

Review Article

Zinc as a Micronutrient in Cellular Function and Nutritional Balance

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***Corresponding author:** Kiran Alluri, College of Dentistry, University of Nebraska Medical Center, Omaha, Nebraska, 68198, USA**Email:** kalluri@unmc.edu or kiran.allurikumar@gmail.com**Received:** April 26, 2025**Accepted:** May 08, 2025**Published:** May 13, 2025**Abstract**

Zinc performs many physiological effects like cell viability, proliferation, differentiation, bone calcification, insulin packaging, brain development, activation of the immune system, and acts as a cofactor for many enzymes and hormones. Dietary zinc deficiency leads to stunting/growth retardation in humans. In circulation, zinc levels are maintained through its distribution from various tissues. In this regard, macrophages and muscle and bone play an important role.

Cellular and consequently the whole body zinc homeostasis is maintained by coordinated regulation of two major families of zinc metal transporters, ZnTs, and ZIPs with opposite functional activities and an intracellular zinc storage protein metallothionein. ZnTs includes 10 (ZnT1-10) transporters, which decrease cytoplasmic zinc concentrations, and the ZIPs includes 14 ZIPs (1-14) transporters and increases cytoplasmic zinc concentrations. Fluctuations in the intracellular zinc levels are tightly regulated by these transporter families depending on their localization and in a tissue, cell, and organelle-specific manners. The expression of zinc transporters determines the zinc levels in tissues or cells, which in turn regulate specific gene expression and physiological functions.

It has been proposed that bone may serve as a passive reserve for zinc and may become available during the normal turnover of osseous tissue. This may be due to the differential role of zinc transporters in tissues that contribute to maintaining zinc homeostasis. The overall balance between ZnTs, ZIPs, and MTs might be the reason for zinc assimilation in a specific tissue. Therefore, the present review was carried out to understand the regulation of zinc in maintaining the homeostasis.

Keywords: Dietary zinc; Zinc depletion; Metallothionein; Zinc transporters**Introduction**

Zinc is the second most abundant transition metal in the human body after iron. The essentiality of zinc as a trace nutrient was first discovered in the fungus *Aspergillus niger* in 1869. However, the significance of zinc as a mineral essential for human life was studied half a century ago by Dr. Ananada Siva Prasad [1]. In 1961, he hypothesized that zinc deficiency could account for human growth retardation, and later he established that subjects from Iran and Egypt suffered zinc deficiency, which could be corrected upon zinc supplementation. Severe zinc deficiency in humans leads to alopecia in children and mild to moderate forms of zinc deficiency has been shown to affect physical growth, development, neurological deficits, and impaired immune system and increased susceptibility to diarrhea in children. Zinc supplementation studies have been shown to have health benefits. Zinc deficiency is a serious global public health problem, affecting ~ 2 billion people because of inadequate zinc in the diet [2]. Zinc is involved in linear growth and is been implicated in stunting in children which are a serious nutritional problem in India. According to the National Family Health Survey, 38.4 % of children under the age of 5 years are stunted in the country [3]. Various studies from India have also shown that zinc deficiency exists in the general population [4].

Zinc participates in several metabolic activities and has three major roles in viz., a) catalytic activity b) structural and c) regulatory mechanisms. Zinc transport proteins at the cellular levels are indispensable for the physiology of zinc and are of two types 1) ZnTs and 2) ZIPs. Zinc is the only metal that is part of all enzyme classes. Zinc also functions as an antioxidant metal and therefore has an important role in the inflammatory process. Animal and in vitro studies using human cell lines have given an enormous understanding of the role of cellular zinc transporters along with the storage protein metallothionein (MT) in maintaining zinc homeostasis. Furthermore, genetically inherited diseases such as Ehlers-Danlos, Acrodermatitis, TNZD (Trans neonatal zinc deficiency) diseases or infection, and inflammation conditions have also revealed the importance of zinc transporters in zinc homeostasis. However, there is a wide lacuna existing in our understanding of tissue and cell specific regulation of zinc under conditions prevailing in humans such as zinc depletion (deficiency), repletion (supplementation) and under conditions of inflammation which is essential to develop strategies to prevent and control zinc deficiency. Therefore, the overall aim of the work is to study changes in various exports and import transporters of zinc under conditions of deficiency, supplementation, and inflammation

in three human cell lines that have contrasting functions of zinc storage and non-storage in humans. Considering the contrasting functions of these three tissues or cells, this study may provide a lead in identifying an early biomarker that can reflect zinc deficiency much before plasma zinc changes.

The following write up is intended to review the literature on various aspects of zinc metabolism in humans, which will identify knowledge gaps about various zinc transporters. There exists certain uncertainty in the distribution of zinc in various tissues and their role in mobilizing zinc under deficiency and inflammation in establishing homeostasis.

Review of Literature

Metabolism of Zinc

Dietary Sources: Intake of zinc through the diet among different age and gender groups in India ranges from 5.2 to 16.2 mg/day [5]. However, zinc content in the total diet depends on the source of the food items and also food processing methods. It is evident from the table below that red meat is a rich source of zinc, while fruits, roots, and tubers contain fewer quantities of zinc (Table 1). Drinking water or other beverages may contain high levels of zinc if they are stored in metal containers. **Dietary Zinc Deficiency:** Reduced intake and impaired absorption of zinc due to autosomal recessive inheritable disease acrodermatitis enteropathica, leads to zinc deficiency in humans. As per the International Zinc Nutrition Consultative Group (IZiNCG) guidelines, if >20% of the population has the plasma levels of zinc lower than cut off values <70 µg/dL, the whole population should be considered at risk of zinc deficiency [6]. In India, various studies carried out in 6 states have revealed the prevalence of zinc deficiency ranging from 36.2-73.3% in children, pregnant and non-pregnant women [7-10]. The prevalence of zinc deficiency in the world based on the childhood growth stunting and absorbable zinc in the food is given in (Figure 1).

Table 1: Zinc content (mg/100g) in Indian foods.

Type of food	Zinc	Type of food	Zinc
Fruits	0.09-0.4	Egg and egg products	1-1.2
Roots and Tubers	0.1-0.3	Poultry	1.4
Condiments and spices	0.1-0.3	Cereals and millets	2-Jan
Marine fish	0.3	Beef	3
Leafy vegetables	0.5	Meat	4
Fresh water fish	0.6	Nuts and oil seeds	5-Mar
Milk and milk products	0.3 – 2.3		

Source: [11].

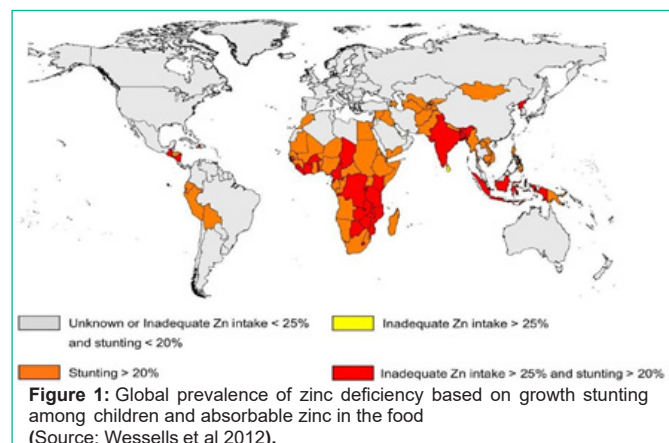


Table 2: Recommended dietary allowance of zinc (mg/day).

Recommended dietary allowance	ICMR-2010	IOM-2001
Man	12	11
Woman	10	8
Pregnant woman	12	8
Boys (13-15 years)	11	11
Girls (13-15 years)	11	9

Recommended Dietary Allowances (RDA) for Zinc in Humans: Zinc requirement varies according to age and gender. The requirement is more in growing children, pregnant and lactating women. According to the Institute of Medicine (IOM) the RDA for zinc is 11 mg per day for men and women [12]. The Indian RDA is comparable to the above recommendations (Table 2). These recommendations have been computed based on dietary intake and absorption of zinc in various target groups [5].

Consequences of Zinc Deficiency

Growth in Children: Growth retardation is the first sign of zinc deficiency condition (Wessells et al 2012). The meta-analysis of studies in developing countries on zinc intervention trials revealed stunted growth in children is due to zinc deficiency [13]. Low levels of maternal zinc decrease the foetal growth, results in low birth weight in new-borns [14]. It was found that growth-promoting response of zinc is due to the coordinate cell signalling mechanism of zinc on the growth-regulating hormone, insulin-like growth factor and insulin-like growth factor-binding protein 3 [15,16].

Development in Children: Zinc levels in mother have propounding impacts on foetus neural development; low levels affect the motor function. Studies of zinc supplementation in school children have improved cognitive behaviour [17]. Further on zinc supplementation has a beneficiary effect on short term memory in children 5-15 years [18].

Pregnancy: In pregnancy, the adverse side effect of zinc deficiency affects both mother and foetus such as placental abruption, prolonged labor, haemorrhage [19]. Zinc supplementation during pregnancy has been shown to reduce about 14%, premature deliveries in low-income women [20]. The most common pre-eclampsia reported in pregnancy is associated with low levels of zinc [21,22].

Diarrhoea: During zinc deficiency susceptible to infection and diarrhoea increases [23]. Zinc as oral rehydration therapy was recommended by the WHO and UNICEF in diarrheal diseases [24]. Besides, the combination of ORS and zinc in acute and persistent diarrhoea lowered the frequency of hospitalization and also the severity of diarrheal stool output [25, 26]. It has been observed that zinc deficiency might potentiate E. coli to produce toxins for causing diarrhoea [27]. However, the mode of action of zinc in diarrhoea to reduce its severity is not yet understood clearly. It is predicted that Na⁺ absorption is elevated, whereas Cl⁻ ions secretion outside is inhibited by zinc [28].

HIV/AIDS: Zinc status of an individual has a major impact on HIV/AIDS patients, increased mortality rate, and progression of the disease was noticed with low serum zinc levels in HIV patients [29]. The importance of zinc further delineated with 45mg/day of zinc to AIDS patients for one month decreased the opportunistic infections [30]. On the other hand, increased zinc intake progressed the disease,

and mortality rate was increased. Thus, there exists ambiguity of zinc dose and the stage at which zinc has to be supplemented in AIDS patients [31].

Assessment of Zinc Status

Although, there are no sensitive and reliable biochemical markers for identifying the zinc status; currently, plasma zinc, metallothionein (MT), and zinc-dependent enzymes have been used for assessing the zinc status. Given the above knowledge gap, it is necessary to develop an early biomarker for zinc.

Plasma/serum Zinc: Plasma zinc can be measured using Atomic Absorption spectrophotometry or inductively coupled plasma mass spectrometry (ICPMS). Plasma zinc levels below the cut off value 70 µg/dL are used for assessing the inadequacy of zinc [6]. However, zinc is a type-II nutrient during zinc starvation condition other tissues such as bone release zinc into the blood and maintain plasma zinc concentration [32]. Moreover, plasma zinc levels vary based on the inflammation, infection, and plasma protein levels (albumin). These confounders make plasma zinc levels an unreliable biomarker for assessing zinc status.

Metallothionein: Metallothionein (MT) is an intracellular zinc storage protein which is ubiquitously expressed in tissues. Studies reported that metallothionein (MT) levels reflect the zinc status, during zinc depletion and repletion in human. RBC metallothionein levels have been shown to correlate with the zinc changes [33]. Similarly, in monocytes also a direct relation of changes to zinc fluctuations in monocytes MT mRNA was reported [34]. The specificity of MT levels is questionable since MT is affected in inflammation and by heavy metals Co, Ag, Cu, and Cd.

Zinc Dependent Enzymes: Many enzymes contain zinc as an essential component, such as carboxy peptidase, alkaline phosphatase, thymidine kinase and have been used as a marker for assessing zinc status [35]. However, none of these enzymes has correlated clearly with zinc levels [36].

Other Markers: Notably, attempts were made to relate hair and skin zinc contents as a possible biomarker. However, all these attempts were failed due to large variations in the distribution of zinc in these organs. Moreover, these tissues are also affected by many other parameters apart from zinc. It has been reviewed that hair zinc concentration has limited application in population surveys [37].

Digestion and Absorption

Dietary zinc in food is present in the bound form complexed with amino acids and nucleic acids. Hydrochloric acid in the stomach acts on zinc- biomolecule complexes and liberate zinc as free ions. Intestinal enzymes, particularly proteases and nucleases, act on proteins and nucleic acid and liberate zinc.

Zinc is absorbed in the lumen of the small intestine and is transported through specific proteins across the cell membrane into the portal circulation through specific transporters. The type of transport and their role in the absorption of zinc in the enterocyte is described elsewhere (Section 1.9.2)

Absorption of free zinc ions liberated from the food in the stomach is affected by dietary ligands that can promote or inhibit

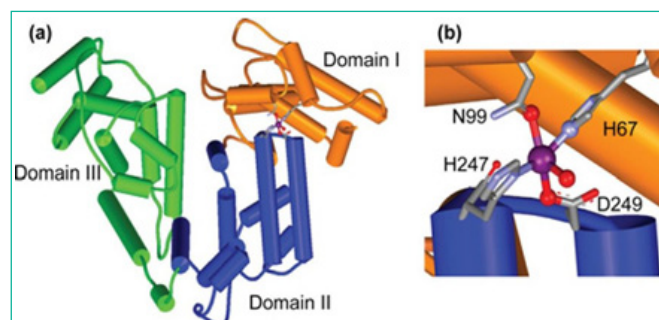


Figure 2: Depiction of zinc binding site on albumin. A) Zinc binding site at the interface of domains I (orange) and II (blue). B) zinc (purple) site composed of (H67 and D 249) in domain I, (H247 and D249) in domain II and a fifth exogenous (water) ligand (Source: Lu et al 2008).

zinc absorption. Cysteine, histidine, phytates reduce the absorption of zinc and ligands like tannic acid promote zinc absorption [38]. The relative proportion of these ligands in the lumen will determine the bioavailability of zinc from a habitual diet. Using stable isotopes of zinc, bioavailability (fractional absorption of zinc) of zinc among Indian adolescent boys and girls have been reported to be about 30% from a rice-based diet [39].

The kinetics of zinc uptake in enterocyte and its resolution into individual components can provide insights into the transport process. Studies have shown that zinc uptake is more complex, as it involves three components [40]. Resolution of this triphasic zinc uptake into individual components shows substantial uptake at concentrations below Km (1) paracellular uptake of about ~30%, (2) mediated uptake and (3) in maintaining steady- state concentrations of cellular zinc by efflux of excess zinc from the cell in human enterocyte Caco-2 cell line.

Among the polyphenols, tannic acid, and components of red grape juice have been shown to increase the zinc uptake in Caco-2 cells [41]. It is not known how the zinc-polyphenols complex enters into enterocytes and suggested non- involvement of specific zinc transporters similar to amino acid zinc complexes [42]. Studies have shown that tannic acid and quercetin significantly enhance zinc uptake and induce metallothionein synthesis, whereas phytates, reduce the zinc absorption by forming indigestible complexes with zinc [41,43].

Circulation, Distribution and Excretion of Zinc

Circulation: The hepatic portal system transfers the absorbed zinc into the liver and distributed to different organs. Zinc in the circulation (plasma zinc ~ 0.1 %) is delivered to tissues in the bound form to albumin (80%) and lesser extent to α2-macroglobulin (20%) [44,45]. Furthermore, zinc (70-80%) in RBCs is bound to the carbonic anhydrase and acts as a source for zinc [46].

Zinc binding site in albumin is located at the domain interface between domains I and II, as shown in (Figure 2). These domains are two amino acids His67 (H), and Asn99 (N) in domain I, and His247 (H) and Asp249 (D) in domain II and a water molecule is included as a fifth ligand [47].

Distribution: Zinc is absorbed and transported from small intestine to the liver by a hepatic portal vein from here it is redistributed to the other tissues. Tissues such as muscle (~60%) and bone (~30%) are

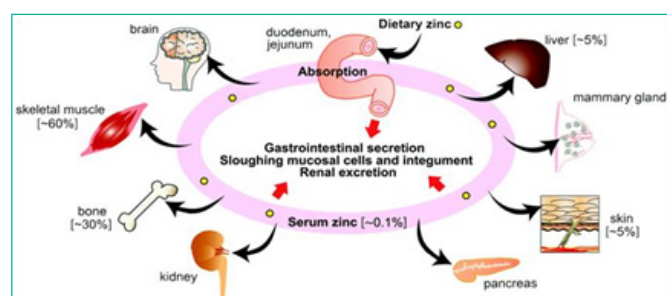


Figure 3: Tissue distribution of zinc in the human body (Source: Golan et al 2017).

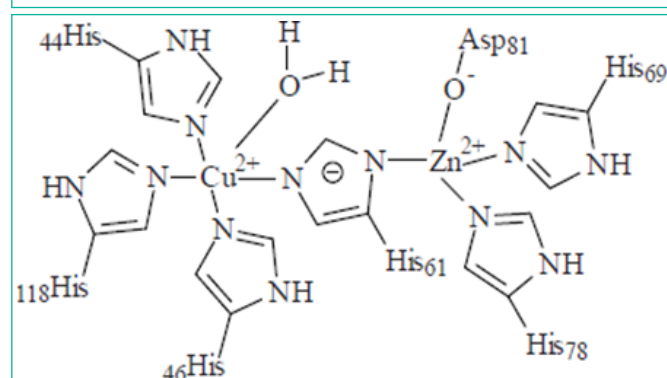


Figure 4: Active site configuration of Cu, Zn-SOD active configuration (Source: Labadi et al 2009).

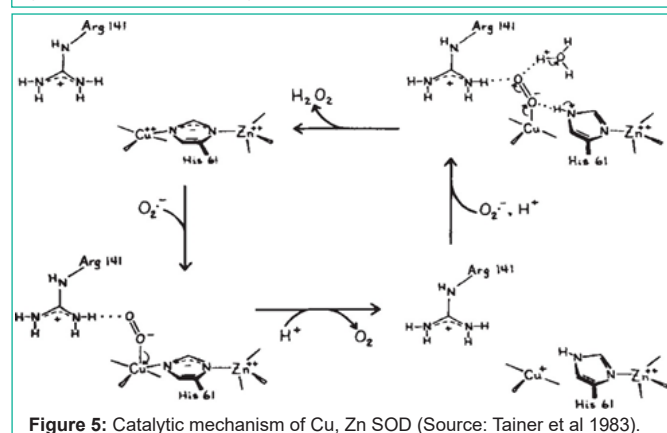


Figure 5: Catalytic mechanism of Cu, Zn SOD (Source: Tainer et al 1983).

the major reservoirs of zinc while skin and liver (~5%) hold relatively fewer concentrations of zinc (Figure 3). Although pancreatic β - cells contain high levels of zinc compared to other soft tissues, they are not the prominent reservoirs of zinc as muscle and bone [48].

Excretion: Zinc is excreted primarily through the gut; unabsorbed zinc is eliminated through faeces. Besides, bile, pancreatic, and intestinal secretions in the lumen along with mucosal sloughing of the gut also contribute to excrete zinc [49]. The renal system regulates zinc loss through urine as per the dietary zinc intakes [50]. The total daily excretion of zinc is estimated to be around 0.63 mg/day [51].

Biochemical Functions of Zinc

The three important biochemical functions of zinc are (1) catalytic (2) structural and (3) regulatory are presented in (Table 1.3). Zinc has certain special physico-chemical characteristics that enable it to function uniquely. It belongs to the "d" block of the periodic table with

electrons completely occupying d and s shells. The natural redox state for zinc is Zn^{2+} and it does not easily undergo oxidation or reduction processes. The chemical features that allow zinc to participate in structural and catalytic roles are as follows:

- Lewis acid which can accept an electron.
- Chemical tendency to form multiple geometries. Zinc can bind with 2 to 8 ligands.

Structural Role: Many enzymes require zinc as a structural component. The enzyme copper-zinc dismutase (Cu, Zn SOD) is the most prominent zinc-containing enzyme. In this enzyme, zinc stabilizes the protein while copper provides catalytic activity [53]. The detailed structure analysis revealed that the zinc is bound to the protein through the amino acids His-61, His-69, His-78, and Asp-81. Whereas copper binds to the four histidine residues His-44, His-46, His-61 and His-118, and a water molecule. The imidazole of the His-61 bridges the two metal ions and are separated by 6.3 \AA away from each other in (Figure 4). Zinc has an important role in the fast protonation of nitrogen of His-61, which disconnects cuprous ion upon reduction [54] as shown in (Figure 5).

Catalytic Role: Carbonic anhydrase, alkaline phosphatase, Ca-ATPase, 5'nucleotidase, and carboxypeptidase enzymes depend on zinc for their catalytic activity. During catalysis of these enzymes, amino acids such as histidine, glutamic acid, and threonine present at the active site form tetra dentate co-ordination with zinc and function as a Lewis acid. One of the best examples of such a process is the hydration of CO_2 to H_2CO_3 by carbonic anhydrase, as shown in (Figure 6). The detailed structure analysis revealed that the HCAII (human carbonic anhydrase) enzyme is a functional 29-kDa monomer consisting of a 10-stranded, twisted β -sheet. The active site is located at the bottom of a 15- \AA cone-shaped cavity that leads to the center of the protein. The active site includes a zinc ion coordinated tetrahedrally by 3 histidine residues (His-94, His-96, and His-119) and a water molecule/hydroxide ion (Wat-263) as a fourth ligand [55]. **Regulatory Role:** Zinc regulates the gene expression at the transcriptional level of various cellular activities such as DNA synthesis, mitosis, cell division

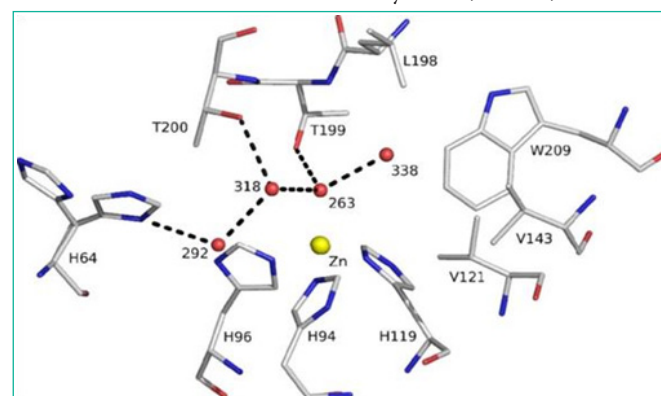


Figure 6: The active site of human carbonic anhydrase, The zinc ion is tetrahedrally coordinated by His-94, His-96, and His-119 and catalytic water (Wat-263). The deep water (Wat-338) sits in a hydrophobic pocket lined by Leu-198, Trp-209, Val-143, and Val-121 at the bottom of the active site. Wat-318 is in a hydrophilic environment toward the mouth of the active site. The proton shuttle His-64, shown in both "in" and "out" positions, is linked via Wat-292 and Wat-318 to the catalytic water. Hydrogen bonds are depicted as dotted lines, and waters are labelled with numbers only. (Source: Sjöblom et al 2009).

Table 3: Examples of biochemical functions of zinc.

Role	Biological Process	Function of Zinc	Reference
Catalytic and Structural	DNA and protein synthesis	Zinc is a cofactor for many enzymes including DNA polymerase, RNA polymerase, and reverse transcriptase	[56]
Regulatory	Gene Expression	MTF-1 a zinc responsive transcription factor regulates the number of genes	[57]
	Inflammation	IL-6 upregulates expression of zinc importer, ZIP14 in hepatocytes which leads to the accumulation of zinc bound to metallothionein in the liver	[58]
	Immunity	Zinc deficiency leads to reprogramming of the immune system	[59]
Catalytic and structural	Enzymes	Zinc acts as a cofactor for all the six classes of enzyme	[60]
	Signalling	Zinc regulates the release of neurotransmitter	[61]

and protein synthesis mediated via zinc finger proteins or zinc finger motifs [56, 62]. MTF-1 (metal transcription factor) is an important zinc finger protein involved during heavy metal stress and activates the transcription of metallothionein, which is a zinc-responsive protein [63]. MTF-1 has a critical role in regulating genes; any abnormal function in MTF-1 leads to catastrophic effects. MTF-1 malfunction in null mutation leads to a lethal effect on foetal development [64]. However, the total number of genes that are under the regulation of MTF-1 is not yet deciphered.

Zinc Finger Motifs: Zinc in several proteins co-ordinate and chelate through cysteine, histidine residues. This stabilizes the protein structure by the formation of zinc finger-like structures known as zinc finger motifs [65]. Several different structural families are known, and they typically function as interaction modules that bind to DNA, RNA, proteins, or small molecules which are involved in the cell growth and differentiation (Figure 7).

Transcription factor-III α (TF-III α) is the first transcription factor containing zinc and identified as zinc finger type regulates the gene expression by binding to DNA. In steroid hormone receptors such as estrogen and glucocorticoid receptors has a zinc twist type of zinc finger motif have been described while in testosterone, and vitamin D zinc has a structural role [46, 66]. In protein (insulin) also zinc co-ordinates with histidine and or cysteine residues for proper hexameric conformation of the protein [67]. Similar, kind of structural motifs exist in viruses also the best notable example is the nucleocapsid protein of HIV [66].

Cell Signalling: Zinc plays an important role in cell signalling mechanisms, in neurons zinc act as signalling ion and reduces the activity of N-methyl-D-aspartate (NMDA) receptors while increasing the activity of gamma-aminobutyric acid (GABA) receptors [68,69]. Zinc can act as an insulin-mimetic signalling molecule and elevates the glucose uptake [70,71]. Similarly, earlier, it was reported that treatment of 3T3-L1 adipocytes with Zn elevated glucose uptake and lipogenesis, by stimulating the tyrosine phosphorylation of insulin receptor [72]. In diabetic mice, zinc deficiency enhances the hepatic injury by the degradation of NrF2, while zinc maintains NrF2 levels, via the Akt activation which prevents the Fyn transportation of NrF2 into the cytoplasm for degradation [73]. Zinc inhibits protein tyrosine phosphatase 1B (PTP1B), a cytoplasmic phosphatase that interacts with the insulin receptor and catalyzes its dephosphorylation resulting in the attenuation of insulin signalling [74]. Thus, zinc supplementation to diabetes can be considered for further studies to determine the possibilities of generating a very rational therapy.

Cellular and Sub Cellular Distribution of Zinc

The cellular and subcellular zinc homeostasis is maintained through zinc uptake, distribution, storage, and efflux (Figure 8). Normally zinc levels in the cells vary from picomolar to micromolar depending on the physiological needs of the tissue. In neurons and HT-29 cells, zinc concentration is 396 μ M and 264 μ M, respectively [75,76] and in mammalian oocytes at the time of maturation zinc levels increases to zillions of atoms [77].

At subcellular level mitochondria contains a low amount of zinc (picomolar range), endoplasmic reticulum contains a maximum of 5 nM while the golgi complex contains the lowest zinc concentration

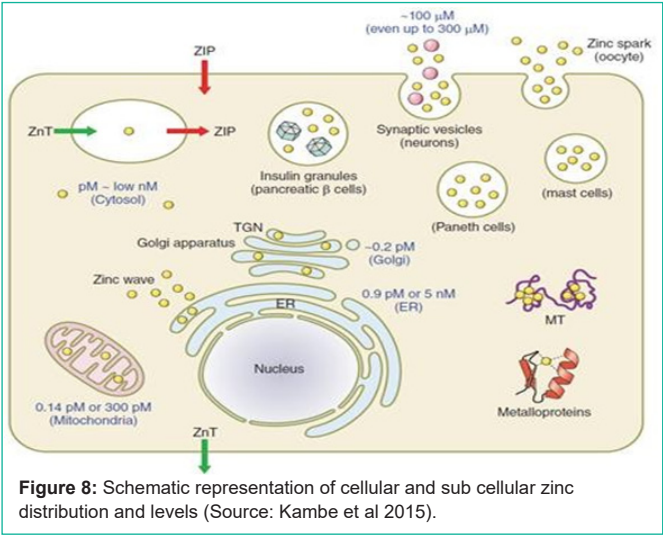
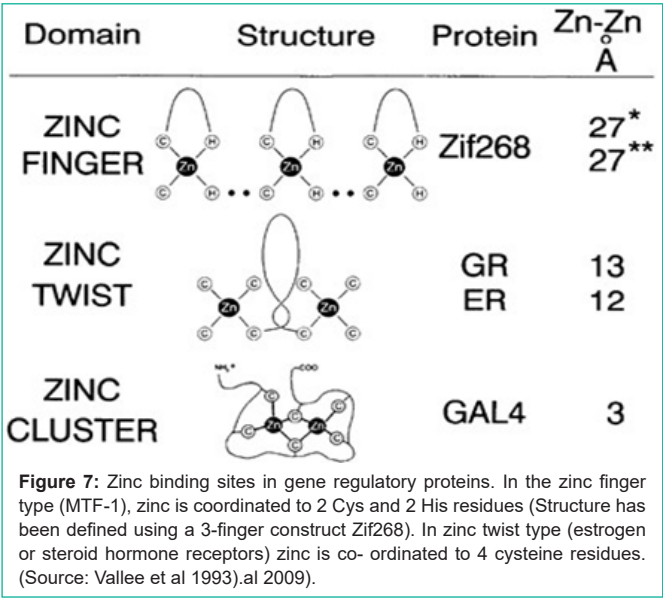


Table 4: Isoforms of MT expression.

Isoform	Tissue
MT-I and MT-II	Ubiquitously expressed majorly on liver and kidney
MT-III	Neurons
MT-IV	Skin and G.I.T

(~0.2pM). All these processes of zinc trafficking across the cells are achieved by the zinc-specific transporters along with co-ordinated regulation of MT, a zinc-binding protein. Collectively, these proteins function together and regulate the intracellular zinc levels between the cytoplasm and cellular organelles known as “buffering” and “muffling” of zinc [78,79].

Cellular Regulation of Zinc Metabolism

Metallothionein: Mammalian metallothionein (MT) is an intracellular zinc-binding protein, and it is a small ~7 kDa cellular protein with a high (~30%) content of cysteine residues and are present in four major isoforms (MT1–MT4) which are tissue-specific in expression (Table 4) [79].

Metallothionein (MT), regulates the zinc absorption and excretion to zinc supplementation and in inflammation [81]. In addition, dietary zinc restriction results in diminished MT synthesis [82]. Structural studies of unsaturated MT have proven to be a challenge, mainly due to the unfolded and highly dynamic structure.

To date, only one X-ray structure of metallothionein (Figure 1.9 A) has been solved: hepatic rat MT-II. Metallothionein folds into two separate domains with two Zn–S clusters, one with 3 Zn(II) and 9 Cys (β-cluster) and the other with 4 Zn(II) and 11 Cys (α-cluster). In both domains, metal ions are present in tetrahedral geometry and tetrathiolate environments, where sulfur donors have bound to one or two (bridging donors) metal ions. Here, the X-ray structure is based on Cd(II)-induced in vivo protein, which has one Cd(II) ion in the β-domain at position IV and four ions in all positions (I, V, VI, and VII) in the α-domain (Figure 9B). MT reconstituted in vitro also binds seven Zn (II) or seven Cd(II) ions. Most of the current knowledge regarding the structural, biophysical and even biochemical properties of MTs is based on cadmium MTs, which are more convenient to study due to their better spectroscopic properties, less dynamic nature or higher protein affinity, in comparison with a zinc counterpart [83].

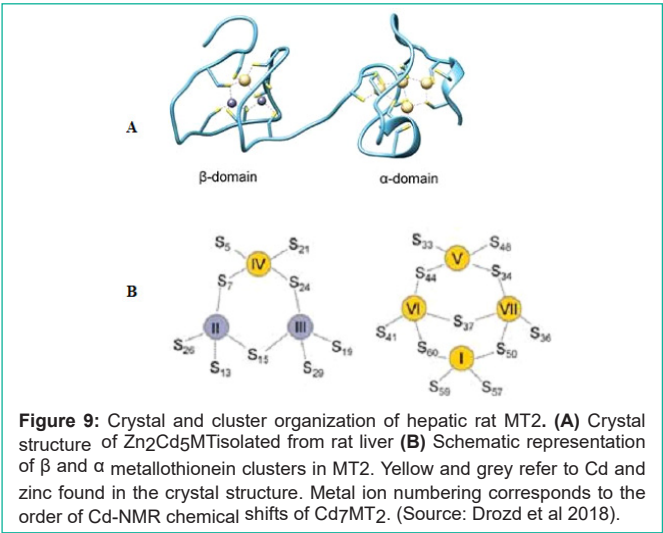


Table 5: Tissue and sub cellular expression and localization of zinc efflux transporters.

Protein	Cellular location	Subcellular localization	Technique used for identification
ZnT-1	Ubiquitous	Plasma membrane	[102]
ZnT-2	Mammary gland, pancreas, prostate, retina, intestine, kidney	Endosome, lysosome, secretory vesicle, plasma membrane	[103]
ZnT-3	Brain, pancreas testis	Synaptic vesicles	[104]
ZnT-4	Mammary gland, placenta, prostate, kidney, brain	Endosome, secretory vesicle, plasma membrane	[105]
ZnT-5	Heart, prostate, ovary, testis, bone	Golgi, vesicles, plasma membrane	[106]
ZnT-6	Brain, lung, intestine	Golgi vesicles	[107]
ZnT-7	Intestine, stomach, pancreas, muscle	Golgi vesicles	[108]
ZnT-8	Pancreas, adrenal gland, thyroid	Secretory granules	[109]
ZnT-9	Brain, muscle, kidney	Endoplasmic reticulum	[110]
ZnT-10	Brain, retina, liver	Golgi, plasma membrane	[111]

Cellular and Sub Cellular Zinc Transporters

Two major families of zinc transporters at cellular and sub-cellular levels belong to the cation diffusion facilitators (CDF), also known as the SLC30 family. These include 10 ZnTs (1-10) involved in exporting zinc and 14 Zrt Irt-like Proteins ZIPs (1-14), known as the SLC39 family involved in importing zinc. Both of these proteins are tissue-specific in expression and have a distinct response to zinc intakes and also for hormones and cytokines [85]. A review of zinc efflux transporters and their tissue specificity subcellular localization and methodology based for their identification is given in (Table 5). Here, ZnTs are the effluxers which decrease the cellular zinc levels based on their localization. Among ZnTs, ZnT-1 has ubiquitously expressed protein which has been localized in the basolateral membrane in enterocytes and renal tubular cells; zinc intake elevated ZnT-1 protein in rats [86]. Hence these observations on zinc intakes might lead to a current consensus that ZnT-1 functions mainly as a zinc exporter in zinc homeostasis under conditions of excess of zinc.

Similarly, another set of zinc transporters ZIPs are the zinc influxers increase the zinc levels depending on their site of localization. A review of zinc efflux transporters their tissue specificity subcellular localization and methodology based for their identification is given in (Table 6). Importantly, ZIP-4 was well studied of all the ZIPs, major transporter involved in the absorption of zinc at the intestinal region to dietary zinc status. Consequently, mutations in ZIP-4 levels leads to low absorption of zinc at the intestine [87]. ZIP-4 is the main transporter involved in the absorption of zinc at the intestine [88] whereas ZIP-5 located at the basolateral membrane of enterocyte involved in the regulation of zinc excretion [89]. An isoform of ZnT-5, ZnT-5 B regulates the zinc absorption and excretion at the apical membrane of enterocyte in either way (exit and entry) of zinc [90]. A schematic representation of zinc absorption at the enterocyte is shown in (Figure 10).

Studies using human enterocyte Caco-2 cell line have shown that ZIP-4 transporter is most responsive to cellular zinc status,

Table 6: Tissue and sub cellular expression and localization of zinc influx transporters.

Protein	Cell location	Subcellular localization	Technique used for identification
ZIP-1	Prostate, small intestine, kidney, liver, pancreatic α cells	Plasma membrane	[112]
ZIP-2	Epithelial cells, ovary, liver, skin, uterine, prostate	Plasma membrane	[113]
ZIP-3	Testes, Pancreatic cells	Plasma membrane	[114]
ZIP-4	Small intestine, stomach, colon, cecum, kidney, pancreatic β cells	Plasma membrane	[115]
ZIP-5	Liver, kidney, spleen, colon, pancreas	Plasma membrane	[116]
ZIP-6	Testis, pancreatic β cells	Plasma membrane	[117]
ZIP-7	Brain, liver, pancreatic β cells	Golgi, ER	[118]
ZIP-8	Pancreas, placenta, lung, liver, testis, thymus, RBC.	Plasma membrane endosomes, Lysosome	[119]
ZIP-9	Prostate	Golgi	[120]
ZIP-10	Testis, kidney, breast, pancreatic α cells, RBCs.	Plasma membrane	[121]
ZIP-11	Mammary gland, testis, stomach, ileum, and cecum	Golgi	[122]
ZIP-12	Neurons, endothelial, smooth muscle, and interstitial cells	Unknown	[93]
ZIP-13	Retinal pigment, epithelial cell line, osteoblasts	Endoplasmic reticulum	[123]
ZIP-14	Liver, heart, placenta, lung, brain, pancreatic α cells.	Plasma membrane, mitochondria, Lysosome	[124]

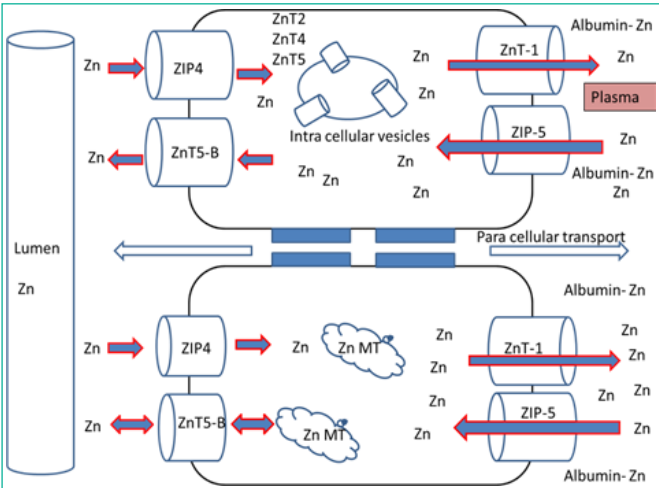


Figure 10: Zinc absorption at the enterocyte; Absorbed zinc is effluxed into circulation by ZnT-1 at the basolateral side while ZIP-5 eliminates the excess zinc in the circulation effluxing into lumen via ZIP-5 and ZnT-5B axis.

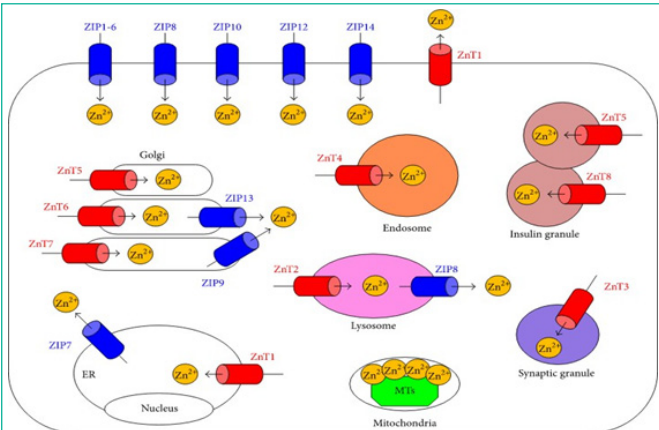


Figure 11: Generalized schematic representation of localization of zinc transporters in cellular and sub cellular organelles (source: Hojyo et al 2016).

while efflux could be through the ZnTs (SLC30) family of efflux transporters, most plausibly ZnT-1 and also zinc may be transported through paracellular route [40]. Thus, absorbed zinc functions either bound to MT or exist as labile zinc. Since zinc is present in all these diverse forms, which makes it difficult to assess accurately the intracellular distribution status of zinc. Earlier studies on human lung adenocarcinoma cells, reported the compartmentalization of total

intracellular zinc in the cytoplasm and nucleus [91]. Fluctuations in intracellular zinc levels regulated by these transporter families or any changes in zinc transporter localization and function results in zinc dyshomeostasis, which affects signalling pathways involved in cell proliferation, differentiation, and death.

Zinc transporters deregulation leads to catastrophic effects on B cell, T cells maturation, and activation via cytokines IL-2, IL-6, IL-10 [59]. Cytokines such as IL-6, IL-10, IL-12, IL-15, GM-CSF, and interferons (IFNs), activate Janus kinases (JAKs) and signal transducer and activator of transcription (STATs) factors to exert their effects [93]. However, zinc inhibits the IL-6 activation of STATs. Further on, ZIP-12 involvement in cell signalling mechanism was observed in the central nervous system (CNS) via activation of CREB cAMP response element binding protein [93].

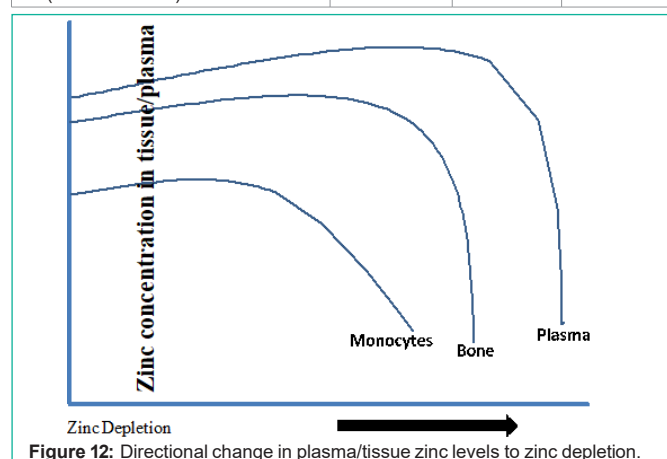
Similarly, ZIP-7 has a key role in glucose metabolism in skeletal muscle, and ZnT-7 knockout mouse have shown suppression of insulin signalling pathway in myocytes [94,95]. In addition, ZnT-3 null mice have decreased insulin gene expression and insulin secretion that resulted in hyperglycemia [96]. Moreover, ZnT-8 plays a critical role in the synthesis and secretion of insulin and therefore represents a pharmacological target for treating disorders of insulin secretion, including diabetes [97]. Thus, zinc transporters completely regulate the cellular and total body zinc levels for maintaining physiological functions. It was found that infection and inflammation also affect the ZIPs expression, of interest ZIP-14 levels, were upregulated in inflammation condition leading to high levels of zinc [98].

Similarly, studies on lipopolysaccharide (LPS) mediated inflammation has shown that zinc transporter ZIP-8 modulate the zinc levels [99,100]. A schematic representation of zinc transporters localization is represented in (Figure 11) 3.10. Functional Role of Zinc in Monocytes, Muscle and Bone

Monocytes: Monocytes differentiate into macrophage clears the pathogens or foreign substances from the body by phagocytosis. In addition, these cells activate other immune cells by secreting cytokines. Lipopolysaccharide (LPS) is a gram-negative bacterial cell wall component, binds to the TLR-4 receptor leads to activation of signalling pathway thereby secretion of proinflammatory cytokines IL-1, IL-6 and tumour necrosis factor [125]. Zinc deficiency in monocytes hampers the production of TNF- α and IL-6 cytokines [126]. However, zinc acts as an anti-inflammatory reduce the cytokines

Table 7: Effect of zinc repletion and depletion on tissue concentration of zinc in rats.

Tissue ($\mu\text{mol g}^{-1}\text{dry wt}$)	Normal	Depleted	Repleted
Liver	1.73 ± 0.05	1.0 ± 0.07	1.63 ± 0.05
Bone	3.54 ± 0.23	1.28 ± 0.16	2.14 ± 0.10
Testes	2.32 ± 0.32	1.09 ± 0.14	2.64 ± 0.24
Hair	1.63 ± 0.16	1.59 ± 0.27	1.61 ± 0.32
Skin	0.69 ± 0.08	0.84 ± 0.03	0.73 ± 0.07
Heart	0.77 ± 0.02	0.73 ± 0.03	0.79 ± 0.04
Skeletal muscle soleus	4.55 ± 0.61	4.14 ± 0.37	3.84 ± 0.19
EDL (Extensor Digitorum longus)	0.73 ± 0.05	0.79 ± 0.04	0.77 ± 0.04
AT (Anterior tibialis)	0.64 ± 0.10	0.56 ± 0.12	0.57 ± 0.13

**Figure 12:** Directional change in plasma/tissue zinc levels to zinc depletion.

levels. It has been shown that zinc influences more than 1000 genes in THP-1 monocytes, which are involved in signal transduction and cytokine production [127].

Muscle: Zinc regulates skeletal muscle contraction and relaxation. It was found that zinc potentiates the twitch tension in sartorius muscle in frogs [128]. In addition, zinc in the small concentrations of 0.001 mM improved the contractility of the glycerinated fiber, while in a high concentration such as 0.25 mM reduced the contractility [129-131]. Thus, zinc levels affect the functional properties of muscle. Zinc has propounding impacts on athletes. Usually, their diet mostly consists of carbohydrate-rich and less of proteins and fats, which leads to a decrease in dietary zinc levels [132].

Bone: Bone remodeling, i.e. formation and absorption of bone, is under precise regulation; osteoblasts deposit calcified bone matrix, and osteoclasts absorb it. Zinc is a potent inhibitor of osteoclastogenesis and tightly regulates the osteoclast function [133]. Any change or abnormal activities of osteoclasts leads to bone deformities, one of the best examples is osteoporosis, in which bone resorption takes place majorly. While bone is the prominent reservoir of zinc, its significant role in zinc homeostasis has been described in many studies [134,135].

Tissue Response During Zinc Depletion and Repletion

Zinc, the most abundant intracellular element, is found in all body tissues, with 85% of the whole body zinc in muscle and bone (Table 7) and remaining minor quantities in the skin and liver, pancreas. Prolonged intake of low dietary zinc changes the homeostatic mechanism of zinc, which no longer maintains the lost zinc, and a negative zinc balance occurs. Earlier observations [136] on low zinc diet, i.e. 0.06 $\mu\text{mol/g}$ leads to a decrease in the whole body zinc content compared to control animals. During this period of zinc depletion,

the loss of zinc is not uniform in hair, skin, heart and skeletal muscle zinc concentrations remained constant, whereas plasma, liver, bone and testes zinc concentrations dropped significantly.

Studies showed that bone releases zinc which composes 10–20% primarily from a rapidly turning over pool whereas the second major pool turns over more slowly and does not release zinc in required conditions [137]. Furthermore, bone accumulates zinc in zinc excess situations and releases zinc in depletion conditions [135]. Therefore, when dietary intakes of zinc are low bone seems to function, as a reserve of zinc by releasing zinc.

Tissue Response of Zinc Under Inflammation: Zinc levels in serum also change during infection and inflammation, redistribution of kidney zinc levels in association with increased MT expression was reported in coxsackievirus Type B infection [138]. During inflammation, the liver sequesters zinc, perhaps as an adaptive response to limit zinc bioavailability to pathogenic microbes [139, 140]. Zinc levels in inflammation were regulated by cytokines (IL-1 α) to tissue- specific zinc distribution into the liver, bone marrow, and bone, skin, intestine [141].

Rationale and Key Research Questions

Zinc is a typical type-II nutrient as there is no onset of clinical symptoms of deficiency, but growth retardation can be observed [142]. A transient mild zinc deficiency may not alter plasma zinc concentration (12-16 μM), whereas persistent deficiency results in decreased plasma Zn levels [143]. In addition, bone and liver also contribute to plasma zinc during zinc depletion [32]. Moreover, plasma levels of zinc vary in pathological conditions [144]. Some of the important studies in these directions are highlighted below.

Zinc supplementation and deficiency have been shown to influence the metabolic functions in monocytes a non-storage tissue, bone, and muscle storage tissues [127,145,146]. Contrasting effects of changes in tissue concentration of zinc have been shown during zinc repletion-depletion in rats. It is interesting to note that bone depletes zinc while muscle retains it during depletion. This contrasting observation in the two major storage tissues of zinc appears to play an important role in zinc homeostasis, especially in maintaining plasma zinc levels during deficiency. A schematic model of depletion of zinc from these two zinc storage organ and plasma concentration is given in (Figure 12). Thus, the contribution of monocytes, muscle, and bone in maintaining zinc homeostasis is still not yet established, and there is scope for studying the role of these tissues in isolation or in tandem in maintaining zinc homeostasis.

During zinc depletion, the loss of zinc is not uniform across all tissues. Zinc levels in skeletal muscle, skin, and heart are maintained to normal levels, while in bone, monocytes, liver, testes, and plasma zinc levels are declined [136]. Bone can apparently accumulate zinc in times of excess and release zinc during depletion [135]. Hence, it has been proposed that bone may serve as a passive reservoir for zinc and may become available during the normal turnover of osseous tissue. This may be due to the differential role of zinc transporters in tissues that contribute to maintaining zinc homeostasis. However, zinc released from bone comes primarily from a rapidly turning over pool that composes 10–20% of the total bone zinc [147]. In vitro studies on mouse pre- osteoblasts cells showed the importance of zinc

transporters such as ZnT-2 on bone mineralization [148]. Notably, ZnT-5 knock out studies reported crucial role in osteopenia [149]. Previous reports on PBMCs, osteoblasts, cancerous tissues, and THP-1 monocyte cell lines have widened our knowledge on whole body zinc regulation. However, there is a wide lacuna in our understanding of the mechanism of zinc homeostasis. Zinc being a type-II nutrient, during zinc deficiency clinical symptoms appear only with severe zinc depletion indicating that there are no conventional zinc reservoirs as like type-I nutrient iron [150]. Unlike iron metabolism, where hepcidin plays a critical role in systemic iron homeostasis [151], biological regulators of zinc homeostasis have not been identified. A low-molecular-weight (2 kDa) zinc-regulated humoral factor, which is induced in zinc deficiency, associated with immune functions and development in smooth muscle cells is reported [152].

The present review was aimed to understand the contribution of zinc storage and non-storage contrasting tissues during zinc sufficiency, depletion, and in inflammation. This will be addressed using suitable human cell lines representing these tissues and experimental system to mimic conditions of zinc repletion, deficiency and in inflammatory conditions in humans. Here, known zinc export and import transporters, ZIPs and ZnTs, were quantified using qRT-PCR. The results of these experiments may lead to the identification of a possible biomarker for zinc.

Future Research Questions

1. Can there be a difference in the expression levels of zinc transporters in zinc storage (bone, muscle) and non-storage (monocyte) cell lines?
2. Can there be a difference in the expression levels of zinc transporters when cells are subjected to depletion and repletion in storage and non-storage cell lines?
3. Can there be a difference in the expression levels of zinc transporters in zinc storage and non-storage cell lines to inflammation?

Funding

The author acknowledges Council Scientific Industrial Research (CSIR)-New Delhi, India for the financial assistance to Mr. Kiran Alluri as JRF and SRF (09/484/(0050)/2012-EMR-1).

Orcid id: 0009-0007-1567-3434.

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