

Zinc Nutrition and Allied Deficiency Syndrome

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Abstract: Zinc (Zn) is a divalent cation and essential trace element with manifold biochemical and physiological functions. It is abundantly required for the activity of over 300 enzymes, and partakes in many enzymatic, metabolic and neuromodulatory functions in the body. Zn is an indispensable component of DNA-binding proteins and is involved in antioxidant defence and DNA repair. Specific and non-specific neuronal carriers afford Zn entry into central neurones, where the metal ion accumulates in synaptic vesicles. The systemic level of Zn distribution is closely regulated with physiological concentration maintained within the range of 10-20 mM. Cellular and subcellular homeostasis is achieved via complex regulation of uptake, distribution, storage, and efflux, in which ZnT and ZIP transporters play primary roles. Zn plays major roles in ion channel modulation, signalling, inhibition of bone loss, anti-inflammation and anti-oxidation. Symptoms associated with Zn deficiency include impairment of prenatal and ante-natal growth and development, neuropsychological performance, diarrhoea, pneumonia, infertility and cancer. Measurement of plasma Zn concentration is essential for diagnosis as less than 50 µg/dl is indicative of severe deficiency of Zn. Enhanced Zn bioavailability and supplementation as single micronutrient or as a component of a multi-micronutrient mix are imperative to prevent Zn deficiency. In conclusion, there is compelling evidence demonstrating detrimental effects associated with Zn deficiency, thus supporting the beneficial role of Zn as a nutraceutical essential for enhancing health and well-being of man and animals.

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Introduction

Zinc (Zn) is the next most abundant and essential trace element in the body after iron. It performs multifunctional biological roles, and up to 10% of all proteins in mammalian cells require Zn for their breakdown, conformational modification or activity (Andreini *et al.*, 2006; Prasad, 2013; 2014). Zn is required for the activity of over 300 enzymes, and is therefore involved in many enzymatic and metabolic functions in the body (Andreini *et al.*, 2012). Zn is a divalent cation with manifold biochemical and physiological functions, and it plays principal neuromodulatory roles in the central nervous system. Specific and non-specific neuronal carriers afford Zn entry into central neurones, where the metal ion accumulates in synaptic vesicles. Modulation of excitatory and inhibitory amino acid transmissions is amongst the many putative roles ascribed to brain Zn (Ghasem *et al.*, 2016). Indeed, Zn is co-released with glutamate, and it modulates glutamatergic excitation by inhibiting N-methyl-D-aspartate (NMDA) receptors, and averts gamma-aminobutyric acid (GABA) inhibition by blocking GABA-A receptor function (Fregoneze *et al.*, 1999). It is also known to be an indispensable component for DNA-binding proteins with Zn fingers, as well as copper/Zn superoxide

dismutase and a variety of proteins involved in DNA repair (Ho and Ames, 2002). Thus, Zn plays a vital role in transcription factor function, antioxidant defense and DNA repair.

The aim of this paper, is to review the biochemical and physiological functions, homeostasis, groups at high-risk, nutritional requirement, causes and consequences of zinc deficiency and as well highlight diagnostic and prevention strategies of zinc deficiency.

Sources of Zinc

A wide variety of foods contain Zn, and good sources usually have 1-2 mg per serving. Oysters contain more than any other food (74 mg/serving), but red meat and poultry provide the majority of Zn in diet. Other food sources with high Zn content include beans, nuts, whole grains, fortified breakfast cereals, and dairy products (Wood, 2000; VNDPG, 2013; Nunes *et al.*, 2017). Other sources include:

Legumes

Tofurky Italian sausage, 1 sausage 9 mg, hummus, ½ cup 2.3 mg, tofu, ½ cup firm, raw 2.0 mg, chickpeas, ½ cup cooked 1.3 mg, lentils, ½ cup cooked 1.3 mg, edamame, ½ cup cooked 1.1 mg, green peas, ½ cup cooked 1 mg, black beans, ½ cup cooked 1 mg, peanut butter, 2 tablespoons 0.9 mg, refried pinto beans, ½ cup cooked 0.9 mg, miso, 1 tablespoon 0.4 mg.

Vegetables

Mushrooms, ½ cup cooked 0.7 mg, spinach, ½ cup cooked 0.7 mg, broccoli, ½ cup cooked 0.4 mg, kale, ½ cup cooked 0.2 mg.

Grains

Total, ¾ cup 15 mg, wheaties, ¾ cup 7.5 mg, wheat germ cereal, ¼ cup 4.7 mg, rice chex, 1 cup 3.8 mg, oatmeal, 1 cup cooked 2.3 mg, corn, 1 cup 0.9 mg, whole-wheat bread, 1 slice 0.6 mg.

Nuts and Seeds

Pumpkin seeds, ¼ cup roasted 2.3 mg, cashews, ¼ cup dry roasted 1.9 mg, sunflower seeds, ¼ cup dry roasted 1.7 mg, almonds, ¼ cup dry roasted 1.1 mg, walnuts, ¼ cup chopped 0.9 mg, nutritional yeast, 1 tablespoon 2 mg.

Biochemistry of Zinc

Zn, distinct from iron and copper, is redox neutral and reactive as a Lewis acid in biological reactions. In view of these features, Zn plays crucial roles as a structural, catalytic, and signalling component. As a structural component in proteins, the involvement of Zn with proteins was first acknowledged by the crystallisation of insulin with Zn (Myers *et al.*, 2013). The “Zn finger” motif was first established in the transcription factor TFIIIA of *Xenopus* (Andreini *et al.*, 2011). Zn fingers are now categorised into more than 20 classes of structurally distinct modules and are recognised as a functional motif that interacts with a variety of proteins, lipids, and nucleic acids, clearly indicating the importance of Zn in cellular biochemistry. In catalytic functions, Zn can activate substrates in enzymes by stabilising negative charges, due to its strong Lewis acid property (Maret *et al.*, 2012). After the first identification of Zn in erythrocyte carbonic anhydrase as a Zn-dependent enzyme, the presence of Zn has been documented in many enzymes in all six classes defined in the Enzyme Commission (EC) system (Valle and Falchuk, 1993; Prasad, 2013, 2014).

Within proteins, Zn can be coordinated by nitrogen, oxygen, and sulphur atoms and can have different coordination numbers (Kochanczyk *et al.*, 2015). The Zn proteome estimates that almost 9% of proteins are Zn proteins in eukaryotes with the number significantly greater in higher organisms. The number of Zn proteins encoded by humans is approximately 10%. In Zn proteomes, the number of Zn-binding motifs is counted predominantly based on mining for intramolecular Zn-binding sites (Andreini *et al.*, 2006).

Zinc Distribution

The human body mass contains 2-3 g of Zn, and 57% is in skeletal muscle, 29%, 6% and 5% in bone, liver and skin, respectively (King *et al.*, 2000). There is only about 0.1% of total Zn circulating in plasma, and yet this minute fraction of total Zn is essential to

maintain Zn homeostasis at a systemic level. Plasma Zn turns over rapidly to meet tissue needs and has to be replenished daily from diet. Once getting into plasma, the dietary Zn is delivered to peripheral tissues. The systemic level of Zn distribution is closely regulated and the physiological concentration of plasma Zn is maintained within the range of 10-20 mM (Meunier *et al.*, 2005; Disilvestro *et al.*, 2015; Brnic *et al.*, 2016). The plasma Zn concentration is normally around 15 mM in Zn adequate adults and about 11.0-13.5 mM in Zn adequate children. Greater than 70% of plasma Zn is weakly bound to albumin and is mostly derived from intestinal absorption with a destination of the liver and soft tissues (Rukganuer *et al.*, 1997; Disilvestro *et al.*, 2015).

Zinc Homeostasis in Cell and in the Body

After entry of Zn into cells, it is further distributed to diverse cellular organelles; about 30-40 % to the nucleus, 10 % to membranes and at least 50 % to the cytoplasm. The intracellular Zn exists in three pools which include those tightly bound to metalloenzymes, metalloproteins, and nucleoproteins, those loosely bound with diverse protein and amino acid ligands and those unbound as the free Zn²⁺ ion, albeit at very low concentrations (Rukganuer *et al.*, 1997; Disilvestro *et al.*, 2015). While the total cellular Zn concentration is estimated to be in mM range, the cytosolic concentration of free Zn²⁺ is predicted to be in the nM-pM range. The precise determination of free Zn²⁺ concentration is of importance to the understanding of many Zn-mediated cellular events, yet the reliable measurement of free intracellular Zn²⁺ and visualisation of its dynamic changes continue to be a technical challenge. This is partly a consequence of the selectivity and sensitivity of Zn probes, which is strongly influenced by the concentrations of Zn and additional cations in living biological systems (Disilvestro *et al.*, 2015).

The adult human body contains 2-3 g of Zn. Approximately 60 % of Zn is stored in skeletal muscle, 5 % in the liver and skin, 30 % in bone, and the remaining 2-3 % in other tissues (Jackson, 1989). Serum Zn accounts for only 0.1 % of the body's Zn, 80 % of which is loosely bound to albumin and 20 % of that is tightly bound to α₂-macroglobulin (Barnett *et al.*, 2014), and 0.1 % of the body Zn is replenished daily via diet. The absorption of Zn in the duodenum and jejunum is strictly regulated; it increases up to 90 % when dietary Zn is restricted (Taylor *et al.*, 1991), whereas Zn release, when in excess, is facilitated by the gastrointestinal secretion, sloughing mucosal cells and integument, and renal excretion (Krebs, 2013).

In general, more than 30 proteins, including ZnT and ZIP transporters, operate under a precisely coordinated regulation for the maintenance of systemic and cellular Zn homeostasis in mammals. No short

peptide hormone hepcidin has been identified in Zn to play a vital role as seen in systemic iron homeostasis (Nameth *et al.*, 2004; Golan *et al.*, 2015). Study findings reveal that a low-molecular-weight (2 kDa) Zn-regulated humoral factor, which is likely induced in Zn deficiency, controls gene expression predominantly associated with immune functions and development in smooth muscle cells. It is suggested that a different short peptide functions as a humoral factor in systemic Zn homeostasis (Golan *et al.*, 2015).

The cellular and subcellular Zn homeostasis is achieved via sophisticated regulation of uptake, distribution, storage, and efflux, in which ZnT and ZIP transporters play primary roles. Zn mobilisation between intracellular vesicles and organelles by these transporters also contributes to buffer perturbations of cytosolic Zn homeostasis, which is termed “buffering” and “muffling”. Buffering and muffling are essential in situations of excess Zn conditions and also play a role in Zn ion fluctuations (Maret, 2013).

Zinc Transporters

ZIPs and ZnTs

Maintenance of intracellular Zn homeostasis is mainly dependent on two families of Zn transporters (Fukunaka and Kambe, 2010; Pan *et al.*, 2017). In particular, ZIP family proteins function in the uptake of Zn into the cytoplasm of the cell from the extracellular space or from intracellular compartments, such as Golgi apparatus, endoplasmic reticulum (ER), and mitochondria; ZnT proteins function in the efflux of Zn from the cytoplasm to the extracellular space or to intracellular compartments. There are about 14 ZIP transporters and 10 ZnT proteins in the human body with differential tissuespecific expression (Cousins *et al.*, 2006; Pan *et al.*, 2017). Depending on their degree of sequence conservation, the 14 ZIP proteins can be further organised into 4 subgroups: the ZIP subfamily I, the ZIP subfamily II, the *guf*-A subfamily, and the LIV-1 subfamily (Gather and Eide, 2006). While the functions of the ZIP subfamily II and the LIV-1 subfamily have been comprehensively investigated, the ZIP subfamily I (ZIP9) and the *guf*-A subfamily (ZIP11) are less well described. The structural homology and variation among these Zn transporters as well as their tissue distributions and functions have been documented (Cousins *et al.*, 2006; Kambe *et al.*, 2015).

Role of Zinc in Ion Channel Modulation

Zn-regulated channels in the central nervous system has been largely investigated (Marger *et al.*, 2014). Many channels in the CNS excitable cells are regulated by Zn, such as NMDA and GABA receptors, glycine receptors (glyR) and serotonin receptors (5-HT₃). The purinergic receptors are adenosine triphosphate (ATP)-gated cation channels at the plasma membrane, with wide cell-type expression, including neurones, cancer and immune cells (Pan *et al.*, 2017).

The physiological concentrations of Zn can strongly inhibit human purinergic receptors P2X₂ (hP2X₂), while Zn enhances current activated by 5 μ M ATP in a voltageindependent manner for P2X₄ (Punthambaker *et al.*, 2012). Acid-sensing ion channel 3 (ASIC3) is a proton-gated, voltage-insensitive Na⁽⁺⁾ channel that plays a key role in pain perception, particularly as a pH sensor following cardiac ischaemia. Zn is reported to be an important regulator of ASIC3 at physiological concentrations, and it inhibits ASIC3 in a pH-and Ca²⁺-independent manner. The inhibition of ASIC3 currents is dependent upon the interaction of Zn with extracellular domain(s) of ASIC3 (Jiang *et al.*, 2010; Pan *et al.*, 2017).

For large-conductance voltage-and Ca²⁺activated Slo1 K (BK) channels, intracellular Zn potently and reversibly activates the channel through the regulator of conductance for K⁽⁺⁾ (RCK1) domain of the channel (Hou *et al.*, 2010; Inoue *et al.*, 2015). Zn is also able to modulate a number of subunits of Ca²⁺ channels, including Cav1.2, Cav1.3, Cav3.1, Cav3.2 and Cav3.3 (Marger *et al.*, 2014). In cultured cells exogenously expressing recombinant T-type Ca²⁺ channels (transient opening Ca²⁺ channels), that is, Cav3.1, Cav3.2 and Cav3.3, the Cav3.2 current is significantly more sensitive to Zn than that of Cav3.1 and Cav3.3 (Traboulsie *et al.*, 2007; Chevallet *et al.*, 2014). Zn can cause a major increase in Cav3.3 current in action potential clamp experiments; while Cav3.1 and Cav3.2 currents are majorly reduced, indicating that Zn exhibits differential modulatory effects on T-type Ca²⁺ channels. In addition to voltage-gated Ca²⁺ channels, other Ca²⁺ channels can be modulated by Zn in general, such as TRP channels. In whole-cell patch-clamp recordings, extracellular application of Zn inhibited TRPM5 currents (Uchida *et al.*, 2013). Using mutagenesis approach, it was demonstrated that inhibition by 30 μ M ZnCl₂ was impaired in TRPM5 mutants in which Histidin at 896, and Glutamine at 926 and/or Glutamine at 939 in the outer pore loop were replaced with Glycine. These data suggest that extracellular Zn inhibits TRPM5 channels through its interaction with the extracellular pore loop domain. Another Zn-modulated unique TRP family member is TRPA1 (Banke and Wickenden, 2009). In this case, the activation of TRPA1 firstly requires Zn influx through the TRPA1 channel which subsequently activates the channel itself. Several intracellular cysteine and histidine residues are required for the Zn activation. TRPA1 is highly sensitive to intracellular Zn and nM concentrations are sufficient to activate the channel (Pan *et al.*, 2017).

Role of Zinc in Inhibition of Bone Loss

The cellular mechanism of Zn action has been shown to stimulate proliferation and differentiation in osteoblastic cells (Masayoshi and Satoshi, 2003). Zn

can stimulate protein synthesis in osteoblastic cells. Zinc has also proven to inhibit the formation of osteoclastic cells from bone marrow cells, indicating that the metal has an inhibitory effect on bone resorption. Consequently, Zn may play a role in the preservation of bone mass by stimulating bone formation and inhibiting bone resorption. A Zn compound, β -alanyl-L-histidinato Zn, has preventive and therapeutic effects on different experimental osteopenia with ageing, skeletal unloading, aluminium toxicity, hydrocortisone treatment, low-calcium and vitamin D-deficient diets, inflammation, and ovariectomy treatment *in vivo*. The anabolic effect of β -alanyl-L-histidinato Zn on bone components has been shown to be more than that of Zn sulphate (Masayoshi and Satoshi, 2003). It has been reported that Zn acexamate has a potent effect on bone formation as compared with that of β -alanyl-L-histidinato Zn *in vitro* (Masayoshi and Satoshi, 2003; Biaggio *et al.*, 2014). Zn compound may be a good tool in therapy of osteoporosis. Results demonstrate that the administration of Zn acexamate has a preventive effect on bone loss in streptozotocind diabetic rats *in vivo*.

Zinc Action as Anti-Inflammatory Agent

Necrotic factor- κ B (NF- κ B) is one of the major immune response transcription factors involved in molecular signalling. Zn plays an essential role in activation of NF- κ B. The regulation of NF- κ B activation by Zn is, however, cell specific (Prasad *et al.*, 2004; Prasad, 2014). It has been reported that Zn is required for NF- κ B DNA binding in purified or recombinant NF- κ B p50 protein in T helper cell lines (Prasad *et al.*, 2001). Prasad *et al.* (2001) stated that normal healthy volunteers who were supplemented with 45 mg elemental Zn daily had a significant reduction in TNF- α and IL-1 β messenger RNAs and TNF- α -induced NF- κ B DNA binding in isolated peripheral blood mononuclear cells in comparison to placebo-treated subjects (Prasad *et al.*, 2004). Zn has been reported to upregulate the expression of A20 in HL-60 cells. It decreases ox-LDL-induced generation of TNF- α , IL-1 β , and VCAM-1, oxidative stress markers in the plasma and activation of NF- κ B, and increases A20 and PPAR- α protein in human monocytic and vascular endothelial cells (Bao *et al.*, 2010; Prasad, 2014). Zn is believed to inhibit NF- κ B activation via A20, a Zn finger transactivating factor that plays an essential role in down regulating IL-1 β and TNF- α -induced NF- κ B activation. A20 was originally thought to protect cells from TNF- α -induced cytotoxicity by inhibiting the activation of NF- κ B, resulting in lower IL-1 β and TNF- α signalling in endothelial cells (Bao *et al.*, 2010). It was shown that A20 inhibits NF- κ B signalling by TNF- α and IL1 β via TNF-receptor-associated factor pathways in endothelial cells (Bao *et al.*, 2010; Prasad, 2014).

The PPAR- α and - γ of nuclear receptors, the mediators of lipoprotein metabolism, inflammation, and glucose homeostasis were shown to play a protective role in the development and progression of atherosclerosis. The mechanism by which Zn may exert athero-protective role is most likely due to its anti-inflammatory effect. Zn-sufficient HAEC cells have been shown to increase PPAR- α concentration compared with Zn-deficient HAEC cells, which suggested that Zn increases the expression of PPAR- α protein, apparently contributing to down-regulation of inflammatory cytokines and adhesion molecules. It is proposed that the down-regulation of NF- κ B activation by Zn via A20-PPAR- α signalling pathways results in decreased generation of inflammatory cytokines, which protects the endothelial cells from atherosclerosis (Prasad, 2014).

Antioxidant Role of Zinc

Ghasem *et al.* (2016), reported that Zn supplementation greatly augmented the fertilisation capacity of male rats exposed to noise stress. The ameliorative effect of Zn has been attributed to its antioxidant potential to scavenge reactive oxygen species produced following exposure to noise and other stress factors (Ghasem *et al.*, 2016).

The effect of Zn on hepatic lipid peroxidation and antioxidative enzymes in ethanol-fed rats has also been demonstrated. Alcoholic liver disease may be caused by reactive oxygen species (ROS) generated during the metabolism of ethanol by a microsomal ethanol-oxidising system. Increased formation of ROS and enhanced hepatic lipid peroxidation have been reported after acute and chronic ethanol consumption, and are linked with the development of hepatic injury (Khalaf *et al.*, 2017). Feeding of ethanol to rats has been confirmed to augment the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) and diminish the activity of catalase in rat liver. Zn supplementation to ethanol-intoxicated rats notably stabilises the increased activities of oxidative stress-related enzymes (*viz.* catalase, SOD and GPx) as well as decreases lipid peroxidation; thus, signifying its antiperoxidative potential. This antioxidative role of Zn appears to be the fundamental process whereby it restores the structural as well as functional integrity of the liver under ethanol toxicity. In addition, to a large extent Zn regulates the activities of enzymes of the antioxidative system as well as reduces lipid peroxidation (Nunnes *et al.*, 2017).

Zn deficiency aggravates the increase in ROS formation in ovariectomised rats, but its supplementation prevents the formation of ROS (Baltaci *et al.*, 2004). It has also been proposed that the augmentation of lipid peroxidation by cadmium in rats is a result of a decrease in SOD and CAT activities. Zn supplementation prior to cadmium administration averts

several of the effects, observed when cadmium is administered alone (Jemaia *et al.*, 2007). Result indicates that one of the mechanisms involved in the protective role of Zn against cadmium-induced toxicity is connected with inhibition of cadmium-induced ROS formation. The role of Zn in protecting biological structures from ROS damage is due to several factors: first, by maintaining an adequate level of metallothionein, which is also a scavenger of ROS; then, as an essential structural component of Cu/Zn SOD; and finally, as a protective agent for thiols and additional chemical groups (Jemaia *et al.*, 2007). It has been shown that cadmium induces a decline in erythrocyte counts, SOD and CAT activities, and an increase in lipid peroxide concentration in plasma. Zn supply on exposure to cadmium shields against the cadmium-induced decrease in SOD activity and might partially protect against Cd-induced lipid peroxidation (Jemaia *et al.*, 2007). Zn is also an incredibly essential supplement during administration of chelating agents in lead treatment, especially when using Ethelenediaminetetraacetic acid and dimacropol as it has also been accounted to scavenge ROS released in the process (Grogorescu *et al.*, 2015).

Mechanism of Zinc Action as an Antioxidant

Zn operates as an antioxidant by different mechanisms. Principally, it competes with iron (Fe) and copper (Cu) ions for binding to cell membranes and proteins, displacing these redox active metals, which catalyses the formation of OH from H₂O₂ (Bao *et al.*, 2013). Zn also binds to (SH) sulfhydryl groups of biomolecules, shielding them from oxidation. It increases the activation of antioxidant proteins, molecules, and enzymes such as glutathione (GSH), catalase, and SOD and also attenuates the activities of oxidant-promoting enzymes, such as inducible nitric acid synthase (iNOS) and NADPH oxidase, and inhibits the generation of lipid peroxidation products. Zinc also instigates the expression of a metal-binding protein metallothionein (MT), which is very rich in cysteine and is an excellent scavenger of OH ions (Bao *et al.*, 2013; Gao *et al.*, 2014). Nuclear factor erythroid 2-related factor 2 (Nrf2), a family member of cap'n' collar/basic leucine zipper (CNC-bZIP) proteins is a vital transcription factor that regulates the gene expression of antioxidant proteins and enzymes such as GSH and SOD, as well as detoxifying enzymes such as glutathione-S-transferase-1 (GSTA1) and haemeoxygenase-1 (HO-1), by binding to an antioxidant responsive element (ARE) in the promoter region of the target gene. Several studies have demonstrated that Zn may have a regulatory role in Nrf2, since it up-regulates Nrf2 activity and attenuates oxidative stress (Bao *et al.*, 2013).

Zinc Signalling

The signalling functions of Zn is very crucial and occurs by increase in Zn (Zn²⁺) concentrations,

triggered by stimuli. Zn-activated signalling is associated with pathophysiological functions (Fakada and Kambe, 2011) and thus, has therapeutic potential. Extracellular release of Zn acts as a signalling mediator in endocrine, paracrine, and autocrine systems. In the CNS, Zn, which is released from presynaptic neurones following excitation into synaptic clefts, modulates synaptic transmission by binding to various transporters and receptor channels on postsynaptic neurones (Takada *et al.*, 2013). Zn, co-released from pancreatic β -cells along with insulin by glucose stimuli, can suppress hepatic insulin clearance and diminish insulin secretion from the β cells (Tamaki *et al.*, 2013). Extracellular release of Zn binds to diverse cell surface proteins, and the most intriguing protein is the Zn receptor, the G protein-coupled receptor 39 (GPR39) (Sharir *et al.*, 2010).

Zn plays vital signalling roles as a second messenger in the cytosol (Fakada and Kambe, 2011); where Zn signalling is instigated by Zn influx, which originates from extracellular sites and from intracellular organelles. "Zn wave" refers to Zn released from the perinuclear area, including the endoplasmic reticulum (ER) which has been shown to be important for cell signalling functions (Taylor *et al.*, 2012). Moreover, Zn, released from cytosolic proteins with oxidation-sensitive Zn-binding sites such as metallothionein (MT) via oxidative stimuli, is also involved in intracellular Zn signalling (Aras *et al.*, 2011; Nunnes *et al.*, 2017). Intracellular Zn signalling is divided into several categories according to the time-scale in which it acts (Haase *et al.*, 2014). "Fast" or "early" Zn signalling occurs within seconds to minutes after stimulation and does not require transcription of proteins (Haase *et al.*, 2008). Conversely, "late" Zn signalling requires biosynthesis of proteins to control cytosolic Zn concentrations and occurs over a period of hours after stimulation (Kitamura *et al.*, 2006). The word "late" Zn signalling is commonly used to contrast with the word "fast" or "early" Zn signalling. This term portrays the downstream effects mediated through changes in gene expression, not signalling events. Prominently, intracellular Zn signalling targets a number of enzymes, involved in cellular signalling, including protein tyrosine phosphatases (PTPs) (Wilson *et al.*, 2012) phosphodiesterases (PDEs) (Hajyo *et al.*, 2011), calcineurin (Aydemir *et al.*, 2009), caspases (Miyai *et al.*, 2014) and diverse kinases such as mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) (Hasson *et al.*, 1996). Physiological levels of intracellular free Zn²⁺ concentrations regulate activities of these enzymes. For example, inhibition of caspase-3, T-cell PTP, and PTP-1B is achieved with IC₅₀ values below 10, 200, and 17 nM, respectively (Haase and Maret, 2003). Inhibition of the activity of PTPs by Zn is commonly operative in intracellular Zn signalling. The

enzymatic activity of caspase-9 is reversibly inhibited by Zn binding to cysteine and histidine residues in the active site, and similar reversible inhibition mechanisms operate in other enzymes such as PTPs (Haase and Maret, 2005). Specifically, inhibition of PTP-1B activity by Zn is possibly mediated via a cysteinyl-phosphate intermediate (Bellomo *et al.*, 2014).

Zinc Level Detection

Lack of ample laboratory biomarkers (Wood, 2000; Wu *et al.*, 2015) and the inadequacy of pathognomonic clinical features of Zn deficiency states are key factors, hampering the detection of Zn deficiency states. Measurements of Zn concentrations in plasma are useful in identifying children, who are more likely to have a growth response to Zn supplements (Brown 1998) or diarrhoea (Bahl *et al.* 1998). The potentially confounding factor of hypozincaemia as part of the acute phase response to infection may not be a foremost detriment. Unfortunately, however, this assay lacks the sensitivity required to give it a strong endorsement as a biomarker of Zn status. The application of other indices, such as hair Zn concentrations, although capable of yielding interesting and useful data (Ferguson *et al.* 1993), has been even less well defined. An example is the activity of a Zn-dependent enzyme. Consequently, elucidation of the prevalence and the clinical effects of milder Zn deficiency states has depended, to a very large extent, on the results of well-designed and executed, controlled, randomised intervention trials with dietary Zn supplements. Such trials have made fundamental contribution to recent progress (Bostanci *et al.*, 2015).

Physiological Changes Associated With Zinc

Inbalance

Hyperzincaemia

Excess Zn is proven to be toxic to cells, especially to neurones. Acute toxicity from Zn poisoning affects the respiratory and gastro-intestinal systems and is typified by nausea, vomiting, loss of appetite, abdominal cramps and headaches. Excess Zn in neuronal and glial cells is associated with a variety of excitotoxicity conditions, such as epilepsy, ischaemia, brain trauma, and neuro-degenerations in Alzheimer's disease. Conversely, the evidence for significant toxic effects of Zn in humans is limited to accidental exposure to compounds of high Zn concentration and these cases are rather rare (Bostanci *et al.*, 2015).

Clinical Manifestations of Zinc Deficiency

The symptoms may be very severe and even fatal if not recognised and treated promptly by Zn, such as seen in patients with acrodermatitis enteropathica (AE). AE is a genetic disorder mainly affecting infants of Italian, Armenian, or Iranian lineage (Bao *et al.*, 2010). The manifestations include bullous pustular dermatitis, particularly around the orifices. Ophthalmic signs

include blepharitis, conjunctivitis, photophobia, and corneal opacities (Prasad, 2013, 2014).

Neuropsychiatric signs include irritability, emotional instability, tremors and cerebellar ataxia, weight loss, growth failure, and male hypodonadism are prominent features. Congenital malformation of fetuses and infants born of pregnant women with AE has been reported frequently (Cavdar *et al.*, 1980; Prasad, 2014).

Acrodermatitis enteropathica patients are very susceptible to infections. Thymic hypoplasia and plasmacytosis in the spleen are commonly seen in experiment animals. T cell-mediated immune disorders are corrected by Zn supplementation. Without Zn supplementation, the clinical course is downhill with failure to thrive and complicated by intercurrent bacterial, viral, fungal, and opportunistic infections. Gastro-intestinal manifestations include diarrhoea, malabsorption, steatorrhoea, and lactose intolerance. Zn supplementation in therapeutic doses (in excess of 50 mg elemental Zn daily) results in complete recovery (Prasad, 2013; 2014).

Allied Zinc Deficiency Syndrome

The deficiency of Zn predisposes the patient to several pathological alterations resulting in severe health impairments in several aspects, some of which have been reviewed below:

Effect of Zinc Deficiency on Pregnancy and Prenatal Development and Child Health

Recent studies are now focused on pregnancy and the effects of maternal Zn status on both prenatal and postnatal development (Caulfield 1999a). Early results of these endeavors indicate that poor maternal Zn status in pregnancy can have detrimental effects on foetal brain function (Merialdi *et al.* 1998). In contrast to recent observations in the United States (Goldenberg *et al.* 1995), the lack of effect of maternal supplementation on foetal growth has been unexplained (Caulfield *et al.*, 1999b).

It has been estimated (Zn for Child Health, 1997) that the beneficial effects of Zn supplements for diarrhoea prevention are of the same magnitude as those achieved by cleaning the water supply and providing quality sanitation. In the case of children under 5 years, the public health benefits of Zn supplementation in the prevention of acute lower respiratory disease and malaria have also been calculated to be superior to any other preventive modalities (Black, 1998). These nutritional diseases are the principal causes of childhood morbidity and mortality globally. The impairment of physical growth and that of neuropsychological development are well recognised related features. The sum of recent evidence indicates that the maintenance of optimal Zn nutrition is perhaps the most effective, even if only partial, preventive measure that can be undertaken to reduce morbidity

rates in young children in the developing world (Alam *et al.*, 2017).

Zinc Deficiency and Impaired Growth

The one clinical feature that is most studied is the impairment of physical growth. As with other features of Zn deficiency, the most definitive investigations have been those based on randomised, controlled studies of dietary Zn supplementation. In the Colorado studies in the 1970s and 1980s, the principal focus was on physical growth. The principal reason for this focus was the earlier, clear demonstration in animal models that Zn had little pharmacological effect on growth (Bostanci *et al.*, 2015). Consequently, the demonstration of an increase in growth velocity associated with modest dietary Zn supplements under double-blind, controlled, randomised study conditions provided convincing evidence for a pre-existing growth-limiting Zn deficiency state. In all, four of such studies were undertaken in Colorado. Subjects ranged from healthy cow's milk formula-fed infants (Walravens and Hambidge, 1976), at a time before Zn fortification of cow's milk formulas became the norm, to older infants, toddlers or young children with non-organic failure to thrive. Consequently, many other studies of

Zn supplementation have included growth measurements. These results have been subjected to rigorous and repeated meta-analysis with confirmation of the effect of Zn supplements in increasing height and weight velocity, when administered to children in many countries (Brown *et al.* 1998).

In some cases, effects have been observed on body composition rather than on weight or on linear growth velocity (Kikafunda *et al.* 1998). These different responses serve as a reminder of how much there remains to be learned about variations in the presentation of Zn deficiency and their responsible factors. They are also a reminder that the biochemical and hormonal factors underlying these effects on growth remain unclear. One of the environmental issues of note is the total diet, including concurrent deficiencies or imbalances of other micronutrients (Solomons *et al.* 1999).

Zinc Deficiency and Diarrhoea.

One of the recent strategies made to minimise the diarrhea-associated mortality in children involves the use of oral Zn in diarrhoea management (Alam *et al.*, 2017). The inadequacy of dietary Zn uptake is aggravated by the net loss of Zn during diarrhoea. Zn is enlisted in WHO essential drug list under medicine for diarrhea, where it is indicated in acute diarrhoea as an adjunct to oral rehydration salts (Alam *et al.*, 2017).

That Zn added to conventional therapy is effective in reducing the duration of acute and persistent diarrhoea has been confirmed via pooled analysis of

data derived from multiple studies (Bhutta *et al.* 1999). The severity of the illness may also be reduced. Pooled analyses have also confirmed that Zn supplementation of children at a community level in the developing world results in significant decrease in the incidence and prevalence of diarrhoea. Losses of Zn via the intestine are likely to be increased in diarrhoeal states and may contribute to Zn deficiency and to a vicious cycle. Supplementation trials have not always included rigorous or perhaps any data on habitual diet, although it seems very likely that dietary intake of bioavailable Zn is typically low in the populations studied. More attention to these factors in future studies will be helpful in determining aetiology as well as paramount prevention strategies (Alam *et al.*, 2017).

Long-term recognition exists that diarrhoea is characteristically, albeit not inevitably, a prominent feature of acrodermatitis enteropathica, which is a severe form of Zn deficiency. The plausible mechanistic explanations for the links between Zn deficiency and diarrhoea, example is the functional impairment of the immune system and of intestinal mucosal cell transport mechanisms (Ghishan 1984). The modest supplements requisite to achieve a beneficial effect; and, perhaps above all, the concurrent increase in growth velocity are all compatible with the conclusion that the favourable effects on diarrhoea are attributable to correction of a Zn deficiency state that is the cause of, or contributing to the diarrhoea (Bostanci *et al.*, 2015).

Zinc Deficiency and Pneumonia

Pooled analyses of the results of community Zn supplementation studies in children in developing countries have demonstrated a very substantial and statistically significant reduction in the prevalence of pneumonia (Bhutta *et al.* 1999).

Infections such as malaria appear to be reduced by Zn supplementation (Black, 1998). **Effect of Zinc Deficiency on Neuropsychological performance**

Evidence of improved brain development attributable to improved Zn status has originated from studies of activity levels in young children in India (Sazawal *et al.* 1996) and Guatemala (Bentley *et al.* 1997). Neuropsychological performance has been reported to improve with Zn supplementation in young Chinese children (Sandstead *et al.* 1998), but only when other micronutrient nutrition is sufficient. Zn has been confirmed to enhance learning and memory due to its antioxidant potential to scavenge ROS and protect neuronal degeneration in animals subjected to acute stress conditions (Prasad, 2013, 2014).

Zinc Deficiency and Infertility

Zn losses have been reported to occur through menstruation in women and ejaculation in males; especially as semen has a high Zn concentration. In males, Zn concentration in seminal plasma is known to

correlate with sperm count, motility and viability, although studies report conflicting findings about the magnitude of these correlations and whether concentrations are higher or lower in subfertile compared to fertile men; probably explained by between-study differences in inclusion criteria (Agarwal *et al.*, 2010). Although the underlying mechanisms by which Zn affects spermatogenesis remain unknown, the positive effects of Zn on sperm count and parameters (morphology and motility) are documented (Ghasem *et al.*, 2016). The ability of Zn to reduce oxidative stress in sperm has also been identified, although this was negatively associated with sperm decondensation (Agarwal *et al.*, 2010). Even when Zn supplementation far surpasses the recommended daily intake, a concurrent increase in circulating or local concentrations of Zn or FSH and testosterone are not always evident; possibly explained by the absence of Zn deficiency or high excretion by the prostate. In females, serum Zn concentrations are almost twice as high as follicular concentrations, although the high expression of Zn transport genes in the oocyte suggests active Zn transport during the first stages of pre-implantation development (Ozkaya *et al.*, 2011; Golan *et al.*, 2015). Similar to studies in males, studies have reported conflicting results as to whether differences exist in serum Zn concentrations between infertile and fertile women (Menezo *et al.*, 2011). Lower follicular fluid and serum Zn and selenium levels were found in IVF patients than in fertile women (Menezo *et al.*, 2011), with normalisation to those of fertile women following multivitamin supplementation (Ozkaya *et al.*, 2011), although the effect on pregnancy rate was not studied. Zn consumption is also very beneficial in reducing the detrimental effects of noise pollution in rats (Ghasem *et al.*, 2016).

Zinc Deficiency and Cancer

Although serum or plasma Zn is not a good biomarker for Zn deficiency, there is compelling evidence that dysregulated Zn homeostasis is indeed associated with many cancers (Pan *et al.*, 2017). Manifold studies show that serum Zn levels are usually low in patients with certain cancers, including malignant prostate cancer (Christudoss *et al.*, 2011), oesophageal squamous cell carcinoma (Abnet *et al.*, 2005; Alam *et al.*, 2017), breast cancer (Vimala *et al.*, 2017) and ovarian cancer (Alam and Kelleher *et al.*, 2012). Normal physiological concentrations of Zn inhibit cancer cell proliferation and migration, maintain balanced metabolism and promote apoptosis in cancer cells (Vimala *et al.*, 2017). In consequence, Zn deficiency appears to be involved in every aspect of cancer cell generation and growth. The anti-cancer function of Zn has been widely studied in prostate cancer. The normal human prostate stores the highest content of Zn of all soft tissues in the body, with a

typical Zn content more than 1000 µg/g dry tissue (Nunnes *et al.*, 2017). The accumulation of Zn by prostate glandular epithelial cells is essential for the specialised function of these cells: to produce and secrete enormously high levels of citrate. During the transformation from prostatic epithelial hyperplasia to carcinoma, a reduction in Zn concentration occurs at a relative early stage and continues throughout tumour progression towards the androgen-resistant stage in prostate cancer patients. Zn contents in the prostate at the malignant stage can decline to as low as 150 µg/g dry tissue, which in turn significantly decreases the production of citrate in the prostate. Concurrently, the plasma Zn levels in patients with prostate carcinoma are much lower than those in healthy subjects or in patients with benign prostatic hyperplasia (Nidia *et al.*, 2016). A number of recent studies suggest that Zn deficiency is more than a by-stander or “passenger” during the carcinogenesis and tumourigenesis. For example, the Akt-Mdm2-p53 signalling axis in human normal prostate epithelial cells (PrEC) was activated as response to Zn deficiency, while the Akt-p21 signalling axis was stimulated in malignant prostate LNCaP cells under the same condition (Han *et al.*, 2009; Pan *et al.*, 2017). As a result, LNCaP cells but not PrEC cells survived better and progressed through the G0/G1 phase of the cell cycle at Zn deficient conditions (Han *et al.*, 2009; Pan *et al.*, 2017). Golovine *et al.* (2008) showed that physiological levels of Zn suppressed NF-κB activity and reduced expression of pro-angiogenic and pro-metastatic cytokines VEGF, IL-6, IL-8, and MMP-9 associated with negative prognostic features in prostate cancer (Golvine *et al.*, 2008). Selective Zn deficiency initiated by the Zn chelator N, N, N', N'-tetrakis (2pyridylmethyl)-ethylenediamine (TPEN) increased activation of NF-κB and up-regulates expression of the NF-κB controlled pro-angiogenic and prometastatic cytokines VEGF, IL-6 and IL-8. Reports support the contention that Zn deficiency may contribute to tumour progression via increased expression of the NF-κB-dependent pro-tumourigenic cytokines (Golvine *et al.*, 2008; Brnic *et al.*, 2016).

Kambe *et al.* (2015) have conducted serial studies using N-nitrosomethylbenzylamine (NMBA) induced esophageal cancer rat or mouse models to understand the impact of dietary Zn deficiency on carcinogenesis and tumour development. In these studies, the animals were fed with either Zn-deficient or Zn-sufficient diet. The data confirmed that the tumour incidence increased from 6% in the Zn-sufficient group to 100% in the Zn-deficient group at 11 weeks after NMBA treatment (Wu *et al.*, 2015). Using *in vivo* bromodeoxyuridine (BrdU) labeling followed by immunohistochemical detection of cells in S-phase, they further showed that Zn deficiency notably increased cell proliferation in oesophageal epithelial cells, with shortened lag time for tumour

induction. In cultured esophageal cancer cells, reports from a number of groups suggest that replenishment of Zn inhibits proliferation and induces apoptosis. In the same vein, the replenishment of Zn in the diet could reduce cell proliferation and induce apoptosis in esophageal epithelia and, thus, greatly reduce both incidence and size of tumour in the oesophagi and fore-stomach of treated animals (Bae *et al.*, 2016).

Studies revealed no difference in serum Zn levels between breast cancer patients and controls (SMD (95%CI):-0.65[-1.42, 0.13]). However, the hair Zn levels were lower in women with breast cancer, compared with those of control subjects (SMD (95%CI):-1.99[-3.46,-0.52]). Using a Zn-deficient mouse model, Bostanci *et al.* (2015), demonstrated that marginal Zn intake creates a toxic microenvironment in the mammary gland impairing breast development, which could increase the risk for breast disease and cancer (Bostanci *et al.*, 2015).

It is meaningful to note that breast biopsies from breast cancer patients contain significantly higher Zn levels, compared with normal breast tissue. Epidemiological studies have established a relationship between high breast tissue Zn levels and development of breast cancer (Cui *et al.*, 2007; Pan *et al.*, 2017). It seems paradoxical since breast cancer patients have low Zn levels in hair or serum, and Zn deficiency is thought to contribute to the development of breast cancer. These studies suggest that the dysregulated Zn homeostasis is complicated, and relates not only to Zn concentrations, but also to its distribution as well as its temporal pattern (Chandler *et al.*, 2016). The high Zn content in breast tumour tissues implies that cancer cells selectively increase Zn uptake or reduce Zn efflux via regulating one or more Zn transporters (Pan *et al.*, 2017).

Diagnosis of Zinc Deficiency

Recommended daily dietary allowances (RDA) for infants up to 1 year is 3–5 mg, for children from 1 to 10 years is 10 mg, and for adults (both males and females) it is 15 mg. For pregnant women, RDA is 20 mg and for lactating women RDA is 25 mg (Prasad, 2013, 2014). Currently, the most widely used test for diagnosing Zn deficiency in humans is the determination of plasma Zn level. Normal levels of plasma Zn in adults and children are 100 ± 10 $\mu\text{g/dl}$. Values below 80 $\mu\text{g/dl}$ will be considered to be in the deficient range. Measurement of plasma Zn by flameless atomic absorption specifically is useful, provided the sample is not haemolysed or contaminated. In patients with acute stress or infection, Zn from the plasma pool may redistribute to other compartments, thus making the diagnosis of Zn deficiency difficult. Intravascular haemolysis would also increase the plasma Zn level, in as much as the Zn in the erythrocytes is much higher than in the plasma (Bao *et al.*, 2010). Plasma Zn level of <50 $\mu\text{g/dl}$ would be indicative of

severe deficiency of Zn (Prasad, 2013; 2014; Chavellet *et al.*, 2014).

Determination of Zn in the lymphocytes, plasma somatomedin activity, and measurement of IL-2 mRNA in PHA stimulated mononuclear cells by reverse transcriptase (RT)-polymerase chain reaction (PCR) are the most sensitive tests for diagnosing Zn deficiency in humans (Prasad *et al.*, 1993).

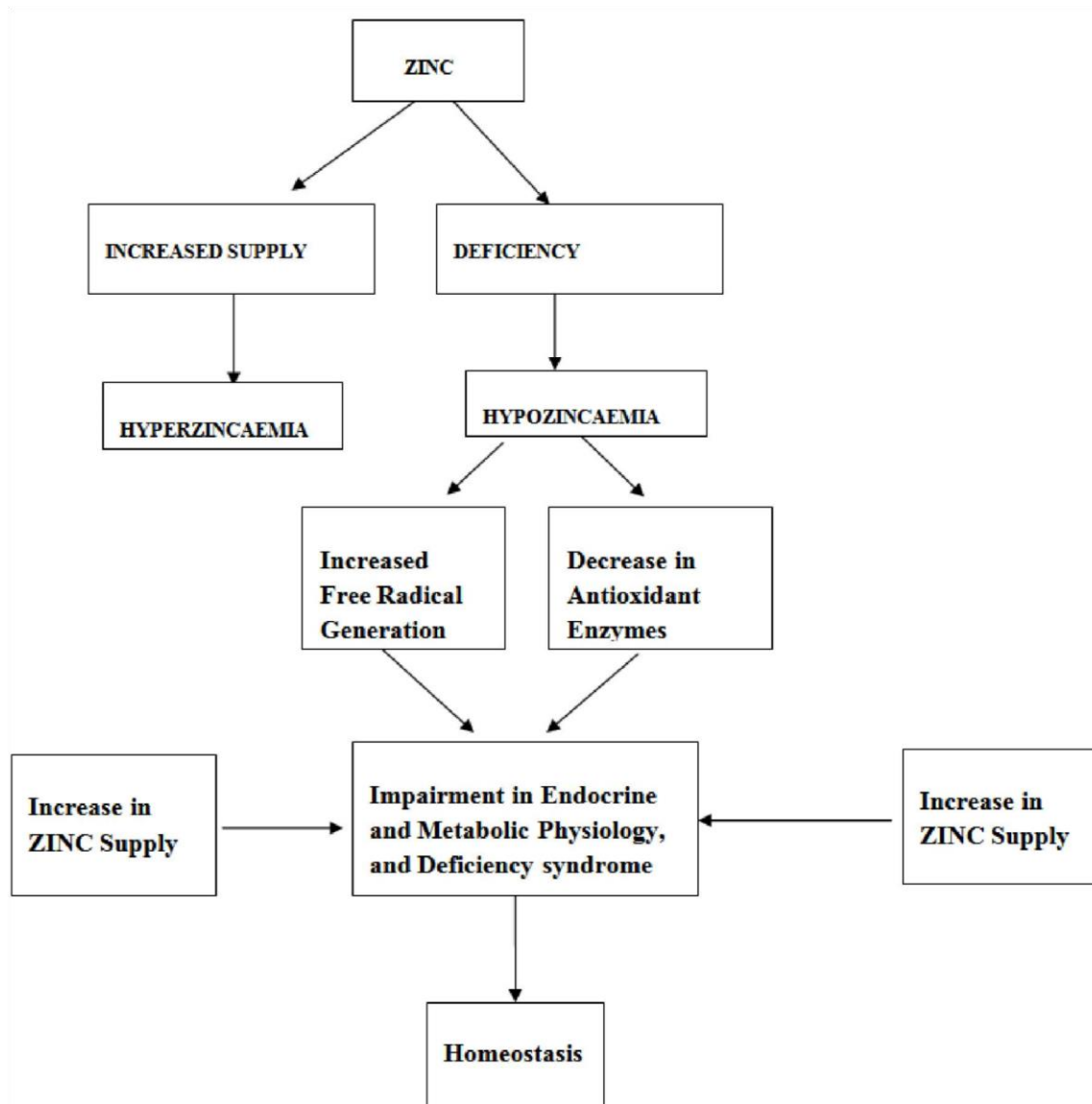
Prevention and Management of Zinc Deficiency

Research studies have emphasised the ubiquity of Zn in biology and the dependence of a wide range of vital metabolic processes on an adequate supply of this metal. As observed in all nutrients, the challenge is to achieve intakes of bioavailable Zn and tissue levels within a physiological range. It has become more apparent that there is a cost to pay for intakes and levels above this range. Presently, this is commonly considered to be a total of 50 mg elemental Zn per day for adults, with levels of intake for children that are less clear. It is imperative to be appropriately sensitive to this upper limit, even if a decision is made to temporarily exceed this for anticipated (but often uncertain) pharmacological benefits. It is even more essential to be aware that these upper limits are for total daily intake, which may be higher than anticipated due to the increasing trend to fortify foods with Zn. Recently, major intervention approaches for tackling zinc deficiency comprises of dietary supplementation, fortification, modification/diversification, and bio-fortification. The use of Zn, as a single micronutrient supplement, for research purposes has proved priceless, and such studies will continue to have an important role in advancing our understanding of the prevalence and effects of human Zn deficiency. There is already, however, also an important role for studies that include arms for multi-nutrient supplements and, even more important, for studies of dietary modifications that affect mineral bioavailability, whether these are at a local community level or involve more global strategies. Meanwhile, widespread Zn supplementation either as a single micronutrient or as a component of a multi-micronutrient mix is a good preventive measure (Prasad, 2013; 2014).

Concluding Remarks

Zn is an essential trace element that plays major biochemical and physiological roles in the body. It is essential for ion channel modulation, signalling, inhibition of bone loss, anti-inflammation, antioxidation and alleviation of symptoms associated with Zn deficiency, manifested by impairment of prenatal and antenatal growth and development, neuropsychological performance, diarrhoea, pneumonia, infertility and cancer. Enhanced Zn bioavailability and supplementation as single micronutrient or as a component of a multimicronutrient mix is imperative to preventive Zn deficiency. Zn is a

nutri-pharmaceutical essential for enhancing health and wellbeing of man and animals.



Schematic Representation of Zinc Nutrition and Deficiency Syndrome.

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