. Excess intracellular iron is stored in the storage protein ferritin; this is done to maintain the labile iron pool (LIP) in specific limits and to avoid toxicity. Ferritin oxidizes and sequesters excess ferrous iron into a ferrihydrite mineral core. Iron sequestered in the Ft of enterocytes is lost after a few days through the sloughing of intestinal epithelial cells [1, 26]. Dietary cytosolic iron is exported into the plasma by ferroportin (Fpn). Exported Fe^{2+} iron is oxidized to Fe^{3+} by the ferroxidase hephaestin (Heph) and ceruloplasmin (Cp) **(Fig. 1)**. In the plasma, Fe^{3+} circulates bound to Tf, a glycoprotein that has two binding sites for ferric iron and maintains iron in a soluble form. Transferrin-bound iron (TBI) can be then recognised by TFR1 and delivered to cells via an endosomal cycle **(Fig. 2)** [26].

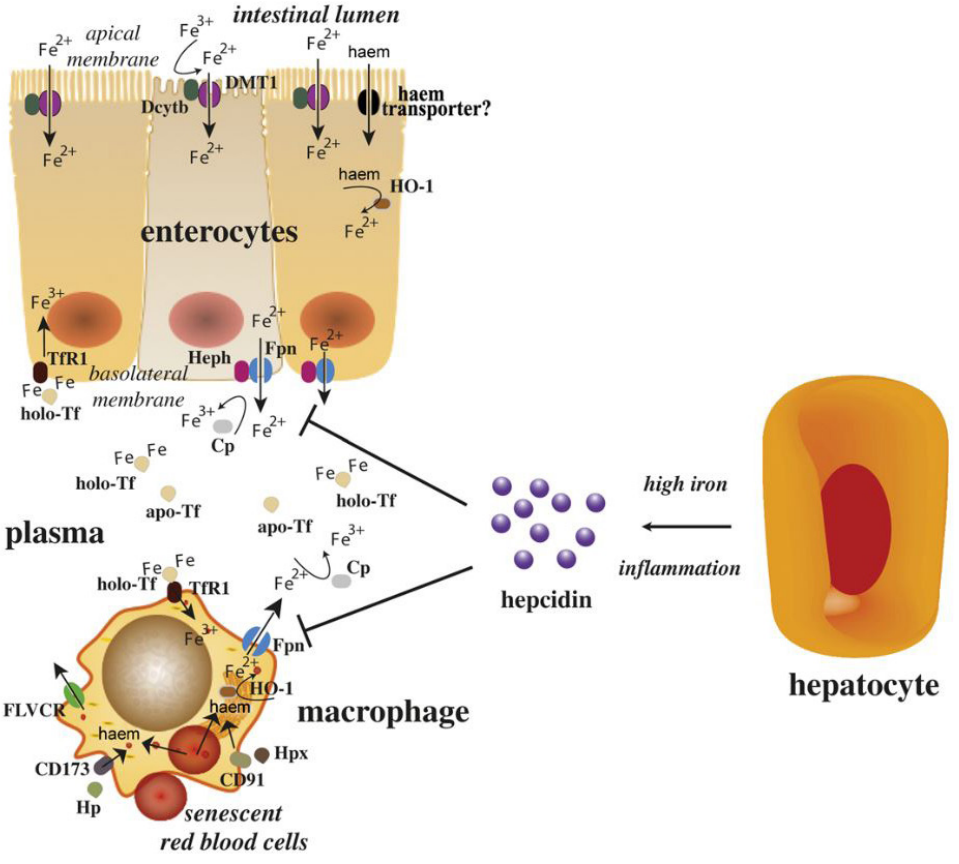

Fig. 1. Iron metabolism and systemic control by hepcidin. Enterocytes absorb inorganic or haem iron from the diet and macrophages phagocytose iron-loaded senescent red blood cells. Both types of cells release Fe^{2+} into the plasma via ferroportin (Fpn), which is incorporated into apo-Tf following oxidation to Fe^{3+} via hephaestin (Heph) or ceruloplasmin (Cp). Hepatocytes generate hepcidin in response to high iron or inflammatory signals, which inhibits the efflux of iron via Fpn and promotes its retention within enterocytes and macrophages [26].

Regulation of Iron Metabolism

Iron metabolism is tightly controlled on two levels, cellular and systemic. The cellular control of iron is done by the iron responsive element (IRE) – iron regulatory protein (IRP) system. The system exerts a post-transcriptional control on proteins associated with storage and transport. In an iron-deficient state, IRP1/2 stabilize TFR1 mRNA, which leads to increase in iron uptake. Additionally, iron storage and export are blocked due to ferritin and ferroportin translation being suppressed. When iron levels are repleted, Fe-S clusters convert IRP1 to cytosolic aconitase and IRP2 undergoes proteasomal degradation **(Fig. 2)** [4].

The systemic control of iron is regulated by the hormone hepcidin, which is produced by the liver. After secretion into the blood stream, hepcidin binds to its target – Fpn, and leads to its internalization and subsequent lysosomal degradation. Fpn degradation prevents the uptake of iron by the gut and prevents macrophages

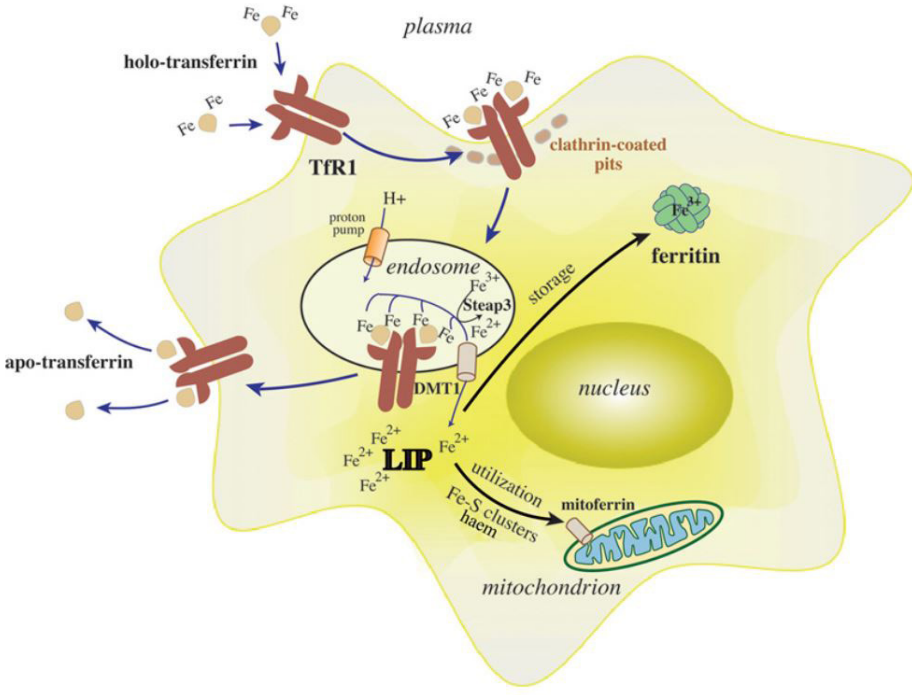

Fig. 2. Cellular iron uptake via the Tf cycle. Iron-loaded holo-Tf binds to TfR1 and is endocytosed via clathrin-coated pits. A proton pump acidifies the endosome, releasing Fe^{3+}, which is then reduced to Fe^{2+} by Steap3 and transported to the cytosol by DMT1. Fe^{2+} is directed to mitochondria via mitoferrin for metabolic utilization and excess iron is stored in ferritin. A cytosolic fraction of Fe^{2+} constitutes the LIP. The apo-Tf–TfR1 complex is recycled to the cell surface, where apo-Tf is released to capture plasma Fe^{3+} [26].

from releasing their iron stores. Circulating and tissue iron are positive regulators of hepcidin production. Endothelial cells in the liver sense an iron increase and produce bone morphogenic proteins 6 and 2 (BMP6/2), which induce hepcidin production by the hepatocytes. BMP6 production is dependent on iron concentrations, and it is believed that is the dominant ligand in high iron conditions. Whereas BMP2 is less iron sensitive and has higher concentrations in normal conditions, leading to the conclusion that it is the main regulator in normal physiology. Hepcidin levels can also be upregulated by inflammatory cytokines, interleukin 6 (IL-6) and macrophages. This crosstalk can potentiate iron sequestration during inflammation. Potent inhibitors of hepcidin production, which leads to increase in plasma iron, are iron deficiency, erythropoiesis, anaemia, and hypoxia **(Fig. 1)** [2, 4, 15].

Renal Function and Iron Metabolism

Nephrons are the functional units of the kidney, they are epithelial tubular structures, with specific interconnected morphological regions. Each region possesses specific functional properties that ensure the primary function of the kidneys. The regions are glomerulus, proximal tubule, loop of Henle, distal tubule, connecting tubule and finally collecting duct **(Fig. 3)**.

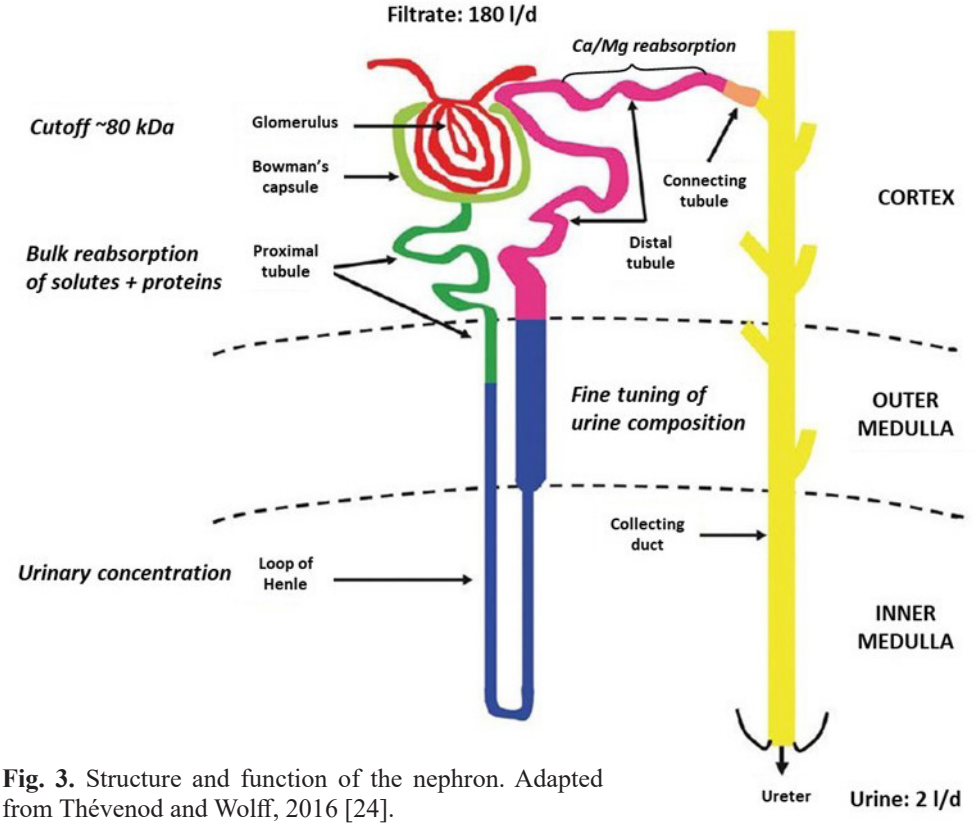

Fig. 3. Structure and function of the nephron. Adapted from Thévenod and Wolff, 2016 [24].

As mentioned previously, the kidney was often overlooked as a major contributor to systemic iron homeostasis, due to TBI exceeding the molecular mass limit of the glomerulus of ~80 kDa [21]. Some of the first indications that this belief was erroneous began with research in Faconi's syndrome, a pathology affecting the reabsorption potential of the proximal tubule (PT) [9]. Patients with this syndrome have an increased urinary excretion of proteins up to 160 kDa and subsequently have higher levels of urinary Tf than healthy individuals [16]. The general presence of Tf in the urine and the fact that in certain pathologies its levels increased led researchers to the conclusion that TBI can pass through the glomerulus. This was further substantiated by the presence of several iron transporters in the PT, such as cubilin, DMT1, TfR1 [29]. Cubilin was originally considered a receptor only for apoliprotein A1 and albumin in the kidney, but Kozyraki et al. 2001, discovered that it is a novel ligand for TBI located at the apical pole of polarized epithelial cells [10]. Due to lacking a transmembrane region, cubilin is dependent on megalin to carry out its functions. Megalin itself, colocalizes and binds to cubilin and the two receptors work together for ligand uptake. Megalin-dependent cubilin-mediated endocytosis is considered one of the main ways by which filtered TBI is reabsorbed and its proposed role is to rescue iron and supply the iron-dependent enzymes in the renal proximal tubules [10]. DMT1 is another iron transporter located in the PT, which further hints towards the importance of the kidneys

in iron homeostasis. In fact in one of the first studies on DMT1, some of the highest levels of DMT1 mRNA were identified in renal tissue [5]. Ultimately, findings like this inspired Smith and Thévenod, 2009 to propose a hypothetical model of renal iron transport **(Fig. 4)** [21].

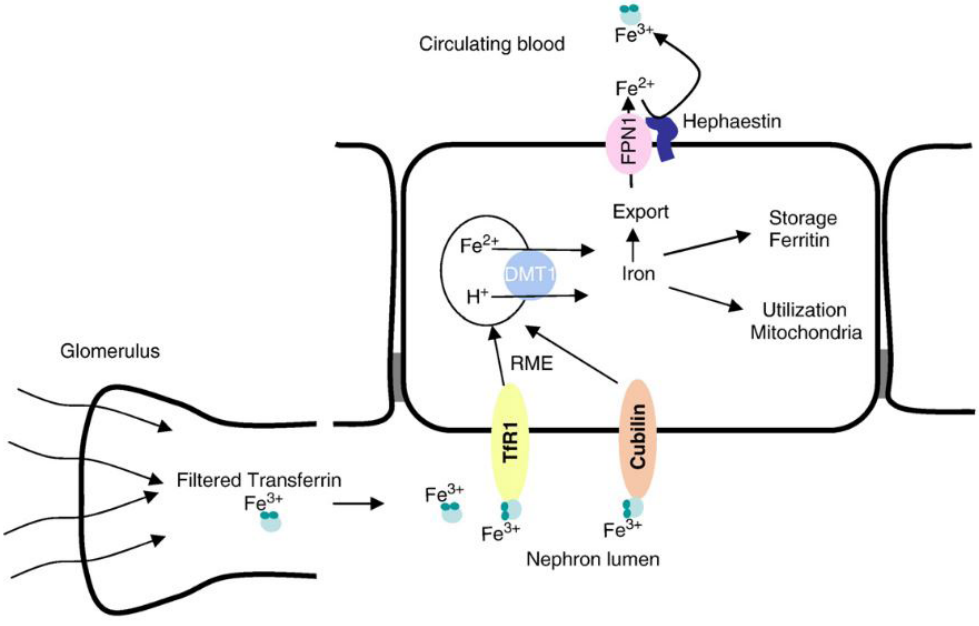

Fig. 4. Hypothetical model of renal iron transport. TBI is filtered and reabsorbed by proximal tubule cells by receptor mediated endocytosis (RME) bound to either to TfR1 or cubilin. TBI is transited to late endosomes/lysosomes where iron dissociates from Tf. Fe^{2+} (potential Dcytb activity) is translocated to the cell cytosol. Smith and Thévenod are not sure of Fe^{2+}'s ultimate fate and so propose three possible outcomes: (1) Fe^{2+} is utilized by PT mitochondria or iron requiring processes; (2) Fe^{2+} is stored in ferritin; (3) Fe^{2+} is exported back into the circulation via FPN1 aided by hephaestin. According to the authors, the three potential fates are not mutually exclusive [21].

Iron, The Kidney and Disease

With the growing interest in the kidney's role in systemic iron homeostasis, researchers have additionally enquired about the effect of renal pathologies on iron homeostasis and vice versa, the pathological effect of iron on the kidney. Kidney diseases do not escape the toxic effects of iron, and ferroptosis is identified as a pathophysiological mechanism that could be a therapeutic target to avoid damage or progression of kidney disease. Various animal models have been developed to study iron overload [18].

Renal Pathologies and Fe metabolism

The body can experience direct loss of iron with urine as a result of proteinuria caused by glomerulopathy. Tf and iron excretion with urine is increased with patients with focal segmental glomerulosclerosis (FSGS), focal glomerulonephritis, mesangioproliferative glomerulonephritis and diabetic nephropathy. These diseases are associated with systemic absolute iron deficiency and iron deficiency anaemia (IDA) [25]. Renal disorders may also affect systemic iron homeostasis indirectly by negatively affecting the production of erythropoietin. EPO is a glycoprotein hormone synthesised by the peritubular cells of the kidney and it stimulates red blood cell production. Renal cell loss and inflammation in chronic kidney disease (CKD) cause low levels of circulating EPO that correlates with the degree of anaemia and decreased erythropoietic activity [13, 27]. In patients with proteinuria, loss of EPO with urine is increased and may lead to lower systemic levels of the hormone, which has been observed in animal and human studies [8, 28].

Kidney pathologies and specifically CKD can affect iron metabolism in another way, by elevating hepcidin levels. Patients with CKD present with chronic inflammation, receive intravenous (IV) iron which raises iron stores and have low glomerular filtration rate (GFR). All of this leads to increase of Hep and decrease in its excretion. The increase in serum Hep impairs absorption of dietary iron and promotes iron sequestration by the reticuloendothelial system. Therefore, patients also present with functional iron deficiency (FID), characterized with impaired release of iron from stores and not meeting the needs of erythropoiesis, low serum Tf saturation (TSAT) levels and normal or high Ft levels [25].

Systemic Fe disorders and the kidney

Disruption of iron homeostasis, iron overload, has been known to exert a negative effect on organs that are heavy iron users and/or involved in iron recycling. Iron overload is primarily caused by hereditary haemochromatosis (HH), a genetical condition, caused by resistance or general deficiency of hepcidin. The causes are inherited genetical defects that affect proteins involved in the production, function, or regulation of hepcidin. This results in an unregulated uptake of iron by the duodenum which eventually leads to systemic iron overload [17]. Systemic iron overload can also be caused as a secondary pathological feature of thalassaemia disorders. They are characterized with reduced production of α-globin or β-globin chains, structural units of haemoglobin. Based on which chain production is disturbed, the disorders are classified as α-thalassaemia or β-thalassaemia. Nevertheless, both syndromes have dysfunctional haemoglobin production and impaired erythropoiesis. Patients with β-thalassaemia are classified as either intermediate or major, based on globin production levels, anaemia severity and clinical presentation. Both have an ineffective and increased erythropoiesis which results in a reduced hepcidin production. Additionally, patients with β-thalassaemia major require life-long, lifesaving blood transfusions, which could further exacerbate any systemic iron overload [14, 23]. Iron overload leads to elevated levels of Ft and TBI, increased iron saturation of Tf and increase in non-Tf bound iron (NTBI) and catalytic iron in the LIP. The LIP is a pool of NTBI, that in normal conditions participates in redox cycling, it does so by converting hydrogen peroxide to free-radical ions (Fenton reactions). Catalytic iron can cause oxidative damage to cell membranes, proteins and DNA [12, 20].

Conclusion

The kidney is potentially one of the biggest contributors to systemic iron homeostasis. This has been shown by the presence of several major iron transports in the nephrons and also by the fact that the kidney synthesises EPO, master regulator of erythropoiesis. Furthermore, renal pathologies have a wide-reaching negative effect on iron homeostasis and like any of the canonical organs involved in iron metabolism, the kidney is very susceptible to iron disorders.

Acknowledgements: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Young scientists and postdoctoral students-2" approved by DCM 206/07.04.2022.

References

1. Arosio, P., F. Carmona, R. Gozzelino, F. Maccarinelli, M. Poli. The importance of eukaryotic ferritins in iron handling and cytoprotection. – *Biochem. J.,* **472**, 2015, 1-15.

2. Aschemeyer, S., B. Qiao, D. Stefanova, E. V. Valore, A. C. Sek, T. A. Ruwe, K. R. Vieth, G. Jung, C. Casu, S. Rivella, M. Jormakka, B. Mackenzie, T. Ganz, E. Nemeth. Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. – *Blood,* **131**, 2018, 899-910.

3. Braymer, J. J., R. Lill. Iron-sulfur cluster biogenesis and trafficking in mitochondria. – J. *Biol. Chem.*, **292**, 2017, 12754-12763.

4. Camaschella, C., A. Nai, L. Silvestri. Iron metabolism and iron disorders revisited in the hepcidin era. – *Haematologica,* **105**, 2020, 260-272.

5. Gunshin, H., B. Mackenzie, U. V. Berger, Y. Gunshin, M. F. Romero, W. F. Boron, S. Nussberger, J. L. Gollan, M. A. Hediger. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. – *Nature,* **388**, 1997, 482-488.

6. Hamza, I., H. A. Dailey. One ring to rule them all: Trafficking of heme and heme synthesis intermediates in the metazoans. – *Biochim. Biophys. Acta (BBA) – Molecular Cell Research*, **1823**, 2012, 1617-1632.

7. Hider, R. C., X. Kong. Iron: Effect of overload and deficiency. – *Met. Ions Life Sci.*, **13,** 2013, 229-295.

8. Inoue, A., T. Babazono, K. Suzuki, Y. Iwamoto. Albuminuria is an independent predictor of decreased serum erythropoietin levels in type 2 diabetic patients. – *Nephrol. Dial. Transplant.*, **22**, 2007, 287-288.

9. Klootwijk, E. D., M. Reichold, R. J. Unwin, R. Kleta, R. Warth, D. Bockenhauer. Renal Fanconi syndrome: taking a proximal look at the nephron. – *Nephrol. Dial. Transplant.,* **30**, 2015, 1456-1460.

10. Kozyraki, R., J. Fyfe, P. J. Verroust, C. Jacobsen, A. Dautry-Varsat, J. Gburek, T. E. Willnow, E. I. Christensen, S. K. Moestrup. Megalin-dependent cubilin-mediated endocytosis is a major pathway for the apical uptake of transferrin in polarized epithelia. – *Proc. Natl. Acad. Sci.,* **98**, 2001, 12491-12496.

11. Le Blanc, S., M. D. Garrick, M. Arredondo. Heme carrier protein 1 transports heme and is involved in heme-Fe metabolism. – *Am. J. Physiol.-Cell Physiol.*, **302**, 2012, C1780-1785.

12. Leaf, D. E., D. W. Swinkels. Catalytic iron and acute kidney injury. – *Am. J. Physiol. – Ren. Physiol.,* **311**, 2016, F871-876.

13. McGonigle, R. J., J. D. Wallin, R. K. Shadduck, J. W. Fisher. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. – *Kidney Int.,* **25**, 1984, 437-444.

14. Nemeth, E. Hepcidin in β-thalassemia. – *Ann. N. Y. Acad. Sci.,* **1202**, 2010, 31–35.
15. Nemeth, E., M. S. Tuttle, J. Powelson, M. B. Vaughn, A. Donovan, D. M. Ward, T. Ganz, J. Kaplan. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. – *Science,* **306**, 2004, 2090-2093.
16. Norden, A. G. W., M. Lapsley, P. J. Lee, C. D. Pusey, S. J. Scheinman, F. W. K. Tam, R. V. Thakker, R. J. Unwin, O. Wrong. Glomerular protein sieving and implications for renal failure in Fanconi syndrome. – *Kidney Int.*, **60**, 2001, 1885-1892.
17. Powell, L. W., R. C. Seckington, Y. Deugnier. Haemochromatosis. – *The Lancet,* **388**, 2016, 706-716.
18. Ríos-Silva, M., Y. Cárdenas, A. G. Ortega-Macías, X. Trujillo, E. Murillo-Zamora, O. Mendoza-Cano, J. A. Bricio-Barrios, I. Ibarra, M. Huerta. Animal models of kidney iron overload and ferroptosis: a review of the literature. – *BioMetals,* **36**, 2023, 1173-1187.
19. Shaw, G. C., J. J. Cope, L. Li, K. Corson, C. Hersey, G. E. Ackermann, B. Gwynn, A. J. Lambert, R. A. Wingert, D. Traver, N. S. Trede, B. A. Barut, Y. Zhou, E. Minet, A. Donovan, A. Brownlie, R. Balzan, M. J. Weiss, L. L. Peters, J. Kaplan, L. I. Zon, B. H. Paw. Mitoferrin is essential for erythroid iron assimilation. - *Nature,* **440**, 2006, 96-100.
20. Slotki, I., Z. I. Cabantchik. The Labile side of iron supplementation in CKD. – *J. Am. Soc. Nephrol.*, **26**, 2015, 2612-2619.
21. Smith, C. P., F. Thévenod. Iron transport and the kidney. - *Biochim. Biophys. Acta BBA – Gen. Subj.,* **1790**, 2009, 724-730.
22. Staroń, R., P. Lipiński, M. Lenartowicz, A. Bednarz, A. Gajowiak, E. Smuda, W. Krzeptowski, M. Pieszka, T. Korolonek, I. Hamza, D. W. Swinkels, R. P. L. V. Swelm, R. R. Starzyński. Dietary hemoglobin rescues young piglets from severe iron deficiency anemia: Duodenal expression profile of genes involved in heme iron absorption. – *PLOS One*, **12**, 2017, e0181117.
23. Taher, A. T., D. J. Weatherall, M. D. Cappellini. Thalassaemia. – *Lancet Lond. Engl.,* **391**, 2018, 155-167.
24. Thévenod, F., N. A. Wolff. Iron transport in the kidney: implications for physiology and cadmium nephrotoxicity. – *Metallomics,* **8**, 2016, 17-42.
25. van Swelm, R. P. L., J. F. M. Wetzels, D. W. Swinkels. The multifaceted role of iron in renal health and disease. – *Nat. Rev. Nephrol.*, **16**, 2020, 77-98.
26. Wang, J., K. Pantopoulos. Regulation of cellular iron metabolism. – *Biochem. J.*, **434**, 2011, 365-381.
27. Weiss, G., L. T. Goodnough. Anemia of chronic disease. – *New Engl. J. Med.,* **352**, 2005, 1011-1123.
28. Yamaguchi-Yamada, M., N. Manabe, K. Uchio-Yamada, N. Akashi, Y. Goto, Y. Miyamoto, M. Nagao, Y. Yamamoto, A. Ogura, H. Miyamoto. Anemia with chronic renal disorder and disrupted metabolism of erythropoietin in ICR-derived glomerulonephritis (ICGN) mice. – *J. Vet. Med. Sci.,* **66**, 2004, 423-431.
29. Zhang, D., E. Meyron-Holtz, T. A. Rouault. Renal iron metabolism: Transferrin iron delivery and the role of iron regulatory proteins. – *J. Am. Soc. Nephrol.,* **18**, 2007, 401.