Mechanism of Bone Loss Induced by Iron Deficiency

Subjects: Public, Environmental & Occupational Health

Contributor: Jiancheng Yang , Qingmei Li , Yan Feng , Yuhong Zeng

Iron is a vital component of various enzymes involved in many biological processes, including oxygen transport, DNA biosynthesis, and cellular energy generation. Moreover, the biological activity of iron depends mainly on its efficient electron transfer properties. It has the ability to accept or donate electrons during the conversion between ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}), thereby functioning as a catalytic cofactor in various biochemical reactions. Therefore, iron is necessary for the growth, proliferation, and differentiation of bone cells, especially for osteoblasts and osteoclasts. In addition, hypoxia caused by iron deficiency can also lead to the activity and function disorder of bone cells. With respect to bone metabolism, iron is involved in the synthetic process of collagen and vitamin D metabolism.

iron deficiency anemia iron metabolism osteoblast

1. Physiological Iron in Bone Homeostasis

The maintenance of bone homeostasis depends on two important cells, osteoblasts that form new bone and osteoclasts that dissolve old and impaired bone. In the process of children's growth and development, bone formation plays a major role, far greater than the speed of bone destruction, termed bone modeling in this state. Once the bone grows to maturity, these two processes will form a roughly balanced state, termed bone remodeling [1]. Appropriate regulation and coordination of the function and differentiation of osteoblasts and osteoclasts is necessary to acquire bone homeostasis. Hematopoietic stem cells, the source cells of osteoclasts, first differentiate into bone marrow mononuclear cells (BMMs) under macrophage colony stimulating factor (M-CSF) stimulation and then differentiate to form multinuclear osteoclasts in the stimulation of NF-kB ligand receptor activator (RANKL) [2]. At the initial stage of bone remodeling, mononuclear precursors are recruited to the bone surface and undergo proliferation, differentiation, and fusion to finally form mature multinucleated cells [3]. Then, osteoclasts decompose or absorb bone and release calcium into the blood. After the completion of the bone resorption process, it was widely accepted in the past that that osteoclasts would undergo apoptosis or programmed cell death [4], but recently it was recognized that osteoclasts divide into daughter cells called osteomorphs, which can fuse and re-form osteoclasts in RANKL stimulation [5]. Osteoclasts secrete a variety of signal molecules during bone resorption, attracting bone marrow mesenchymal stem cells (BMSCs) to migrate to newly excavated pits, where they proliferate and differentiate into osteoblasts [3], or activate the quiescent bone lining cells on the bone surface to form osteoblasts [6]. In the process of bone formation, osteoblasts proliferate and differentiate to maturity, and then secrete a variety of matrix proteins, such as type I collagen (COL1), alkaline phosphatase (ALP), and osteocalcin,

to form osteoid [7]. Subsequently, hydroxyapatite crystals were deposited into the osteoid to form new mineralized bone. Once the mineralization process is completed, most osteoblasts are eliminated through apoptosis, and the residual are transformed into osteocytes embedded in the bone matrix and bone lining cells on the bone surface [8]. So far, the whole bone remodeling process is completed. There is no doubt that the activity and function of osteoblasts and osteoclasts are crucial in the maintenance of bone homeostasis.

Both osteoclast formation and bone absorption activity of mature osteoclasts require high energy; thus, osteoclasts contain large amounts of mitochondria. Mitochondrial respiratory complex I and peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β, key mitochondrial transcription regulator) are essential for osteoclast differentiation [9][10]. Mitochondrial reactive oxygen species (ROS) also are important components that stimulate osteoclastic differentiation and resorption of bone tissue [11]. Iron plays an important role in mitochondrial metabolism, ROS production, and the biosynthesis of heme and Fe-S clusters, which are the key components of the mitochondrial respiratory complex [12][13]. Therefore, iron is crucial for the differentiation of osteoclasts and the activation of bone resorption activity. Indeed, deferoxamine (DFO), an iron chelator, inhibits osteoclastogenesis, and bone resorption has been reported extensively in vitro [10][14][15]. Zhang et al. [14] found that DFO inhibited iron-uptake-stimulated osteoclast differentiation, negatively affected mitochondrial function through decreasing electron transport chain activity, and suppressed mitogen-activated protein kinase (MAPK) activation independently of ROS stimulation. However, the findings in vivo seem different from the results of cell experiments. In vivo, serum levels of bone turnover markers, such as tartrate-resistant acid phosphatase (TRAP), C-telopeptide of type I collagen (CTX-I), and N-telopeptide of type I collagen (NTX-I), can reflect the activity of osteoclasts [16]. Díaz-Castro et al. [17] demonstrated that severe nutritional IDA increased the levels of serum TRAP and CTX-I in rats. Toxqui et al. [18] showed that serum NTX-I levels were significantly higher in healthy iron-deficient women compared with iron-sufficient women.

Similarly to osteoclasts, osteoblasts also have a high demand for energy in osteogenic differentiation; thus, mitochondria play an essential role in this process [19][20]. Iron deficiency induced by treatment with an iron chelator deferoxamine (DFO) in primary cell cultures isolated from fetal rat calvaria was studied, the results of which demonstrated that low iron suppresses osteoblast phenotypic development [21]. Mild low iron induced by DFO promoted osteoblast activity, but serious low iron inhibited osteoblast activity [22]. Mouse and human progenitor cells, differentiated under standard osteoblast protocols in the presence of DFO, ALP activity, mineralization, and osteogenic genes (e.g., osterix, Col1a1, Bglap, and Dmp1), were reduced significantly [23]. Interestingly, the same dose of DFO had no significant effect on adipogenic differentiation, suggesting that osteoblasts maintain a higher iron requirement for differentiation and function compared with adipocytes [23]. In vivo, a zebrafish model of FPN gene mutant showed severe iron deficiency, resulting in a reduction in the number of calcified vertebrae in the larvae and a reduction in the expression of bone formation genes (such as alpl, runx2a, and col1a1a) and BMP signal genes (bmp2a and bmp2b), indicating that iron deficiency may inhibit bone formation through the BMP signal pathway [24]. Serum bone formation makers, procollagen type I N-terminal propeptide (P1NP) and osteocalcin, were markedly decreased by dietary iron deficiency in rats [17][25]. These parameters were recovered after supplying a normal or high-iron diet [26].

In brief, iron is involved in the regulation of the basic cell activity and function of osteoblasts and osteoclasts, and is an essential mineral element for maintaining bone homeostasis. However, iron must be controlled at the physiological level because excessive iron can lead to the over-activation of osteoclasts and the toxic effect of osteoblasts, resulting in the imbalance of bone homeostasis, and ultimately induce osteopenia and even osteoporosis [27][28].

2. Iron-Deficiency-Induced Hypoxia in Bone Homeostasis

Iron is closely associated with oxygen sensing in cells. Since iron is indispensable to oxygen transport, iron deficiency can create low-oxygen conditions (hypoxia) as a result of decreased oxygen delivery to cells and tissues. Hypoxia-inducible factors (HIFs), the heterodimeric transcription factors composed of an oxygen-sensitive α subunit and a stable β subunit, are critical mediators of the cellular response to hypoxia [29]. HIF1-α and HIF2-α are proposed to contribute to the functions of osteoblasts and osteoclasts in bone homeostasis [30][31], which can potentially be attributed to the hypoxic condition in bone microenvironments, such as the endosteal areas of bone medullary cavity and the epiphyseal growth plate [32].

Hypoxia stimulates angiogenesis-dependent bone formation during bone regeneration, mainly via VEGF-dependent promotion of neoangiogenesis through the HIF-1α pathway [33]. Mice overexpressing HIF-1α in osteoblasts increases bone modeling in developing bone [34]. Mice lacking HIF-1α in osteoblasts markedly decreases trabecular bone volume, reduces bone formation rate, and alters cortical bone architecture in developmental long bone [35]. Prolyl hydroxylases (PHDs) and Von Hippel–Lindau (VHL) are the upstream regulators of HIF-α. Under normoxia, PHDs hydroxylate HIF-α on two proline residues, inducing VHL-mediated poly-ubiquitination and proteasomal degradation [36]. Therefore, knockdown of PHDs or VHL in mesenchymal progenitors leads to elevated HIF-1α, which further stimulates osteogenesis [37][38]. However, another study showed that conditional knockout of PHD2 in osteoblast lineage cells resulted in reduced bone mineral content, bone area, and BMD in femurs and tibias of mice [39]. Bone marrow stromal cells derived from PHD2 knockout mice formed fewer mineralized nodules when cultured with a mineralized medium, suggesting that PHD2 plays an important role in regulating bone formation [39]. Several studies have shown that hypoxia and HIF-1α promote osteoclastogenesis and subsequent bone resorption during bone remodeling, in contrast to the anabolic role of HIF-1α in bone modeling. For example, hypoxia and HIF-1α overexpression inhibit BMP-2-induced osteoblast differentiation and stimulate osteoclastogenesis [40]. Osteoclast-specific HIF-1α inactivation antagonizes bone loss in ovariectomy (OVX) mice and osteoclast-specific estrogen receptor α-deficient mice, whereas oral HIF-1α inhibitors protect OVX mice from osteoclast activation and bone loss [41]. Similarly, HIF-1α protein accumulates in osteoclasts following orchidectomy (ORX) in mice, and administration of a HIF-1α inhibitor abrogated ORX-induced osteoclast activation and bone loss [42]. The reasons for potential differences regarding the role of HIF-1α in bone modeling and remodeling are unclear but may reflect the combined coordinated or coupled effects of multiple cell types in the bone microenvironment in vivo.

In contrast to the role of HIF-1α in promoting bone formation, HIF-2α inhibits the differentiation of osteoblasts [43]. HIF-2α deficiency in mice enhances bone formation in vivo and overexpression of HIF-2α inhibits osteoblast

differentiation of mouse calvarial preosteoblasts by targeting *Twist2* [44]. Although both HIF-1α and HIF-2α activate osteoclasts, the former mainly mediates the bone resorption capacity of osteoclasts, while the latter is mainly involved in the process of osteoclast formation [45]. HIF-2α deficiency in mice stimulates osteoclast formation in vivo, and overexpression of HIF-2α enhances osteoclast differentiation of mouse-bone-marrow-derived macrophages (BMMs) via regulation of Traf6 [44]. In addition, HIF-2α contributes to the interaction between osteoblasts and osteoclasts by directly targeting RANKL in preosteoblasts [44]. Osteoblast-specific or osteoclast-specific conditional knockout of HIF-2α in male mice enhances bone mass and reverses age-related bone loss [44] [46]. Consequently, HIF-2α may regulate bone homeostasis through its effects on osteoblasts and osteoclasts during bone remodeling.

3. Iron in Collagen Synthesis

Iron participates in a variety of enzymatic systems in the body, including the enzymes involved in collagen synthesis. In mammals, approximately 28 types of collagen have so far been identified [47]; among these types, the most prevalent is type I collagen that was found in the extracellular matrix (ECM), particularly in tissues such as tendon and bone [48]. Bone is a complex assembly of type I collagen fibers filled in with mineral crystal of hydroxyapatite [49]. Type I collagen constitutes 90% of the organic matrix, and together with the mineral, governs the biomechanical properties and functional integrity of bone [50].

For collagen synthesis, a three-dimensional stranded structure with the amino acids glycine and proline as its principal components is assembled primarily. This is called procollagen and is precursor of collagen. Procollagen is then modified by adding the hydroxyl groups to proline and lysine. This reaction requires α-ketoglutarate, molecular oxygen, ferrous iron, and a reducing agent, and it is an important step to later glycosylation and the formation of the triple-helix structure of collagen [51]. In this process, ascorbate and molecular oxygen reduce the inactive Fe^{3+} to the active Fe^{2+} [52], and α-ketoglutarate is decarboxylated oxidatively to produce succinate and CO_2 [53]. Two different enzymes, prolyl-hydroxylase and lysyl-hydroxylase, catalyze these hydroxylation reactions, and iron is essential in this pathway. Moreover, iron-containing prolyl hydroxylases are also involved in the regulation of hypoxia-inducible factors (HIF), which sense oxygen status in the body. When oxygen is sufficient, HIFs are transcriptionally restrained through ubiquitination by prolyl hydroxylation [54]. Therefore, it is a hypothesis that in an iron deficiency status, there may be less iron available to the prolyl and lysyl hydroxylases which could result in decreased cross-linking activity and, subsequently, weaker collagen fibers [55].

C-terminal propeptide of type I procollagen (P1CP) and P1NP, the byproducts of the process of type I collagen synthesis, are cleaved from type I procollagen by proteases outside the osteoblast [16]. Serum P1NP and P1CP levels reflect the rate of type I collagen synthesis and osteoblast activity. CTX-I and NTX-I are the degradation products of collagen, and serum CTX-I and NTX-I levels reflect the rate of type I collagen degradation. Iron deficiency can decrease serum P1NP or P1CP levels and increase serum CTX-I or NTX-I levels, which has been shown in human and animal studies. Wright et al. [56] indicated that serum NTX-I levels were significantly higher and serum P1NP levels tended to be lower in woman with iron deficiency anemia (IDA). Similarly, the lower amount of serum P1NP and increased serum CTX-I were found in IDA rats by Díaz-Castro et al. [17]. Scott et al. [57] also

demonstrated that iron deficiency increased the concentration of serum CTX-I in rats. Therefore, iron deficiency may reduce the synthesis and increase the degradation of type I collagen in bone tissue.

4. Iron in Vitamin D Metabolism

Iron is involved in bone metabolism, and another mechanism may be through vitamin D activation and deactivation [58][59]. Vitamin D plays a major role in the regulation of mineral homeostasis and affects bone metabolism [60]. The vitamin D hormone maintains a constant level in serum calcium concentrations. Active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] promotes the absorption of calcium and phosphorous in the intestine, phosphate reabsorption in the kidney, and calcium and phosphate release from the bone [61]. Moreover, active vitamin D has a direct regulating effect on the activity and function of osteoblast and osteoclast [62][63][64]. Therefore, vitamin D deficiency in the body will cause osteoporosis; in contrast, vitamin D supplementation can increase bone density and is widely used in the treatment of osteoporosis [65][66].

Iron is an essential element involved in the cytochrome P450 superfamily, which catalyzes single or multiple hydroxylation reactions in pathways by vitamin D substrate at specific carbons using a heme-bound iron [59]. Vitamin D from the diet and skin has two steps of hydroxylation for its activation. Firstly, it is hydroxylated in the liver into 25-hydroxyvitamin D [25(OH)D] by the cytochrome P-450 25-hydroxylase (CYP2R1) [67]. In addition, further hydroxylases, such as CYP27A1 and CYP3A4, have been found involved in the anabolism of vitamin D in the liver [68][69]. In the second step, 25(OH)D is transported to the kidney and is hydroxylated into 1,25$(OH)_2$D, the active form of the vitamin D, by the 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1). In the inactivation process, the 1,25$(OH)_2$D is inactivated by the 1α,25-hydroxyvitamin D 24-hydroxylase (CYP24A1) through multiple oxidations of the sterol side chain [70]. In case of iron deficiency in tissues, the activity of iron-containing enzymes is decreased [71].

Numerous studies have revealed a relationship between clinical iron deficiency and low vitamin D levels [18][72]. Blanco-Rojo et al. [72] demonstrated that vitamin D deficiency or insufficiency is very high in women with iron deficiency. However, the recovery of iron status by an iron-fortified diet did not affect 25(OH)D levels. Grindulis et al. [73] showed that there is a significant association between iron deficiency and lower vitamin D levels in Asian children. Qader et al. [74] revealed that serum vitamin D levels were lower in Iraqi children with iron deficiency in contrast with children with normal iron. El-Adawy et al. [75] certified that vitamin D deficiency had a higher frequency in Egyptian adolescent females with IDA than the healthy control. Jin et al. [76] found that vitamin D deficiency existed in 67% of infants with IDA. Thus, vitamin D supplementation is important particularly in IDA infants. Ferritin, an iron-containing protein with high molecular weight, plays a key role in the body as an iron storage compound [77]. Serum ferritin concentration has been proposed as an index of iron deficiency and iron overload [78]. Kang et al. [79] showed that there is an association between lower serum ferritin and vitamin D in breastfed infants and their mothers that had anemia during pregnancy.

In animal models, an iron-deficient diet has been shown to reduce serum vitamin D levels. Rats were fed on an iron-deficient diet for 6 weeks, and the serum levels of 25-(OH)D and 1,25-$(OH)_2$D were significantly lower than

those in rats with a normal diet [80]. Moreover, Western blotting, immunofluorescence, and q-PCR assay revealed that the protein and gene expressions of CYP2R1, CYP27A1, and CYP24A1 in the iron-deficient diet group were down-regulated compared to control group, and the expression of CYP27B1 protein and gene was up-regulated in rats with a low-iron diet. However, another study indicated that an iron-deficient diet reduced renal CYP27B1 activity, accompanied by a decreased serum 1,25-$(OH)_2D_3$ concentration and bone formation in rats [81]. These data demonstrate that iron may be involved in the metabolism of vitamin D by regulating the expression of vitamin D hydroxylase, suggesting that appropriate iron supplementation might activate vitamin D.

In addition, iron deficiency regulates vitamin D metabolism potentially through fibroblast growth factor 23 (FGF23), which is secreted by osteocytes [82]. The main target organ of FGF23 is the kidney, where FGF23 inhibits transcription of the key enzyme in vitamin D hormone activation, CYP27B1, and activates transcription of the key enzyme responsible for vitamin D degradation, CYP24A1 [83]. In the absence of FGF23 signaling, tight control of renal 1α-hydroxylase fails, leading to overproduction of 1,25-$(OH)_2D$ in mice and humans [84][85]. Therefore, FGF-23 is a crucial modulator of vitamin D metabolism. Iron deficiency stimulated the transcription of FGF23 in osteocytes and the increase in serum levels of intact, biologically active FGF23 (iFGF23) and C-terminal fragments, a degradation product; biologically inactive FGF23 (cFGF23) in mice has been reported [86][87]. However, several studies revealed that serum iFGF23 levels did not change, whereas cFGF23 levels were significantly increased in iron-deficient mice [86][88]. Similar alteration of serum FGF23 levels was also found in patients with iron deficiency or IDA [89][90]. FGF23 is metabolized and eliminated by the kidneys [91].

In conclusion, iron deficiency can directly or indirectly regulate the activity and function of osteoblasts and osteoclasts by inducing hypoxia and disorder of vitamin D metabolism, ultimately destructing bone homeostasis. Reduced active vitamin D also leads to disturbances in calcium and phosphorus metabolism and thus affects bone metabolism. In addition, iron deficiency potentially inhibits new bone formation by deranging collagen synthesis.

References

1. Langdahl, B.; Ferrari, S.; Dempster, D.W. Bone modeling and remodeling: Potential as therapeutic targets for the treatment of osteoporosis. Ther. Adv. Musculoskelet. Dis. 2016, 8, 225–235.
2. Kubatzky, K.F.; Uhle, F.; Eigenbrod, T. From macrophage to osteoclast - How metabolism determines function and activity. Cytokine 2018, 112, 102–115.
3. Kenkre, J.S.; Bassett, J. The bone remodelling cycle. Ann. Clin. Biochem. 2018, 55, 308–327.
4. Manolagas, S.C. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr. Rev. 2000, 21, 115–137.
5. McDonald, M.M.; Khoo, W.H.; Ng, P.Y.; Xiao, Y.; Zamerli, J.; Thatcher, P.; Kyaw, W.; Pathmanandavel, K.; Grootveld, A.K.; Moran, I.; et al. Osteoclasts recycle via osteomorphs during RANKL-stimulated bone resorption. Cell 2021, 184, 1330–1347.

6. Matic, I.; Matthews, B.G.; Wang, X.; Dyment, N.A.; Worthley, D.L.; Rowe, D.W.; Grcevic, D.; Kalajzic, I. Quiescent Bone Lining Cells Are a Major Source of Osteoblasts During Adulthood. Stem Cells 2016, 34, 2930–2942.
7. Amarasekara, D.S.; Kim, S.; Rho, J. Regulation of Osteoblast Differentiation by Cytokine Networks. Int. J. Mol. Sci. 2021, 22, 2851.
8. Eghbali-Fatourechi, G.Z.; Lamsam, J.; Fraser, D.; Nagel, D.; Riggs, B.L.; Khosla, S. Circulating osteoblast-lineage cells in humans. N. Engl. J. Med. 2005, 352, 1959–1966.
9. Jin, Z.; Wei, W.; Yang, M.; Du, Y.; Wan, Y. Mitochondrial complex I activity suppresses inflammation and enhances bone resorption by shifting macrophage-osteoclast polarization. Cell Metab. 2014, 20, 483–498.
10. Ishii, K.A.; Fumoto, T.; Iwai, K.; Takeshita, S.; Ito, M.; Shimohata, N.; Aburatani, H.; Taketani, S.; Lelliott, C.J.; Vidal-Puig, A.; et al. Coordination of PGC-1beta and iron uptake in mitochondrial biogenesis and osteoclast activation. Nat. Med. 2009, 15, 259–266.
11. Agidigbi, T.S.; Kim, C. Reactive Oxygen Species in Osteoclast Differentiation and Possible Pharmaceutical Targets of ROS-Mediated Osteoclast Diseases. Int. J. Mol. Sci. 2019, 20, 3576.
12. Xu, W.; Barrientos, T.; Andrews, N.C. Iron and copper in mitochondrial diseases. Cell Metab. 2013, 17, 319–328.
13. Dixon, S.J.; Stockwell, B.R. The role of iron and reactive oxygen species in cell death. Nat. Chem. Biol. 2014, 10, 9–17.
14. Zhang, J.; Hu, W.; Ding, C.; Yao, G.; Zhao, H.; Wu, S. Deferoxamine inhibits iron-uptake stimulated osteoclast differentiation by suppressing electron transport chain and MAPKs signaling. Toxicol. Lett. 2019, 313, 50–59.
15. Guo, J.P.; Pan, J.X.; Xiong, L.; Xia, W.F.; Cui, S.; Xiong, W.C. Iron Chelation Inhibits Osteoclastic Differentiation In Vitro and in Tg2576 Mouse Model of Alzheimer's Disease. PLoS ONE 2015, 10, e0139395.
16. Eastell, R.; Szulc, P. Use of bone turnover markers in postmenopausal osteoporosis. Lancet Diabetes Endocrinol. 2017, 5, 908–923.
17. Diaz-Castro, J.; Lopez-Frias, M.R.; Campos, M.S.; Lopez-Frias, M.; Alferez, M.J.; Nestares, T.; Ojeda, M.L.; Lopez-Aliaga, I. Severe nutritional iron-deficiency anaemia has a negative effect on some bone turnover biomarkers in rats. Eur. J. Nutr. 2012, 51, 241–247.
18. Toxqui, L.; Perez-Granados, A.M.; Blanco-Rojo, R.; Wright, I.; de la Piedra, C.; Vaquero, M.P. 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, 441–448.

19. Shum, L.C.; White, N.S.; Mills, B.N.; Bentley, K.L.; Eliseev, R.A. Energy Metabolism in Mesenchymal Stem Cells During Osteogenic Differentiation. Stem Cells Dev. 2016, 25, 114–122.
20. Lee, W.C.; Guntur, A.R.; Long, F.; Rosen, C.J. Energy Metabolism of the Osteoblast: Implications for Osteoporosis. Endocr. Rev. 2017, 38, 255–266.
21. Messer, J.G.; Cooney, P.T.; Kipp, D.E. Iron chelator deferoxamine alters iron-regulatory genes and proteins and suppresses osteoblast phenotype in fetal rat calvaria cells. Bone 2010, 46, 1408–1415.
22. Zhao, G.Y.; Zhao, L.P.; He, Y.F.; Li, G.F.; Gao, C.; Li, K.; Xu, Y.J. A comparison of the biological activities of human osteoblast hFOB1.19 between iron excess and iron deficiency. Biol. Trace Elem. Res. 2012, 150, 487–495.
23. Edwards, D.F., 3rd; Miller, C.J.; Quintana-Martinez, A.; Wright, C.S.; Prideaux, M.; Atkins, G.J.; Thompson, W.R.; Clinkenbeard, E.L. Differential Iron Requirements for Osteoblast and Adipocyte Differentiation. JBMR Plus 2021, 5, e10529.
24. Bo, L.; Liu, Z.; Zhong, Y.; Huang, J.; Chen, B.; Wang, H.; Xu, Y. Iron deficiency anemia's effect on bone formation in zebrafish mutant. Biochem. Biophys. Res. Commun. 2016, 475, 271–276.
25. 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, 2547–2550.
26. Diaz-Castro, J.; Ramirez Lopez-Frias, M.; Campos, M.S.; Lopez-Frias, M.; Alferez, M.J.; Nestares, T.; Ortega, E.; Lopez-Aliaga, I. Goat milk during iron repletion improves bone turnover impaired by severe iron deficiency. J. Dairy Sci. 2011, 94, 2752–2761.
27. Balogh, E.; Paragh, G.; Jeney, V. Influence of Iron on Bone Homeostasis. Pharmaceuticals 2018, 11, 107.
28. Ledesma-Colunga, M.G.; Weidner, H.; Vujic Spasic, M.; Hofbauer, L.C.; Baschant, U.; Rauner, M. Shaping the bone through iron and iron-related proteins. Semin. Hematol. 2021, 58, 188–200.
29. Semenza, G.L. Hypoxia-inducible factors in physiology and medicine. Cell 2012, 148, 399–408.
30. Qin, Q.; Liu, Y.; Yang, Z.; Aimaijiang, M.; Ma, R.; Yang, Y.; Zhang, Y.; Zhou, Y. Hypoxia-Inducible Factors Signaling in Osteogenesis and Skeletal Repair. Int. J. Mol. Sci. 2022, 23, 11201.
31. Mendoza, S.V.; Genetos, D.; Yellowley, C. Hypoxia inducible factor-2α signaling in the skeletal system. JBMR Plus 2023, e10733.
32. Hiraga, T. Hypoxic Microenvironment and Metastatic Bone Disease. Int. J. Mol. Sci. 2018, 19, 3523.

33. Wan, C.; Gilbert, S.R.; Wang, Y.; Cao, X.; Shen, X.; Ramaswamy, G.; Jacobsen, K.A.; Alaql, Z.S.; Eberhardt, A.W.; Gerstenfeld, L.C.; et al. Activation of the hypoxia-inducible factor-1alpha pathway accelerates bone regeneration. Proc. Natl. Acad. Sci. USA 2008, 105, 686–691.

34. Wang, Y.; Wan, C.; Deng, L.; Liu, X.; Cao, X.; Gilbert, S.R.; Bouxsein, M.L.; Faugere, M.C.; Guldberg, R.E.; Gerstenfeld, L.C.; et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J. Clin. Investig. 2007, 117, 1616–1626.

35. Shomento, S.H.; Wan, C.; Cao, X.; Faugere, M.C.; Bouxsein, M.L.; Clemens, T.L.; Riddle, R.C. Hypoxia-inducible factors 1alpha and 2alpha exert both distinct and overlapping functions in long bone development. J. Cell. Biochem. 2010, 109, 196–204.

36. Strowitzki, M.J.; Cummins, E.P.; Taylor, C.T. Protein Hydroxylation by Hypoxia-Inducible Factor (HIF) Hydroxylases: Unique or Ubiquitous? Cells 2019, 8, 384.

37. Wu, C.; Rankin, E.B.; Castellini, L.; Alcudia, J.F.; LaGory, E.L.; Andersen, R.; Rhodes, S.D.; Wilson, T.L.; Mohammad, K.S.; Castillo, A.B.; et al. Oxygen-sensing PHDs regulate bone homeostasis through the modulation of osteoprotegerin. Genes Dev. 2015, 29, 817–831.

38. Mangiavini, L.; Merceron, C.; Araldi, E.; Khatri, R.; Gerard-O'Riley, R.; Wilson, T.L.; Rankin, E.B.; Giaccia, A.J.; Schipani, E. Loss of VHL in mesenchymal progenitors of the limb bud alters multiple steps of endochondral bone development. Dev. Biol. 2014, 393, 124–136.

39. Cheng, S.; Xing, W.; Pourteymoor, S.; Mohan, S. Conditional disruption of the prolyl hydroxylase domain-containing protein 2 (Phd2) gene defines its key role in skeletal development. J. Bone Miner. Res. 2014, 29, 2276–2286.

40. Hiraga, T.; Kizaka-Kondoh, S.; Hirota, K.; Hiraoka, M.; Yoneda, T. Hypoxia and hypoxia-inducible factor-1 expression enhance osteolytic bone metastases of breast cancer. Cancer Res. 2007, 67, 4157–4163.

41. Miyauchi, Y.; Sato, Y.; Kobayashi, T.; Yoshida, S.; Mori, T.; Kanagawa, H.; Katsuyama, E.; Fujie, A.; Hao, W.; Miyamoto, K.; et al. HIF1α is required for osteoclast activation by estrogen deficiency in postmenopausal osteoporosis. Proc. Natl. Acad. Sci. USA 2013, 110, 16568–16573.

42. Tando, T.; Sato, Y.; Miyamoto, K.; Morita, M.; Kobayashi, T.; Funayama, A.; Kanaji, A.; Hao, W.; Watanabe, R.; Oike, T.; et al. Hif1α is required for osteoclast activation and bone loss in male osteoporosis. Biochem. Biophys. Res. Commun. 2016, 470, 391–396.

43. Merceron, C.; Ranganathan, K.; Wang, E.; Tata, Z.; Makkapati, S.; Khan, M.P.; Mangiavini, L.; Yao, A.Q.; Castellini, L.; Levi, B.; et al. Hypoxia-inducible factor 2α is a negative regulator of osteoblastogenesis and bone mass accrual. Bone Res. 2019, 7, 7.

44. Lee, S.Y.; Park, K.H.; Yu, H.G.; Kook, E.; Song, W.H.; Lee, G.; Koh, J.T.; Shin, H.I.; Choi, J.Y.; Huh, Y.H.; et al. Controlling hypoxia-inducible factor-2α is critical for maintaining bone

homeostasis in mice. Bone Res. 2019, 7, 14.

45. Knowles, H.J. Distinct roles for the hypoxia-inducible transcription factors HIF-1α and HIF-2α in human osteoclast formation and function. Sci. Rep. 2020, 10, 21072.
46. Lee, S.Y.; Park, K.H.; Lee, G.; Kim, S.J.; Song, W.H.; Kwon, S.H.; Koh, J.T.; Huh, Y.H.; Ryu, J.H. Hypoxia-inducible factor-2α mediates senescence-associated intrinsic mechanisms of age-related bone loss. Exp. Mol. Med. 2021, 53, 591–604.
47. Gelse, K.; Poschl, E.; Aigner, T. Collagens—Structure, function, and biosynthesis. Adv. Drug Deliv. Rev. 2003, 55, 1531–1546.
48. Currey, J.D. The structure and mechanics of bone. J. Mater. Sci. 2012, 47, 41–54.
49. Ferreira, A.M.; Gentile, P.; Chiono, V.; Ciardelli, G. Collagen for bone tissue regeneration. Acta Biomater. 2012, 8, 3191–3200.
50. Viguet-Carrin, S.; Garnero, P.; Delmas, P. The role of collagen in bone strength. Osteoporos. Int. 2006, 17, 319–336.
51. Tuderman, L.; Myllylä, R.; Kivirikko, K.I. Mechanism of the prolyl hydroxylase reaction. Role of co-substrates. Eur. J. Biochem. 1977, 80, 341–348.
52. De Jong, L.; Kemp, A. Stoicheiometry and kinetics of the prolyl 4-hydroxylase partial reaction. Biochim. Biophys. Acta 1984, 787, 105–111.
53. Gorres, K.L.; Raines, R.T. Prolyl 4-hydroxylase. Crit. Rev. Biochem. Mol. Biol. 2010, 45, 106–124.
54. Masson, N.; Willam, C.; Maxwell, P.H.; Pugh, C.W.; Ratcliffe, P.J. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J. 2001, 20, 5197–5206.
55. Toxqui, L.; Vaquero, M.P. Chronic iron deficiency as an emerging risk factor for osteoporosis: A hypothesis. Nutrients 2015, 7, 2324–2344.
56. Wright, I.; Blanco-Rojo, R.; Fernández, M.C.; Toxqui, L.; Moreno, G.; Pérez-Granados, A.M.; de la Piedra, C.; Remacha, Á.F.; Vaquero, M.P. Bone remodelling is reduced by recovery from iron-deficiency anaemia in premenopausal women. J. Physiol. Biochem. 2013, 69, 889–896.
57. Scott, J.M.; Swallow, E.A.; Metzger, C.E.; Kohler, R.; Wallace, J.M.; Stacy, A.J.; Allen, M.R.; Gasier, H.G. Iron deficiency and high-intensity running interval training do not impact femoral or tibial bone in young female rats. Br. J. Nutr. 2022, 128, 1518–1525.
58. Al-Daghri, N.M.; Yakout, S.; Ghaleb, A.; Hussain, S.D.; Sabico, S. Iron and 25-hydroxyvitamin D in postmenopausal women with osteoporosis. Am. J. Transl. Res. 2022, 14, 1387–1405.
59. Jones, G.; Prosser, D.E.; Kaufmann, M. Cytochrome P450-mediated metabolism of vitamin D. J. Lipid Res. 2014, 55, 13–31.

60. Burt, L.A.; Billington, E.O.; Rose, M.S.; Raymond, D.A.; Hanley, D.A.; Boyd, S.K. Effect of High-Dose Vitamin D Supplementation on Volumetric Bone Density and Bone Strength: A Randomized Clinical Trial. JAMA 2019, 322, 736–745.
61. Goltzman, D. Functions of vitamin D in bone. Histochem. Cell Biol. 2018, 149, 305–312.
62. van Driel, M.; Pols, H.A.; van Leeuwen, J.P. Osteoblast differentiation and control by vitamin D and vitamin D metabolites. Curr. Pharm. Des. 2004, 10, 2535–2555.
63. Kikuta, J.; Kawamura, S.; Okiji, F.; Shirazaki, M.; Sakai, S.; Saito, H.; Ishii, M. Sphingosine-1-phosphate-mediated osteoclast precursor monocyte migration is a critical point of control in antibone-resorptive action of active vitamin D. Proc. Natl. Acad. Sci. USA 2013, 110, 7009–7013.
64. Li, A.; Cong, Q.; Xia, X.; Leong, W.F.; Yeh, J.; Miao, D.; Mishina, Y.; Liu, H.; Li, B. Pharmacologic Calcitriol Inhibits Osteoclast Lineage Commitment via the BMP-Smad1 and IκB-NF-κB Pathways. J. Bone Miner. Res. 2017, 32, 1406–1420.
65. Polzonetti, V.; Pucciarelli, S.; Vincenzetti, S.; Polidori, P. Dietary Intake of Vitamin D from Dairy Products Reduces the Risk of Osteoporosis. Nutrients 2020, 12, 1743.
66. Hou, Y.-C.; Wu, C.-C.; Liao, M.-T.; Shyu, J.-F.; Hung, C.-F.; Yen, T.-H.; Lu, C.-L.; Lu, K.-C. Role of nutritional vitamin D in osteoporosis treatment. Clin. Chim. Acta 2018, 484, 179–191.
67. Cheng, J.B.; Levine, M.A.; Bell, N.H.; Mangelsdorf, D.J.; Russell, D.W. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc. Natl. Acad. Sci. USA 2004, 101, 7711–7715.
68. Sawada, N.; Sakaki, T.; Ohta, M.; Inouye, K. Metabolism of Vitamin D3 by Human CYP27A1. Biochem. Biophys. Res. Commun. 2000, 273, 977–984.
69. Roizen, J.D.; Li, D.; O'Lear, L.; Javaid, M.K.; Shaw, N.J.; Ebeling, P.R.; Nguyen, H.H.; Rodda, C.P.; Thummel, K.E.; Thacher, T.D.; et al. CYP3A4 mutation causes vitamin D-dependent rickets type. J. Clin. Investig. 2018, 128, 1913–1918.
70. Schlingmann, K.P.; Kaufmann, M.; Weber, S.; Irwin, A.; Goos, C.; John, U.; Misselwitz, J.; Klaus, G.; Kuwertz-Bröking, E.; Fehrenbach, H.; et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. N. Engl. J. Med. 2011, 365, 410–421.
71. Galan, P.; Hercberg, S.; Touitou, Y. The activity of tissue enzymes in iron-deficient rat and man: An overview. Comp. Biochem. Physiol. B 1984, 77, 647–653.
72. Blanco-Rojo, R.; Perez-Granados, A.M.; Toxqui, L.; Zazo, P.; de la Piedra, C.; Vaquero, M.P. Relationship between vitamin D deficiency, bone remodelling and iron status in iron-deficient young women consuming an iron-fortified food. Eur. J. Nutr. 2013, 52, 695–703.
73. Grindulis, H.; Scott, P.H.; Belton, N.R.; Wharton, B.A. Combined deficiency of iron and vitamin D in Asian toddlers. Arch. Dis. Child. 1986, 61, 843–848.

74. Qader, E.A.; Alkhateeb, N.E. Vitamin D Status in Children with Iron Deficiency and/or Anemia. Int. J. Pediatr. 2016, 4, 3571–3577.
75. El-Adawy, E.H.; Zahran, F.E.; Shaker, G.A.; Seleem, A. Vitamin D Status in Egyptian Adolescent Females with Iron Deficiency Anemia and Its Correlation with Serum Iron Indices. Endocr. Metab. Immune Disord. Drug Targets 2019, 19, 519–525.
76. Jin, H.J.; Lee, J.H.; Kim, M.K. The prevalence of vitamin D deficiency in iron-deficient and normal children under the age of 24 months. Blood Res. 2013, 48, 40–45.
77. Mancias, J.D.; Wang, X.; Gygi, S.P.; Harper, J.W.; Kimmelman, A.C. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature 2014, 509, 105–109.
78. Garcia-Casal, M.N.; Pasricha, S.R.; Martinez, R.X.; Lopez-Perez, L.; Peña-Rosas, J.P. Serum or plasma ferritin concentration as an index of iron deficiency and overload. Cochrane Database Syst. Rev. 2021, 5, Cd011817.
79. Kang, Y.S.; Kim, J.H.; Ahn, E.H.; Yoo, E.G.; Kim, M.K. Iron and vitamin D status in breastfed infants and their mothers. Korean J. Pediatr. 2015, 58, 283–287.
80. Qiu, F.; Li, R.; Gu, S.; Zhao, Y.; Yang, L. The effect of iron dextran on vitamin D3 metabolism in SD rats. Nutr. Metab. 2022, 19, 47.
81. Katsumata, S.; Katsumata, R.; Matsumoto, N.; Inoue, H.; Takahashi, N.; Uehara, M. Iron deficiency decreases renal 25-hydroxyvitamin D3-1α-hydroxylase activity and bone formation in rats. BMC Nutr. 2016, 2, 33.
82. Noonan, M.L.; Ni, P.; Solis, E.; Marambio, Y.G.; Agoro, R.; Chu, X.; Wang, Y.; Gao, H.; Xuei, X.; Clinkenbeard, E.L.; et al. Osteocyte Egln1/Phd2 links oxygen sensing and biomineralization via FGF23. Bone Res. 2023, 11, 7.
83. Latic, N.; Erben, R.G. FGF23 and Vitamin D Metabolism. JBMR Plus 2021, 5, e10558.
84. Shimada, T.; Kakitani, M.; Yamazaki, Y.; Hasegawa, H.; Takeuchi, Y.; Fujita, T.; Fukumoto, S.; Tomizuka, K.; Yamashita, T. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J. Clin. Investig. 2004, 113, 561–568.
85. Araya, K.; Fukumoto, S.; Backenroth, R.; Takeuchi, Y.; Nakayama, K.; Ito, N.; Yoshii, N.; Yamazaki, Y.; Yamashita, T.; Silver, J.; et al. A novel mutation in fibroblast growth factor 23 gene as a cause of tumoral calcinosis. J. Clin. Endocrinol. Metab. 2005, 90, 5523–5527.
86. Farrow, E.G.; Yu, X.; Summers, L.J.; Davis, S.I.; Fleet, J.C.; Allen, M.R.; Robling, A.G.; Stayrook, K.R.; Jideonwo, V.; Magers, M.J.; et al. Iron deficiency drives an autosomal dominant hypophosphatemic rickets (ADHR) phenotype in fibroblast growth factor-23 (Fgf23) knock-in mice. Proc. Natl. Acad. Sci. USA 2011, 108, E1146–E1155.

87. Clinkenbeard, E.L.; Farrow, E.G.; Summers, L.J.; Cass, T.A.; Roberts, J.L.; Bayt, C.A.; Lahm, T.; Albrecht, M.; Allen, M.R.; Peacock, M.; et al. Neonatal iron deficiency causes abnormal phosphate metabolism by elevating FGF23 in normal and ADHR mice. J. Bone Miner. Res. 2014, 29, 361–369.

88. David, V.; Martin, A.; Isakova, T.; Spaulding, C.; Qi, L.; Ramirez, V.; Zumbrennen-Bullough, K.B.; Sun, C.C.; Lin, H.Y.; Babitt, J.L.; et al. Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. Kidney Int. 2016, 89, 135–146.

89. Wolf, M.; Koch, T.A.; Bregman, D.B. Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. J. Bone Miner. Res. 2013, 28, 1793–1803.

90. Imel, E.A.; Peacock, M.; Gray, A.K.; Padgett, L.R.; Hui, S.L.; Econs, M.J. Iron modifies plasma FGF23 differently in autosomal dominant hypophosphatemic rickets and healthy humans. J. Clin. Endocrinol. Metab. 2011, 96, 3541–3549.

91. Sharma, S.; Katz, R.; Ginsberg, C.; Bullen, A.; Vallon, V.; Thomson, S.; Moe, O.W.; Hoofnagle, A.N.; de Leeuw, P.W.; Kroon, A.A.; et al. Renal Clearance of Fibroblast Growth Factor-23 (FGF23) and its Fragments in Humans. J. Bone Miner. Res. 2022, 37, 1170–1178.