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Review

Iron and bones: effects of iron overload, deficiency and anemia treatments on bone

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Abstract

Iron is a vital trace element and exerts opposing effects on bone in both iron overload and iron deficiency situations. Remarkably, iron supplementation through intravenous infusion in patients with iron deficiency can also have detrimental effects on bone in special cases. The diverse mechanisms underlying these effects and their manifestations contribute to the complexity of this relationship. Iron overload impacts both bone resorption and formation, accelerating bone resorption while reducing bone formation. These effects primarily result from the direct action of reactive oxygen species (ROS), which influence the proliferation, differentiation, and activity of both osteoclasts and osteoblasts differently. This imbalance favors osteoclasts and inhibits the osteoblasts. Simultaneously, multiple pathways, including bone morphogenic proteins, RANK ligand, and others, contribute to these actions, leading to a reduction in bone mass and an increased susceptibility to fractures. In contrast, iron deficiency induces low bone turnover due to energy and co-factor deficiency, both of which require iron. Anemia increases the risk of fractures in both men and women. This effect occurs at various levels, reducing muscular performance and, on the bone-specific level, decreasing bone mineral density. Crucially, anemia increases the synthesis of the phosphaturic hormone iFGF23, which is subsequently inactivated by cleavage under physiological conditions. Thus, iFGF23 levels and phosphate excretion are not increased. However, in specific cases where anemia has to be managed with intravenous iron treatment, constituents—particularly maltoses—of the iron infusion suppress the cleavage of iFGF23. As a result, patients can experience severe phosphate wasting and, consequently, hypophosphatemic osteomalacia. This condition is often overlooked in clinical practice and is often caused by ferric carboxymaltose. Ending iron infusions or changing the agent, along with phosphate and vitamin D supplementation, can be effective in addressing this issue.

Keywords: iron overload, anemia, osteomalacia, FGF23, iron, bone

Lay Summary

Iron is a trace element that plays an important role in the human body and is crucial for maintaining the whole-body functions. However, both excess and insufficient levels of iron can have detrimental effects on bone. Iron overload overstimulates bone-resorbing cells, leading to increased bone breakdown. Moreover, iron overload inhibits the formation of bone-forming cells, thereby preventing the restoration of lost bone. These effects are driven by reactive oxygen species induced by iron overload. However, iron deficiency can also cause detrimental effects on bone and increase fracture susceptibility. Iron is needed not only as a cofactor for collagen formation but also for other anabolic processes within the cell, maintaining bone mass. Additionally, the resorption of damaged bone is hampered by the low activity of bone-resorbing cells caused by iron deficiency. Interestingly, anemia as a result of iron deficiency may be treated with iron infusions. Such infusions, however, can cause a severe form of osteomalacia due to increased phosphate excretion caused by iFGF23. iFGF23 is induced by iron deficiency, and its inactivation is disabled by intravenous iron infusions, such as e.g. ferric carboxymaltose.

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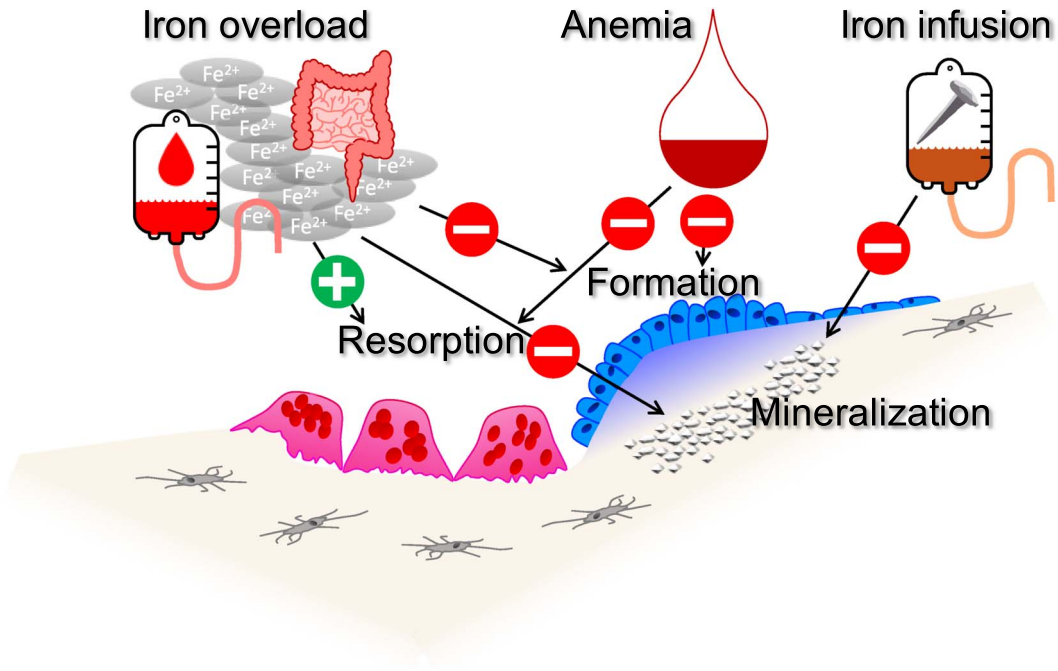
Graphical Abstract



Introduction

In 2014, Looker[1] reported that there is an increased risk of hip fractures in patients with both elevated and low hemoglobin levels compared to individuals with normal serum hemoglobin (Hb) levels.[1] Various mechanisms that increase the risk of fracture in iron-deficient individuals may include (i) the supply of oxygen to the tissue at low iron values,[2] (ii) the frequent combination of iron deficiency and an overall poor health status,[3] and (iii) frailty as a risk factor for an increased occurrence of fractures. Thus, in the case of iron deficiency or anemia, there is a primary reduction in both the bone formation and resorption processes, inducing a low-turnover bone metabolism. Additionally, reduced muscle performance and overall posture increase the risk of falls, which increases the risk of fractures.

In contrast, iron overload has been shown to affect the bone through other mechanisms.[4] Iron overload is primarily linked to the toxicity of iron and reactive oxygen species (ROS), which act as resorption-accelerating factors of bone metabolism. Thus, catabolic processes are favored,[4,5] and anabolic processes and cell formation (osteoblasts formation) decrease,[5] inducing a decreasing bone mineral density (BMD) over time.[4,5]

This review aimed to enhance the general awareness and clinical attention given to the interaction between iron and bone metabolism and to its treatment for both iron deficiency and overload.

Iron overload

Iron overload is known to cause oxidative stress through the formation of ROS, which can damage molecular components such as lipids, DNA, and proteins.[5-7] The negative effects of these substances on bone have been described since 1997.[4,8]

The main reasons for iron overload may be attributed to diverse etiologies,[4] such as hemochromatosis[4-8] and hemoglobinopathies,[4,6,9,10] which lead to primary or secondary iron overload, respectively. The primary form of hemochromatosis is hereditary, as it is caused by pathogenic variants in the hepcidine gene (*HAMP*) or ferroportin gene (*SLC40AI*, *FPN*). It is referred to as hereditary hemochromatosis (HH).[5,11,12] Other causes of iron overload include mutations in the bone morphogenic protein (BMP) signaling cascade,[5] which were discussed in depth by Ledesma-Colunga et al.[5] and Baschant et al.[7] Iron overload in hemoglobinopathies, such as thalassemia major and sickle cell disease, is the result of treatment with repetitive blood transfusions causing secondary hemochromatosis.

Thalassemia is a disease of particular interest since anemia (see below) is known to induce fractures and low BMD. However, iron overload, such as that induced by thalassemia treatment, also leads to bone complications.[13] In particular, bone marrow expansion affects the bone in thalassemia.[13] This was reviewed by Dede et al.[13] Thalassemia is caused by pathogenic variants in the gene encoding hemoglobin chains.[14] Depending on the severity of the disease, blood transfusions are needed. Non–deletional hemoglobin H disease (HbH), β-thalassemia major, and severe HbE/β-thalassemia (hemoglobin E, a β-hemoglobin variant), hereafter all referred to as thalassemia, are considered transfusion-dependent subtypes since transfusions are needed to address the life-threatening symptoms of severe anemia.[9] However, since the body lacks any mechanisms to avert iron excess, transfusions inevitably increase the iron load in the body, leading to iron overload.[9,15] Hence, when the body stores iron beyond the capacity of iron-binding proteins, excess iron can induce damage within cellular and tissue compartments due to its redox potential.[7] Therefore, iron chelation is part of the treatment protocol for these patients[9] to reduce iron toxicity. However, iron overload is inevitable and leads to bone disease in individuals with β-thalassemia.[10] Thalassemia-associated osteoporosis is the most common

comorbidity in thalassemia patients.[4,13] In this condition, iron interrupts bone formation by decreasing osteoblast activity and inducing osteoclast activity due to ROS. Furthermore, iron chelation itself, as part of the treatment, is known to affect bone, as is the case for desferrioxamine-induced bone dysplasia in young patients.[16] However, in addition to these negative effects in young patients, positive effects, such as increased spinal[17,18] and femoral[17] BMD, are observed in patients chelated with deferasirox[17,18] but no other chelating agents.[17] In addition to the direct effects on bone metabolism, iron overload can cause different endocrinopathies, such as hypogonadism, hypothyreoidism, and hypoparathyroidism, as well as interactions with glucose metabolism and growth hormones (such as GH and IGF-1).[13] All of the aforementioned effects, in turn, decrease bone quality and mass. This clearly indicates the tremendous effects of thalassemia on bone, which can induce thalassemia-associated osteoporosis.

Sickel cell disease (SCD), which is known to be protective against malaria,[19,20] is caused by a single nucleotide variant in the Hb β-chain coding gene (*HBB*).[4] Several acute and chronic features concerning the bone have been described in these patients.[21] The acute symptoms include osteomyelitis, stress fractures, vertebral fractures, and bone marrow necrosis.[21] Chronic bone problems include osteonecrosis, osteoporosis, and impaired bone growth.[21] However, the results regarding SCD and iron load are conflicting.[4] Primarily, a significantly larger portion of patients have anemia and thus no iron overload *per se*.[22] However, iron overload can be induced after repeated transfusions for SCD treatment,[22] which leads to an elevated iron load in up to 70% of patients.[23] This leads to a manifestation of reduced BMD in approximately 50%[24] of SCD patients. Since most SCD patients exhibit bone manifestations, even without transfusion treatment, mechanisms other than iron overload and treatment[22] must play a role in bone pathologies such as rheological and vascular complications.[21,22]

In summary, the aforementioned diseases are predominantly associated with iron overload resulting from iatrogenic blood transfusions. The challenge arises not from the transfusions themselves but rather from the body lacking an effective mechanism to excrete iron. Instead, the regulation of iron levels in the body is primarily mediated through iron uptake and recycling processes.[9,25]

While iron overload in the aforementioned diseases is primarily induced by parenteral blood transfusions, HH is a primary cause of iron overload. In the case of HH, dietary iron absorption in the gut becomes uncoupled from the body's iron requirements due to specific mutations. Pathogenic variants of genes involved in irion uptake (such as *HFE*, hemojuvelin, hepcidine, transferrin receptor type II, and ferroportin)[26] cause iron overload through the abnormally high uptake of iron from the gut. In addition, highlighting the connection between these pathways and bone, several links exist between HH and BMPs.[5] Further review on this topic was addressed by Baschant & Altamura et al.[7] and Ledesma-Colunga et al.[5]

Approximately 25%-34% of HH patients are affected by osteoporosis,[4] while 40%-80% have a BMD in the range of osteopenia,[27,28] which is associated with microstructural deterioration.[29] The incidence of fractures is correlated with iron overload, as patients with ferritin levels $\geq$1000 μg/l exhibit more wrist and vertebral fractures and a greater incidence of osteoporosis than patients with lower levels (18% vs. 29.7%).[30] All of these findings may be linked to the iron toxicity itself but also to secondary effects caused by iron overload-induced endocrinopathies, such as hypogonadism and its related decreases in testosterone, LH and FSH levels, diabetes mellitus, cardiac dysfunction, and elevated parathyroid hormone.[7]

Mechanistically, iron overload affects both parts of the bone remodeling process: it suppresses bone formation and exacerbates bone resorption (Figure 1).[5] In both cases, the proliferation, differentiation, and activity of the involved cell types, namely, osteoclasts and osteoblasts, as well as their precursors, are affected, as shown in vitro and in vivo animal model studies.[5]

Osteoblasts are restricted in their differentiation, proliferation and function by iron overload, as evidenced by decreases in the levels of osteoblast markers in vitro and in vivo,[5] such as alkaline phosphatase (ALP) and osteocalcin in cell culture.[31] Furthermore, mineralization is inhibited in vitro and animal models,[4] as is the activation of vitamin D in vitro,[5] limiting the calcium available for sufficient bone mineralization. Additionally, limited calcium access by low active vitamin D increases bone resorption through increased parathyroid hormone levels.

In contrast to osteoblasts, osteoclasts play a crucial role in bone remodeling at the catabolic site. Their normal function is indispensable for maintaining bone mass by preventing excessive bone loss associated with overactivity. Iron, however, does favor the overactivity of osteoclasts. This becomes clear when considering the role of iron in energy production in cells. Bone resorption is an energy-intensive process, and iron is necessary for mitochondrial energy production and the subsequent resorptive performance of osteoclasts.[32] In particular, Transferrin receptor 1 (TfR1) induction and osteoclast induction seem to be coupled, and TfR1 iron uptake facilitates osteoclast differentiation and resorbing activity, highlighting the connection between iron overload and bone resorption.[4] In particular, ROS formation induced by iron overload is central to osteoclast differentiation, as shown in vitro,[33] and thereby increases bone resorption. This is indicated by an elevated RANKL/OPG ratio in patients,[34] which favors bone resorption and osteoclast activity. Furthermore, TRAP, which is an essential molecule for osteoclast action, is an iron-dependent molecule that is favored in the presence of ferric iron. Furthermore, in vivo studies have shown an increased number of osteoclasts in patients with iron overload, which increases bone resorption.[4]

Taken together, these findings show that iron overload favors bone resorption through several interactions with osteoclastic pathways, such as osteoclast proliferation, differentiation and activity pathways; thus, iron increases osteoclast numbers and activity, lowering bone mass. This may be considered the main effect.[4] However, inactivation of bone formation also plays a crucial role in iron overload. Both effects are detrimental to bone and lead to iron overload-induced osteoporosis mediated by ROS and specific molecules involved in iron metabolism, such as TfR1.

In addition, high levels of iron can be observed in the osteoid in particular conditions such as the Itai-Itai disease.[35] However, an accumulation of iron at the mineralization front was also seen in patients diagnosed with osteomalacia and pathological fractures exhibiting extremely high ferritin concentration.[36] It therefore is suggested that iron may inhibit calcium deposition in osteoid.[35] Iron has been demonstrated to promote the formation of less structured hydroxyapatite

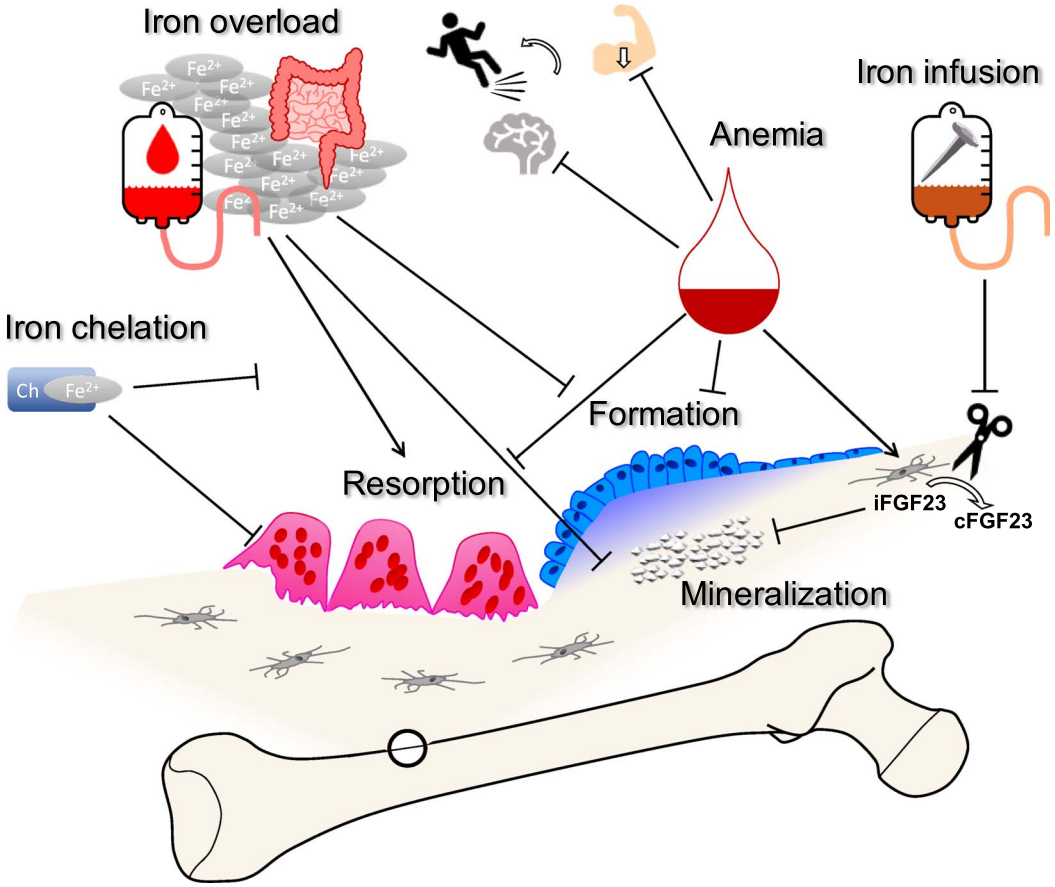


Figure 1. Schematic diagram of the interactions between iron supply and bone metabolism. From left to right: while iron chelation can inhibit osteoclast action, iron overload induces osteoclast activity and inhibits osteoblasts. Anemia, in turn, inhibits both bone resorption and bone formation, thus inducing low turnover. Additionally, anemia induces high levels of iFGF23, which is directly cleaved and thereby inactivated under physiological conditions. However, specific maltoses from intravenous iron infusion therapy interfere with cleavage, thus inducing high iFGF23 levels and thereby increasing phosphate excretion, leading to hypophosphatemia and subsequently osteomalacia.

and reduce the deposition of calcium and phosphate in the bone matrix, resulting in decreased bone mineralization in vitro. Among the explanations for this mineralization-inhibiting effect of iron (Fe^{3+}) is the irons high affinity for phosphate ions, which may hinder the formation of hydroxyapatite, a compound composed of phosphate and calcium (Ca^{2+}), by forming iron-phosphate complexes.[37] Other authors suggest a direct competition of iron with calcium inhibiting the hydroxyapatite formation besides decreasing the collagen production in vitro.[26]

It is inherent that chelation is effective for patients suffering from iron overload and is therefore part of the treatment regimen. Indeed, positive effects have been reported on both osteoblasts and osteoclasts when iron overload is treated with these regimens. Ferric chelators can inhibit TRAP activity, thus slowing osteoclast-derived bone resorption,[4] which becomes inherently clear when considering that TRAP is iron dependent.[38]

Iron insufficiency

Approximately 40% of pregnant women but also 30% of non–pregnant women suffer from iron deficiency.[6] Iron deficiency is the most common cause of anemia.[6] The main reasons for iron deficiency are chronic bleeding, malnutrition, and malabsorption syndrome.[6] However, vegetarian and vegan diets may also cause iron deficiency.[6]

Research on the relationship between low iron intake and its negative impact on bone density is complex, given that conclusions are drawn from the use of various serum iron markers in studies. For example, iron intake,[39,40] Hb,[41] serum iron,[42] and ferritin[42,43] are used as dichotomous markers for anemia,[41,42,44-48] but each marker has a different meaning. In addition, many findings are extrapolated from correlation studies.

Conclusions regarding the adverse effects of iron deficiency or anemia on BMD are also drawn from the observation that there is a clear positive correlation between dietary iron intake and bone density[39] in women; thus, a negative effect is expected for low levels of iron intake. Accordingly, Harris et al. showed, when dividing patients into quartiles of iron uptake, that female patients in the highest quartile of iron intake had the best bone density (Table 1).[39] Moreover, Liu et al. demonstrated a decreasing risk of osteopenia/osteoporosis with increasing iron uptake (Table 1).[40] However, it should be noted that many factors, such as a healthy lifestyle, sporting and muscular activity, a healthy diet, and other factors, may also play a role and are particularly difficult to account for in observational studies. Therefore, no significant correlation remained after stratification for multiple cofactors by Liu et al. (Table 1).[40] Yet the nutritional iron supply is not a direct measure of the body's iron balance, as iron absorption from the gastrointestinal tract depends on multiple factors.[49-51]

Therefore, it is not surprising that serum iron levels and BMD do not interact according to Kim et al. (Table 1).[42]

In clinical practice, hemoglobin levels are routinely used to assess the iron supply. Several studies have shown correlations between anemia or hemoglobin levels and bone density in selected diseases, such as sickle cell disease, chronic inflammatory diseases, or renal failure.[52-54] However, they all share a commonality—an underlying disease or deficiency. This is why it is difficult to dismiss the possibility of interaction through this underlying condition.

Stratification for all covariates is complicated in clinical observational studies. For older men and women, Cesari et al. showed a correlation between low bone density and low Hb (Table 1). According to their results, this effect is primarily due to reduced cortical bone density.[48] These findings remained valid even after adjusting for the effects of inflammation, which, on its own, can lower BMD.[55] A positive effect of Hb serum levels has also been shown by Jørgensen et al. for women and men,[44] yet an increased fracture risk was only present for men with anemia but not for women.[44] However, given the sex-specific nature of bone metabolism, it is important to look at this topic comprehensively.

In a cohort of Turkish women, anemia was shown to serve as an independent predictor of low bone mass,[45] even if the direct correlation with $r = 0.164$ was rather weak (Table 1). A similar situation was observed in women from the Women's Health Initiative (WHI) cohort, where the risk of fractures was increased in those with anemia.[47] Even in young women, having better iron levels was shown to be a positive factor in lowering bone breakdown.[56] However, the r^2 of 0.079 was small and was noticeable only when vitamin D was administered alongside.

To yield a positive effect of iron, a adequate calcium intake is required, as indicated by Harris et al. Only women with an optimal calcium supply of 800-1200 mg/d exhibited a positive association between iron intake and bone density, while no clear association was found for those with levels above or below this range.[39] This shows that iron interacts with calcium and plays an important role in its effects. Nevertheless, even after adjustment for protein supply, covariants, and calcium, it became clear that a correlation existed between iron and bone density.[39]

Ferritin is one of the key indicators of iron supply, where elevated ferritin levels indicate abundant iron. In the KNHANES 2008-2010 cohort study by Kim et al.,[42] it was revealed that high ferritin levels in women, particularly those aged >45 years, corresponded with decreased BMD compared to women with lower ferritin levels.[42] Notably, this correlation was not evident in men according to the study. Intriguingly, optimal bone density was observed at low ferritin levels, specifically in the lowest quartile. However, it is essential to consider the 95% confidence interval, which ranged from 55.8 to 60.4 in the corresponding age group. This finding suggested that only a few patients likely fell within a deficiency value range. The specified ferritin deficiency range was 1.1-28.0 ng/ml, and the minimum of the normal range was 15.0 ng/ml.[42]

Most of the studies in men have shown an association between decreasing Hb levels and decreasing bone mineral density.[44,46,48] However, in the MrOS cohort, no significant association was detected between BMD and anemia in males.[41] Nonetheless, in the same cohort, Hb serum levels were shown to be positively associated with BMD, yet including sex hormone levels led to a non–significant association.[57] One year later, the MrOS cohort was used to clearly show an increased fracture risk for men suffering from anemia.[41] When distinguishing between patients with and without anemia, no direct association between BMD and anemia was shown. This, according to the authors, may be explained by the limited number of patients rather than the lack of interaction between anemia and BMD.[41]

In addition to general anemia due to iron deficiency, chronic illness, or bleeding, there are specific causes of anemia, as mentioned above. Interestingly, a correlation between Hb and BMD was also found in hemodialysis patients. However, according to the authors, this was more of an indicator of sporting activity.[54] When exploring the link between increasing hemoglobin (Hb) levels and a concurrent increase in BMD or decrease in fracture rate, a critical factor influencing this association, beyond molecular and genetic factors, appears to be increased vulnerability to fractures due to diminished muscle performance,[58] hindering an adequate and secure posture and gait. Consequently, reduced mechanical stimulation of the bone may accompany reduced BMD due to decreased mechanical impact, such as in the case of immobilization.[59] This implies a deficiency in the ability to prevent a fall, absorb impact during a fall, and prevent bone-muscle crosstalk, which is crucial for preserving bone mass, especially when viewing the bone as a mechano-reactive organ.

Interestingly, iron deficiency is also very frequently linked to chronic kidney disease (CKD), especially in those treated by dialysis,[60] were vascular calcifications are very frequent.[60,61] Moreover, CKD patients suffer from ROS accumulation,[62] thus combining the effects of iron insufficiency and increased ROS, usually seen at iron overload, leading to a pronounced loss of bone. For review of the interaction of CKD, vascular calcification, and iron, see Neven et al.[60]

In summary, existing evidence suggests that a diminished iron supply increases the vulnerability of bones to fractures and contributes to a reduced BMD in both men and women. It is crucial to carefully consider the chosen laboratory parameters regarding differentiating the influence of iron on bone, as each may imply different conditions. For instance, elevated ferritin levels, associated with infections, reflect iron availability within cells, while hemoglobin (Hb) measures a protein bound to iron rather than iron itself. Among the crucial aspects influencing the interaction between low iron levels and compromised bone quality, a lack of physical activity often observed in individuals with low Hb levels may be one key factor. Notably, several studies have thoroughly adjusted for physical activity[43,48] and have still been able to demonstrate significant differences associated with anemia.

While many of the clinical observations are linked to anemia and an interaction of bone and muscle function as well as neuronal function, there are several specific reasons why an iron deficit may hamper bone mass and quality through reduced bone mass or fracture resistance after correction for muscular performance (Table 1). Specifically, iron acts as a cofactor for collagen synthesis[63-65] and is important for collagen maturation,[66] enabling collagen crosslinking.

At the molecular level and when focusing on deficiency, it is important to first acknowledge the main needs for iron at the cellular level. First, and of utmost importance, iron is needed for oxygen transport in erythrocytes, as it is coupled to hemoglobin. However, at the local intracellular level, iron is needed for the electron transport chain (ETC), which is a

Table 1. Interaction of laboratory indicators of iron metabolism and BMD/fractures.

				Fractures						BMD							
	First author	Cohort	Year	All	(*n*)	Women	(*n*)	Men	(*n*)	all	(*n*)	Women	(*n*)	Men	(*n*)	Inclusion	Exclusion
Diatary iron																	
	M.M. Harris	–	2003									↑[a]	242			Healthy, non–smoking postmenopausal women	Women with implausible or missing data
	X. Liu	NHANES	2023							↑	11 690					USA residents	Missing values, osteopenia or osteoporosis, aged <20 years
Hb																	
	H.L. Kristjansdottir	MrOS	2021			N/A		N/A		N/A		N/A		↑[b]	1550	Men ages 69-81	Missing blood parameters
Ferritin																	
	B.J. Kim	KNHANES	2013							N/A		↓[c]	2712	N/A		Civilian Korean, adult population	Missing values, cronic liver diseases, chronic renal diseases, neoplastic diseases, increased serum liver enzymes, increased creatinine, abnormal leukocyte counts
	K.S. Lee	KNHANES	2013									↔	1569	↑[d]	1374	Patients >64 years	Current thyroid disease, chronic hepatitis B or C, liver cirrhosis, chronic kidney disease, malignancy, pulmonary and extrapulmonary tuberculosis, current osteoporosis treatment, hormone replacement therapy, hysterectomy
Serum iron																	
	B.J. Kim	KNHANES	2013									↔	7200	↔	6817	Civilian Korean, adult population	Missing values, cronic liver diseases, chronic renal diseases, neoplastic diseases, increased serum liver enzymes, increased creatinine, abnormal leukocyte counts
Anemia (by Hb)																	
	L. Jørgensen	Tromsø IV	2010			↔	2775	↑	2511			↑	5286	↑	2775	Male and female inhabitants of Tromsø aged from 55 to 74	Pathological fractures, missing BMD or Hb
	H.L. Kristjansdottir	MrOS	2022			N/A		↑[g]	1005			N/A		↔	1005	Man aged 70–80 years	Men without Hb value
	U. Korkmaz	–	2012									↑	371			Patients with osteopenia or osteoporosis, postmenopausal patients	Known chronic infectious or inflammatory disease, known coronary heart disease, thyroid disease, severe liver or renal disease, neoplasm, hematological disorders, taking anti- coagulant therapy, severe physical inactivity, hormone replacement therapy
	R.J. Valderrábano	MrOS	2017			N/A		↑[h]	3632							Men > 64, able to talk, did not have bilateral hip replacement, complete white blood cell count	Men with extreme total white blood cell counts
	Z. Chen	WHI	2010			↑[i]	160 080									Women aged 50–79 years, postmenopausal, not likely to die within 3 years after study enrollement	Dependent on WHI clinical trials
	R.J. Valderrábano	MrOS	2017									N/A		↑[e]	2571	Men > 64, able to talk, did not have bilateral hip replacement, complete white blood cell count	Men with extreme total white blood cell counts
	E. A. Lee	NHANES III	2019			↑[$]	37 857	↑[$]	34 274							Community-dwelling Korean older adults aged 65 years and older	Missing values, fractures in the year before the baseline examination, aplastic or hemolytic anemia, hematologic malignancy, other blood disorders
	M. Cesari	InCHIANTI	2005			N/A		N/A		N/A		↑[f]	530	↑[f]	420	Subjects aged 65–102 years from the Chianti geographic area	Subjects without hemoglobin levels or pQCT measured
	A. C. Looker	NHANES III	2014	↑[j]	2122											Non–Hispanic white individuals aged 65 and above	Missing values, prior hip fractures, other previous fractures, other bone diseases
	T. P. van Staa	GPRD	2002	↑	231 778											Patients aged > 17 that had a fracture during study period	Fractures prior to study
	N. A. Merriman	GPRD	2010	↑[k]	47 530											Patients that visited a general practioner in the United Kingdom	Patients at an age < 40 and > 90, hip fractured patients

[a]For women with a dietary calcium intake of 800–1200 mg/d. [b]Disappeared after adjustment for sex-hormone estradiol. [c]Risk for OPO as defined by the WHO. [d]Independent of physical activity, Vit. D, PTH, ALP, BMI, education, income, smoking status. [e]At lumbar spine (greater risk for anemia at high BMD loss). [f]Also after adjustment for muscle power, Vit. D, age, stature, and education. [g]For any fracture; [h]Prospective occurrence of a fracture predicted by anemia; [i]Anemic patient had a significantly lower physical function. [j]After adjustment for age, sex, ever smoked, femur neck BMD, iron/folate deficiency, inflammation, renal insufficiency, BMI, and timed chair stand. [k]With adjustment for sex and age, the hazard ratio (HR) depended on the number of years after diagnosis of pernicious anemia. The HR for 2 years after diagnosis was 0.95 and was not included in the range. The range is specified for 1-5 years after diagnosis of pernicious anemia; $Adjusted hazard ratio, ↓: decreases with respective parameter, ↑: increases with respective parameter, ↔: no interaction, stays constant

series of protein complexes located in the inner mitochondrial membrane. The ETC is the final stage of aerobic respiration and involves the breakdown of glucose to produce energy. Therefore, iron acts as an essential cofactor for proteins involved in electron transfer, helping to facilitate the movement of electrons from one protein to another. Iron plays a key role in the production of ATP, which is the energy transport molecule of the cell. It participates in the oxidative phosphorylation of cytochromes via electron transfer.[67] These processes, in turn, are needed to deliver energy for protein biosynthesis.

Bone growth and remodeling are both anabolic processes involving biosynthesis. As bone is composed of collagen, hydroxyapatite, and several other molecules, iron is urgently needed to provide energy for collagen synthesis. Furthermore, iron is important for cell functions, such as ribosome synthesis, and serves as a cofactor for many enzymes. Specifically, iron is an essential cofactor for enzymes involved in the hydroxylation of proline and lysine residues within collagen molecules.[65,68,69] Thus, iron deficiency may subsequently lead to reduced bone structure.[70]

The hydroxylation of these amino acids is a critical step in collagen synthesis.[69] This step occurs in the endoplasmic reticulum, where the iron-dependent enzymes prolyl and lysyl hydroxylases[64,68] catalyze the addition of hydroxyl groups to specific proline and lysine residues. However, this process does not occur for free proline but requires at least a triplet of amino acids (X is an interchangeable amino acid), namely, -X-Pro-Gly-,[66] which are thus parts of collagen molecules. This hydroxylation process is necessary for the stabilization and proper formation of collagen triple helices and fibrils.[71] Thus, an iron deficiency can lead to diminished collagen and thereby bone quality.

Interestingly, the renal conversion of cholecalciferol into active vitamin D[72] involves ferredoxin and cytochrome P-450, both of which are dependent on iron.[72] This clarifies why iron deficiency can impact bone mineral density.[73] Iron deficiency not only impacts bone quality with respect to collagen but also with respect to mineral content, acknowledging that active vitamin D is vital for calcium homeostasis and thus the formation of hydroxyapatite to mineralize bone tissue. Subsequently, a lack of mineralization leads to reduced mechanical competence in the bone,[74] which explains why a calcium-deficient diet further exacerbates the manifestations of poor bone mass and mineralization when combined with iron deficiency.[75]

Furthermore, on the catabolic side, iron deficiency, which reduces energy availability, inhibits osteoclast action.[73,75,76] Therefore, in the case of anemia, a low turnover rate is associated with low bone formation and low bone resorption.[76] Thus, reduced bone quality results in a decrease in the forces needed to break the bones of individuals with low serum iron levels.[75]

Intravenous iron treatment

As presented above, anemia and low iron levels are disadvantageous for skeletal health. Anemia is primarily characterized by fatigue,[77] pale skin color, cardiopulmonary symptoms such as shortness of breath, a feeling of weakness, and possibly an irregular pulse.[78] Iron deficiency also manifests as brittle nails, thin hair and possibly restless leg syndrome.[78] The reasons for anemia can include gastrointestinal bleeding, a history of other severe bleeding events, such as heavy uterine bleeding or inflammatory bowel disease, malnutrition/malabsorption, chronic heart failure, cancer, or bariatric surgery.[79,80] Thus, intravenous iron substitution is an individual medical decision. The treatment of anemia, as outlined above (Table 1), is generally believed to be valuable for general and bone health.

However, an increasing number of case studies have reported that intravenous iron substitution induces hypophosphatemia with subsequent osteomalacia.[79,80] The reported substitutes are ferric carboxymaltose (FCM), iron sucrose, saccarated ferric oxide, iron polymaltose, iron isomaltoside/-ferric derisomaltose, ferumoxytol and low-molecular-weight iron dextran,[79,80] some of which more or less frequently exhibit the complication of hypophosphatemia. Risk factors for hypophosphatemia include the type of substitute used (FCM is associated with a significantly greater risk),[81] the severity of iron deficiency (pronounced iron deficiency is associated with higher FGF23 expression and thus the potential for hypophosphatemia), and kidney function.[78] Specifically, compared with other patients, 47% of FCM patients develop hypophosphatemia, while only 4% of IIM patients exhibit hypophosphatemia.[81] Notably, intravenous iron infusion-induced osteomalacia is less common in patients suffering from CKD.[79,81,82] This may be caused by the inability of kidneys to excrete the phosphate due to kidney insufficiencies or malfunctions.

Patients affected by intravenous iron infusion-induced hypophosphatemia clinically present with fatigue, bone pain caused by osteomalacia, respiratory symptoms, nausea, vomiting and diarrhea, pseudofractures, and pronounced muscle weakness.[78] Thus, reoccurring fractures, pseudofractures, or bone marrow edema at intravenous iron substitution should increase the suspicion of induced phosphate wasting, and further diagnosis should be performed. This includes monitoring of serum phosphate levels, X-ray imaging, subsequent MRI, and fracture occurrence.[79]

Biochemical testing is of paramount importance for diagnosing, monitoring, and treating this disease. Patients with renal phosphate loss exhibit low phosphate levels and increased urinary phosphate excretion caused by increased intact FGF23 (iFGF23). Consequently, active vitamin D levels are often low, and calcium is within the lower range of normal levels. Correspondingly, increased parathyroid hormone levels can be measured, as can increased ALP levels, emphasizing the effect on bone.

Therapy wise, in case of a fracture or moderate to severe hypophosphatemia (moderate: 0.6 mmol/l, severe: 0.3 mmol/l),[79] a termination of iron substitution or a switch of the intravenous iron substitute is needed if possible.[79] In patients with mild hypophosphatemia and no fracture, phosphate monitoring may be sufficient.[79] Furthermore, to address osteomalacia and the lagging effect of iron infusion, oral phosphate substitution should be considered, as should active vitamin D application,[79] to account for the downregulation of 1,25-vitamin D due to the effect of iFGFG23.[82] When $25(OH)D_3$ deficiency is detected, cholecalciferol should also be used.

The serum phosphate concentration in the human body is primarily regulated by active vitamin D, PTH, and FGF23, which is produced by osteocytes.[78,79,82,83] Interestingly, iron deficiency increases the levels of iFGF23[83] through the hypoxia-inducible factors HIF1a and HIF1b.[79] However, in the case of iron deficiency, induced iFGF23 is cleaved within osteocytes and does not interact with phosphate

metabolism.[79] Physiologically, iFGF23 levels increase with increasing serum phosphate levels and inhibit the phosphate cotransporter NaPi-2a and NaPi-2c in the proximal tubule, inducing urinary phosphate excretion. Furthermore, iFGF23 inhibits the activation of 25 vitamin D to 1,25-vitamin D and increases the inactivation of 1,25 vitamin D to 24,25-vitamin D. Therefore, iFGF23 decreases serum phosphate and calcium levels.

Specific intravenous iron substitutes, however, inhibit the cleavage of iFGF23, thus increasing the serum level of active iFGF23.[79,82,83] This inhibition is especially common in the case of FCM, which increases iFGF23 serum levels and thus high urinary phosphate excretion. Due to kidney malfunction in CKD patients, CKD patients exhibit a significantly lower proportion of hypophosphatemia induced by FCM (0.27) than patients with normal kidney function and FCM (0.51).[81]

Simultaneously, the inactivation of 1,25 vitamin D and the decreased activation of 25 vitamin D decrease serum calcium concentrations.[78] Thus, secondary hyperparathyroidism occurs with increasing PTH levels, where the need for calcium is especially high due to osteomalacia. This in turn accelerates phosphate excretion and induces further iFGF23 production.

Over time, ALP levels increase. Newly formed osteoid is subsequently deposited into resorption pits; however, it cannot mineralize by incorporating hydroxyapatite due to low phosphate and calcium serum levels. The missing mineralization of the collagenous matrix of bone, in turn, favors fractures due to a decreased resistance to even physiological loadings.

Conclusion

In conclusion, an interaction between iron and bone metabolism is evident, and clinical consequences of both iron overload and deficiency have been shown. The interactions are multifactorial and not yet fully elucidated, but both conditions can affect the bone, negatively increasing the risk for fractures. Thus, a high level of awareness is important for both clinical strategies and basic research. Although addressing the clinical needs of such patients by treating the underlying disease and its bone manifestations is crucial, treatment itself can significantly affect bone metabolism. This is exemplified by iron overload caused by transfusions or intravenous iron infusion causing hypophosphatemic osteomalacia.

To comprehensively address the various aspects of these complex interactions and their clinical manifestations, a coordinated and interdisciplinary approach in diagnostics and therapy is imperative.

Author contributions

Felix von Brackel (Data curation, Investigation, Methodology, Validation, Visualization, Writing—original draft, Writing—review & editing) and Ralf Oheim (Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing)

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Conflicts of interest

F.v.B. has no conflict of interest. R.O. has served as a speaker and advisory board member for Kyowa Kirin, Inozyme, Ipsen, Pharmacosmos, and UCB, and has received an institutional research grant from Kyowa Kirin and UCB.

Data availability

No new data were generated or analyzed in support of this research.

References

1. Looker AC. Hemoglobin and hip fracture risk in older non-Hispanic white adults. *Osteoporos Int*. 2014;25(10):2389–2398. https://doi.org/10.1007/s00198-014-2769-3
2. Chaves PHM. Functional outcomes of anemia in older adults. *Semin Hematol*. 2008;45(4):255–260. https://doi.org/10.1053/j.seminhematol.2008.06.005
3. Merchant AA, Roy CN. Not so benign haematology: anaemia of the elderly. *Br J Haematol*. 2012;156(2):173–185. https://doi.org/10.1111/j.1365-2141.2011.08920.x
4. Jeney V. Clinical impact and cellular mechanisms of iron overload-associated bone loss. *Front Pharmacol*. 2017;8(8):77. https://doi.org/10.3389/fphar.2017.00077
5. Ledesma-Colunga MG, Weidner H, Vujic Spasic M, Hofbauer LC, Baschant U, Rauner M. Shaping the bone through iron and iron-related proteins. *Semin Hematol*. 2021;58(3):188–200. https://doi.org/10.1053/j.seminhematol.2021.06.002
6. Gattermann N, Muckenthaler MU, Kulozik AE, Metzgeroth G, Hastka J. The evaluation of iron deficiency and iron overload. *Dtsch Arztebl Int*. 2021;118(49):847–856. https://doi.org/10.3238/arztebl.m2021.0290
7. Baschant U, Altamura S, Steele-Perkins P, et al. Iron effects versus metabolic alterations in hereditary hemochromatosis driven bone loss. *Trends Endocrinol Metab*. 2022;33(9):652–663. https://doi.org/10.1016/j.tem.2022.06.004
8. Sinigaglia L, Fargion S, Fracanzani AL, et al. Bone and joint involvement in genetic hemochromatosis: role of cirrhosis and iron overload. *J Rheumatol*. 1997;24(9):1809–1813
9. Farmakis D, Porter J, Taher A, et al. 2021 Thalassaemia International Federation guidelines for the management of transfusion-dependent thalassemia. *Hemasphere*. 2022;6(8):e732. https://doi.org/10.1097/HS9.0000000000000732
10. De Sanctis V, Soliman AT, Elsefdy H, et al. Bone disease in β thalassemia patients: past, present and future perspectives. *Metabolism*. 2018;80(80):66–79. https://doi.org/10.1016/j.metabol.2017.09.012
11. Pietrangelo A. The ferroportin disease. *Blood Cell Mol Dis*. 2004;32(1):131–138. https://doi.org/10.1016/j.bcmd.2003.08.003
12. De Gobbi M, Roetto A, Piperno A, et al. Natural history of juvenile haemochromatosis. *Br J Haematol*. 2002;117(4):973–979. https://doi.org/10.1046/j.1365-2141.2002.03509.x
13. Dede AD, Trovas G, Chronopoulos E, et al. Thalassemia-associated osteoporosis: a systematic review on treatment and brief overview of the disease. *Osteoporos Int*. 2016;27(12):3409–3425. https://doi.org/10.1007/s00198-016-3719-z
14. Weatherall DJ. The thalassaemias. *BMJ*. 1997;314(7095):1675–1678. https://doi.org/10.1136/bmj.314.7095.1675
15. Hajimoradi M, Haseli S, Abadi A, Chalian M. Musculoskeletal imaging manifestations of beta-thalassemia. *Skeletal Radiol*. 2021;50(9):1749–1762. https://doi.org/10.1007/s00256-021-03732-9
16. Chan Y-L, Pang L-M, Chik K-W, Cheng JCY, Li C-K. Patterns of bone diseases in transfusion-dependent homozygous thalassaemia major: predominance of osteoporosis and desferrioxamine-induced bone dysplasia. *Pediatr Radiol*. 2002;32(7):492–497. https://doi.org/10.1007/s00247-002-0664-0

17. Poggi M, Sorrentino F, Pugliese P, et al. Longitudinal changes of endocrine and bone disease in adults with β-thalassemia major receiving different iron chelators over 5 years. *Ann Hematol*. 2016;95(5):757–763. https://doi.org/10.1007/s00277-016-2633-y
18. Casale M, Citarella S, Filosa A, et al. Endocrine function and bone disease during long-term chelation therapy with deferasirox in patients with β-thalassemia major. *Am J Hematol*. 2014;89(12):1102–1106. https://doi.org/10.1002/ajh.23844
19. Beet EA. Sickle cell disease in the Balovale District of Northern Rhodesia. *East Afr Med J*. 1946;23:75–86
20. Allison AC. Protection afforded by sickle-cell trait against subtertian malarial infection. *Br Med J*. 1954;1(4857):290–294. https://doi.org/10.1136/bmj.1.4857.290
21. Almeida A, Roberts I. Bone involvement in sickle cell disease. *Br J Haematol*. 2005;129(4):482–490. https://doi.org/10.1111/j.1365-2141.2005.05476.x
22. Porter J, Garbowski M. Consequences and management of iron overload in sickle cell disease. *Hematology*. 2013;2013(1):447–456. https://doi.org/10.1182/asheducation-2013.1.447
23. Sadat-Ali M, Sultan O, Al-Turki H, Alelq A. Does high serum iron level induce low bone mass in sickle cell anemia ? *Biometals*. 2011;24(1):19–22. https://doi.org/10.1007/s10534-010-9391-4
24. Shah FT, Chatterjee R, Owusu-Asante M, Porter JB. Adults with severe sickle cell anaemia and iron overload have a high incidence of osteopenia and osteoporosis. *Blood*. 2004;104(11):1684. https://doi.org/10.1182/blood.V104.11.1684.1684
25. Wallace DF. The regulation of iron absorption and homeostasis. *Clin Biochem Rev*. 2016;37(2):51–62
26. Borriello A, Caldarelli I, Speranza MC, et al. Iron overload enhances human mesenchymal stromal cell growth and hampers matrix calcification. *Biochim Biophys Acta*. 2016;1860(6):1211–1223. https://doi.org/10.1016/j.bbagen.2016.01.025
27. Guggenbuhl P, Deugnier Y, Boisdet JF, et al. Bone mineral density in men with genetic hemochromatosis and HFE gene mutation. *Osteoporos Int*. 2005;16(12):1809–1814. https://doi.org/10.1007/s00198-005-1934-0
28. Valenti L, Varenna M, Fracanzani AL, Rossi V, Fargion S, Sinigaglia L. Association between iron overload and osteoporosis in patients with hereditary hemochromatosis. *Osteoporos Int*. 2009;20(4):549–555. https://doi.org/10.1007/s00198-008-0701-4
29. Jandl NM, Rolvien T, Schmidt T, et al. Impaired bone microarchitecture in patients with hereditary hemochromatosis and skeletal complications. *Calcif Tissue Int*. 2020;106(5):465–475. https://doi.org/10.1007/s00223-020-00658-7
30. Richette P, Ottaviani S, Vicaut E, Bardin T. Musculoskeletal complications of hereditary hemochromatosis: a case-control study. *J Rheumatol*. 2010;37(10):2145–2150. https://doi.org/10.3899/jrheum.100234
31. Zarjou A, Jeney V, Arosio P, et al. Ferritin ferroxidase activity: a potent inhibitor of osteogenesis. *J Bone Miner Res*. 2010;25(1):164–172. https://doi.org/10.1359/jbmr.091002
32. Roodman GD. Osteoclasts pump iron. *Cell Metab*. 2009;9(5):405–406. https://doi.org/10.1016/j.cmet.2009.04.005
33. Jia P, Xu YJ, Zhang ZL, et al. Ferric ion could facilitate osteoclast differentiation and bone resorption through the production of reactive oxygen species. *J Orthop Res*. 2012;30(11):1843–1852. https://doi.org/10.1002/jor.22133
34. Morabito N, Gaudio A, Lasco A, et al. Osteoprotegerin and RANKL in the pathogenesis of thalassemia-induced osteoporosis: new pieces of the puzzle. *J Bone Miner Res*. 2004;19(5):722–727. https://doi.org/10.1359/jbmr.040113
35. Hiratsuka H, Katsuta O, Toyota N, et al. Iron deposition at mineralization fronts and osteoid formation following chronic cadmium exposure in ovariectomized rats. *Toxicol Appl Pharmacol*. 1997;143(2):348–356. https://doi.org/10.1006/taap.1996.8101
36. Pierides AM, Myli MP. Iron and aluminum osteomalacia in hemodialysis patients. *N Engl J Med*. 1984;310(5):323. https://doi.org/10.1056/NEJM198402023100517
37. Guggenbuhl P, Filmon R, Mabilleau G, Baslé MF, Chappard D. Iron inhibits hydroxyapatite crystal growth in vitro. *Metabolism*. 2008;57(7):903–910. https://doi.org/10.1016/j.metabol.2008.02.004
38. Alcantara O, Reddy SV, Roodman GD, Boldt DH. Transcriptional regulation of the tartrate-resistant acid phosphatase (TRAP) gene by iron. *Biochem J*. 1994;298(Pt 2):421–425
39. Harris MM, Houtkooper LB, Stanford VA, et al. Dietary iron is associated with bone mineral density in healthy postmenopausal women. *J Nutr*. 2003;133(11):3598–3602. https://doi.org/10.1093/jn/133.11.3598
40. Liu X, An J. Dietary iron intake and its impact on osteopenia/osteoporosis. *BMC Endocr Disord*. 2023;23(1):154. https://doi.org/10.1186/s12902-023-01389-0
41. Kristjansdottir HL, Mellström D, Johansson P, et al. Anemia is associated with increased risk of non-vertebral osteoporotic fractures in elderly men: the MrOS Sweden cohort. *Arch Osteoporos*. 2022;17(1):85. https://doi.org/10.1007/s11657-022-01130-9
42. Kim B-J, Lee SH, Koh J-M, Kim GS. The association between higher serum ferritin level and lower bone mineral density is prominent in women ≥45 years of age (KNHANES 2008-2010). *Osteoporos Int*. 2013;24(10):2627–2637. https://doi.org/10.1007/s00198-013-2363-0
43. Lee KS, Jang JS, Lee DR, et al. Serum ferritin levels are positively associated with bone mineral density in elderly Korean men: the 2008-2010 Korea National Health and Nutrition Examination Surveys. *J Bone Miner Metab*. 2014;32(6):683–690. https://doi.org/10.1007/s00774-013-0540-z
44. Jørgensen L, Skjelbakken T, Løchen M-L, et al. Anemia and the risk of non-vertebral fractures: the Tromsø study. *Osteoporos Int*. 2010;21(10):1761–1768. https://doi.org/10.1007/s00198-009-1131-7
45. Korkmaz U, Korkmaz N, Yazici S, et al. Anemia as a risk factor for low bone mineral density in postmenopausal Turkish women. *Eur J Intern Med*. 2012;23(2):154–158. https://doi.org/10.1016/j.ejim.2011.11.009
46. Valderrábano RJ, Lee J, Lui L-Y, et al. Osteoporotic Fractures in Men (MrOS) Study Research Group. Older men with anemia have increased fracture risk independent of bone mineral density. *J Clin Endocrinol Metab*. 2017;102(7):2199–2206. https://doi.org/10.1210/jc.2017-00266
47. Chen Z, Thomson CA, Aickin M, et al. Short list of Women's Health Initiative Investigators. The relationship between incidence of fractures and anemia in older multiethnic women. *J Am Geriatr Soc*. 2010;58(12):2337–2344. https://doi.org/10.1111/j.1532-5415.2010.03183.x
48. Cesari M, Pahor M, Lauretani F, et al. Bone density and hemoglobin levels in older persons: results from the InCHIANTI study. *Osteoporos Int*. 2005;16(6):691–699. https://doi.org/10.1007/s00198-004-1739-6
49. Rusu IG, Suharoschi R, Vodnar DC, et al. Iron supplementation influence on the gut microbiota and probiotic intake effect in iron deficiency-a literature-based review. *Nutrients*. 2020;12(7):1993. https://doi.org/10.3390/nu12071993
50. Collings R, Harvey LJ, Hooper L, et al. The absorption of iron from whole diets: a systematic review. *Am J Clin Nutr*. 2013;98(1):65–81. https://doi.org/10.3945/ajcn.112.050609
51. Beck KL, Conlon CA, Kruger R, Coad J. Dietary determinants of and possible solutions to iron deficiency for young women living in industrialized countries: a review. *Nutrients*. 2014;6(9):3747–3776. https://doi.org/10.3390/nu6093747
52. Sarrai M, Duroseau H, D'Augustine J, Moktan S, Bellevue R. Bone mass density in adults with sickle cell disease. *Br J Haematol*. 2007;136(4):666–672. https://doi.org/10.1111/j.1365-2141.2006.06487.x
53. Gasche C. Complications of inflammatory bowel disease. *Hepatogastroenterology*. 2000;47(31):49–56

54. Taal MW, Masud T, Green D, Cassidy MJD. Risk factors for reduced bone density in haemodialysis patients. *Nephrol Dial Transpl*. 1999;14(8):1922–1928. https://doi.org/10.1093/ndt/14.8.1922
55. Leng S, Chaves P, Koenig K, Walston J. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *J Am Geriatr Soc*. 2002;50(7):1268–1271. https://doi.org/10.1046/j.1532-5415.2002.50315.x
56. Toxqui L, Pérez-Granados AM, Blanco-Rojo R, Wright I, de la Piedra C, Vaquero MP. Low iron status as a factor of increased bone resorption and effects of an iron and vitamin D-fortified skimmed milk on bone remodelling in young Spanish women. *Eur J Nutr*. 2014;53(2):441–448. https://doi.org/10.1007/s00394-013-0544-4
57. Kristjansdottir HL, Mellström D, Johansson P, et al. High platelet count is associated with low bone mineral density: the MrOS Sweden cohort. *Osteoporos Int*. 2021;32(5):865–871. https://doi.org/10.1007/s00198-020-05766-6
58. Cesari M, Penninx BWJH, Lauretani F, et al. Hemoglobin levels and skeletal muscle: results from the InCHIANTI study. *J Gerontol A*. 2004;59(3):M249–M254. https://doi.org/10.1093/gerona/59.3.M249
59. Rolvien T, Milovanovic P, Schmidt FN, et al. Long-term immobilization in elderly females causes a specific pattern of cortical bone and osteocyte deterioration different from postmenopausal osteoporosis. *J Bone Miner Res*. 2020;35(7):1343–1351. https://doi.org/10.1002/jbmr.3970
60. Neven E, De Schutter TM, Behets GJ, Gupta A, D'Haese PC. Iron and vascular calcification. Is there a link? *Nephrol Dial Transpl*. 2011;26(4):1137–1145. https://doi.org/10.1093/ndt/gfq858
61. Persy V, D'Haese P. Vascular calcification and bone disease: the calcification paradox. *Trends Mol Med*. 2009;15(9):405–416. https://doi.org/10.1016/j.molmed.2009.07.001
62. Ling XC, Kuo K-L. Oxidative stress in chronic kidney disease. *Ren Replace Ther*. 2018;4(1):53. https://doi.org/10.1186/s41100-018-0195-2
63. Hutton JJ, Tappel AL, Udenfriend S. Cofactor and substrate requirements of collagen proline hydroxylase. *Arch Biochem Biophys*. 1967;118(1):231–240. https://doi.org/10.1016/0003-9861(67)90302-5
64. Hausmann E. Cofactor requirements for the enzymatic hydroxylation of lysine in a polypeptide precursor of collagen. *Biochim Biophysica Acta*. 1967;133(3):591–593. https://doi.org/10.1016/0005-2795(67)90566-1
65. O'Dell BL. Roles for iron and copper in connective tissue biosynthesis. *Philos Trans R Soc Lond Ser B Biol Sci*. 1981;294(1071):91–104. https://doi.org/10.1098/rstb.1981.0091
66. Tuderman L, Myllylä R, Kivirikko KI. Mechanism of the prolyl hydroxylase reaction. 1. Role of co-substrates. *Eur J Biochem*. 1977;80(2):341–348. https://doi.org/10.1111/j.1432-1033.1977.tb11888.x
67. Conway D, Henderson MA. Iron metabolism. *Anaesth Intensive Care Med*. 2019;20(3):175–177. https://doi.org/10.1016/j.mpaic.2019.01.003
68. Hutton JJ, Trappel AL, Udenfriend S. Requirements for alpha-ketoglutarate, ferrous ion and ascorbate by collagen proline hydroxylase. *Biochem Biophys Res Commun*. 1966;24(2):179–184. https://doi.org/10.1016/0006-291X(66)90716-9
69. Salo AM, Myllyharju J. Prolyl and lysyl hydroxylases in collagen synthesis. *Exp Dermatol*. 2021;30(1):38–49. https://doi.org/10.1111/exd.14197
70. Medeiros DM, Stoecker B, Plattner A, Jennings D, Haub M. Iron deficiency negatively affects vertebrae and femurs of rats independently of energy intake and body weight. *J Nutr*. 2004;134(11):3061–3067. https://doi.org/10.1093/jn/134.11.3061
71. Schmidt FN, Zimmermann EA, Campbell GM, et al. Assessment of collagen quality associated with non-enzymatic crosslinks in human bone using Fouriertransform infrared imaging. *Bone*. 2017;97:243–251. https://doi.org/10.1016/j.bone.2017.01.015.
72. DeLuca HF. Metabolism of vitamin D: current status. *Am J Clin Nutr*. 1976;29(11):1258–1270. https://doi.org/10.1093/ajcn/29.11.1258
73. Katsumata S, Tsuboi R, Uehara M, Suzuki K. Dietary iron deficiency decreases serum osteocalcin concentration and bone mineral density in rats. *Biosci Biotechnol Biochem*. 2006;70(10):2547–2550. https://doi.org/10.1271/bbb.60221
74. Medeiros DM, Ilich J, Ireton J, Matkovic V, Shiry L, Wildman R. Femurs from rats fed diets deficient in copper or iron have decreased mechanical strength and altered mineral composition. *J Trace Elem Exp Med*. 1997;10(3):197–203. https://doi.org/10.1002/(SICI)1520-670X(1997)10:3<197::AID-JTRA7>3.0.CO;2-8
75. Medeiros DM, Plattner A, Jennings D, Stoecker B. Bone morphology, strength and density are compromised in iron-deficient rats and exacerbated by calcium restriction. *J Nutr*. 2002;132(10):3135–3141. https://doi.org/10.1093/jn/131.10.3135
76. Katsumata S, Katsumata-Tsuboi R, Uehara M, Suzuki K. Severe iron deficiency decreases both bone formation and bone resorption in rats. *J Nutr*. 2009;139(2):238–243. https://doi.org/10.3945/jn.108.093757
77. Ryblom H, Hast R, Hellström-Lindberg E, Winterling J, Johansson E. Self-perception of symptoms of anemia and fatigue before and after blood transfusions in patients with myelodysplastic syndromes. *Eur J Oncol Nurs*. 2015;19(2):99–106. https://doi.org/10.1016/j.ejon.2014.10.011
78. Schaefer B, Tobiasch M, Wagner S, et al. Hypophosphatemia after intravenous iron therapy: comprehensive review of clinical findings and recommendations for management. *Bone*. 2022;154:116202. https://doi.org/10.1016/j.bone.2021.116202
79. Vilaca T, Velmurugan N, Smith C, Abrahamsen B, Eastell R. Osteomalacia as a complication of intravenous iron infusion: a systematic review of case reports. *J Bone Miner Res*. 2022;37(6):1188–1199. https://doi.org/10.1002/jbmr.4558
80. Glaspy JA, Wolf M, Strauss WE. Intravenous iron-induced hypophosphatemia: an emerging syndrome. *Adv Ther*. 2021;38(7):3531–3549. https://doi.org/10.1007/s12325-021-01770-2
81. Schaefer B, Tobiasch M, Viveiros A, et al. Hypophosphataemia after treatment of iron deficiency with intravenous ferric carboxymaltose or iron isomaltoside-a systematic review and meta-analysis. *Br J Clin Pharmacol*. 2021;87(5):2256–2273. https://doi.org/10.1111/bcp.14643
82. Zoller H, Schaefer B, Glodny B. Iron-induced hypophosphatemia: an emerging complication. *Curr Opin Nephrol Hypertens*. 2017;26(4):266–275. https://doi.org/10.1097/MNH.0000000000000329
83. Wolf M, Koch TA, Bregman DB. Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. *J Bone Miner Res*. 2013;28(8):1793–1803. https://doi.org/10.1002/jbmr.1923