JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2021, 72, 1, 23-33
www.jpp.krakow.pl | DOI: 10.26402/jpp.2021.1.03

Review article

M. WOJCIECHOWSKA[1], O.W. WISNIEWSKI[2], P. KOLODZIEJSKI[3], H. KRAUSS[4]

ROLE OF HEPCIDIN IN PHYSIOLOGY AND PATHOPHYSIOLOGY. EMERGING EXPERIMENTAL AND CLINICAL EVIDENCE.

[1]Department of Mother and Child Health, Poznan University of Medical Sciences, Poznan, Poland; [2]Students' Scientific Society, Poznan University of Medical Sciences, Poznan, Poland; [3]Department of Animal Physiology, Biochemistry and Biostructure, Poznan University of Life Sciences, Poznan, [4]Poland; Preventive Research Institute, The President Stanislaw Wojciechowski State University of Applied Sciences in Kalisz, Kalisz, Poland

Normal iron metabolism is an inherent feature of maintaining homeostasis. There is a wide range of iron disorders, which arise from iron deficiency or overload. In addition, disturbances in iron metabolism are observed in the course of numerous chronic diseases. Since iron is an essential constituent of hemoglobin, different types of anemia are clinical manifestations of both iron deficit or excess. This seemingly contradictory statement may be elucidated by the presence of hepcidin. Hepcidin is a primary regulator of iron metabolism in the human body. By promoting ferroportin degradation, hepcidin decreases the amount of iron in the circulation due to iron sequestration in the tissues and reduced intestinal absorption. Altered hepcidin concentration is a compensatory mechanism aimed at restoring iron homeostasis in various physiologic states, including pregnancy. However, hepcidin may also participate in the pathophysiologic background of hereditary hemochromatosis, anemia of chronic disease, myelodysplastic syndromes or β-thalassemia. Moreover, hepcidin is an acute-phase protein involved in innate immunity reactions. In our paper, we provide a comprehensive review of the physiologic and pathophysiologic functions of hepcidin. We present current knowledge on the structure, physiologic role and its expression control, as well as demonstrate the contribution of hepcidin in a state of illness. We also summarize the significance of hepcidin in normal and complicated pregnancy. Emphasizing the alterations in hepcidin upon treatment of specific diseases and their position in certain pathomechanisms, we support clinicians with practical aspects related to hepcidin.

Key words: *hepcidin, iron, iron disorders, iron homeostasis, iron metabolism, pregnancy, innate immunity, hereditary hemochromatosis, chronic kidney disease*

INTRODUCTION

The desire to discover the role of Ferrum in the human body has been persistent among researchers for centuries. Thanks to their efforts and commitment, we already know that iron is a key component of many particles that are crucial for the functioning of the whole organism. Not only does the iron build up the molecules of hemoglobin and myoglobin supplying oxygen to the tissues, but also it is an essential constituent of the wide range of enzymes participating in oxidation-reduction reactions, including those of the respiratory chain. Thus, iron is an indispensable element in maintaining homeostasis, since apart from transporting oxygen and carbon dioxide, it opens the way for oxidative phosphorylation and the effective release of energy. Furthermore, iron is also involved in the biosynthesis or neutralization of numerous substances by occupying the active sites of enzymes.

However, an excess of free iron in the body may have pernicious consequences. Then the unbound iron becomes the initiating factor for the production of reactive oxygen species (ROS), which results in tissue damage and loss of their function (1, 2). Hence, it is not surprising that blood iron concentration and the saturation of its intracellular stores are tightly controlled. This regulation is guided mainly by hepcidin, initially associated only with its antimicrobial activity (3, 4).

The aim of our paper is to present a comprehensive review of hepcidin functions. Starting from the hepcidin structure, we provide the current knowledge on the physiologic role of hepcidin, including the molecular level. Furthermore, we describe the contribution of hepcidin alterations in the pathophysiology, yielding a better understanding of pathomechanisms. Emphasizing the changes in hepcidin upon treatment of specific diseases and their influence on the pathophysiologic background, we support clinicians with practical aspects related to hepcidin. Our paper is addressed primarily to internal medicine and infectious diseases specialists as well as obstetricians. In addition, the presented role of hepcidin in pathophysiology and its position in certain pathomechanisms may indicate the targets of novel therapeutic strategies and inspire further research.

HEPCIDIN STRUCTURE

Hepcidin is a 25 amino acid peptide hormone produced by the *HAMP* gene (19q13) (3, 4). It contains 8 disulfide residues, which form 4 disulfide bridges, keeping the peptide molecule in a hairpin tertiary structure (*Fig. 1*) (5). This structure is also strengthened by hydrogen bonds of antiparallel peptide fragments, which additionally results in its amphipathic nature (6, 7). In addition, hepcidin is characterized by a high content of arginine and lysine, with positively charged chains, which enables it to bind negatively charged cell membranes of bacteria, among others (3, 4).

Hepatocytes are the main source of hepcidin synthesis in the human body, although its mRNA has also been found in adipocytes, macrophages, small intestine, kidneys, pancreas, placenta, heart and lungs (3, 6, 8-12). Hepcidin is transcribed as an 84 amino acid preprohormone, of which 24 N-terminal are the signal sequence targeting the prepropeptide to the endoplasmic reticulum, and another 35 amino acids from the N-terminal comprise the proregion responsible for the correct folding of the exact peptide (3, 6). Thus, functional hepcidin is constructed with 25 C-terminal amino acids. Nevertheless, not all of them determine the biological activity of hepcidin - only 9 amino acids located at the N-terminus of the peptide play a key role (13). They are responsible for the binding of hepcidin to ferroportin and are sufficient to achieve biological effects identical to full-length hepcidin in regulating iron metabolism.

Interestingly, in addition to the 25 amino acid hepcidin molecule, shorter 20 or 22 amino acid particles were also detected (7, 14). Due to their monomeric form, they could potentially differ in biological activity from full-length hepcidin, which in turn forms aggregates in solutions, especially in terms of biocidal capacity.

PHYSIOLOGICAL ROLE OF HEPCIDIN IN IRON METABOLISM

Hepcidin is the primary iron metabolism regulator in the human body (1). It acts indirectly by binding to ferroportin, a unique membrane protein functioning only as an exporter of bivalent iron (15). Ferroportin expression occurs especially in cells of the reticuloendothelial system, in the erythrocyte precursors in the red bone marrow, in the basolateral membrane of enterocytes, and, to a lesser extent, in hepatocytes (8, 15, 16).

After hepcidin binds to ferroportin, the resulting complex is internalized and ferroportin undergoes lysosomal degradation (17). Thus, hepcidin reduces the number of ferroportin molecules exposed on cell membranes. As a result, a reduced outflow of iron from cells and its increased intracellular storage are observed. Despite the short biological half-life of hepcidin, this effect lasts up to 48 hours due to the needed resynthesis of degraded ferroportin molecules.

The other purpose of hepcidin is a reduction in dietary iron absorption, which results both from the blocking of ferroportin activity in enterocytes and the indirect influence of hepcidin on the divalent metal transporter 1 (DMT1) (15, 17, 18). DMT1 is a protein located in the apical enterocyte membrane that is responsible, among others, for the absorption of FE^{2+} cations (19). This is extremely important since there are no mechanisms in the human body that could increase iron excretion in the case of its excess (1). Physiological iron losses arise almost exclusively as a result of microhemorrhages and desquamation of skin and small intestine cells, mainly the duodenum (20). In women of childbearing age, menstrual bleeding is also a significant source of iron loss. The effect of hepcidin on enterocytes is, therefore, the only mechanism that prevents iron overload.

So far, three mechanisms have been proposed by which hepcidin could reduce the expression or accelerate the degradation

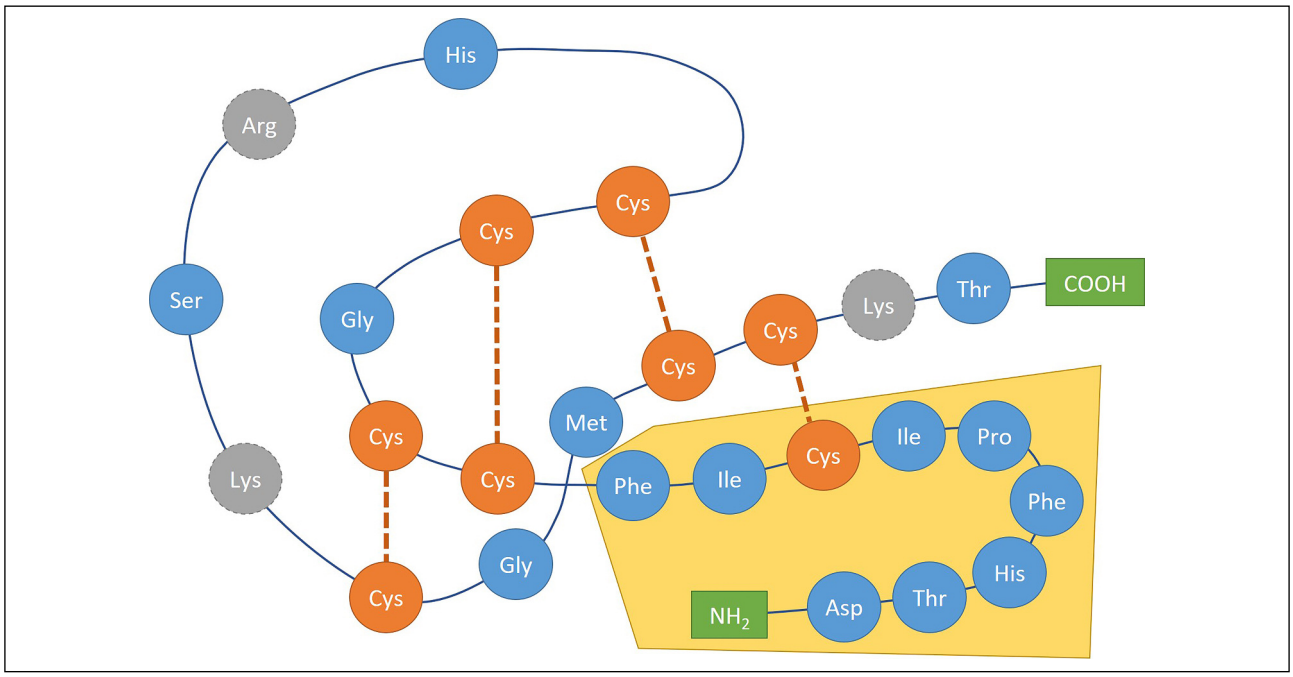

Fig. 1. Hepcidin structure. Hepcidin has a hairpin tertiary structure. The crucial component in its formation corresponds to 8 cysteine residues (orange), bound to each other with disulfide bridges (orange dashed lines). Amino acids with a positive side-chain charge, which are particularly important in the hepcidin interaction with bacterial cell membranes, are marked in gray. The yellow polygon surrounds the 9 N-terminal amino acids determining the biological activity of hepcidin in iron metabolism (hepcidin bound to ferroportin).
Arg, arginine; Asp, aspartic acid; Cys, cysteine; Gly, glycine; His, histidine; Ile, isoleucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine.

of DMT1 molecules in enterocytes. The first one concerns increased concentration of Fe^{2+} ions in the cytoplasm of enterocytes due to the inhibition of its outflow by ferroportin. Then the excess iron ions are used by prolyl hydroxylases, leading to the proteolysis of the hypoxia-inducible factor 2α (HIF2α), which is an activator of DMT1 expression (21). Furthermore, some authors claim that the binding of hepcidin to ferroportin inactivates the iron regulatory proteins 1 and 2 (IRP1 and IRP2), involved in the stabilization of *DMT1* mRNA (19). Thus, hepcidin can reduce the translation of this transporter molecule. Finally, the formation of the hepcidin-ferroportin complex may be a signal for DMT1 ubiquitination and subsequent proteasomal degradation (22).

To summarize, hepcidin reduces blood iron by both, inhibiting its absorption and promoting its sequestration in macrophages, monocytes, red cell precursors and hepatocytes (*Fig. 2*). Thus, hepcidin prevents the iron excess and, therefore, restricts the occurrence of transferrin-unbound iron and consequently the production of ROS (20).

THE ROLE OF HEPCIDIN IN INNATE IMMUNITY MECHANISMS

In addition to regulating iron metabolism, another relevant function of hepcidin is the modulation of the immune system. Hepcidin has antibacterial, antifungal and antiparasitic activity (3, 23). Its antimicrobial properties are tightly connected with iron metabolism, as it is one of the key growth factors for numerous microorganisms (23). Thus, hepcidin-induced intracellular iron sequestration protects the human body against the development of infections or their more severe course, by limiting iron concentration in the circulation and the tissue fluid.

The beneficial effects of hepcidin are confirmed by the results of studies conducted on murine models of sepsis, which showed significantly higher mortality in mice with knockout hepcidin gene than in wild-type (24, 25). Moreover, the administration of exogenous hepcidin eliminated this difference and equally protected against sepsis death in hepcidin-deficient mice. Similarly, increased mortality with concomitant elevation in serum iron levels was observed in mice with silenced hepcidin transcription (26, 27). The above data correspond adeptly to the results of a clinical trial, which evidenced a raised hepcidin concentration in the course of sepsis and its decrease in the recovery period (28).

Similar results apply to infections with siderophilic bacteria, *e.g.*, with certain species from the genera *Vibrio* and *Yersinia* (29, 30). It has been shown that mice deficient of hepcidin are more susceptible to infection due to the higher concentration of iron in the extracellular fluid and the bloodstream. Comparable conclusions can also be drawn from studies on different species of *Plasmodium*. High hepcidin concentrations determine a milder course of malaria and even resistance to re-infection (31-33). However, increased hemolysis in the course of *P. falciparum* disease may reduce hepcidin expression (34), marginalizing its positive role (see also next chapter). Promising effects of hepcidin have also been

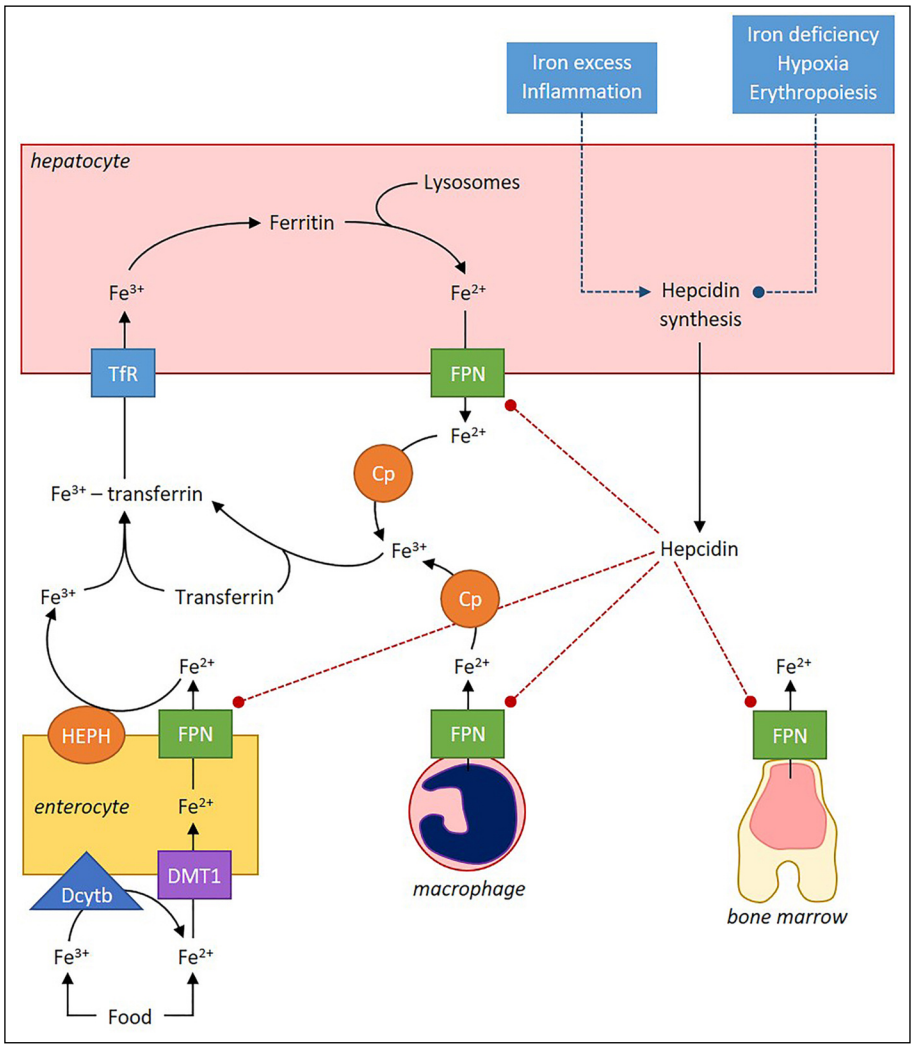

Fig. 2. The role of hepcidin in iron metabolism. Dashed arrows with a sharp point indicate the stimulation of the process, dashed arrows with a round tip indicate inhibition of the process or surface protein expression. Hepcidin inhibits iron absorption in duodenal villi enterocytes and the initial part of the jejunum, as well as the release of iron accumulated in hepatocytes, macrophages and bone marrow cells by reducing the number of active ferroportin molecules anchored within cell membranes. Hepcidin synthesis occurs in the liver and is stimulated by excess iron and inflammation. In turn, inhibition of its synthesis is the result of iron deficiency, hypoxia and erythropoiesis.
Cp, ceruloplasmin; Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; FPN, ferroportin; HEPH, hephaestin; TfR, transferrin receptor.

described in fungal infections, including *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus Niger* (3).

However, the presence of hepcidin is not always beneficial for the organism. Iron sequestration in macrophages promoted by hepcidin encourages the development of intracellular bacteria that occupy this niche after entering the human body. So far, this relationship has been described for mycobacteria (35-37), *Salmonella enterica* s. Typhimurium (38, 39), *Chlamydia* sp. and *Legionella* sp. (40). Interestingly, similar results were obtained for parasites penetrating macrophages, *e.g.*, *Leishmania amazonensis* (41).

Apart from limiting the availability of iron ions for the development of pathogens, hepcidin may also be characterized by its own antimicrobial activity, which results from the structure of its molecule, resembling the structure of defensins and protegrins, proteins involved in the mechanisms of innate immunity (4). Nonetheless, there is no conclusive evidence confirming the direct antimicrobial activity of hepcidin (42).

Summarizing, the participation of hepcidin in the regulation of iron metabolism also increases the immune system's defensive capacity in bacterial (especially siderophilic bacteria), fungal and parasitic infections, except for those with intracellular bacteria inhabiting macrophages.

REGULATION OF HEPCIDIN SECRETION

Hepcidin production is controlled by complex regulatory mechanisms, of which iron saturation status plays a key role. The change in hepcidin secretion depends not only on the current blood iron concentration and transferrin saturation but may also be affected by fluctuations in intracellular iron levels (43). Since the vast majority of synthesized hepcidin comes from the liver, the iron concentration in hepatocytes has a pivotal impact on the latter controlling mechanism.

The expression of hepcidin enhances with an increase in the amount of transferrin bound to Fe^{3+} ions (holotransferrin) and decreases as transferrin saturation declines (43). Thus, the blood concentration of hepcidin negatively correlates with the total iron binding capacity (TIBC) under the physiologic state. Similarly, the synthesis of hepcidin elevates in the case of intracellular iron excess and lowers upon its deficiency.

At the molecular level, the mechanism of stimulating hepcidin secretion is based on the induction of the *HAMP* gene expression through the SMAD signaling pathway, which is dependent on activation of the bone morphogenetic protein receptor (BMPR) by appropriate ligands (44). Bone morphogenetic protein 6 (BMP6), mainly synthesized in epithelial cells of the hepatic sinuses, appears to play a major role in this interaction, although the participation of BMP2, 4, 5, 7 and 9 was also revealed in murine experimental models (44, 45).

In the state of iron excess, increased BMPR stimulation by BMP6 promotes hepcidin expression (46). The higher concentration of holotransferrin affects the transferrin receptors 1 and 2 (TfR1 and 2), leading to BMPR sensitization and facilitating its binding with BMP6 (43, 47, 48). Two accessory proteins are also involved in this process: human homeostatic iron regulator (HFE), which is an adapter of transferrin receptors (48), and hemojuvelin, a coreceptor of BMP6 (49). The presence of both proteins is necessary for the signal transduction on the current iron

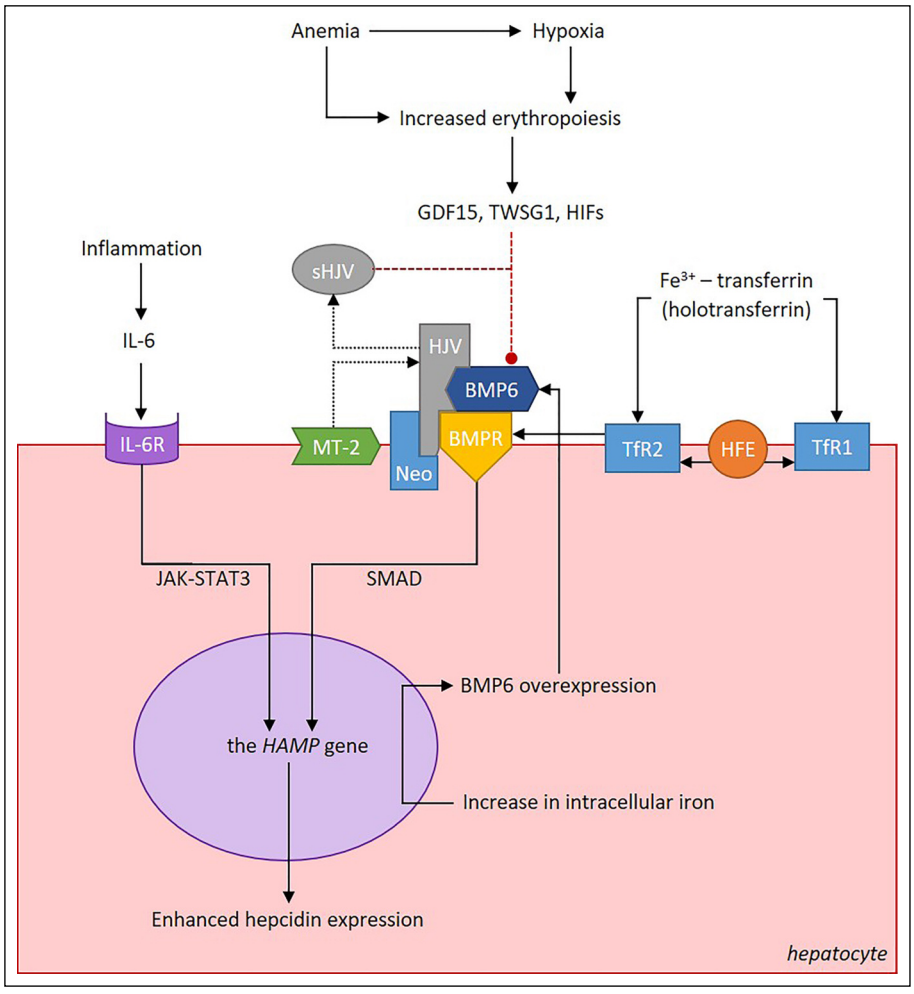

Fig. 3. Regulation of hepcidin expression (description in the text). Dashed red arrows with a circular end indicate the process is inhibited. BMP6, bone morphogenetic protein 6; BMPR, bone morphogenetic protein receptor; GDF15, growth/differentiation factor 15; HFE, human homeostatic iron regulator protein; HIFs, hypoxia-inducible factors; HJV, hemojuvelin; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; JAK-STAT3, the JAK-STAT3 pathway; MT-2, matriptase 2; Neo, neogenin; sHJV, soluble hemojuvelin; SMAD, the SMAD pathway; TfR1, transferrin receptor 1; TfR2, transferrin receptor 2; TWSG1, twisted gastrulation 1.

concentration in the blood and the extracellular fluid (48, 49). Conversely, the decrease in transferrin saturation changes the interplay between both transferrin receptors, reducing the activity of the BMPR and the associated SMAD signaling pathway, which results in diminished hepcidin expression (47, 48).

Moreover, the described mechanism can be additionally modulated by neogenin as well as by transmembrane serine protease 6 (TMPRSS6), also known as matriptase 2 (MT-2). Neogenin stimulates the expression of hepcidin by stabilizing hemojuvelin in the cell membrane (50). On the contrary, TMPRSS6 reduces the formation of hepcidin, promoting the detachment of hemojuvelin molecules from the cell membrane and thus increasing the concentration of its soluble form, which further disrupts the BMPR signaling (51, 52).

The regulatory mechanism of hepcidin expression in response to the level of intracellular iron is slightly different, although BMP6 still maintains its crucial role. Increasing amounts of stored iron stimulate the synthesis of *Bmp6* mRNA, which directly enhances signal transmission *via* BMPR and SMAD proteins, contributing to the induction of hepcidin secretion (53). In the state of iron deficiency, the synthesis of BMP6 decreases, thus decreasing downstream signaling in the BMPR and the SMAD pathways, as well as the production of hepcidin. Interestingly, the modulation of *Bmp6* expression is not the only link between intracellular iron concentration and hepcidin transcription (43). It is postulated that iron-dependent prolyl hydroxylases and ubiquitin ligases may also contribute (54, 55), although this requires confirmation in future research.

Apart from the status of iron metabolism, the synthesis of hepcidin is also affected by inflammation (1). Hepcidin concentration increases significantly under inflammation, which is related to its positive effect in limiting the growth of microorganisms (56). Hepcidin is, therefore, an acute-phase protein, just like C-reactive protein, fibrinogen, ferritin and procalcitonin. Interleukin 6 (IL-6), one of the major pro-inflammatory cytokines, is believed to be the main stimulator of hepcidin synthesis (57). The binding of IL-6 to a specific receptor (IL-6R) activates the JAK-STAT3 signaling pathway, which induces the expression of hepcidin (58, 59). Once the infection is controlled, the concentration of IL-6 steadily declines, bringing hepcidin secretion back to earlier levels.

Hepcidin expression is also influenced by exaggerated erythropoiesis, an essential component of the compensatory responses to hypoxia or bleeding (1). This is due to the need to ensure an adequate supply of iron for red blood cell production. In the case of increased erythropoiesis, hepcidin synthesis is reduced, which elevates the number of iron ions released from intracellular stores and absorbed in the gastrointestinal tract (60). As a result, a large pool of iron can be delivered as quickly as possible to the forming erythrocyte precursors. The exact mechanism by which erythropoiesis suppresses the production of hepcidin is unknown. Nevertheless, it appears that this may be mediated by bone marrow secreted growth factors, such as growth/differentiation factor 15 (GDF15) or twisted gastrulation 1 (TWSG1) (61, 62), as well as hypoxia-inducible factors (HIFs) (63).

In summary, the increase in iron saturation and inflammation stimulate hepcidin expression, leading to a decline in serum iron concentration, while augmented erythropoiesis and iron deficiency reduce hepcidin production, raising the availability of iron ions in the blood (*Fig. 3*).

HEPCIDIN IN PATHOPHYSIOLOGY

Alterations in hepcidin levels have already been observed in the course of many diseases, especially hematological and metabolic, among which myelodysplastic syndromes and hereditary hemochromatosis should be acknowledged. There is also ongoing research on the role of hepcidin in the pathogenesis of endocrine diseases, such as acromegaly and Graves' disease. Moreover, since almost the entire pool of hepcidin is excreted by the kidneys, hepcidin accumulates in patients with chronic kidney disease, which is one of the mechanisms leading to anemia (64). The role of hepcidin in particular nosological entities is briefly described in the following sections.

HEREDITARY HEMOCHROMATOSIS

Hemochromatosis is a genetic disease of iron metabolism that inevitably leads to an iron overload. The excess of iron ions accumulates especially in the liver, endocrine glands and heart muscle, which is closely related to the clinical manifestations of the disease (65). Characteristic features include hepatomegaly, gastroenterological symptoms, cardiac arrhythmias and hormonal imbalances. The increased production of ROS promoted by the surplus of iron is a mechanism of tissue damage and organ dysfunction.

There are several types of hereditary hemochromatosis, depending on different gene mutations. The utterly rare type 2b is the consequence of a mutation in the *HAMP* gene, which results in the production of a defective hepcidin molecule devoid of biological activity (6). In the classic type of hereditary hemochromatosis, in turn, a mutation of the *HFE* gene encoding HFE protein precludes the regulation of hepcidin secretion in response to the current iron level in the body, leading to hepcidin deficiency (66, 67). The same transmission problem is also caused by the transferrin receptor abnormalities (68). Likewise, mutations in the hemojuvelin gene disrupt the BMP6-associated pathway for promoting hepcidin expression (67). In this case, however, the symptoms appear several decades earlier than in the classic subtype. Interestingly, mutations in ferroportin may also be responsible for the development of hemochromatosis, making ferroportin resistant to the effects of hepcidin (69). Then, despite the presence of correctly synthesized hepcidin, the membrane ferroportin molecules do not internalize, and therefore hepcidin cannot fulfill its physiological function (17, 69).

Regardless of the gene mutation, excess iron is accumulated in the body due to dysfunction or deficiency of hepcidin, as a result of its abnormal structure, disturbed signal transduction of regulatory mechanisms or ferroportin resistance (6, 66-69). The deficit of properly functioning hepcidin also affects the immune system and increases susceptibility to infections (23).

It should be noted that bloodletting, which is one of the few methods of hemochromatosis treatment, leads to hepcidin suppression by secondary erythropoiesis stimulation (20). Thus, a vicious circle is created, as enhanced hepcidin deficiency results in an increasing accumulation of iron in the body, which requires even more frequent phlebotomies to maintain a given iron concentration and remission of disease symptoms.

ANEMIA OF CHRONIC DISEASE

Anemia of chronic disease, also known as anemia of inflammation, arises from chronic inflammation accompanying the underlying illness. As a result of the increased production of pro-inflammatory cytokines, the proliferation of erythroid lineage precursors in the bone marrow is disturbed, the secretion of erythropoietin by the kidneys is reduced, and the survival time of circulating erythrocytes is shortened (70). Moreover, the presence of inflammation stimulates the expression of hepcidin, which leads to iron sequestration in the cells and limits its availability to erythropoiesis (56, 67, 71, 72). Impaired

erythropoiesis and faster disintegration of mature erythrocytes contribute to the development of anemia, mainly normocytic and normochromic (71).

Thus, the rise in hepcidin concentration in response to the inflammatory process is one of the mechanisms involved in the pathogenesis of anemia of chronic disease (67, 72-74). Elevated hepcidin concentration is accompanied by a decline in transferrin saturation and the amount of iron in the blood, while the concentration of ferritin, which is also an acute-phase protein, increases.

A similar mechanism may also generate anemia in the course of neoplastic diseases, especially in multiple myeloma and Hodgkin's lymphoma (67, 75, 76).

CHRONIC KIDNEY DISEASE

Physiologically, the vast majority of hepcidin is filtered into primary urine and excreted by the kidneys (8). As a result of the loss of active nephrons in the course of chronic kidney disease (CKD), the mechanism of hepcidin excretion becomes ineffective (64). High blood hepcidin precludes the adequate supply of iron for erythropoiesis and is one of the mechanisms of anemia in CKD (64, 67), in addition to the reduced secretion of erythropoietin and the effect of uremic toxins on circulating erythrocytes. An additional factor that elevates hepcidin levels in the course of CKD is the inflammatory component (77).

Importantly, the increase in hepcidin production in CKD necessitates the use of high doses of erythropoiesis-stimulating agents during the treatment of anemia (78).

Of note, iron-induced ROS production has been recently linked with asymptomatic intraperitoneal inflammation in peritoneal dialysis patients (79). Intravenous iron isomaltoside supplementation resulted in raised dialysate IL-6 and monocyte chemoattractant protein-1 (MCP1) as well as reduced fibrinolytic activity of mesothelial cells. Interestingly, these changes were attenuated by the simultaneous infusion of N-acetylcysteine. Since IL-6 promotes hepcidin expression, intraperitoneal inflammation may further enhance hepcidin concentration and complicate the treatment of CKD-associated anemia.

MYELODYSPLASTIC SYNDROMES AND β-THALASSEMIA

The role of hepcidin has also been proven in the pathogenesis of myelodysplastic syndromes (MDS) with ineffective erythropoiesis, mainly in various types of refractory anemia (80). Disturbances in the production of red blood cells result in the development of anemia and, therefore, lead to the stimulation of erythropoiesis, which is a signal for the inhibition of hepcidin synthesis. The decrease in hepcidin secretion contributes to the rise in serum iron concentration and the overload of its intracellular stores (81). Nonetheless, the elevation in the amount of available iron does not solve the problem of ineffective erythropoiesis associated with increased apoptosis of erythroid lineage precursors and their impaired maturation (82). Thus, the treatment of anemia requires transfusions of red blood cells, which additionally multiplies the amount of iron in the body and enhances the hepcidin deficit.

A very similar mechanism is also observed in β-thalassemia, a genetic disorder that partially or wholly disrupts the synthesis of β-globin chains, which results in ineffective erythropoiesis and the production of abnormal forms of erythrocytes (83). These, in turn, are quickly captured by spleen macrophages and removed. As a result, anemia develops, followed by stimulation of erythropoiesis and secondary inhibition of hepcidin production (61). Then the absorption of iron in the gastrointestinal tract increases, gradually leading to iron overload (83). As in MDS, symptomatic treatment of anemia with red blood cell transfusions aggravates hepcidin deficiency.

To recapitulate, anemia can be associated with both increased (CKD, anemia of chronic disease) and decreased (MDS, β-thalassemia) levels of hepcidin. The explanation for this phenomenon lies in the pathogenesis of individual disease entities, as described in detail above.

THE ROLE OF HEPCIDIN IN PREGNANCY

Changes in hepcidin concentration are also observed in the course of physiologic pregnancy, which is strongly associated with the significantly elevated demand for iron. This is not only due to the need to ensure an adequate pool of iron for the proper development of the fetus but also to create iron stores in the placenta and for the physiological increase in the number of maternal erythrocytes (84). It is estimated that 1040 mg of iron is consumed throughout pregnancy (85). Because of pregnancy-related amenorrhea, the mother’s iron requirement in the first trimester is lower than that of a non-pregnant. However, starting from the second trimester, it rises gradually as a result of the ever-increasing needs of the developing fetus, reaching the peak value at the end of the third trimester. This means that during pregnancy, the systemic iron reserves, estimated at 1 g, would be fully utilized (20). However, this does not happen since the expanding needs of the pregnant woman’s body are counterbalanced by increased iron absorption and, most often, its higher supply. These adaptative mechanisms are mediated by hepcidin (86).

With more advanced pregnancy, the concentration of hepcidin in the blood decreases gradually (87), which reduces the degradation of ferroportin, thereby increasing the number of active iron exporter molecules bound to the cell membranes (17). As a result, the amount of iron transported from enterocytes into the bloodstream and tissues increases, and thus the amount of iron absorbed in the intestines. Therefore, the declining hepcidin concentration adapts the mother's body to the increasing demand on iron during pregnancy (87). However, the mechanism responsible for the decreased hepcidin expression in pregnancy has not been identified yet (84). Nevertheless, most likely due to the lack of this unknown factor, maternal hepcidin levels elevate rapidly after delivery (87, 88).

Moreover, hepcidin influences placental iron transfer (*Fig. 4*), as ferroportin is also expressed in syncytiotrophoblast (89). Initially, maternal transferrin-bound iron integrates to TfR1 located in the apical membrane of the syncytiotrophoblast cells. The complex is then internalized in the process of clathrin-dependent endocytosis (90). After a while, the content of the endocytic vesicle acidifies, resulting in the detachment of the Fe^{3+} ion from the holotransferrin-TfR1 complex (91). Subsequently, Fe^{3+} is reduced to Fe^{2+} by ferroreductase (91) and removed from the endosome into the syncytiotrophoblast cytoplasm with the participation of DMT1 (92). Iron-depleted transferrin (apotransferrin) as well as TfR1 return to the apical membrane and can be reused (91). In turn, Fe^{2+} ions are further exported through the basolateral syncytiotrophoblast membrane by ferroportin to the connective tissue surrounding the endothelium of the fetal blood vessels (92). After being oxidized to Fe^{3+}, iron is transferred to the fetal circulation in an unidentified way (92, 93).

Notably, both maternal and fetal hepcidin are involved in placental iron transfer regulation (94-96). Despite the proximity of the fetal circulation to the basolateral syncytiotrophoblast membrane with incorporated ferroportin molecules, it seems that maternal hepcidin plays a major role in this regulation. In the physiologic state, it is produced in much higher amounts by the

liver of the mother than that of the fetus. Isotope methods have also shown a direct effect of the reduced maternal hepcidin concentration on the increase in placental iron transfer (96). Moreover, it has been shown that the iron status of the fetus correlates with the level of both fetal (96) and maternal hepcidin (97), albeit some studies have not proved the latter relationship (98). On the other hand, hepcidin levels in maternal blood and fetal circulation are not interrelated (96, 98). Nonetheless, lower hepcidin concentrations are evidenced in the mother and higher in the fetus (measurements from cord blood in the third stage of labor) (96, 98, 99).

Thus, decreasing hepcidin expression in pregnant women not only determines higher iron absorption from food but also increases the amount of iron delivered to the fetus from maternal circulation. Physiologically, both mechanisms are able to provide the appropriate amount of iron for the proper growth of the fetus. However, in the presence of iron deficiency in the mother before pregnancy, as in iron-deficiency anemia, the above adaptative mechanisms may transpire insufficient (100).

Fetal iron deficiency leads primarily to the birth defects of the central nervous system, which may manifest in learning difficulties, behavioral and hearing impairment, as well as dysfunction of the hypothalamic-pituitary axis and its subordinate endocrine glands (101, 102). Furthermore, the risk of low birth weight and premature delivery increases significantly (101). In murine experimental models, higher mortality and incidence of cardiovascular diseases, type 2 diabetes and obesity in offspring of mothers with iron-deficiency anemia have also been described (102).

Compared to healthy pregnant women, augmented levels of maternal hepcidin have also been noted in the pathology of pregnancy with an increased inflammatory component, *e.g.*, in preeclampsia or obese mothers (97, 103). The rise in hepcidin led to a significant reduction in iron inflow to the fetus and emphasized the role of inflammation as a hepcidin expression-inducing factor, also in pregnancy. The elevated concentration of maternal hepcidin is also explained by the higher concentration of pro-inflammatory IL-6 in mothers delivering naturally or as a result of emergency cesarean section, compared to elective cesarean section (98). According to some researchers, this relationship is reversed as early as three days after elective cesarean section, when the maternal hepcidin concentration exceeds its level in mothers after

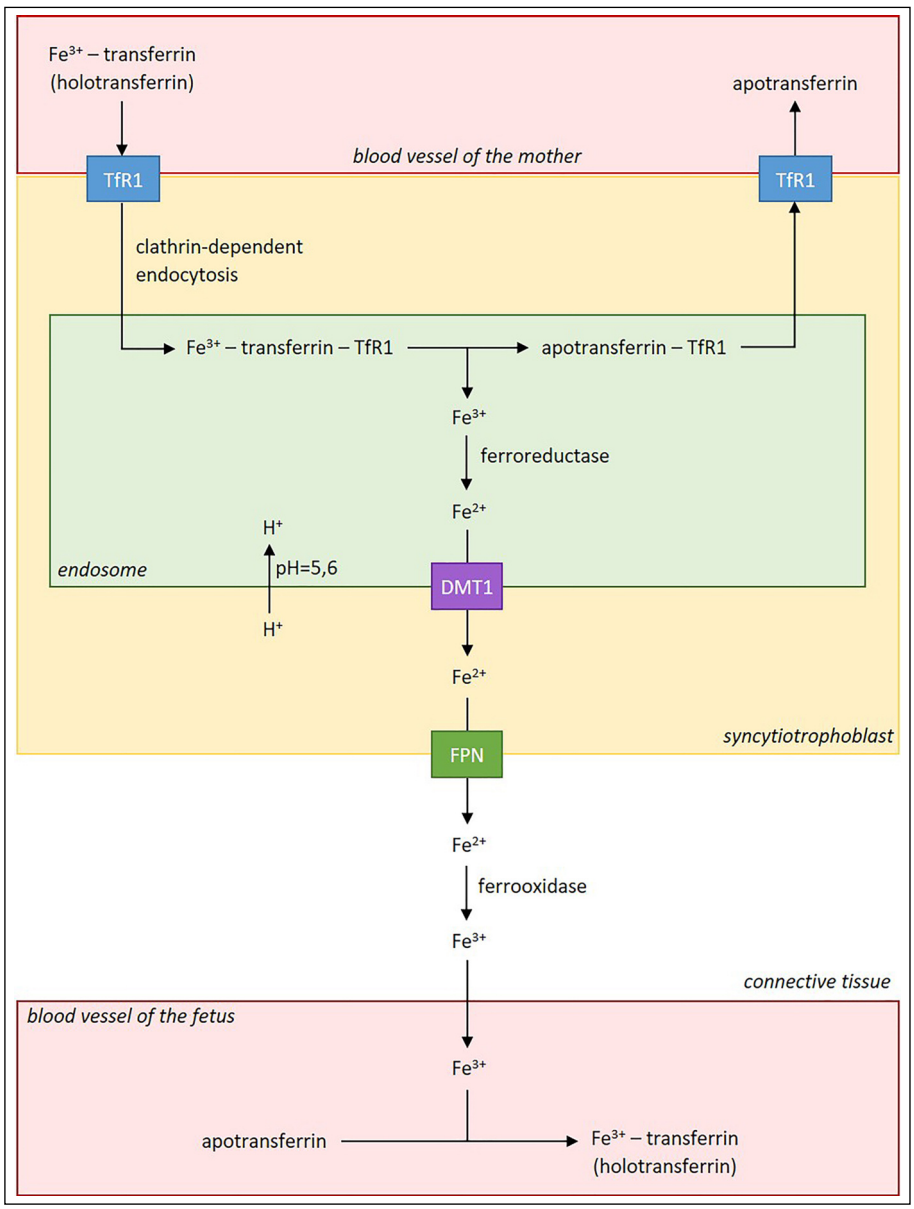

Fig. 4. Placental transfer of iron (description in the text).
DMT1, divalent metal transporter 1; FPN, ferroportin; TfR1, transferrin receptor 1.

vaginal delivery. Like abdominal surgeries, a cesarean section is accompanied by an inflammatory response that lasts longer than the temporary stress stimulus during vaginal delivery (104).

Iron deficiency was also observed in children of pregnant smoking mothers (105). Hypoxia accompanying smoking increased maternal erythropoiesis, thus inhibiting hepcidin synthesis. However, despite the daily supplementation with iron preparations by every woman and the best conditions of placental iron transfer provided by utterly low concentrations of maternal hepcidin, smoking led to a depletion of maternal iron resources, as well as significantly lower birth weight, limited height and head circumference of newborns. It appears that smoking may limit the absorption of iron in the apical part of the enterocytes or is associated with its utilization in ROS-producing reactions. Interestingly, no increased inflammatory parameters were found in the group of smoking mothers.

CONCLUSIONS

Hepcidin is a primary regulator of iron metabolism with additional antimicrobial properties resulting not only from its biological activity but also from the structure of the molecule. By promoting the degradation of ferroportin, hepcidin reduces the outflow of iron from the tissues and the intestinal iron absorption, and hence the concentration of iron in the blood and extracellular fluid. The expression of hepcidin is stimulated primarily by the decreasing reserves of systemic iron and inflammation, and inhibited in situations of excess iron or increased erythropoiesis, as well as in pregnancy. Thanks to this regulation, changes in hepcidin concentration are a compensatory mechanism aimed at restoring iron homeostasis in the body, increasing its supply in response to the growing needs of the developing fetus, or defense against pathogens. However, in disease states associated with the non-infectious stimulation of an inflammatory response, an elevation in hepcidin production is undesirable as it leads to iron deficiency and its numerous consequences.

Abbreviations: Arg, arginine; Asp, aspartic acid; BMP6, bone morphogenetic protein 6; BMPR, bone morphogenetic protein receptor; CKD, chronic kidney disease; Cp, ceruloplasmin; Cys, cysteine; Dcytb, duodenal cytochrome B; DMT1, divalent metal transporter 1; FPN, ferroportin; GDF15, growth/differentiation factor 15; Gly, glycine; HEPH, hephaestin; HFE, human homeostatic iron regulator protein; HIFs, hypoxia-inducible factors; HIF2α, hypoxia-inducible factor 2α; His, histidine; HJV, hemojuvelin; IL-6, interleukin 6; IL-6R, interleukin 6 receptor; IRP1, iron regulatory protein 1; IRP2, iron regulatory protein 2; Ile, isoleucine; JAK-STAT3, the JAK-STAT3 pathway; Lys, lysine; MCP1, monocyte chemoattractant protein-1; MDS, myelodysplastic syndromes; Met, methionine; MT-2, matriptase 2; Neo, neogenin; Phe, phenylalanine; Pro, proline; ROS, reactive oxygen species; Ser, serine; sHJV, soluble hemojuvelin; SMAD, the SMAD pathway; TfR1, transferrin receptor 1; TfR2, transferrin receptor 2; Thr, threonine; TIBC, total iron binding capacity; TMPRSS6, transmembrane serine protease 6; TWSG1, twisted gastrulation 1.

Authors' contribution: M. Wojciechowska and O.W. Wisniewski contributed equally to this work.

Source of funding: This study has not been supported by any funding.

Acknowledgements: The authors thank Ms. Edeth M.G. Edwin for English language editing.

Conflict of interests: None declared.

REFERENCES

1. Nicolas G, Chauvet C, Viatte L, *et al.* The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002; 110: 1037-1044.
2. Kwiecien S, Jasnos K, Magierowski M, *et al.* Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress - induced gastric injury. *J Physiol Pharmacol* 2014; 65: 613-622.
3. Krause A, Neitz S, Magert HJ, *et al.* LEAP-1 a novel highly disulfide-bonded peptide, exhibits antimicrobial activity. *FEBS Lett* 2000; 480: 147-150.
4. Park CH, Valore EV, Waring AJ, T Ganz. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem* 2001; 276: 7806-7810.
5. Wolff F, de Verneuil H, Rucheton B, *et al.* Hepcidin: immunoanalytic characteristics. *Ann Biol Clin* (*Paris*) 2018; 76: 705-715.
6. Jordan JB, Poppe L, Haniu M, *et al.* Hepcidin revisited, disulfide connectivity, dynamics, and structure. *J Biol Chem* 2009; 284: 24155-24167.
7. Hunter HN, Fulton DB, Ganz T, Vogel HJ. The solution structure of human hepcidin, a peptide hormone with antimicrobial activity that is involved in iron uptake and hereditary hemochromatosis. *J Biol Chem* 2002; 277: 37597-37603.
8. Kulaksiz H, Theilig F, Bachmann S, *et al.* The iron-regulatory peptide hormone hepcidin: expression and cellular localization in the mammalian kidney. *J Endocrinol* 2005; 184: 361-370.
9. Liu XB, Nguyen NB, Marquess KD, Yang F, Haile DJ. Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. *Blood Cells Mol Dis* 2005; 35: 47-56.
10. Kulaksiz H, Fein E, Redecker P, Stremmel W, Adler G, Cetin Y. Pancreatic beta-cells express hepcidin, an iron-uptake regulatory peptide. *J Endocrinol* 2008; 197: 241-249.
11. Bekri S, Gual P, Anty R, *et al.* Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. *Gastroenterology* 2006; 131: 788-796.
12. Tabbah SM, Buhimschi CS, Rodewald-Millen K, *et al.* Hepcidin, an iron regulatory hormone of innate immunity, is differentially expressed in premature fetuses with early-onset neonatal epsis. *Am J Perinatol* 2018; 35: 865-872.
13. Preza GC, Ruchala P, Pinon R, *et al.* Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. *J Clin Invest* 2011; 121: 4880-4888.
14. Bansal SS, Halket JM, Bomford A, *et al.* Quantitation of hepcidin in human urine by liquid chromatography-mass spectrometry. *Anal Biochem* 2009; 384: 245-253.
15. Donovan A, Lima CA, Pinkus JL, *et al.* The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab* 2005; 1: 191-200.
16. Zhang DL, Hughes RM, Ollivierre-Wilson H, Ghosh MC, Rouault TA. A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. *Cell Metab* 2009; 9: 461-473.
17. Nemeth E, Tuttle MS, Powelson J, *et al.* Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; 306: 2090-2093.
18. Mena NP, Esparza A, Tapia V, Valdes P, Nunez MT. Hepcidin inhibits apical iron uptake in intestinal cells. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: 192-198.

19. Gunshin H, Allerson CR, Polycarpou-Schwarz M, *et al.* Iron-dependent regulation of the divalent metal ion transporter. *FEBS Lett* 2001; 509: 309-316.
20. Ganz T, Nemeth E. Hepcidin and iron homeostasis. *Biochim Biophys Acta* 2012; 1823: 1434-1443.
21. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. *Cell Metab* 2009; 9: 152-164.
22. Brasse-Lagnel C, Karim Z, Letteron P, Bekri S, Bado A, Beaumont C. Intestinal DMT1 cotransporter is down-regulated by hepcidin via proteasome internalization and degradation. *Gastroenterology* 2011; 140: 1261-1271.
23. Michels K, Nemeth E, Ganz T, Mehrad B. Hepcidin and host defense against infectious diseases. *PLoS Pathog* 2015; 11: e1004998. doi: 10.1371/journal.ppat.1004998
24. Huang YH, Yang YL, Tiao MM, Kuo HC, Huang LT, Chuang JH. Hepcidin protects against lipopolysaccharide-induced liver injury in a mouse model of obstructive jaundice. *Peptides* 2012; 35: 212-217.
25. De Domenico I, Zhang TY, Koening CL, *et al.* Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. *J Clin Invest* 2010; 120: 2395-2405.
26. Zeng C, Chen Q, Zhang K, Chen Q, Song S, Fang X. Hepatic hepcidin protects against polymicrobial sepsis in mice by regulating host iron status. *Anesthesiology* 2015; 122: 374-386.
27. Chen QX, Song SW, Chen QH, *et al.* Silencing airway epithelial cell-derived hepcidin exacerbates sepsis induced acute lung injury. *Crit Care* 2014; 18: 470. doi: 10.1186/s13054-014-0470-8
28. van Eijk LT, Kroot JJ, Tromp M, van der Hoeven JG, Swinkels DW, Pickkers P. Inflammation-induced hepcidin-25 is associated with the development of anemia in septic patients: an observational study. *Crit Care* 2011; 15: R9. doi: 10.1186/cc9408
29. Arezes J, Jung G, Gabayan V, *et al.* Hepcidin-induced hypoferremia is a critical host defense mechanism against the siderophilic bacterium Vibrio vulnificus. *Cell Host Microbe* 2015; 17: 47-57.
30. Robins-Browne RM, Prpic JK. Effects of iron and desferrioxamine on infections with Yersinia enterocolitica. *Infect Immun* 1985; 47: 774-779.
31. Portugal S, Carret C, Recker M, *et al.* Host-mediated regulation of superinfection in malaria. *Nat Med* 2011; 17: 732-737.
32. Wang HZ, He YX, Yang CJ, Zhou W, Zou CG. Hepcidin is regulated during blood-stage malaria and plays a protective role in malaria infection. *J Immunol* 2011; 187: 6410-6416.
33. de Mast Q, Nadjm B, Reyburn H, *et al.* Assessment of urinary concentrations of hepcidin provides novel insight into disturbances in iron homeostasis during malarial infection. *J Infect Dis* 2009; 199: 253-262.
34. Casals-Pascual C, Huang H, Lakhal-Littleton S, *et al.* Hepcidin demonstrates a biphasic association with anemia in acute Plasmodium falciparum malaria. *Haematologica* 2012; 97: 1695-1698.
35. Olakanmi O, Schlesinger LS, Britigan BE. Hereditary hemochromatosis results in decreased iron acquisition and growth by Mycobacterium tuberculosis within human macrophages. *J Leukoc Biol* 2007; 81: 195-204.
36. Gomes-Pereira S, Rodrigues PN, Appelberg R, Gomes MS. Increased susceptibility to mycobacterium avium in hemochromatosis protein HFE-deficient mice. *Infect Immun* 2008; 76: 4713-4719.
37. Johnson EE, Sandgren A, Cherayil BJ, Murray M, Wessling-Resnick M. Role of ferroportin in macrophage-mediated immunity. *Infect Immun* 2010; 78: 5099-5106.
38. Chlosta S, Fishman DS, Harrington L, *et al.* The Iron efflux protein ferroportin regulates the intracellular growth of Salmonella enterica. *Infect Immun* 2006; 74: 3065-3067.
39. Nairz M, Theurl I, Schroll A, *et al.* Absence of functional Hfe protects mice from invasive Salmonella enterica Serovar Typhimurium infection via induction of lipocalin-2. *Blood* 2009; 114: 3642-3651.
40. Paradkar PN, De Domenico I, Durchfort N, Zohn I, Kaplan J, Ward DM. Iron depletion limits intracellular bacterial growth in macrophages. *Blood* 2008; 112: 866-874.
41. Ben-Othman R, Flannery AR, Miguel DC, Ward DM, Kaplan J, Andrews NW. Leishmania-mediated inhibition of iron export promotes parasite replication in macrophages. *PLoS Pathog* 2014; 10: e1003901. doi: 10.1371/journal.ppat.1003901
42. Maisetta G, Petruzzelli R, Brancatisano FL, *et al.* Antimicrobial activity of human hepcidin 20 and 25 against clinically relevant bacterial strains: effect of copper and acidic pH. *Peptides* 2010; 31: 1995-2002.
43. Ramos E, Kautz L, Rodriguez R, *et al.* Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology* 2011; 53: 1333-1341.
44. Shanmugam NK, Cherayil BJ. Serum-induced up-regulation of hepcidin expression involves the bone morphogenetic protein signaling pathway. *Biochem Biophys Res Commun* 2013; 441: 383-386.
45. Xia Y, Babitt JL, Sidis Y, Chung RT, Lin HY. Hemojuvelin regulates hepcidin expression via a selective subset of BMP ligands and receptors independently of neogenin. *Blood* 2008; 111: 5195-5204.
46. Andriopoulos B Jr, Corradini E, Xia Y, *et al.* BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat Genet* 2009; 41: 482-487.
47. Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Andrews NC. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. *Cell Metab* 2008; 7: 205-214.
48. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. *Cell Metab* 2009; 9: 217-227.
49. Niederkofler V, Salie R, Arber S. Hemojuvelin is essential for dietary iron sensing, and its mutation leads to severe iron overload. *J Clin Invest* 2005; 115: 2180-2186.
50. Lee DH, Zhou LJ, Zhou Z, *et al.* Neogenin inhibits HJV secretion and regulates BMP-induced hepcidin expression and iron homeostasis. Blood 2010; 115: 3136-3145. Erratum in: Blood 2010; 116: 151.
51. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. *Cell Metab* 2008; 8: 502-511.
52. Babitt JL, Huang FW, Xia Y, Sidis Y, Andrews NC, Lin HY. Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. *J Clin Invest* 2007; 117: 1933-1939.
53. Kautz L, Meynard D, Monnier A, *et al.* Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad7, Id1, and Atoh8 in the mouse liver. *Blood* 2008; 112: 1503-1509.
54. Salahudeen AA, Thompson JW, Ruiz JC, *et al.* An E3 ligase possessing an iron-responsive hemerythrin domain is a regulator of iron homeostasis. *Science* 2009; 326: 722-726.

55. Ivan M, Haberberger T, Gervasi DC, *et al.* Biochemical purification and pharmacological inhibition of a mammalian prolyl hydroxylase acting on hypoxia-inducible factor. *Proc Natl Acad Sci USA* 2002; 99: 13459-13464.
56. Nemeth E, Rivera S, Gabayan V, *et al.* IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004; 113: 1271-1276.
57. Rodriguez R, Jung C-L, Gabayan V, *et al.* Hepcidin induction by pathogens and pathogen-derived molecules is strongly dependent on interleukin-6. *Infect Imm* 2014; 82: 745-752.
58. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. *Blood* 2006; 108: 3204-3209.
59. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, Muckenthaler MU. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. *Blood* 2007; 109: 353-358.
60. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. *Blood* 2006; 108: 3730-3735.
61. Tanno T, Bhanu NV, Oneal PA, *et al.* High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nat Medic* 2007; 13: 1096-1101.
62. Tanno T, Porayette P, Sripichai O, *et al.* Identification of TWSG1 as a second novel erythroid regulator of hepcidin expression in murine and human cells. *Blood* 2009; 114: 181-186.
63. Peyssonnaux C, Zinkernagel AS, Schuepbach RA, *et al.* Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *J Clin Invest* 2007; 117: 1926-1932.
64. Zaritsky J, Young B, Wang HJ, *et al.* Hepcidin - a potential novel biomarker for iron status in chronic kidney disease. *Clin J Am Soc Nephrol* 2009; 4: 1051-1056.
65. Crownover BK, Covey CJ. Hereditary hemochromatosis. *Am Fam Physician* 2013; 87: 183-190.
66. Laine F, Jouannolle AM, Morcet J, *et al.* Phenotypic expression in detected C282Y homozygous women depends on body mass index. *J Hepatol* 2005; 43: 1055-1059.
67. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. *Blood* 2008; 112: 4292-4297.
68. Wallace DF, Summerville L, Crampton EM, Frazer DM, Anderson GJ, Subramaniam VN. Combined deletion of Hfe and transferrin receptor 2 in mice leads to marked dysregulation of hepcidin and iron overload. *Hepatology* 2009; 50: 1992-2000.
69. Wallace DF, Harris JM, Subramaniam VN. Functional analysis and theoretical modeling of ferroportin reveals clustering of mutations according to phenotype. *Am J Physiol Cell Physiol* 2010; 298: C75-C84.
70. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood* 2019; 133: 40-50.
71. Theurl I, Aigner E, Theurl M, *et al.* Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. *Blood* 2009; 113: 5277-5286.
72. Yacoub MF, Ferwiz HF, Said F. Effect of interleukin and hepcidin in anemia of chronic diseases. *Anemia* 2020; 2020: 3041738. doi: 10.1155/2020/3041738
73. Roy CN, Mak HH, Akpan I, Losyev G, Zurakowski D, Andrews NC. Hepcidin antimicrobial peptide transgenic mice exhibit features of the anemia of inflammation. *Blood* 2007; 109: 4038-4044.
74. den Elzen WP, de Craen AJ, Wiegerinck ET, Westendorp RG, Swinkels DW, Gussekloo J. Plasma hepcidin levels and anemia in old age. The Leiden 85-Plus Study. *Haematologica* 2013; 98: 448-454.
75. Mei S, Wang H, Fu R, *et al.* Hepcidin and GDF15 in anemia of multiple myeloma. *Int J Hematol* 2014; 100: 266-273.
76. Hohaus S, Massini G, Giachelia M, *et al.* Anemia in Hodgkin's lymphoma: the role of interleukin-6 and hepcidin. *J Clin Oncol* 2010; 28: 2538-2543.
77. Sonkar SK, Singh NK, Sonkar GK, *et al.* Association of hepcidin and anemia in early chronic kidney disease. *Saudi J Kidney Dis Transpl* 2019; 30: 315-324.
78. Elliott J, Mishler D, Agarwal R. Hyporesponsiveness to erythropoietin: causes and management. *Adv Chronic Kidney Dis* 2009; 16: 94-100. Erratum in: *Adv Chronic Kidney Dis* 2010; 17: 111.
79. Misian M, Baum E, Breborowicz A. N-acetylcysteine modulates effect of the iron isomaltoside on peritoneal mesothelial cells. *J Physiol Pharmacol* 2020; 71: 365-371.
80. Santini V, Girelli D, Sanna A, *et al.* Hepcidin levels and their determinants in different types of myelodysplastic syndromes. *PLoS One* 2011; 6: e23109. doi: 10.1371/journal.pone.0023109
81. Winder A, Lefkowitz R, Ghoti H, *et al.* Urinary hepcidin excretion in patients with myelodysplastic syndrome and myelofibrosis. *Br J Haematol* 2008; 142: 669-671.
82. Urbanowicz I, Stacherzak-Pawlik J, Mazur G, Wozniak M. Hepcidin in myelodysplastic syndromes. *Diagn Lab* 2010; 46: 395-401.
83. Origa R, Galanello R, Ganz T, *et al.* Liver iron concentrations and urinary hepcidin in beta-thalassemia. *Haematologica* 2007; 92: 583-588.
84. Fisher AL, Nemeth E. Iron homeostasis during pregnancy. *Am J Clin Nutr* 2017; 106: 1567S-1574S.
85. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. *Am J Clin Nutr* 2000; 72: 257S-264S.
86. Koenig MD, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. *Nutrients* 2014; 6: 3062-3083.
87. van Santen S, Kroot JJ, Zijderveld G, Wiegerinck ET, Spaanderman ME, Swinkels DW. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. *Clin Chem Lab Med* 2013; 51: 1395-1401.
88. Gyarmati B, Szabo E, Szalay B, *et al.* Serum maternal hepcidin levels 3 days after delivery are higher compared to those measured at parturition. *J Obstet Gynaecol Res* 2011; 37: 1620-1624.
89. Bastin J, Drakesmith H, Rees M, Sargent I, Townsend A. Localisation of proteins of iron metabolism in the human placenta and liver. *Br J Haematol* 2006; 134: 532-543.
90. Bialasek M, Kubiak M, Gorczak M, *et al.* Exploiting iron-binding proteins for drug delivery. *J Physiol Pharmacol* 2019; 70: 675-685.
91. Dautry-Varsat A, Ciechanover A, Lodish HF. pH and the recycling of transferrin during receptor-mediated endocytosis. *Proc Natl Acad Sci USA* 1983; 80: 2258-2262.
92. McArdle HJ, Lang C, Hayes H, Gambling L. Role of the placenta in regulation of fetal iron status. *Nutr Rev* 2011; 69: S17-22.
93. Chen H, Attieh ZK, Syed BA, *et al.* Identification of zyklopen, a new member of the vertebrate multicopper ferroxidase family, and characterization in rodents and human cells. *J Nutr* 2010; 140: 1728-1735.
94. Nicolas G, Bennoun M, Porteu A, *et al.* Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proc Natl Acad Sci USA* 2002; 99: 4596-4601.
95. Willemetz A, Lenoir A, Deschemin JC, *et al.* Matriptase-2 is essential for hepcidin repression during fetal life and

postnatal development in mice to maintain iron homeostasis. *Blood* 2014; 124: 441-444.
96. Young MF, Griffin I, Pressman E, *et al.* Maternal hepcidin is associated with placental transfer of iron derived from dietary heme and nonheme sources. *J Nutr* 2012; 142: 33-39.
97. Dao MC, Sen S, Iyer C, Klebenov D, Meydani SN. Obesity during pregnancy and fetal iron status: is hepcidin the link? *J Perinatol* 2013; 33: 177-181.
98. Rehu M, Punnonen K, Ostland V, *et al.* Maternal serum hepcidin is low at term and independent of cord blood iron status. *Eur J Haematol* 2010; 85: 345-352.
99. Van Santen S, de Mast Q, Luty AJ, Wiegerinck ET, Van der Ven AJ, Swinkels DW. Iron homeostasis in mother and child during placental malaria infection. *Am J Trop Med Hyg* 2011; 84: 148-151.
100. Basu S, Kumar N, Srivastava R, Kumar A. Maternal and cord blood hepcidin concentrations in severe iron deficiency anemia. *Pediatr Neonatol* 2016; 57: 413-419.
101. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr* 2007; 85: 614S-620S.
102. Slomka A, Zekanowska E, Piotrowska K, Kwapisz J. Iron metabolism and maternal-fetal iron circulation. *Postepy Hig Med Dosw* (*Online*) 2012; 66: 876-887.
103. Toldi G, Stenczer B, Molvarec A, *et al.* Hepcidin concentrations and iron homeostasis in preeclampsia. *Clin Chem Lab Med* 2010; 48: 1423-1426.
104. Park KH, Sawada T, Kosuge T, *et al.* Surgical inflammation induces hepcidin production after abdominal surgery. *World J Surg* 2012; 36: 800-806.
105. Chelchowska M, Ambroszkiewicz J, Gajewska J, Jabłonska-Glab E, Maciejewski TM, Oltarzewski M. Hepcidin and iron metabolism in pregnancy: correlation with smoking and birth weight and length. *Biol Trace Elem Res* 2016; 173: 14-20.

Received: March 28, 2021
Accepted: February 26, 2021

Author's address: Oskar Wojciech Wisniewski, Students' Scientific Society, Poznan University of Medical Sciences, 10 Fredry Street, 61-701 Poznan, Poland.
e-mail: wisniewski.oskar@outlook.com