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REVIEW

Intestinal calcium transport and its regulation in thalassemia: interaction between calcium and iron metabolism

Kornkamon Lertsuwan[1,2] · Kannikar Wongdee[2,3] · Jarinthorn Teerapornpuntakit[2,4] · Narattaphol Charoenphandhu[2,5,6]

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Abstract

Osteoporosis and derangement of calcium homeostasis are common complications of thalassemia. Despite being an important process for bone and calcium metabolism, little is known about intestinal calcium transport in thalassemia. Recent reports of decreases in both intestinal calcium transport and bone mineral density in thalassemic patients and animal models suggested that defective calcium absorption might be a cause of thalassemic bone disorder. Herein, the possible mechanisms associated with intestinal calcium malabsorption in thalassemia are discussed. This includes alterations in the calcium transporters and hormonal controls of the transcellular and paracellular intestinal transport systems in thalassemia. In addition, the effects of iron overload on intestinal calcium absorption, and the reciprocal interaction between iron and calcium transport in thalassemia are elaborated. Understanding the mechanisms underlining calcium malabsorption in thalassemia would lead to development of therapeutic agents and mineral supplements that restore calcium absorption as well as prevent osteoporosis in thalassemic patients.

Keywords Calcium transport · Iron transport · Osteoporosis · Thalassemia · Vitamin D

Introduction

Calcium is one of the most important minerals in the body since it plays a crucial role in many physiological processes such as muscle contraction, neurotransmission, inflammation, blood clotting, intracellular signaling, and lactation. Maintenance of calcium homeostasis involves hormonal regulation of intestinal calcium absorption, bone remodeling process, and renal calcium excretion. Since calcium is obtained only through intestinal absorption, the amount of calcium absorbed partly determines the serum level of calcium, and consequently, bone mineral content and density. Calcium absorption is under the control of classical calciotropic hormones, i.e., parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], as well as some other humoral factors, such as calcitonin, prolactin, growth hormone, estrogen, and fibroblast growth factor (FGF)-23 [1–5]. A decrease in calcium absorption over a period of time can lead to a low level of serum calcium, and subsequently bone defects, which has been reported in many conditions and diseases including thalassemia.

Thalassemia is an inherited disease with hypochromic and microcytic anemia from defective α- or β-globin production [6]. It affects approximately four out of every 10,000 people globally [7], and more than 50% of β-thalassemic patients develop osteoporosis and osteopenia as well as bone deformity [8]. Other major problems in thalassemic patients and mutant animals consist of iron overload, splenomegaly, abnormal heart rhythm, diabetes mellitus, and growth retardation (for review, please see Nienhuis and

✉ Narattaphol Charoenphandhu
naratt@narattsys.com

1 Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

2 Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

3 Office of Academic Management, Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand

4 Department of Physiology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

5 Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

6 Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand

Nathan [9]). A significant decrease in intestinal calcium transport has also been reported in thalassemic patients [10]. Severely impaired intestinal calcium absorption has also been observed in thalassemic mice, and was found to be associated with their lower bone mineral density (BMD) [11, 12]. Although the improvement of calcium absorption has been shown to be an effective way of preventing and relieving osteoporosis [13, 14], it is not known how calcium absorption is affected by thalassemic condition, and whether it could be the cause of bone defect. This review will discuss changes in the intestinal calcium-transport mechanisms and possible cause of intestinal calcium malabsorption in thalassemia with evidence from both human and animal studies.

Calcium transport and its hormonal control in healthy individuals

In human and other mammals, calcium enters the body mainly through ingestion. Dietary calcium is absorbed across the intestinal epithelial cells by two pathways, i.e., transcellular and paracellular pathways. Although both calcium-transport mechanisms take place along the entire length of the small intestine, the transcellular calcium transport is predominant in the proximal part, particularly the duodenum. Free-ionized calcium diffuses across the apical plasma membrane via transient receptor potential vanilloid calcium channel (TRPV) 5 and 6 and L-type voltage-dependent calcium channel (e.g., $Ca_v1.3$) [15]. Cytoplasmic calcium is then translocated by binding to calbindin-D_{9k} and probably also to calbindin-D_{28k}, parvalbumin, and calmodulin, to be extruded at the basolateral membrane through plasma membrane Ca^{2+}-ATPase (PMCA) subtype 1b and Na^+/Ca^{2+}-exchanger (NCX)-1 [16–18]. Some intracellular vesicles can help ferry intracellular calcium and certain ions (e.g., iron) from the apical side to the basolateral side for extrusion [5].

Regarding the paracellular pathway, transepithelial transport of calcium occurs through space between neighboring two epithelial cells. Calcium movement is driven by the free energy of electrochemical gradient (passive diffusion) or by solvent drag [15, 19, 20]. For solvent drag-induced calcium transport, the basolateral Na^+/K^+-ATPase (NKA) pumps sodium into the paracellular space creating Na^+-rich hyperosmotic microenvironment that, in turn, draws water from lumen to the plasma side simultaneously with ionized calcium [21, 22]. Paracellular calcium diffusion is indeed regulated by tight junction proteins, such as claudins, which possess size- and charge-selective properties [15, 19]. The expression of some claudins, particularly claudin-2 and -12, is dependent on $1,25(OH)_2D_3$ and may be responsible for the $1,25(OH)_2D_3$-induced calcium transport across the paracellular pathway [23]. Generally, under normal diets, the level of ionized calcium in the duodenal lumen is relatively high (~ 5 mmol/l) as compared with plasma ionized calcium (1.1–1.3 mmol/l) [19]. Thus, there is a calcium gradient across the duodenal epithelium, which can be a driving force for the paracellular calcium absorption. Our previous study has shown that luminal calcium concentration of ~ 5 mmol/l is substantial to induce the paracellular transport of calcium across the intestinal epithelium, and it can contribute up to 80% of the total calcium absorption, especially in the distal small intestine [19]. Moreover, the impaired paracellular pathway might also reduce the intestinal absorption of some other minerals, such as magnesium [24].

The relatively constant levels of plasma calcium are regulated by an integrative response of the calcium-regulating organs organized as the parathyroid–kidney–intestinal axis [25, 26]. For instance, decreases in plasma calcium stimulate the parathyroid gland to secrete PTH, which raises the plasma calcium level by (i) enhancing calcium reabsorption in the thick ascending limb of the Henle's loop and distal renal tubule within minutes, (ii) stimulating bone resorption within minutes to hours, and (iii) stimulating 1α-hydroxylase in the proximal renal tubule to increase the production of $1,25(OH)_2D_3$, which, in turn, potently stimulates intestinal calcium absorption within 24 h. PTH has also been reported to directly stimulate intestinal calcium absorption via L-type voltage-dependent calcium channel [27, 28]. On the other hand, an increase in plasma free-ionized calcium then induces negative feedback to inhibit PTH secretion through calcium-sensing receptor (CaSR), followed by a decrease in $1,25(OH)_2D_3$ production, thereby reducing calcium absorption [29, 30]. Moreover, certain local and systemic humoral factors, e.g., FGF-23, may negatively regulate the duodenal calcium transport in a calcium and/or $1,25(OH)_2D_3$-dependent manner.

FGF-23 was originally known as osteocyte/osteoblast-derived phosphatonin—i.e., a phosphorus-regulating hormone—and has recently been recognized as a new calcium-regulating hormone [31–33]. Khuituan et al. [31, 32] demonstrated a novel role of FGF-23 as a negative feedback regulator of the $1,25(OH)_2D_3$-enhanced duodenal transcellular and paracellular calcium absorption in rodents. This finding provides an alternative explanation of how the duodenal enterocytes restrict excessive calcium transport and thus prevent lethal hypercalcemia. Meanwhile, an increase in serum phosphate level induces PTH and FGF-23 release that enhance phosphate excretion by suppressing 1α-hydroxylase and $1,25(OH)_2D_3$ production [34, 35]. Calcium is also a potent stimulator of FGF-23 production via a vitamin D receptor (VDR)-independent manner [36].

Evidence of calcium malabsorption and osteoporosis in thalassemia

Osteoporosis and osteopenia are among the most common complications in thalassemia, and are found in > 50% of β-thalassemic patients, especially patients with thalassemia major, the most severe form of β-thalassemia caused by β^o/β^o genotype (i.e., no β-globin chain and no hemoglobin A) [8, 37, 38]. A study in prepubertal children (age 8–9 years old) showed a significant decrease in bone mineral density (BMD) in thalassemia major patients as compared to the age- and sex-matched constitutional short statue control [39]. Similarly, studies in children and adolescents (age 8–25 years old) with both transfusion-dependent and transfusion-independent β-thalassemia major showed that most patients experienced osteopenia/osteoporosis, bone pain and short stature related to impaired bone formation and growth compared to the age-matched controls [40–43]. The prevalence of bone fracture was approximately 12% in all types of thalassemic patients, including β-thalassemia major, β-thalassemia intermediate, thalassemia E/β and α-thalassemia, with an equal distribution between both sexes [44]. Another study covering patients with a variety of thalassemia genotypes and age range (6–75 years old) showed a high incidence of bone fracture, bone pain, and increased bone turnover that were correlated with decreased BMD [45]. Studies in animal models confirmed a high incidence of osteopenia/osteoporosis in thalassemia. Specifically, data from our studies using mice with C → T mutation at nucleotide 654 of intron 2 ($\beta^{IVSII-654}$) and hemizygous knockout of β-globin gene (BKO) as β-thalassemic animal models showed that both hemizygous $\beta^{IVSII-654}$ knockin and BKO mice manifested a significant reduction in BMD, bone mineral content (BMC), bone volume, and bone thickness, as compared to the wild-type controls [46–48].

A potential cause of decreased BMD leading to osteopenia/osteoporosis could result from an imbalance in bone remodeling process, i.e., elevated bone resorption and/or reduced bone formation. Bone histomorphometric analysis in thalassemic mice revealed that osteoclast surface, eroded surface, and osteoclast function were elevated in both hemizygous $\beta^{IVSII-654}$ knockin and BKO thalassemic mice [46, 48]. Consistent with high bone resorption, higher circulating levels of osteoclastogenic factors including interleukin (IL)-1α, IL-1β, receptor activator of nuclear factor-κB ligand (RANKL), and tumor necrosis factor (TNF)-α were reported in thalassemic animals and patients, and were well associated with their decreased BMD [8, 47, 49–51]. These osteoclastogenic cytokines could also suppress osteoblast differentiation and activity, which in turn decrease bone formation. Furthermore, the known osteogenic factor, insulin-like growth (IGF)-1, was decreased in the serum of β-thalassemic patients together with lower BMD [39, 41, 42, 52, 53]. In contrast, serum levels of osteoblast differentiation inhibitors, namely Dickkopf-1 (a negative regulator of Wnt signaling) and sclerostin, were significantly higher in thalassemic patients [54, 55].

Other than the imbalanced bone remodeling process, hypocalcemia—possibly due to a decrease in calcium absorption—has been reported in both thalassemic animals and patients. Significant decreases in serum calcium and intestinal calcium absorption were observed in patients with thalassemia major together with the lower BMD [10, 43, 56, 57]. Evidence from animal studies, such as hemizygous $\beta^{IVSII-654}$ knockin and BKO thalassemic mice, showed a marked decrease in calcium absorption across the small intestinal epithelium [11, 12, 58]. The phenomenon was present in both sexes of animals, and the daily injection of 1 μg/kg $1,25(OH)_2D_3$ failed to restore normal intestinal calcium absorption in thalassemic mice despite being effective in enhancing calcium absorption in wild-type mice—presumably a sign of $1,25(OH)_2D_3$ resistance [11]. Our recent study also found that thalassemia-induced calcium malabsorption could be rescued by a long-time treatment with lower dose of $1,25(OH)_2D_3$ or treatment with hepcidin [12], which will be discussed further in the following section.

The alterations of the intestinal calcium-transport mechanisms in thalassemia

Direct evidence of thalassemia-induced changes in calcium transporters and/or the related proteins involved in intestinal calcium absorption is still limited. A study from our group showed downregulation of the transcellular calcium transporters and calcium transport-related proteins, i.e., TRPV5, TRPV6, calbindin-D_{9k}, and $PMCA_{1b}$, in thalassemic mice (Fig. 1) [11]. This impairment probably accounted for the reduction in intestinal calcium absorption. Besides these calcium transporters, other membrane-transporting proteins may be indirectly involved in calcium transport. The study in pernicious anemia showed the decreased activity of jejunal NKA, which is important for stabilization of the intracellular Na^+ necessary for the extrusion of absorbed calcium via the basolateral NCX1 [59]. Furthermore, calcium-binding proteins, which take part in the cytoplasmic calcium translocation, also play an important role in intestinal calcium transport. In addition to our report of decrease in the intestinal expression of calbindin-D_{9k} in thalassemic mice [11], there was another report of early life iron deficiency anemia that induced a decrease in calcium-binding protein parvalbumin in the rat hippocampus [60]. Even though the reported

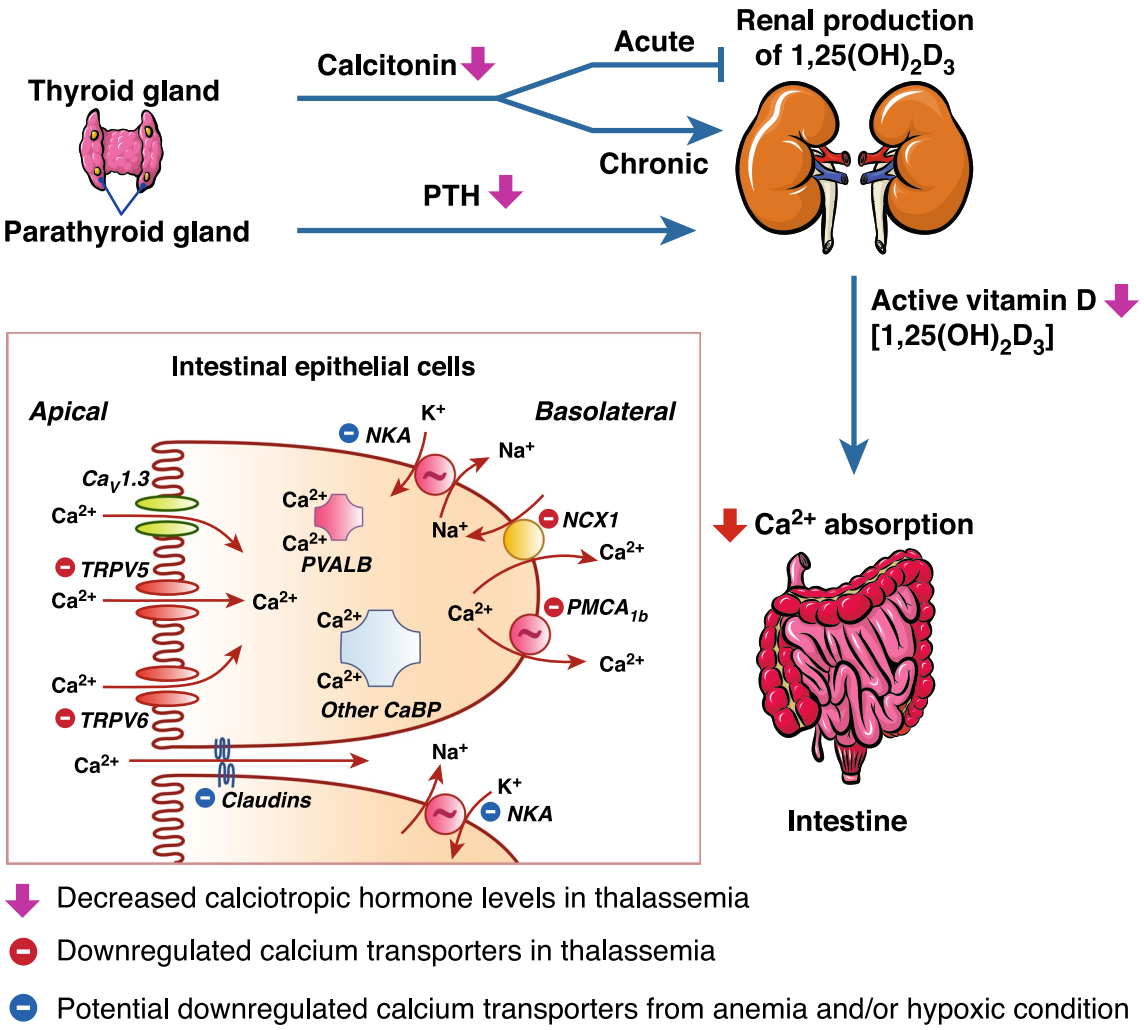

Fig. 1 Schematic diagram shows possible mechanisms of calcium regulatory pathway and intestinal calcium absorption in thalassemia. Reduction of calcium-regulating hormones, i.e., parathyroid hormone (PTH), calcitonin, and $1,25(OH)_2D_3$, in thalassemia leads to a decrease in the intestinal calcium absorption. *Inset* The decreased intestinal calcium absorption in thalassemia is due to downregulation of transcellular calcium transport-related proteins (TRPV5/6, $PMCA_{1b}$, and NCX1). The possible mechanism may also involve downregulation of NKA and tight junction proteins, such as claudins, thereby compromising the paracellular calcium transport (for details, please see text). PVALB, parvalbumin; CaBP, calcium-binding proteins (e.g., calbindin-D_{9k}); $Ca_v1.3$, voltage-dependent calcium channel 1.3

decreased parvalbumin in iron deficiency anemia came from a different organ system, it suggested a possibility of anemia-induced downregulation of calcium-binding protein expression in thalassemia. Taken together, the reduced levels of calcium transporters and related proteins could account for the impaired intestinal calcium transport as can be seen in thalassemic patients and animal models.

Paracellular intestinal calcium transport, another calcium transport system, represents the selective transportation of calcium through tight junction proteins, particularly claudins and occludin [5]. As mentioned earlier, $1,25(OH)_2D_3$ has been shown to upregulate claudin-2 and -12, leading to the enhanced paracellular calcium transport in vitro and in vivo [5, 23]. Interestingly, hypoxia resulting from chronic anemia could lead to the activation of hypoxia-inducible factor (HIF)-1, which was also upregulated in the placentae of women with iron deficiency anemia and β-thalassemia trait carriers [61–63], and has been shown to suppress the expression of occludin and claudin-1 in human intestinal cells and rat duodenum [64, 65]. This body of evidence strongly supports the negative effect of thalassemia on the intestinal calcium transport (Fig. 1). However, more studies are needed to further provide the detailed molecular mechanisms of intestinal calcium transport impairment in thalassemia.

Thalassemia-induced calciotropic endocrinopathies

As shown in Fig. 1, hormonal control plays an important role in the regulation of intestinal calcium absorption. Therefore, the defects or abnormalities of these hormones in many diseases, including osteoporosis and thalassemia, could greatly affect intestinal calcium transport. One of the key regulators for intestinal calcium absorption is $1,25(OH)_2D_3$, which directly stimulates calcium absorption by upregulating the expression and activities of several calcium transporters, e.g., TRPV5, TRPV6, calbindin-D_{9k} [2, 31, 66]. $PMCA_{1b}$ expression and activity were also upregulated by $1,25(OH)_2D_3$ in a human intestinal cell line and vitamin D-deficient mice, respectively [2, 67]. Furthermore, $1,25(OH)_2D_3$ enhanced NCX activity in chick duodenum and NCX expression in duodenum of vitamin D-replete mice [31, 32, 68]. Taken together, it is clear that $1,25(OH)_2D_3$ has a crucial role in intestinal calcium transport; therefore, decreases in $1,25(OH)_2D_3$ level and/or its receptor can negatively affect the intestinal calcium transport.

In β-thalassemic patients, the level of serum 25-hydroxyvitamin D [25(OH)D] was significantly reduced as compared to the healthy individuals [42, 43, 69–71]. Impaired $1,25(OH)_2D_3$ synthesis was also reported in β-thalassemia major patients [72]. In $\beta^{IVSII-654}$ knockin thalassemic mice, vitamin D receptor was downregulated in the duodenal epithelial cells as compared to their wild-type littermates [11]. Other than thalassemia, patients with sickle cell anemia also showed a decreased serum vitamin D level [73]. The reduced serum $1,25(OH)_2D_3$ in thalassemic patients and animals was correlated with low serum calcium levels and BMD, indicating that an impaired $1,25(OH)_2D_3$ production and function could potentially cause intestinal calcium malabsorption in thalassemia.

PTH has been known to increase serum calcium level by reducing urinary calcium reabsorption, stimulating bone resorption, and indirectly enhancing intestinal calcium absorption by stimulating $1,25(OH)_2D_3$ production. However, Picotto and coworkers showed that PTH might directly stimulate intestinal calcium transport, which was inhibited by Ca_v inhibitor [27]. Many studies reported the decreased serum PTH or hypoparathyroidism in patients with thalassemia major regardless of their age or blood transfusion status [40–43, 56, 69, 72, 74, 75]. Specifically, a reduction in serum PTH level is probably due to thalassemia-induced iron overload and iron deposit in the parathyroid gland, thus leading to parathyroid chief cell dysfunction and impairment of calcium homeostasis [76–78]. Decreased PTH was shown to correlate with lower serum and urine calcium levels and lower BMD in thalassemia [40, 43, 56, 57, 75]. Nevertheless, there was a paradox that low levels of PTH with its well-known bone resorption-stimulating activity were present with lower BMD. Taken together, thalassemic patients demonstrated reductions in serum calcium level and BMD that were associated with decreased PTH level, suggesting a possibility of intestinal calcium malabsorption from hypoparathyroidism in thalassemia. Furthermore, since PTH is a potent stimulator of renal $1,25(OH)_2D_3$ production, the thalassemia-induced reduction in PTH level may cause a lower serum $1,25(OH)_2D_3$, thereby reducing intestinal calcium transporter expression and transcellular calcium absorption [5, 79, 80]. A decrease in PTH level may directly aggravate calcium malabsorption in thalassemia because it can exert a direct stimulatory effect on the intestine by increasing cellular calcium uptake and extrusion [27, 28, 81].

Another calcium-regulating hormone, calcitonin, was demonstrated to negatively regulate intestinal calcium absorption in several studies [82, 83]. However, some studies found positive effects of calcitonin on intestinal calcium absorption. Specifically high-dose calcitonin could induce intestinal calcium absorption [84], and chronic calcitonin treatment was able to increase serum calcium level through the stimulation of $1,25(OH)_2D_3$ production in rats [3, 85]. β-thalassemic patients were reported to have decreased levels of calcitonin and chronic calcitonin treatment could improve osteoporosis in these patients [52, 86], presumably due to an inhibitory effect of calcitonin on osteoclast function. Accordingly, the inappropriately decreased calcitonin levels in thalassemic patients could also contribute to intestinal calcium malabsorption possibly from lower $1,25(OH)_2D_3$ production. Moreover, thalassemia-induced iron overload and iron deposit in the gonads further impaired production of sex steroids, particularly 17β-estradiol [12, 87, 88], which is one of the potent positive regulators of intestinal calcium absorption [89]. Thus, thalassemic patients experienced thalassemia-induced calciotropic endocrinopathies, leading to decreased levels of calcium transport-regulating hormones including $1,25(OH)_2D_3$, PTH and calcitonin.

Regarding FGF-23, although it has been reported to negate intestinal calcium absorption in mice [31, 32], there are limited studies on the roles of FGF-23 on calcium homeostasis in thalassemia. Most studies focused on the role of FGF-23 on iron metabolism [90–92]. For example, Bożentowicz-Wikarek and coworkers reported a low level of circulating iron being associated with an increase in FGF-23 levels [90]. Thus, more understanding about FGF-23 and thalassemia would help in improving bone health in thalassemic patients.

Effects of thalassemia-induced iron hyperabsorption, iron metabolism dysregulation, and iron overload on the intestinal calcium transport

Iron overload in thalassemic patients could have resulted from treatment involving repeated blood transfusion as well as ineffective erythropoiesis and the anemia-induced compensation iron hyperabsorption in the small intestine. In iron-overloading conditions, iron deposit in the solid organs (e.g., liver) and endocrine organs (e.g., pancreas and gonads) could lead to organ damage and endocrine disturbance, respectively [93–102]. As depicted in Fig. 2, upregulation of iron transporters, and subsequent increased iron absorption were reported in thalassemic patients [103]. Studies in non-transfusion-dependent thalassemic patients [104] and thalassemic mice with moderate anemia [102, 105–107] also showed increased intestinal iron absorption. The iron transporters and related proteins that were upregulated in thalassemic mice included divalent metal transporter (DMT)-1 (an apical transporter for iron uptake), ferroportin-1 (a basolateral transporter for iron efflux from the enterocytes), neutral gelatinase-associated

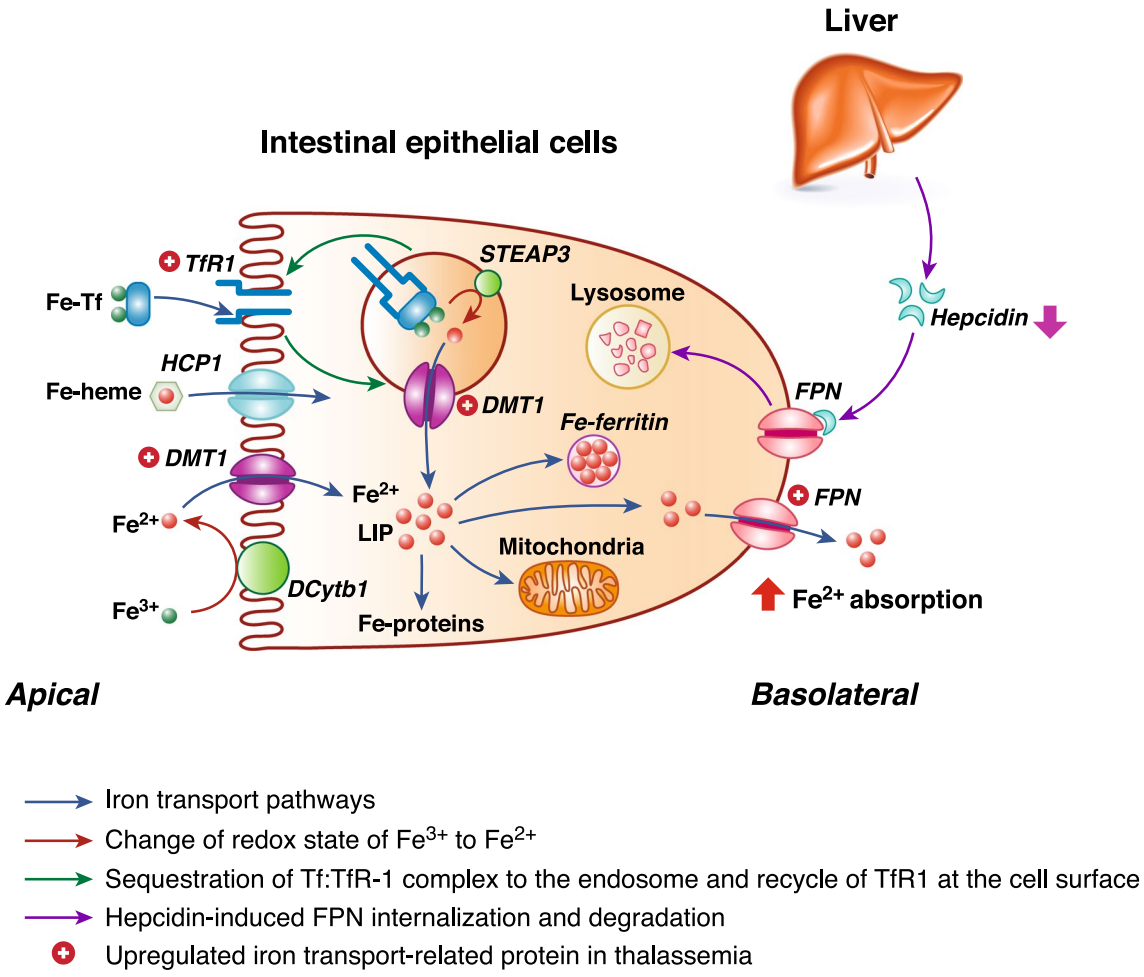

Fig. 2 Cellular mechanisms of iron transport in thalassemia. Under normal conditions, dietary iron in the intestinal lumen can traverse the apical membrane by several pathways, such as transferrin receptor 1 (TfR1)-mediated uptake of iron-bound transferrin (Tf), divalent metal transporter 1 (DMT1)-mediated uptake of Fe^{2+}, and Fe-heme uptake by heme carrier protein (HCP)-1. Although Fe^{3+} (non-heme iron) is more abundant than Fe^{2+}, DMT1 predominantly transports Fe^{2+} in the presence of H^+ in the lumen; therefore, it needs DCytb1 to change the iron redox state. Meanwhile, after endocytosis, iron ions are liberated from Fe-Tf-TfR1 by a metalloreductase, six-transmembrane epithelial antigen of the prostate (STEAP)-3, before being transported into the cytoplasm via DMT1 to join the cellular labile iron pool (LIP). Iron in LIP can be distributed into mitochondria and ferritin, or extruded across the basolateral membrane by ferroportin-1 (FPN). FPN is also a receptor for hepcidin, which can induce FPN internalization and degradation. In thalassemia, the upregulated expressions of DMT1 and TfR1 enhance apical iron uptake into the intestinal epithelial cells. In addition, a reduction in the hepcidin levels decreases FPN internalization and degradation, which can, in turn, increase the number of FPN proteins in the basolateral membrane, and then enhances the intestinal iron absorption

lipocalin (NGAL), and transferrin receptor (TfR)-1 [11, 12, 105, 107]. In contrast, the expression of the negative regulator of intestinal iron transport, namely hepcidin, which is normally produced by the liver and binds to ferroportin-1, was decreased in thalassemic mice, and hepcidin treatment could alleviate iron overload in these mice [96, 102, 105–108]. Thus, the upregulation of iron transporters as well as the downregulation of hepcidin could contribute to an increase in intestinal iron absorption, which would worsen the iron overload condition in thalassemia.

While iron absorption was upregulated in thalassemia [105], many studies showed a significant decrease in calcium absorption as mentioned previously [11, 12]. Recently, an inverse correlation between duodenal calcium transport and iron absorption was demonstrated in thalassemic mice [12]. Subcutaneous administration of hepcidin thus increased calcium transport in these thalassemic mice. In this study, hepcidin showed its potential to effectively alleviate intestinal calcium malabsorption as well as to relieve iron overload by inhibiting intestinal iron transport in thalassemic mice. The mechanism(s) underlying this inverse correlation is not completely understood. Since iron and calcium did not share apical or basolateral transporters, it was likely that the interaction resided in the cytoplasm of intestinal epithelial cells, i.e., the intracellular translocation of their binding proteins or the membrane-bound vesicles [109]. In the duodenums of thalassemic mice, abolishment of hepcidin effects on calcium absorption by a chemical (e.g., chloroquine) that disrupted the function of intracellular vesicles and vesicular transport suggested the possible interaction between calcium and iron transport systems in the vesicles [12]. These intracellular vesicles are believed to rapidly shuttle both calcium and iron from the apical side to basolateral side of the enterocyte. It has been suggested that the lysosome-like intracellular vesicles are able to accommodate iron [110], and they may use Ca^{2+}/H^{+} exchanger (CAX) to accumulate calcium in exchange with H^{+} efflux into the cytoplasm [111]. Since the vesicular H^{+} efflux is dependent on cytoplasmic pH (i.e., acidic pH in the vesicle vs. more alkaline pH in the cytoplasm), an impairment of cellular pH balance may also diminish both calcium and iron absorption. Recently, an inhibitor of Na^{+}/H^{+} exchanger (NHE)-3, which is essential for cellular pH regulation, was found to hinder the hepcidin-induced calcium transport in the duodenums of BKO mice [58].

Other than thalassemic mice, the reciprocal correlation between calcium absorption and extracellular iron concentration has been shown in human intestinal epithelial Caco-2 cells [112]. Data from this cell line model showed that cellular calcium absorption was increased with the decrease in the extracellular iron concentration, and the opposite trend could be seen when extracellular iron concentration was elevated [112]. Furthermore, the negative effects of iron overload on intestinal calcium transport could also occur through the decreases in calciotropic hormone levels. Levels of vitamin D were apparently lower by ~ 90% in iron-overloaded and multiple transfused thalassemic patients [113]. This suggested another possible consequence of iron overload-induced decrease in intestinal calcium absorption in thalassemic patients through the downregulation of calciotropic hormone, as depicted in Fig. 3. Consequently, bone loss occurs as a result of low blood calcium levels. Moreover, high iron levels can suppress osteoblast activity [114]. Although the reciprocal interaction between intestinal calcium absorption and iron absorption in thalassemia has been elaborated in many studies, more studies are needed to elucidate the connecting mechanisms between the two minerals. Manipulation of the iron transport system by using inhibitors (e.g., DMT1 inhibitor or recombinant hepcidin as a negative regulator of ferroportin-1 function) could be a potential novel intervention to assuage both intestinal calcium malabsorption and iron overload for thalassemic patients.

An iron chelator is often prescribed to mitigate iron overload in thalassemic patients. Up till now, there has been no study to investigate the direct effect of iron chelator [e.g., desferoxamine (DFO)] on serum calcium level. The potential association between DFO and hypocalcemia was reported in an infant with parenteral nutrition-associated aluminum overload, which could lead to impaired bone metabolism. The patient failed to respond to any calcium or vitamin D supplement, especially when the level of blood aluminum was high. However, shortly after DFO treatment, the urinary and blood calcium decreased. Accordingly, it was postulated that a decreased serum calcium level could indirectly come from an increased calcium accretion into bone as the level of aluminum was reduced during DFO treatment [115].

On the other hand, some studies have suggested the potential positive effects of iron chelator treatment that capable of improving hypoparathyroidism and hypothyroidism in thalassemia patients [56, 116]. One has shown no correlation between hypothyroidism and the regularity of iron chelation treatment [117]. Others reported no significant side effects on calcium level or calciotropic hormones from oral intake of deferiprone, DFO, or a combination of both in thalassemia patients [118–120]. Thus, the effects of iron chelators, particularly DFO, remain controversial and need more investigation.

Conclusions and perspectives

Although thalassemia is a complex genetic disease affecting several organs, including intestine and bone, it is a good model for investigating an association between iron and calcium transport across the intestinal epithelium. Generally,

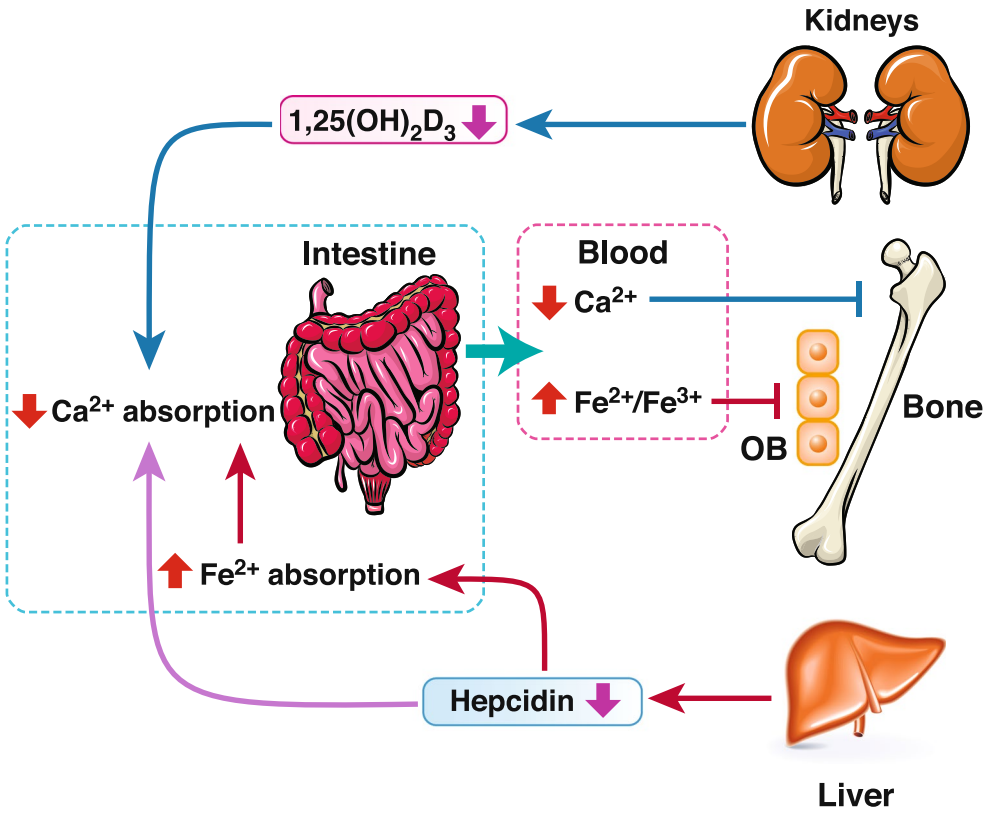

Fig. 3 Reciprocal interaction between calcium and iron absorption in thalassemia induces bone loss. Decreased calcium absorption in thalassemia is caused by a reduction in $1,25(OH)_2D_3$ levels and also by iron hyperabsorption/overload. Low hepcidin levels in thalassemia can basically enhance intestinal iron absorption, which decreases calcium absorption. Since hepcidin has been found to enhance calcium transport in BKO mice, a reduction in its level or action possibly diminishes calcium absorption. Consequently, bone loss occurs as a result of low blood calcium levels. Moreover, high iron levels can suppress osteoblast (OB) activity, thus compromising bone formation

thalassemia with iron hyperabsorption leads to impaired calcium absorption. Negative correlation between iron and calcium transport has recently been demonstrated in the duodenum of thalassemic mice [12], consistent with the general recommendation that iron and calcium supplements should not be administered simultaneously. The thalassemia-induced impairment in calcium transport is caused by several factors, i.e., impaired calciotropic hormone production and response as well as low transcellular calcium uptake. Iron hyperabsorption results, in part, from aberrant hepcidin release and response, and overexpression of DMT1 and/or ferroportin-1. Correlations between serum hepcidin, iron, and other negative regulators of calcium absorption, e.g., FGF-23 [121], remain elusive. Understanding of the underlying mechanism by which iron hinders calcium transport across the intestinal epithelium is crucial for development of better calcium/iron supplement products, particularly for pregnant women who normally need both minerals for fetal development. Finally, hepcidin and iron transport blockers (e.g., DMT1 inhibitor) may be useful for thalassemic patients, who require reduction of iron absorption and restoration of intestinal calcium uptake.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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