Review Article

Iron and zinc interactions: Does entero-pancreatic-zinc excretion cross-talk with intestinal iron absorption?

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Abstract: Iron and zinc are essential micronutrients required for growth and health. Deficiencies of these nutrients are highly prevalent among populations, but can be alleviated by supplementation. Cross-sectional studies in humans showed positive association of serum zinc levels with hemoglobin and markers of iron status.   Dietary restriction of zinc or intestinal specific conditional knock out of ZIP4 (SLC39A4), an intestinal zinc transporter, in experimental animals demonstrated iron deficiency anemia and tissue iron accumulation. Similarly increased iron accumulation has been observed in cultured cells exposed to zinc deficient media. These results together suggest a potential role of zinc in modulating whole body iron metabolism.   Studies in intestinal cell culture models demonstrate that zinc induces iron uptake and transcellular transport via induction of divalent metal iron transporter-1 (DMT1) and ferroportin (FPN) expression, respectively. It is interesting to note that intestinal cells are exposed to very high levels of zinc through pancreatic secretions, which is a major route of zinc excretion from the body.   Therefore, zinc appears to be modulating the iron metabolism possibly via regulating the DMT1 and FPN1 levels.   Herein we critically reviewed the available evidence to hypothesize novel mechanism of Zinc-DMT1/FPN axis in regulating intestinal iron absorption and tissue iron accumulation to facilitate future research aimed at understanding the yet elusive mechanisms of iron and zinc interactions.

Keywords: Iron, Zinc, Interactions, DMT1, ZIP4, Pancreas, Metabolism, Homeostasis, Intestine, Caco-2 cells.

1. Introduction

Iron and zinc are essential micronutrients required for growth and sustained health. Physiologically iron is defined as type 1 nutrient, while zinc is a type 2 nutrient [1]. Type 1 nutrient (i.e. iron, calcium, iodine, vitamins A and B) inadequacy manifests in reductions in stores followed by functional plasma components, whereas in type-2 nutrient (i.e. zinc, protein, sodium and water) deficiencies the clinical symptoms such as impaired growth precedes decline in functional plasma levels. Therefore, assessment of hemoglobin or serum ferritin/transferrin receptor serve as early diagnostic markers of anemia and iron deficiency, while there are no established biomarkers of zinc deficiency [2]. Poor density and bioavailability of iron from typical vegetarian foods is the major

etiological factor for the high prevalence of anemia in general population [3,4]. Phytic acid, an abundant secondary metabolite of plant foods, chelates dietary iron and limits its intestinal absorption. Since phytic acid also inhibits the zinc absorption, higher risk of zinc deficiency is expected in populations with high prevalence of anemia, stunting, and high phytate content of staple diets [2,5]. Given the impact of these deficiencies on general health, particularly in children and pregnant women, correction of these deficiencies through therapeutic or food fortification approaches should be considered.

Iron and zinc combined supplementation trials in humans and animal models have revealed negative interactions, but there are conflicting data on direction and magnitude of these interactions [6,7]. It has been hypothesized that iron-zinc interactions occur through competition at a specific transport protein during intestinal absorption; however, the exact mechanisms remains elusive. On the other hand, zinc deficiency in humans, experimental animal models and in vitro studies gives rise to iron deficiency anemia and tissue and cellular iron accumulation [8-11]. Cross-sectional studies in humans reveal a positive association of serum zinc levels with hemoglobin and markers of iron status [12-15]. Studies in intestinal cell culture and experimental animal models have also demonstrated modulation of iron transporter expression and iron regulatory proteins by zinc [11,16-19]. We have demonstrated that zinc induces iron uptake and transcellular transport in intestinal cells via induction of DMT1 and FPN1 expression [16,17,19,20]. Therefore, zinc appears to be a key modulator of intestinal iron absorption and tissue iron distribution possibly mediated via regulating the DMT1 and FPN1 levels.

Here, we have reviewed the mechanism of iron and zinc homeostasis and their interactions both at the level of intestinal absorption and tissue mobilization in the context of zinc status. From the available evidence, it appears that zinc-DMT1/FPN1 axis is a critical determinant of zinc deficiency-induced changes in iron homeostasis and helps understand the mechanism of iron and zinc interactions.

2. **Iron homeostasis:** In mammals, iron is highly conserved and there are no obligatory pathways for its excretion. The basal losses of iron through shedding of intestinal cells, sweat, urine, and increased demand due to infant and adolescent growth spurts and pregnancy is compensated by concurrent modulation of intestinal iron absorption. In addition, iron is stored and recycled in the

body and thus counters short term dietary inadequacies [21]. The mechanistic aspects of iron absorption, transport, storage, recycling and their regulation are depicted in **Figure 1** and described below.
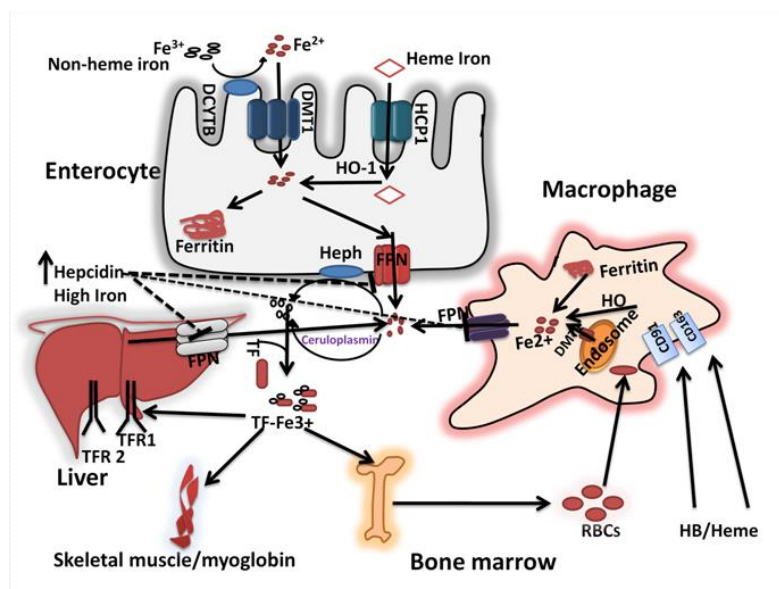

Figure 1. Iron absorption and homeostasis: The dietary non-heme iron is first reduced to ferrous form by DcytB, and is taken up via DMT1 at the apical surface of the enterocytes. Dietary heme iron is taken up via HCP 1 and degraded inside the cell by HO-1 to release iron. Within the enterocyte the iron is either stored in ferritin or transported to the circulation. At the basolateral membrane, the ferrous iron transported via FPN1, coupled with its oxidation by HEPH. The senescent erythorcytes are phagocytosed by macrophages via CD91/CD163, and is degraded in lysosomal compartments to release iron, which then are excreted in to cytosol via DMT1. The iron is then transported out of the macrophage via FPN possibly coupled with ceruloplasmin dependent iron oxidation. In the blood ferric iron is transported bound to Tf, and delivered to the target tissues, the bone marrow, liver and muscle via TfR dependent mediated endocytosis pathway. When the iron stores are adequate, the hepcidin released from the liver in to the blood, which in turn inhibits the ferroportin mediated iron release from intestinal cells and other tissues involved in iron mobilization.

2.1. Intestinal iron absorption: The dietary iron exists in either heme- (animal foods) or non-heme-forms (plant foods), but the latter is predominant source of iron in the diet [22,23]. Villus epithelial cells, known as enterocytes, in the duodenum and upper jejunum are specialized in rapidly transporting both heme- and non-heme iron and movement across the lumen to the blood occurs rapidly. The heme- iron absorption is mediated by heme- carrier protein (HCP1), which undergoes intracellular degradation by heme- oxygenase (HO1) to release iron within the enterocyte [21]. Non-heme iron absorption depends on the solubility of ferric iron in the gastric milieu and its

reduction to ferrous form by compounds such as ascorbic acid in the duodenum [21,24]. In addition soluble ferric iron, possibly bound to peptides, organic acids or amino acids is first reduced by Duodenal Cytochrome B (Dcyt B) [25], before it is taken up by enterocytes via DMT-1, a proton-coupled solute carrier protein [26].

After iron has entered the enterocyte across the apical membrane of the enterocyte, two pathways for its handling are available; transfer across the basolateral membrane or binding to specific cytoplasmic protein, ferritin.   The path taken is governed by the body's demands for iron. In conditions of iron excess, the metal is oxidized to Fe^{3+} at the ferritin shell before being stored [21,24]. There is evidence that iron stored in ferritin can be re-mobilized by targeting ferritin to autolysosomes in a process called ferritinophagy [27].   When demands are high, iron preferentially passes across the basolateral membrane into portal circulation via sequential action of ferroportin 1 (Fpn-1) and hephaestin (HEPH) [28,29].   Fpn-1, a transmembrane protein, abundant at basolateral membrane of polarized enterocytes exports the cellular iron in to circulation. Indeed, Fpn-1 is the only known iron exporter identified to date [28,30,31].   Hephaestin, a transmembrane protein with copper-dependent ferroxidase activity, is predominantly present at the basolateral side of polarized enterocytes converts ferrous iron (Fe^{2+}) to the ferric (Fe^{3+}) form.   In the circulation the apo-transferrin (Tf), binds to the ferric iron and assists in its transport in the blood plasma and delivery to the target tissues. Studies in intestinal cells suggests that apo-Tf may also modulate iron efflux from intestinal cells [32].

Transferrin-bound iron is delivered to the target tissues, most goes to the bone marrow, via transferrin receptor (TfR) mediated endocytosis [33]. The acidic environment of endosome releases the iron from transferrin, and transported to the cytoplasm via DMT1 while the transferrin and TfR are recycled back to plasma membrane [33,34]. The iron released into the cytoplasm is either stored as ferritin or incorporated into various iron-containing molecules, including hemoglobin, myoglobin or cytochromes, depending on the cell type and requirements [35].

2.2. Iron recycling: In addition to dietary iron, recycling of iron by spleen, liver and bone marrow derived macrophages also contributes to iron homeostasis [31,36]. Macrophages engulf senescent erythrocytes via phagocytosis. Subsequently, heme is released from the phagosomes via HRG-1 (Heme-responsive gene 1 protein homolog) and is degraded in the endoplasmic reticulum by

hemeoxygenase [37]. The iron released from the heme is excreted in to the cytosol, and is either stored as ferritin or released in to the circulation via ferroportin-1. This process requires plasma ceruloplasmin; another copper-dependent ferroxidase, to mediate the oxidation of iron during its release from tissues [36]. The observation that genetic inactivation or genetic mutations in Fpn-1 or ceruloplasmin are associated with tissue iron accumulation infers an important role of these proteins in the mobilization of iron from macrophages [38,39].

2.3. Regulation of cellular iron homeostasis by Iron Regulatory Proteins: Intracellular iron levels are regulated by iron regulatory protein 1 and 2 (IRP1 and IRP2) by translational control mechanisms. IRP1 is a bifuctional protein, which requires disassembly of a 4Fe-4S cluster for activation. In contrast, IRP2 expression is inducible, and its levels are controlled by regulating proteosomal degradation [40,41]. Induction of IRP-2 expression and/or activation of IRP-1 during iron deficiency ensure increased iron absorption and mobilization from intestinal cells. IRPs bind to iron-responsive elements (IRE), step loop RNA secondary structures, present in the 3' and 5' untranslated regions (UTR) of mRNA transcripts for iron metabolic proteins, including DMT1, ferritin, FPN and TFR1 [42]. When intracellular iron concentrations are low, the binding with IRPs is high, which results in the stabilization of transcripts containing IRE in the 3' UTR (e.g. TFR1 and DMT1), and repression of translation of transcripts containing an IRE in the 5' UTR (e.g. ferritin and FPN1) (Muckenthaler, 2008). The net effect is increased TFR1/DMT1 and decreased ferritin and FPN expression, thus increasing the labile iron concentrations to normal levels. Conversely, when labile iron concentrations are high, the binding of IRPs to IREs is decreased, resulting in reductions in TFR1, DMT1 and increased ferritin and FPN1 expression resulting in reduced absorption and increased mobilization (Muckenthaler, 2008).

2.4. Regulation of iron homeostasis by systemic factors: It is estimated that about 20 mg/day of iron is required for erythropoiesis, must be met from newly absorbed dietary iron and iron recycled from senescent RBCs [36]. The typical absorbable dietary iron is in the range of 1.2 - 2 mg (12-20 mg intake with 10% bioavailability), thus recycling of RBC derived iron is critical in maintaining iron balance. However, during chronic low intake of dietary iron, intestinal iron deficiency stimulates both iron absorption and its transport to the circulation while the tissue iron (liver and spleen) is mobilized for metabolic needs. During iron sufficiency, reduction in intestinal iron absorption and storage of iron

in ferritin prevents excess iron delivery to the plasma.   Since the sites of iron storage (liver and spleen) are different from that of its entry (intestine), these tissues needs to cross talk to regulate iron absorption and mobilization.   Hepcidin, a cysteine-rich 25 amino acid cationic peptide synthesized and secreted by the liver, is identified as the key regulator of mammalian iron homeostasis [43-45]. Hepcidin regulates iron levels by reducing the efflux of iron from storage tissues and from enterocytes. Hepcidin accomplishes this by binding to the iron exporter FPN1, leading to its endocytosis and intracellular degradation, resulting in decreased FPN-mediated iron transport into extracellular fluids and increased cellular iron retention [45,46]. In addition to iron status, hepcidin expression is also modulated by   inflammation, ineffective erythropoietin and hypoxia [44].

3. **Mechanisms of zinc homeostasis:** Homeostatic regulation of zinc metabolism is orchestrated through a balance of absorption and excretion involving adaptive mechanisms programmed by zinc status (**Figure 2**) [47]. Zinc is ubiquitously present in all tissues, with highest levels found in muscle and bone followed by liver.   The whole body zinc content found to be stable over a wide range of dietary zinc concentrations indicating efficient homeostatic mechanisms [48]. Zinc absorption and excretion in the gastrointestinal tract are the primary mechanisms for maintaining zinc homeostasis. The zinc absorption takes place throughout the small intestine, predominantly in the jejunum and ileum [49].   However, endogenous zinc can be secreted into the intestine and excreted in feces [48]. The balance of intestinal absorption and endogenous losses of zinc through feces are thus two important pathways that regulate the zinc homeostasis. During zinc deficiency or limited dietary zinc intakes, fecal zinc excretion falls with concurrent increase in intestinal absorption, thus conserves the zinc concentration in the tissues/plasma [48,50,51]. On the other hand during zinc excess, fecal zinc excretion increases while the fractional zinc absorption falls. Therefore, an exquisite balance of endogenous losses coupled with modulation of intestinal zinc absorption regulates the whole body zinc homeostasis, such that the plasma zinc levels remain at steady state except under severe zinc deficiency [51]. The mechanisms involved in intestinal zinc absorption and specific role of pancreas in endogenous zinc excretion are described below.
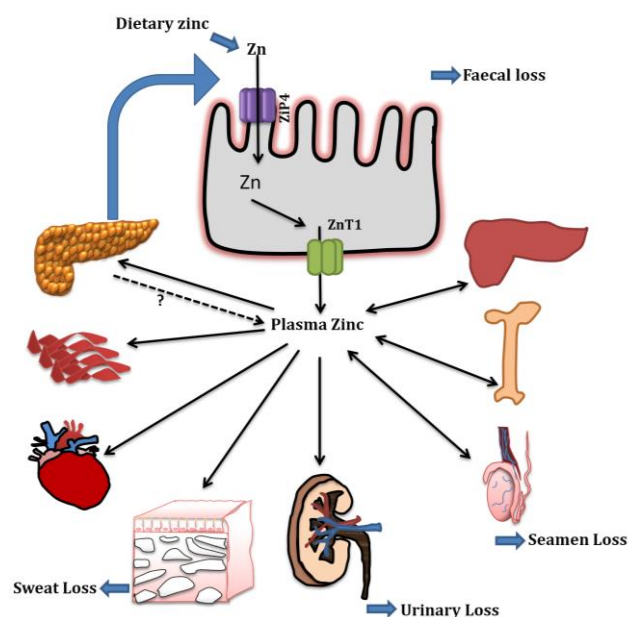
Figure 2. Zinc absorption and homeostasis: Dietary zinc and zinc excreted through pancreatic secretions is absorbed via ZiP4 at the apical surface of the enterocyte, and is transported in to circulation via ZnT1. The zinc in the plasma bound to albumin (major portion) or in free form is taken up by the peripheral tissues such as liver, bone marrow, testis, kidney, skin, heart, skeletal muscle and pancreas. The absorbed zinc is lost through faeces, urine, seamen and sweat, among which faecal excretion is sensitive to zinc status of the host. In addition, during zinc insufficiency, the plasma zinc levels are maintained via secretion of zinc from only from specific tissues such as liver, bone marrow and testes while it is strictly conserved in heart, skeletal muscle, skin and kidney. Thus entero-pancreatic axis in maintains the zinc balance via modulation of both absorption and excretion, while specific tissues contributes to plasma zinc pool maintenance during inadequate intakes or deficiency.

3.1. Intestinal zinc absorption: The identification of two families of zinc transporters namely ZIP (increases cytosolic zinc) and the ZnT proteins (decreases cytosolic zinc) has contributed considerably to the understanding of both intestinal and systemic zinc homeostasis [52-54]. ZIP proteins transport zinc from the extracellular space and intracellular organelles into the cytoplasm while ZnT proteins function as exporters of intracellular zinc. In humans 14 members of ZIP and 10 members of ZnT family protein have been identified, and are expressed in tissue-specific manner [53]. Although multiple ZIP family proteins have been identified in the intestine, ZIP4 is the predominant zinc transporter in enterocytes. Genetic mutations in humans or specific knock down in animal models unequivocally indicate the role of ZIP4 in mediating the intestinal zinc absorption [9,55,56]. In addition, up regulation of ZIP4 expression during zinc deficiency and its internalization

followed by degradation during zinc supply, indicates that ZIP4 expression is in tune with whole body zinc status [51,57]. The zinc absorbed by the enterocytes is either stored as metallothionein or is transported across the basolateral membrane via ZnT 1 [58,59]. The abundant expression of ZnT1 at the basolateral surface of the intestine and the severe zinc deficiency due to its mutations infer a specific role for this protein in mediating zinc exit from enterocytes in to the circulation [60]. The functional significance of other ZnT and ZIP family members in trafficking cytosolic zinc, and its storage in cell organelles in the intestine remains an active area of research.

3.2. Zinc excretion: Early studies using radio-tracer methods identified that the exocrine pancreas plays a functional role in zinc excretion. Indeed, pancreas, intestine and liver have been identified to have very high turnover rates of zinc [61]. In addition, pancreatic acinar cells possess much higher zinc concentrations compared to islet tissue. It is estimated that under normal dietary conditions, 1–2 mg/d Zn enters the digestive tract via zymogen granules secreted from pancreatic acinar- cells [51,62]. These zymogen granules contain enzymes necessary for digestion and for many, their activity is Zn dependent [47]. Dietary Zn restriction markedly decreases the Zn concentration in both pancreatic tissue and secretions [48,63]. Further, progressive decline in the expression of ZnT1 and ZnT2, the pancreatic zinc exporters was found during feeding of zinc restricted diets in mice, and the effect is reversed by zinc repletion [51]. These observations together with increased intestinal expression of ZIP4 and Zn absorption during deficiency, led to the understanding of the importance of the entero-pancreatic axis in maintaining whole body zinc homeostasis [51]. Removal of the pancreas and duodenum results in severe zinc deficiency manifesting in *acrodermatitis enteropathica*-like symptoms that is responsive to zinc therapy. This infers an important role for pancreatic zinc in whole body zinc homeostasis [64]. Therefore, concomitant and inverse modulation of dietary zinc absorption and pancreatic zinc excretion appears to regulate whole body zinc homeostasis.

3.3. Exchangeable zinc pool: Studies in experimental animals indicated that whole body zinc levels and plasma zinc remain unchanged over a wide range of dietary zinc concentrations. But severe dietary zinc restriction (1/10 of normal levels) in experimental animals leads to reduced whole body zinc content [65]. Interestingly, under these conditions only liver, plasma, testis and pancreatic zinc content was reduced but other tissues such as muscle and heart were unaffected. It is consistently observed that dietary zinc restriction is associated with immediate onset of growth

retardation and reduced appetite in animal models, despite unaltered tissue or plasma zinc levels [48,50]. It has been hypothesized that the rapid onset of the clinical features of experimental zinc deficiency could be due to depletion of these rapidly exchangeable zinc pools (EZP). Insufficient dietary intakes leads to rapid depletion of EZP in growing animals and in humans results in reduced growth, skin lesions, and infection [66]. A strong positive correlation of EZP and habitual dietary zinc intake also has been demonstrated [67]. In addition, it appears that when zinc is sufficiently abundant in the diet, the size of EZP is partially dependent on the magnitude of zinc intake rather than on homeostatic control mechanisms. Therefore, EZP could function as a mobilizable zinc pool that is sensitive to dietary zinc intakes, thus explaining the rapid onset of diet induced zinc deficiency symptoms during restriction of intake [68].

4. Iron and zinc interactions during absorption: As described above the homeostatic mechanisms controlling iron and zinc metabolism compensate for short term dietary inadequacies. However, chronic consumption of foods that have low levels of these dietary metals and/or abundant concentrations of dietary inhibitors leads to negative balance or deficiency. Considering the high prevalence of anemia and zinc deficiency in populations, supplementing iron and zinc together could be an ideal strategy. Studies in fasting human subjects have indicated a dose-dependent decrease in zinc absorption (25 mg dose) as measured by area under the curves during 4h time period post-dosing, when supplemented along with non-heme iron, at 2-3 fold molar excess, but not with heme iron   [69]. The inhibitory effect of iron on zinc absorption was also found to be higher with ferrous iron than its ferric counterpart. Further, prior administration of therapeutic doses of iron, had no impact on zinc absorption [70].   Together these studies suggest competitive interaction between iron and zinc during intestinal absorption. However, other studies measuring the zinc absorption at more appropriate doses (2.6mg) by whole body counting found significant negative interaction of iron on zinc absorption only at 25:1 but not at 2.5:1 ratio [71]. Further, the extent of interaction was either decreased or disappeared when minerals are supplemented with histidine (a zinc chelator) or a test meal, respectively. In agreement, multiple studies reported negative interaction of iron on zinc absorption only when given liquid form (cola or water) but not from meal [6]. Further, consumption of iron fortified foods had no impact on zinc absorption among adult human subjects or infants [72,73].   In addition, a study among pregnant women consuming therapeutic iron doses did not find changes in either zinc status or EZP (dependent on dietary zinc intake) measured using stable isotopes [74]. It is evident from all these observations

that negative effect of iron on zinc absorption if any, is significant only when therapeutic doses (2-3 fold excess, or higher, iron relative to zinc), given to fasting human subjects.

If the interactions of iron and zinc occur at a specific protein site, zinc would also be expected to inhibit the absorption of iron.   Indeed, zinc in high doses reduces the absorption of iron among adult human subjects when fed with water, but no such effects were seen when given in a meal [75]. A review of randomized controlled trials of iron and zinc supplementation in human subjects concluded that there is no strong evidence for negative interactions between these minerals [7]. In fact this review concluded that iron had no impact on zinc status, but zinc appears to have marginal negative impact on iron status, particularly on ferritin levels, a marker of iron stores. In contrast, a study in Peruvian children showed improved hemoglobin, iron status and reduced diarrhea when iron and zinc are supplemented with a 1 hour time gap between zinc and iron doses [76]. Therefore, it is likely that spacing iron and zinc doses augments the response to iron therapy, possibly via reducing the interactions or by increasing the intestinal absorption of iron, as explained later.

If zinc negatively interacts with iron absorption, one would expect reduced iron status during prolonged zinc treatment. However, a meta-analysis of zinc supplementation trials indicated no impact of zinc on hemoglobin [77]. Further, zinc supplementation also did not influence the ferritin levels in children [78]. In addition, this study also suggested that in individuals with severe zinc deficiency or with baseline infections, zinc supplementation is associated with hematological benefits. Similarly, supplementation of iron and zinc together was reported to augment the response to iron supplementation and to reduce the prevalence of diarrhea and to improve motor development and exploratory behaviour in children [7,79].   Studies in animal models also found no negative interactions of iron and zinc when supplemented with food [80]. In addition, zinc appears to counter iron-induced oxidative stress in animal models [81,82]. Therefore, the existing evidence suggests that addition of zinc to iron supplementation regimens has no significant negative impact on iron status, and in children at risk of nutritional deficiencies zinc appears to influence the iron status favorably.   But this effect could also be indirect, as zinc supplementation is associated with reduced morbidity, which otherwise has independent negative effect on iron absorption and status.

It was thought initially that iron and zinc, due to their similar atomic radius and oxidation state might compete for intestinal absorption at divalent metal ion transporter-1 (DMT1), a proton coupled apical iron transporter in intestinal cells [83]. However, iron but not zinc uptake in intestinal cell models is induced by acidic pH and neutralizing antibody of DMT1 had no effect on zinc absorption in Caco-2 cells, implying that zinc is not a substrate for DMT1 [84]. A kinetic analysis of iron and zinc uptake in intestinal cells indicated the presence of yet elusive receptor that probably mediate iron and zinc interaction during their intestinal uptake [19]. In addition, treatment of intestinal cells with zinc has been shown to increases the iron absorption via induction of mRNA and protein expression of DMT1 [16,19]. Similarly zinc also increased the expression of ferroportin and basolateral exit of iron, and metal transcription factor-1 (MTF1) appears to mediate these effects [16,17,85]. In addition, iron and zinc interactions were no longer evident when cells were pretreated with zinc [19]. In a more recent study we have demonstrated that zinc induced intestinal iron absorption, which is mediated by IRP2 mediated DMT1 mRNA stabilization and protein expression. Further we also demonstrated that zinc induces intestinal iron uptake, IRP2 and DMT1 expression that require activation of Pi3K signaling pathway [20]. In support of these results, zinc deficiency induced by specific chelators reduced the expression of DMT1 in Caco-2 cells [86]. Further we also demonstrated that oxidative stress induced intestinal cell death in inhibited by zinc, via reducing labile iron pool [87]. Interestingly, zinc reduced the oxidative stress-induced iron uptake and DMT1 expression, but increased these parameters under normal conditions. This implies that effect of zinc on intestinal iron homeostasis is complex and is modulated by underlying patho-physiological status [87]. From these results it appears that under non-stress conditions, zinc appears to positively modulate iron absorption in intestinal cell culture models by inducing the expression of iron metabolic proteins, particularly that of DMT1 and FPN1.

5. Impact of zinc deficiency on iron status: Clinical studies in human subjects indicated that serum zinc levels correlate with hemoglobin and other iron status markers. Hemoglobin, plasma ferritin, MCV and RDW were found to be higher in zinc sufficient (>100 μg/dL) compared to zinc deficient (<100 μg/dL) subjects [12]. Similarly, a large cross-sectional study among pregnant women (n=1185) found low serum zinc levels among anemic subjects, and furthermore, serum zinc levels were significantly and positively correlated with hemoglobin [13]. Other studies have shown that

the plasma zinc levels were significantly lower among subjects with iron deficiency anemia [88].   In addition, serum zinc reported to be an independent risk factor for anemia among school age children in New Zealand [14]. These studies together indicate that zinc status is associated with iron metabolism among human subjects, but its causality cannot be established in these settings. Interestingly concurrent iron deficiency anemia has been reported among people with *acrodermatitis enteropathica*, a rare genetic disease characterized by zinc deficiency [89], therefore a causal role of underlying zinc deficiency in development of anemia or iron deficiency cannot be excluded.

Studies in experimental animal's demonstrated development of iron deficiency anemia and tissue iron accumulation during zinc deficiency. For instance, studies in rats given low zinc diets led to dose-dependent reduction in iron status parameters such as hemoglobin and RBC number, which could be either due to reduced erythropoiesis or increased catabolism [8,82]. In addition, increased plasma and testicular iron levels with concurrent oxidative stress secondary to zinc deficiency has been reported in rat models [90]. The fact that zinc deficiency also leads to reduce transferrin concentrations might increase levels of non-transferrin bound iron in serum and thus increase oxidative stress [91]. Similarly, dietary zinc restriction of rats led to iron accumulation across multiple tissues, and this is reversed by supplementation with zinc [10]. Furthermore, maternal zinc restriction also resulted in higher tissue accumulation of iron in both maternal and fetal tissues, which appears to be stored in the ferritin-hemosiderin fraction [92].

In intestinal specific conditional ZIP4 knockout mice, the intestinal iron and zinc concentrations are significantly reduced at day 4 compared to wild type control while the liver iron remained similar. Interestingly, at day 8, the liver iron concentration in knockout mice was markedly higher despite the fact that liver zinc levels remained unchanged [9]. Adipocyte cell lines grown in zinc deficient medium accumulate iron as a result of increased TfR1 and ferritin and reduced DMT1 levels [11].   Similarly zinc supplementation has been reported to induce the ferroportin expression in zebra fish gills [93]. In rat models, repeated psychological stress resulted in reduced absorption, tissue iron accumulation, iron deficiency anemia, and oxidative stress, which could be due to increased hepcidin levels [94]. Interestingly, zinc supplementation reversed these changes and improved iron absorption and reduced the tissue iron levels induced by stress [94].   These results clearly suggest that zinc has a profound impact on whole body iron metabolism, particularly the

intestinal iron absorption and distribution of iron between tissues. It is also possible that zinc induced changes in iron homeostasis could be mediated indirectly via reduced inflammation and/or oxidative stress.

Surprisingly, there are no studies measuring iron absorption in relation to zinc status in humans, possibly due to lack of reliable biomarker of zinc deficiency. However, a study in suckling rat pups demonstrated that during early infancy (at day 10 of parturition) zinc supplementation increases the DMT1 and FPN1 mRNA and protein levels, but these effects are not observed in late infancy (at day 20 of parturition) [18]. These results further suggest that zinc modulates the expression of iron metabolic proteins, but these effects could be varied by age and other physiological factors.

6. Does pancreas-zinc-DMT1/FPN axis play a role in intestinal iron absorption?   As described above zinc appears to influence the iron homeostasis by modulating expression of DMT1 and ferroportin in intestinal cells and animal models. Studies in animal models indicate iron deficiency anemia occurs secondary to zinc deficiency. In addition, cross-sectional studies in human subjects have demonstrated a positive association between serum zinc levels and markers of iron status. Though the impact of zinc status on intestinal iron absorption remains to be addressed more systematically, the observations in intestinal cell culture models suggest that zinc induces intestinal iron absorption via increasing DMT1 and ferroportin expression. The fact that intestinal-specific knockdown of the zinc transporter Zip4 led to decreased intestinal iron and zinc content and increased liver iron, suggests that the zinc content of intestinal cells appears to be strong modulator of iron absorption and status. It's important to note that pancreatic secretions constitute a major route of endogenous zinc secretion and is regulated by whole body zinc status [62]. Studies indicated that the endogenous zinc loss is related to both recent and long term zinc status. It is estimated that 2 mg of zinc is lost through pancreatic secretions per day which corresponds to ~12 μmol/L zinc considering the 2.5 L juice produced per day [62,95]. In addition the dietary intake of zinc is approximately 12 mg/day [96], though the bioavailability of this source of zinc varies significantly depending on other dietary components. The available evidence clearly indicates that fecal zinc excretion is predominantly a function of pancreatic juice [48]
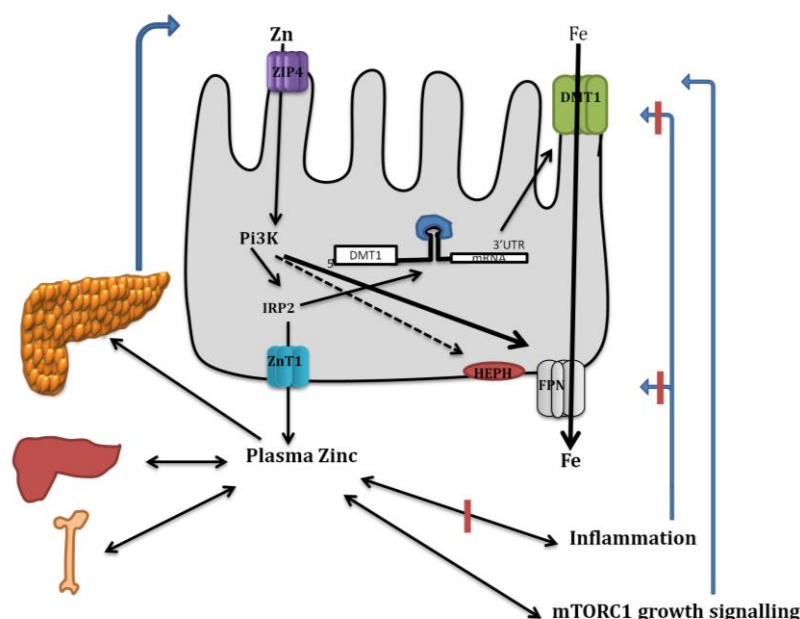
Figure 3. Hypothetical model for direct and indirect effects of zinc on intestinal iron absorption and iron homeostasis: During adequate zinc status and dietary intakes, the pancreatic zinc secreted in to the intestinal lumen stimulates the intestinal iron transport via induction of DMT1 and ferroportin. During inadequate zinc intake reduced pancreatic zinc levels reduces intestinal iron transporter DMT1 and FPN expression leading increased retention and inhibition of absorption. Similarly, excretion of zinc from tissues such as liver and bone marrow, results in declined tissue zinc and as a consequence reduced FPN1 expression leading to reduced secretion for erythropoietic needs. Alternately, zinc might prevent inflammation and thus negate its inhibitory effect on iron absorption. During zinc sufficiency growth signalling mediated by mTORC1 pathway might increase the iron requirements and thus improve iron absorption.

and can be reduced both by decreased pancreatic tissue zinc levels [63] and expression of zinc exporters [51]. Therefore, pancreatic zinc, which in turn is regulated by whole body zinc status, may serve as a stimulus for regulating DMT1 and Fpn-1 expression and thus intestinal iron absorption (**Figure 3**). We hypothesize that during states of zinc deficiency reduced pancreatic zinc secretions resulting from decreased tissue zinc levels, particularly the liver, might negatively impact intestinal iron absorption and could induce iron accumulation in liver. If true, this might account for the observed changes in iron metabolism during zinc deficiency described above.

7. Physiological advantages of zinc modulating iron homeostasis: It is clear from the above observations that zinc status has a marked impact on iron absorption and metabolism. It is possible that this has physiological relevance and metabolic advantage. It's known that iron and zinc compete with various metabolic proteins due to their similar physico-chemical properties. It's

established that iron is a pro-oxidant while zinc is seen as an antioxidant [87]. Therefore, excess iron entry into the body needs to be checked to keep the redox status in balance. Regulation of iron absorption and mobilization, by zinc might help to ensure appropriate redox- balance. On the other hand, zinc is a type 2 nutrient, whose deficiency immediately manifests in reduction in new tissue growth, and adaptation to a lower basal metabolic rate [66]. Zinc has been demonstrated to induce the activity of mTORC1 pathway,

a master regulator of growth in mammals [97]. Therefore, direct regulation of iron metabolism by zinc could reflect changing tissue iron requirements depending on growth at different stages of the life course.

8. Conclusions: The available evidence from experimental animals, cross-sectional studies in human subjects and genetic studies, clearly point to an association of whole-body zinc status with iron homeostasis. Particularly, underlying zinc deficiency appears to induce iron deficiency by mechanisms that block either intestinal absorption or mobilization of iron from tissues. The *in vitro* studies in intestinal cell culture models and studies in animals point to a role for zinc in modulating DMT1 and FPN1 expression, respectively. It is therefore possible that compromised zinc status leads to a reduction in pancreatic zinc content, which in turn reduces intestinal iron absorption via a decrease in DMT1 and FPN1 expression. On the other hand, tissue zinc deficiency in liver (which contributes to plasma zinc during states of zinc deficiency) may also result in reduced DMT1 and FPN1 expression, leading the accumulation of iron in the tissues. Thus, the net effect of zinc deficiency is mechanistically linked with development of iron deficiency, induced by both reduced intestinal iron absorption and decreased mobilization of iron from storage sites.

Although, this review highlights the cross-talk between zinc with iron metabolism at multiple levels, further investigations are warranted to understand the nexus between these important minerals. For example, there is no direct demonstration of the impact of zinc deficiency on iron absorption in humans. The interpretation of these studies is likely to be complicated by the marked differences in body and tissue weights during zinc deficiency. In addition, studies are also needed to understand both short and long term consequences of dietary zinc restriction on iron metabolism, particularly the mRNA and protein expression of iron metabolic proteins and the effects on distribution and concentrations of metals between different tissues.

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