

The Influence of Iron on Bone Metabolism Disorders: A Systematic Review

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ABSTRACT

Iron is a necessary trace element in the human body, and it participates in many physiological processes. Disorders of iron metabolism can cause lesions in many tissues and organs, including bone. Recently, iron has gained attention as an independent factor influencing bone metabolism disorders, especially the involvement of iron overload in osteoporosis. The aim of this review was to summarize the findings from clinical and animal model research regarding the involvement of iron in bone metabolism disorders and to elucidate the mechanisms behind iron overload and osteoporosis. Lastly, we aimed to describe the association between bone loss and iron overload. We believe that a reduction in iron accumulation can be used as an alternative treatment to assist in the treatment of osteoporosis, to improve bone mass, and to improve the quality of life of patients.

ABBREVIATIONS

DMT1: Divalent Metal Transporter 1; HJV: Haemojuvelin; FPN: Ferroportin; BMP: Bone Morphogenetic Protein; PD: Parkinson's Disease; AD: Alzheimer's Disease; ROS: Reactive Oxygen Species; MSC: Mesenchymal Stem Cell; GDF: Growth Differentiation Factor; AMH: Anti-Mullerian Hormone; MMP: Matrix Metalloproteinase; BMPR: Bone Morphogenetic Protein Receptor; M-CSF: Macrophage Colony-Stimulating Factor; Hh: Hedgehog; Ptch: Patched-1; Smo: Smoothed; Ihh: Indian Hedgehog; Dsh/Dvl: Dishevelled; GSK-3 β : Glycogen Synthetase Kinase 3 β ; APC: Adenomatous Polyposis Coli; TNF: Tumor Necrosis Factor; TRAF: TNF Receptor Linker; PI3K: Phosphatidylinositol 3-Kinase; BMD: Bone Mineral Density; HH: Hereditary Hemochromatosis; TfR2: Transferrin Receptor 2; Hbs: Sickle Hemoglobin; HbA: Normal Hb; NAC: N-acetyl-L-cysteine; DFO: Desferoxamine

INTRODUCTION

Iron is a necessary trace element in the human body, and it participates in many physiological processes such as oxygen transportation, electron transportation in the respiratory chain, and production of metabolism-related coenzymes [1-3]. Iron metabolism is affected by intaking, transporting, distribution, storage and excreting [4]. Iron is obtained from nutritional sources. Thus, its quantity is affected by the level of nutritional intake, which is also crucial for the regulation of iron metabolism. Iron metabolism disorders can cause lesions in many tissues and organs, such as the heart, kidneys, liver, and nerve tissue [5-8]. Recent studies have found that iron metabolism disorders may also be directly related to bone metabolism defects [9-13]. Both iron deficiency and iron overload in the body can result in bone metabolism disorders, and

there is an association between iron overload and osteoporosis. Furthermore, research has shown that the role of iron in osteoporosis cannot be ignored. In this article, we review recent literature on iron metabolism and the opinions on bone loss associated with iron overload from teams within our institution.

NUTRITION AND IRON METABOLISM

As a major source of nutrition, food is also the main source of iron intake. Animal-derived foods in daily diets, such as fish, meat, and poultry, contain a large amount of heme iron, a rich source of iron. This part of iron absorption is not affected by other factors, but also promotes absorption of non-heme iron, which accounts for about 40% of the total iron intake in this process [14]. Cereals are rich in non-heme iron and are a staple food for many people in the world, making cereals one of the main sources of iron. Because cereals can be processed in a solid form, they are often fortified with iron [15]. In addition, when beer was brewed using iron cans, the acidity of the beer allowed a large amount of iron to enter the wine, and the iron intake of the consumer was increased [16]. Even with the current improvement in wine-making technology, the iron content in wine is reduced. However, alcohol can still promote the absorption of iron, evident by the increased iron levels of many alcohol consumers [17, 18]. Similarly, cooking food in iron skillets and pots can also increase the iron content of food by 3-400% [19]. Interestingly, some herbs, such as mint leaves, mint roots, black bean roots, garbanzo beans, and gold roots, have a higher iron content than other foods [19]. Details regarding the nutritional sources of iron and its involvement in many physiological processes are not described in this article, but can be obtained from other reviews.

RELATED DISEASES CAUSED BY IRON METABOLISM DISORDERS

The iron transport process and regulation mechanism *in vivo*

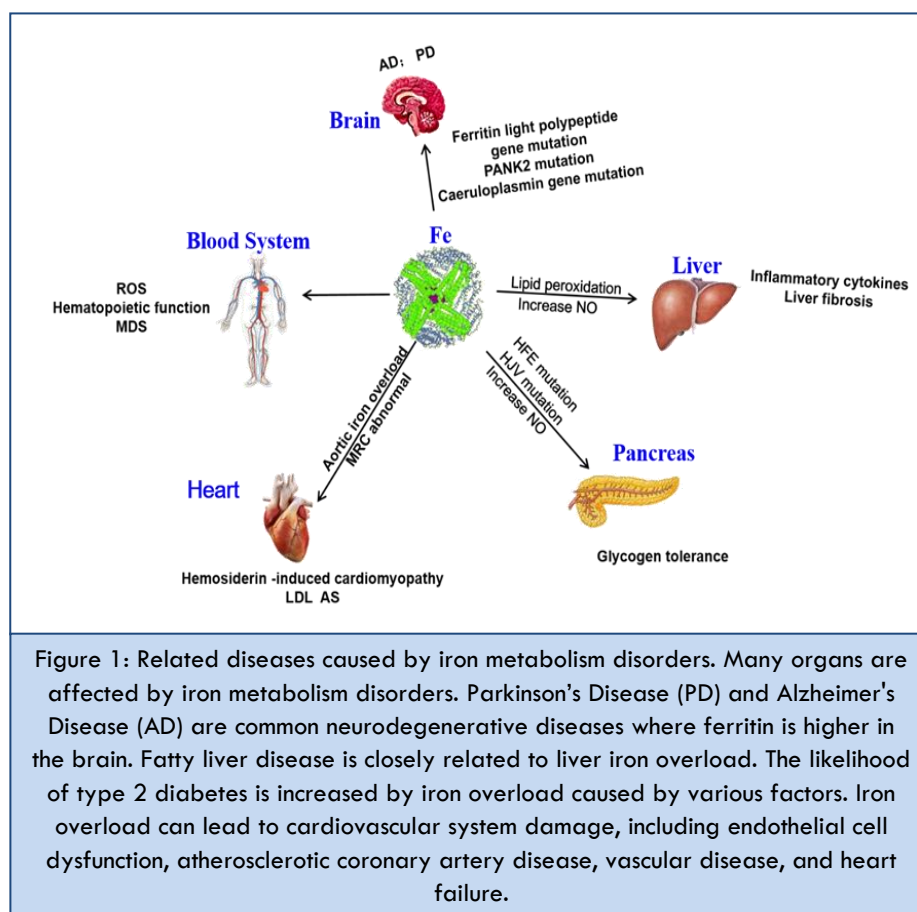
The absorption and homeostasis of iron in the body is precisely regulated, and the absorption of iron is mainly accomplished through the intestinal tract. Divalent Metal Transporter 1 (DMT1) in the small intestinal cell membrane transfers the ferrous particles into the cytosol [20]. DMT1 can be used not only as a carrier transporter for small-intestine chorion, but also as a carrier of iron in many other tissues in the body that are

involved in intracellular metastasis of iron. There are two pathways for the iron that enters the intestinal mucosal cells. One is to enter the blood circulation, and the other is to bind to ferritin in the cytosol as storage iron. The ferrous particles absorbed into the blood are oxidized by caeruloplasmin to ferric ions, which bind to plasma transferrin and are transported to tissue cells.

The homeostasis of iron, including iron absorption, transport, release, and storage, is primarily regulated by protein, transporters, and hormones. In recent years, with the continuous development of molecular biology technology, gene knockout and over-expression technology have become more and more sophisticated. Scientists have found that many genes and proteins, including Hemojuvelin (HJV), hepcidin, and Ferroportin (FPN), are involved in regulating the homeostasis of iron [21]. Hepcidin is a hormone secreted mainly by the liver. It is encoded by the hepcidin gene, which plays a central role in the process of negative regulation of iron. The action of hepcidin is accomplished by FPN1. FPN1 is expressed in the cell membrane and transports intracellular iron out of the cells. When the iron concentration is increased, hepcidin binds to FPN1, which induces internalization and degradation. Thus, the iron in the cells cannot be transported out, and this increases the absorption of iron by the cells. As a result, intracellular iron is increased and the iron concentration is maintained. The regulation of hepcidin has been demonstrated in many studies, and it is mainly regulated by the HJV-Bone Morphogenetic Protein (BMP)/SMAD signaling pathway, Tmprss6, inflammation, and interleukin (IL)-6 [11,22,23]. The details are described in other review articles.

Tissue and organ diseases caused by iron metabolism disorders

Disorders of iron metabolism include both iron deficiency and iron overload in the body. Iron deficiency is mainly caused by insufficient intake of iron or excessive iron loss. Iron overload is mainly caused by iron regulation of metabolic disorders or the termination of iron emission pathways. Iron deficiency or iron overload cause many diseases and even organ lesions, such as brain neurological diseases, liver disease, diabetes, heart damage, and osteoporosis (Figure 1).



PD, Parkinson's disease; AD, Alzheimer's disease; ROS, Reactive Oxygen Species; Fe, Ferritin; MDS, ; MRC, ; HJV, Haemojuvelin; LDL, Low-Density Lipoprotein.

OVERVIEW OF BONE METABOLISM

Bone is a highly metabolic organ. It consists mainly of bone cells, collagen fibers, and matrices. It has certain morphology. It is surrounded by periosteum and contains bone marrow and abundant blood vessels, lymphatic vessels, and nerve tissue. Although bone does not grow during adulthood, it still has a strong ability to repair and regenerate, and this is called bone re-modelling.

Osteogenesis

Types of bone formation can be divided into endochondral ossification and intramembranous ossification. Endochondral ossification can be seen in trunk bones, limb long bones, and parts of the skull base. Its mode of growth and development is based on the pre-formed cartilage, which gradually calcifies and is replaced by bone tissue. In the human body, only a few bones are formed by intramembranous ossification, which has no cartilage template in the process of development. It occurs

mainly in some flat bones, such as the parietal bone and frontal bone. Initially, the connective tissue membrane and mesenchyme form the original embryonic form of bone, then the intramembranous osteogenesis begins at one or more ossification centers, and osteoblasts appear. The osteoblasts deposit materials to form parallel dense bone plates.

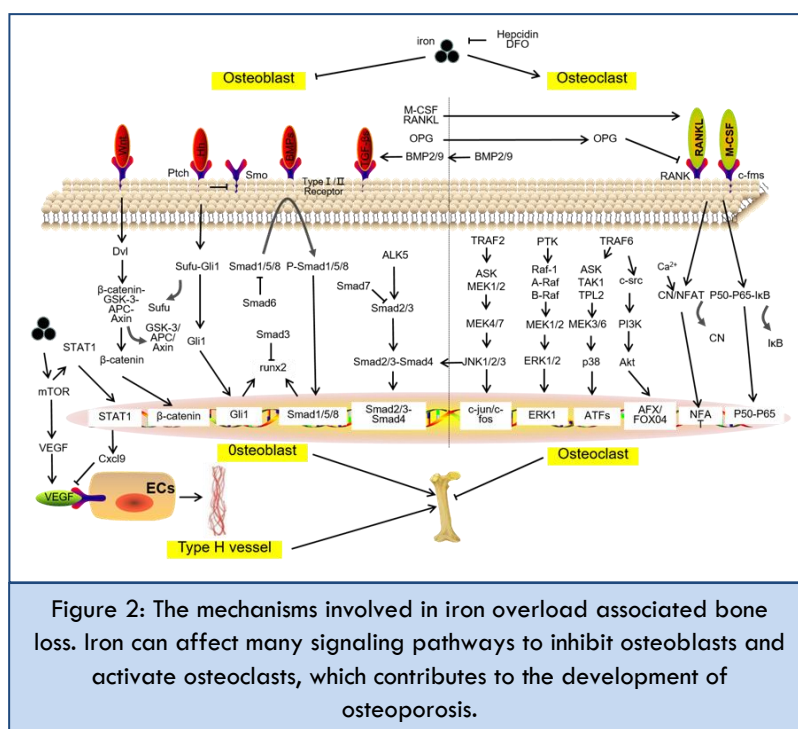
Bone tissue cell types

Bone is mainly composed of bone cells and interstitial bone cells. The cells in bone tissue can be divided into three types according to cell function: osteoclasts, osteoblasts, and osteocytes. During the process of bone destruction and remodeling, these three types of cells work together to absorb old bone and generate new bone. Osteoblasts are different from progenitor cells, which are also known as pre-osteoblasts. Mature osteoblasts can synthesize and secrete bone matrices to increase bone strength and toughness. They are the cells that form bone tissue, participate in bone calcification, and regulate

the amount of calcium and phosphorus in bone and other tissues. Osteoblasts often exist on the surface of new bone and are arranged in a single layer. Osteoblasts are embedded into osteoid tissue and transformed into osteocytes. Osteoclasts are giant multinucleated cells that play a major role in bone resorption. Normal bone metabolism depends on the dynamic balance between osteoclast absorption and osteoblast bone formation. The main functions of osteoclasts are bone resorption, regulation of the activity of osteoblasts, and participation in the migration of hematopoietic stem cells. Osteocytes are the primary cells in mature bone tissue. They are derived from osteoblasts and located in bone lacunae and the protrusions in bone canaliculi. Osteocytes can produce new matrices, change crystal fluid, and maintain calcium and phosphorus depositions, which are released in a stable state in order to maintain the blood calcium balance. Osteocytes play an important role in bone resorption and bone formation, and are the primary cells involved in the maintenance of mature bone metabolism. The balance of bone metabolism and bone is maintained mainly by osteoblasts and osteoclasts. Under normal physiological conditions, the number and function of the two types of cells are in a real-time dynamic balance, thus

maintaining the balance of bone metabolism, which is more important for maintaining the normal renewal of bone. Once the balance is disturbed, this leads to an imbalance in bone homeostasis, resulting in osteopenia, and eventually osteoporosis.

Osteoblasts and bone formation: Osteoblasts originate from bone marrow mesenchymal stem cells (BMSCs). BMSCs are multifunctional stem cells founded by Frieden, a German pathologist, in 1968 [24]. They are located in muscle, bone and adipose tissue and can differentiate into osteoblasts, chondrocytes, and adipocytes. Subsequent studies have revealed that MSCs are the main source of osteoblast differentiation during bone tissue maturation and remodeling. They gradually differentiate into osteoblast progenitor cells, preosteoblasts, and osteoblasts. The differentiation process of osteoblasts can be divided into several stages, including proliferation and deposition in the extracellular matrix. The process of differentiation of BMSCs into osteoblasts involves multiple signaling pathways and is regulated by a series of cytokines, including Transforming Growth Factor (TGF)- β , BMP, Hedgehogs, and Wnt signaling pathway (Figure 2). The details of this can be found in other reviews.



DFO: Desferoxamine; M-CSF: Macrophage Colony-Stimulating Factor; VEGF: Vascular Endothelial Growth Factor; ECs: Endothelial Cells

Osteoclasts and bone resorption: It is now generally accepted that osteoclasts are derived from CD34-positive bone marrow hematopoietic stem cells, which are prerequisite cells for mononuclear/macrophage cell lines in the bone marrow. Undifferentiated bone marrow hematopoietic stem cells are transformed into myeloid precursor cells by some early factors such as transcription factor PU.1 and Melanocyte Inducing Transcription Factor (MITF), which combination with Colony-Stimulating Factor (M-CSF) and its receptor c-Fos to transformed osteoclast precursor cell. Under the action of NF- κ B and NFATc1 transcription factors, osteoclast precursor cells are activated and exposed to RANKL stimulation through osteoclast differentiation and maturation factors and multiple signaling pathways, and they are finally differentiated and developed to form a fusion of mature osteoclasts. Several important signaling pathways, such as OPG/RANKL/RANK signaling pathway, NF- κ B classical signaling pathway, C-SRC-PI3K-AKT signaling pathway, Mitogen-Activated Protein Kinases (MAPK) signaling pathway, and calcineurin/nuclear factor of activated T cells (CN/NFAT) signaling pathway, are involved in osteoclast differentiation (Figure 2), the details of which are presented in a previous review.

CLINICAL STUDIES ON IRON METABOLISM DISORDERS AND RELATED BONE METABOLISM DISORDERS

Thalassemia

Thalassemia is a hereditary hemolytic anemia disease. The anemia, the pathological condition resulting from the loss or deficiency of one or more globin chains in hemoglobin, is due to genetic defects. Patients with thalassemia are treated with regular blood transfusions to maintain adequate hemoglobin levels. The human body lacks a mechanism for excreting excess iron, and repeated blood transfusions cause iron overload in these patients. Excess iron will deposit in organs of the body, especially the pancreas, liver, pituitary glands, and heart [24]. Despite iron chelation therapy, the main manifestation of this disease is iron overload. Osteoporosis, and its resulting fractures, are one of the common complications of thalassemia. Baldini reported bone mass assessment in 70 patients with thalassemia (37 males and 33 females), 53 of whom developed osteoporosis, showing symptoms of reduced bone mass [25]. In a cross-sectional study of 80 patients with thalassemia, it was revealed that serum ferritin and cardiac

iron load (T2*) were inversely correlated with Bone Mineral Density (BMD). The study revealed that serum ferritin and cardiac iron levels were good indicators of BMD in patients with thalassemia [26]. In another five-year population study on thalassemia chelation therapy, treatment with iron chelators instead of the other four chelator treatments, had a significant effect on BMD improvement [27]. The above studies show that the main complication in patients with thalassemia is osteoporosis, and patients have significant bone metabolism problems. Furthermore, osteoporosis has a significant correlation with serum ferritin levels, and iron chelation therapy can improve the symptoms of osteoporosis more than other chelator treatments.

Hereditary hemochromatosis

Hereditary Hemochromatosis (HH) is an autosomal hereditary disease that is a congenital iron metabolism disorder of the tissues of the body. The most common HH is caused by mutations in the Cys282Tyr HFE gene in the Caucasian population [28]. Subsequently, a large number of clinical studies have found that mutations in any one of the HJV, Transferrin Receptor 2 (TfR2), ferritin, and hepcidin genes can also result in HH, and different gene mutations exhibit different clinical symptoms [29-32]. A mouse model of hemochromatosis was constructed using HFE knockout mice. The results showed that the iron-overloaded mice had a reduced bone formation rate, resulting in damage to the bone microstructure [33]. Other studies have shown that iron-rich diets do not contribute to bone loss in wild-type male rats, leading to an osteoporosis phenotype in HFE knockout mice [34]. Other studies have shown that iron overload caused by hemochromatosis affects bone metabolism by affecting the S1P/S1PR and Wnt signalling pathways [35,36].

Sickle cell anemia

Sickle cell anemia is an autosomal dominant Hemoglobin (Hb) disease. Glutamic acid, which is the sixth amino acid of the β -peptide chain, is replaced by proline to form sickle hemoglobin (HbS), which replaces normal Hb (HbA). Clinical manifestations of chronic hemolytic anemia including the susceptibility to infections and recurrent pain crises lead to chronic tissue ischemia, ultimately leading to organ damage. Sickle cell anemia is an inherited hemoglobin disorder. Studies have found that low bone mass is prevalent in children with sickle cell

anemia, and bone mass is inversely related to hemoglobin [37]. Sickle cell anemia also has an effect on the cementum. The calcification of the pulp and the external absorption of the root are the most common changes in the dentin size and the frequency of changes in morphology are higher in the periapical area and root [38]. Another clinical study showed that in patients with sickle cell anemia, more than 65% of men and 65.2% of women had osteoporosis, with a high rate of lumbar osteoporosis [39]. A retrospective study analyzed the prevalence and predisposing factors of low BMD in adult patients with sickle cell anemia. Three patients with lumbar spine, femoral neck, and distal radius fractures were treated with double x-ray absorptiometry. A total of 79.6% of patients had abnormal bone density, especially in the lumbar vertebrae. BMD abnormalities are associated with lower body mass index, lower hemoglobin levels, and higher ferritin levels [40].

Post-menopausal osteoporosis

With increasing age, iron accumulates in the human body (the iron content stored in the body is reflected by serum ferritin), especially after menopause in women. This is due to the decrease in uterine blood discharge. The iron in the body increases rapidly, with the average ferritin value rising to 106 ng/mL, which is twice the concentration of iron in women who are still menstruating [41]. In 2006, Weinberg first proposed the idea that iron is a risk factor for osteoporosis [42]. Since then, more and more studies have shown that iron accumulation is directly related to osteoporosis, especially to post-menopausal osteoporosis. In 2012, Kim et al. conducted a three-year large-scale clinical study of 1,729 subjects, including 789 middle-aged men and 940 post-menopausal women. The results showed that the prevalence of vertebral fractures in post-menopausal women was related to serum ferritin levels, whereas this relationship did not exist in male subjects [43]. In addition, Kim et al. also found that in healthy individuals, elevated serum ferritin levels were positively correlated with the rate of bone loss. Furthermore, elevated ferritin levels were most pronounced in women over 45 years of age [44]. Our findings also support these observations. Xu et al. found that ferritin levels in elderly women with hip fractures were significantly higher than those in older women who had

not experienced fractures, and in these two groups, BMD and ferritin content in the body were negatively related [45].

MECHANISM OF THE EFFECT OF IRON OVERLOAD ON BONE METABOLISM

Iron is an indispensable trace element in the human body, but iron accumulation is an independent risk factor for osteoporosis [42,43]. Studies on the effects of iron accumulation on bone metabolism have been carried out at the cellular level and individual level (studies involving mouse and zebrafish models and clinical studies on humans have been described above). These studies have helped to reveal the mechanisms by which iron overload causes osteopenia or osteoporosis (Figure 2).

Research in animal models

Mouse research: The effect of iron overload on mice was established as early as 2010. By injecting iron dextran (1 g/kg/wk) into C57/BL6 mice for 2 months, it was found that the trabecular bone and cortical bone of the iron-overload mice were significantly thinner, and the concentrations of IL6 and Tumor Necrosis Factor Alpha (TNF α) were increased, resulting in an increased production of ROS. These studies also found that antioxidant N-Acetyl-L-Cysteine (NAC) can rescue this phenotype (Figure 2) [46]. In our research, we injected FAC using the same approach, to construct a model of iron overload in mice, to explore the mechanism behind bone loss after iron-overload. Our results showed that in the absence of estrogen, iron can deepen the damage to bone, and osteoclasts play an important role in this process [47]. Further studies have shown that ROS, produced by iron overload, promotes osteoclast differentiation through the NF- κ B signaling pathway [48]. In addition, we found that the H-type blood vessel is a type of blood vessel specifically expressed by bone tissue, and its abundance is an indicator of bone mass [49]. Iron overload can inhibit bone formation and angiogenesis by increasing the mammalian target of rapamycin. Rapamycin is used to improve blood vessels and bone mass (Figure 2) [50]. In addition to the exogenous iron overload model, studies also found that HFE and hepcidin knockout mice exhibited significant endogenous iron overload, and HFE knockout mice also developed hemochromatosis [51-53]. In HFE knockout mice, there was a low bone mass and bone microstructural disorder similar to the osteoporotic phenotype in humans [51]. Hepcidin is a major hormone, which functions to regulate iron metabolism. The

knock-out of hepcidin also leads to a mouse model of endogenous iron overload. In 2014, our teams found that iron content and ferritin concentrations were significantly elevated in hepcidin knockout mice. The bone density and microstructure of bone changed significantly and the osteogenesis activity related to gene expression decreased [52]. It has recently been found that in hepcidin knocked out mice, the activity of ROS is increased, and the function of osteoblasts is weakened [53].

Zebrafish research: Zebrafish is a very good biological model for use in biomedical research, especially research on bone development and bone metabolism. In our research group, in 2014, the iron overloaded zebrafish model was first constructed, and iron overload inhibited the expression of the osteogenic marker gene (*runx2*, *gblap*, *spp1*, *alpl*, and *acp5b*). Desferrioxamine (DFO), a chelating agent for iron, has a therapeutic effect on the inhibition of bone development caused by zebrafish iron overload [54]. Using MO technology, we knocked down the *hepcidin* gene of zebrafish and found that it resembled the osteoporosis phenotype of mice, similar to the mouse endogenous iron overload model [55]. We also found that zebrafish mutants showed decreased bone mineralization, increased ROS, and decreased osteogenic-specific gene expression. Over expression of *hepcidin* also protected bone development delays caused by iron overload [56]. In addition, we also found that in the zebrafish FPN mutant, bone development showed a markedly low change [57]. The osteoporosis model caused by iron overload in zebrafish can also facilitate high-throughput screening of osteoporosis drugs in the future.

The Negative effect of iron overload on osteoblasts

Osteoblasts are one of the cells targeted for iron action, and a series of experiments have found that iron can inhibit osteoblast function and differentiation. In 2009, the fetal rat calvaria cultures cell line was used for 0–10 μM ferrous sulfate treatment. It was found that iron caused down-regulation of the Transferrin Receptor (TrfR) expression, up-regulation of ferritin expression, down-regulation of osteogenic differentiation, and decreased mineralization ability [58]. In other clinical models on osteoblast cell lines, it has been found that iron overload can increase the concentration of ferritin in cells, suggesting that ferritin plays a central role in the process of iron-induced

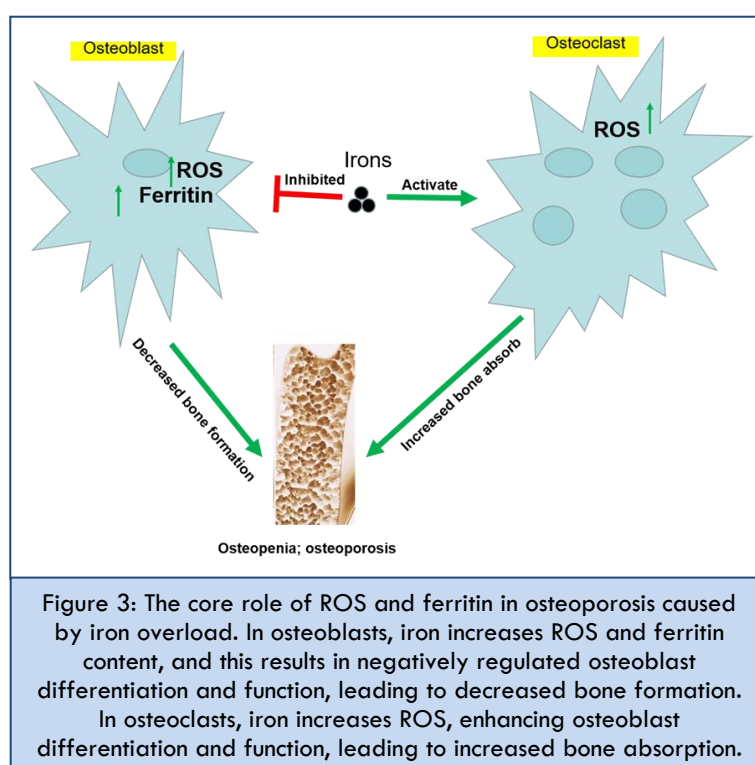
osteoblast inhibition (Figure 3) [59-61]. Ferritin has iron oxidase properties [62]. The inhibitory effect of iron on osteoblasts is due to the inhibition of osteogenic gene expression by iron oxidase activity; however, it is still unclear just how ferritin affects the expression of osteoblast-associated genes and inhibits osteogenic formation. Using the MG-63 osteoblast cell line, it was found that iron can reduce the activity of osteoblasts through HHIPL2 [63]. It is now believed that osteoblasts differentiate from MSCs, and iron can increase ROS levels and apoptosis in mouse mesenchymal stem cells by enhancement of the Caspase-3 dependent pathway [64]. During the differentiation of MSCs and mitochondrial complexes 1 and 2, NADPH oxidase is the main source of ROS, and ROS can initiate the inhibition and apoptosis of MSCs through signaling pathways such as Wnt, Hedgehog, and FOXO, thereby initiating adipogenesis, and chondrocytes of MSCs reduce differentiation into osteoblasts [65,66]. The iron chelator DFO can rescue the phenotype of the above osteoblasts and pre-osteoblasts [67,68]. In pre-osteoblasts, treatment with DFO enabled initiation of osteoblast differentiation in a time- and dose-dependent manner, and DFO was found to mediate osteogenic differentiation via WNT5a. This study also found that lowering basal level iron content with DFO seemed to accelerate the differentiation of osteoblasts, which suggests that low concentrations of iron may be a process or a necessary condition for osteoblast differentiation [68]. In summary, iron can inhibit osteoblast differentiation and mineralization to affect osteogenesis.

Iron overload activates osteoclast activity

Abnormal bone resorption is a key reason for the development of osteoporosis. Bone resorption involves an increased ability of osteoclast differentiation and activity. In patients with hemochromatosis, there is a negative correlation between TRAP and phosphoric acid concentration and bone density [69]. In RAW264.7 cells, iron can initiate RANKL expression and ROS production, and the latter is also a factor in promoting osteoclast differentiation [70]. After intraperitoneal injection of iron citrate in ovariectomized mice, the production of ROS increased in osteoclasts, the expression of RANKL-induced genes such as *Trap-5b*, *Ctsk* and *Mmp9* increased, the expression of phosphorylated I κ -B α increased, and the NF- κ B signaling pathway was activated, enhancing osteoclast

differentiation and bone resorption capacity, eventually leading to more serious bone loss in mice [47]. In our recent study, iron was able to influence osteoclast differentiation through the NF- κ B signaling pathway [48]. Previous studies have also found that osteoclasts have an increased demand for iron during differentiation. Increasing iron intake synergizes with PGC-1 β to up-regulate the number and function of mitochondria in osteoclasts. Increased mitochondria leads to more ROS, which enhances the expression of PGC-1 β and stimulates osteoclast differentiation [71]. However, if FPN1 is specifically knocked out in the bone marrow macrophages, it specifically binds to hepcidin. Iron accumulation occurs in the cells, and the expression of NFATc1 and phosphorylated ERK is

increased, which stimulates osteoclast differentiation and increases the number of TRAP-positive osteoclasts. The bone resorption capacity is enhanced, while the activity of mature osteoclasts is not affected by FPN1 knockout. It has been shown that endogenous iron elevation is a systemic regulatory process, and increasing intracellular iron levels can promote osteoclast differentiation [72]. In addition to the activation of osteoclasts by the above pathways, iron can also directly regulate the transcription of osteoclast-associated genes through the iron response element of the TRAP promoter region [73]. Thus, iron can promote the absorption of bone tissue by promoting osteoclast activation.



ROS: Reactive Oxygen Species

The core role of ROS in the process of bone loss caused by iron overload

Iron is a coenzyme in many intracellular metabolic processes, especially in the processes of the aerobic respiratory chain. In cells, aerobic respiration mainly occurs in the mitochondria. The ATP produced by this process is the main source of energy for each cell, and is also the main pathway and site for intracellular ROS production. In clinical patients with iron

overload, as well as in mouse models and cell lines, ROS are found to be significantly elevated by iron, and this increase is directly related to bone mass loss and abnormal bone metabolism [47,48,69-71,74,75]. Studies have also found that there is a significant increase in ROS in both iron overloaded osteoblasts and osteoclasts, suggesting that ROS may play a role in iron-affecting bone metabolism [48,53,70]. The way ROS acts on osteoblasts and osteoclasts are different (Figure

3). The results of the study found that iron-induced ROS can cause osteogenesis and osteoclasts to develop in the opposite direction. ROS can promote the differentiation and function of osteoclasts, but it can inhibit the differentiation and function of osteoblasts (Figure 3) [53,70]. The combination of these two functions enhances the loss of bone mass and aggravates the development of osteoporosis. ROS in osteoclasts can synergize with PGC-1 β to increase mitochondria and promote osteoclast differentiation and function [71]. In osteoblasts, ROS can suppress osteoblast genes expression and extracellular matrix mineralization [50,51,58,63]. The use of iron chelators (DFO) or inhibitors of ROS (NAC) can effectively remove ROS, thereby restoring the function of osteoblasts and enhancing the osteoblast's ability and bone mass [67,68]. Thus, reducing ROS is one of the most effective ways of treating osteoporosis caused by iron overload in the clinical setting (Figure 3).

CLINICAL APPLICATION OF IRON REDUCTION IN THE TREATMENT OF OSTEOPOROSIS

As research technology has progressed, the mechanism by which iron overload causes bone loss has been further clarified, but the detailed mechanisms require more research. The role of ROS in inhibiting osteoblasts and promoting osteoclasts, as well as the role of ferritin in iron inhibition of osteoblast differentiation and its key genes, requires more research. In post-menopausal women, the serum ferritin levels increase year by year, but bone density decreases with the increase of ferritin [76]. Thus, reducing the concentration of iron and ferritin in the body is one of the ways to prevent osteoporosis. In recent times, drugs such as deferoxamine, deferiprone, and deferasirox, have been tried in the clinical setting [77]. In addition, endogenous iron reduction, especially the enhancement of hepcidin expression, has also been shown to have a protective effect on osteoporosis. Furthermore, hepcidin can be used as a new target to improve the bone mass of patients with osteoporosis [52-56, 78]. Osteoporosis is the result of multiple factors in the process of bone metabolism. At present, intervention methods for osteoporosis have focused on the regulation of osteogenesis and osteoclast function, but this intervention does not help all patients. Reducing iron accumulation can be used as an individual treatment to assist in the treatment of osteoporosis, to improve bone mass, and to improve the quality of life of patients.

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