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Review

Zinc, oxidant-triggered cell signaling, and human health

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Abstract

Zinc (Zn) deficiency, a frequent condition in human populations, induces oxidative stress and subsequently activates/inhibits oxidant-sensitive transcription factors that can affect cell function, proliferation and survival leading to disease. Zn deficiency-triggered oxidative stress could affect cell signaling, including: (1) transcription factors containing Zn finger motifs, and (2) other oxidant-sensitive transcription factors (NF-κB and AP-1). The Zn finger motif in the Zn finger transcription factors is mainly a DNA binding domain. Cysteine residues coordinate the Zn ion folding structural domains that participate in intermolecular interactions. Oxidative stress can impair the DNA-binding activity of Zn finger transcription factor, by oxidizing the cysteine residues and therefore altering the secondary structure of the protein. AP-1 is generally activated in Zn deficiency that can occur secondary to an increase in cellular H_2O_2, followed by activation of MAPKs p38 and JNK. The role of AP-1 in Zn deficiency-associated pathology remains to be established. The cytosolic steps of the NF-κB cascade are activated by oxidants in Zn deficiency. However, an impaired nuclear transport of the active transcription factor leads to a low expression of NF-κB-dependent genes that could be involved in multiple aspects of Zn deficiency associated pathology. In summary, Zn deficiency induces

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oxidative stress that can both, lead to tissue oxidative damage and/or to the modulation of select signaling cascades. Their role in the pathology of Zn deficiency remains to be defined.

Abbreviations: MAPKs, mitogen activated kinases; NO, nitric oxide; RNS, reactive nitrogen species; ROS, reactive oxygen species; Zn, zinc

Keywords: Zinc; Oxidative stress; Zinc deficiency; AP-1; NF-κB; Cell signaling

Contents

1. Introduction

Both zinc (Zn) excess and Zn deficiency can lead to oxidative stress and subsequently activate/inhibit oxidant-sensitive transcription factors that can affect cell function, proliferation and survival leading to disease. These aspects of Zn toxicity were recently addressed (Oteiza et al., 2004), thus, the present article reviews the consequences of Zn deficiency in the induction of an oxidative stress condition and the subsequent modulation of redox-sensitive signaling cascades. The possible relevance of those events on human health is discussed.

2. Zinc in biological systems

Zn has multiple relevant functions in biological systems. Among them: (1) Zn is present in a large number of proteins, in which it can have a structural function or can participate in catalysis, (2) Zn could be a physiological constituent of the oxidant defense system, (3) membrane-bound Zn could have structural, regulatory and antioxidant functions and also constitute a pool of rapidly available Zn, (4) Zn is necessary for the maintenance of the normal structure of the cytoskeleton, and (5) Zn plays a major role in cellular signaling. In this regard, Zn-binding proteins

account for nearly half of the transcription regulatory proteins in the human genome (Tupler et al., 2001). These include co-activators, chromatin-modifying and -remodeling enzymes, DNA-binding transcription factors, members of the general transcription machinery and multisubunit RNA polymerases.

3. Zinc deficiency and human health

Based on the numerous physiological functions of Zn, Zn deficiency leads to multiple symptoms that depend on the severity of the deficiency and other associated factors (reviewed in King and Keen, 1999).

Severe Zn deficiency is associated with a genetic condition, acrodermatitis enteropathica, and is also observed in patients fed total parenteral nutrition solutions lacking Zn. Much more frequent conditions are mild to marginal Zn deficiencies, which can occur without apparent symptoms Mild to marginal Zn deficiencies particularly affect individuals with the increased needs associated with growth or reproduction, with altered Zn absorption (children with acute or persistent diarrhea, malabsorption disorders, Crohn's disease, short bowel syndrome, celiac sprue, patients with intestinal bypass surgery, the elderly), increased Zn losses (diabetes, alcoholism), and the treatment with certain drugs. Average Zn intakes in the USA and Europe range from 8 to 14 mg/d and in developing countries from 5 to 11 mg/d. In the general population, FAO national food balance data hve estimated that 48% of the global population is at risk for Zn deficiency (Caulfield et al., 1998). The World Health Report 2002 estimated the global prevalence of Zn deficiency as 31% ranging from 6% to 73% across WHO mortality sub regions.

An early manifestation of Zn deficiency is the loss of appetite. When Zn deficiency becomes a chronic condition; other symptoms appear that also depend on the severity of the deficiency, such as growth retardation, dermatitis, impaired immune function, delayed sexual maturation, pregnancy complications, delayed healing of wounds, behavioral abnormalities and eye lesions. Zn deficiency-induced diarrhea and dermatitis, due to alterations in the barrier elements of the innate immune system and defects in cell-mediated immunity, cause an enhanced susceptibility to numerous pathogens. Through the improvement of immune status or of pathological changes in gut integrity, Zn supplementation can decrease the incidence and duration of diarrhea in children. The analysis of nine trials performed in low-income countries showed that in six of them, there were a significantly lower incidences of diarrhea in Zn-supplemented children than in controls (Black, 2003). Results from 8 randomized controlled trials of women receiving Zn supplements during pregnancy in developing countries show a beneficial effect of maternal Zn supplementation on neonatal immune status, early neonatal morbidity and infant infections (Osendarp et al., 2003).

Furthermore, Zn deficiency could constitute a risk factor for diseases such as cancer and atherosclerosis and Zn supplementation is currently under investigation as a potential therapeutic strategy against different pathologies such as alcoholic liver disease (see review in this volume), cancer, atherosclerosis and pro-inflammatory processes.

4. Zinc deficiency and oxidative stress

Extensive experimental evidence has demonstrated that a low Zn status leads to a condition of oxidative stress (Burke and Fenton, 1985; Ho and Ames, 2002; Ho et al., 2003; Kraus et al., 1997; Oteiza et al., 1995, 1996, 2000, 2001; Sullivan et al., 1980; Virgili et al., 1999). Zn deficiency alters the activity and concentration of enzymes and other components of the oxidant defense system. In animal and cell models of Zn deficiency, the activity of CuZn superoxide dismutase and the activity and expression of Mn superoxide dismutase are increased (Oteiza et al., 1996; Virgili et al., 1999) and the concentration of glutathione is decreased (Bagchi et al., 1998; Kraus et al., 1997; Mackenzie et al., 2004) compared to Zn adequate controls. Zn deficiency leads to tissue oxidative damage, including increased lipid, protein and DNA oxidation (Ho and Ames, 2002; Olin et al., 1993; Oteiza et al., 1995). After 14 d of feeding a Zn deficient diet to developing male rats, a reduced testes growth was observed in association with lipid oxidation (high ratio of 2-thiobarbituric acid-reactive substances/peroxidation index), protein oxidation (high concentrations of protein-associated carbonyls and low glutamine synthetase activity) and DNA oxidation (high 8-oxo-2′-deoxyguanosine levels). DNA damage was also evidenced by the finding of high levels of 8-oxo-2′-deoxyguanosine levels and single strand breaks in liver from Zn deficient infant monkeys and of single strand breaks in C6 glioma cells in association with impaired DNA repair mechanisms, such as p53 and apurinic endonuclease (Ho and Ames, 2002).

Zn deficiency leads to a rapid increase in cellular oxidants. Increased levels of global oxidants (Oteiza et al., 2000; Zago et al., in press), reactive oxygen species (ROS) (Zago et al., in press) and reactive nitrogen species (RNS) (Ho and Ames, 2002) have been observed in association with Zn deficiency in neuroblastoma and glioma cells. A marked increase in H_2O_2 release was observed after only 6 h of incubating IMR-32 cells in Zn deficient media that continued for the subsequent 24 h of incubation (Zago et al., in press). This increase in cellular ROS was prevented by the simultaneous incubation of Zn deficient neuroblastoma cells in the presence of catalase (Zago et al., in press) or the antioxidant substances α-lipoic acid and N-acetylcysteine (Mackenzie et al., 2004).

The mechanisms involved in the increased levels of ROS and RNS when cellular Zn decreases are still poorly understood. The induction of a condition of oxidative stress by Zn deficiency can involve both short term and long term mechanisms. The cause of the rapid increase in cellular ROS (Zago et al., in press) when cellular Zn decreases remains unknown, although preliminary evidence suggests that NADPH oxidase could be involved (unpublished results). In the long term multiple mechanisms could act jointly such as: (1) the requirement of Zn as a physiological antioxidant, (2) altered mitochondrial function leading to increased ROS formation, either by an impaired expression of components of the respiratory chain or other mechanisms, and (3) altered activity/expression of enzymes involved in RNS/ROS metabolism (Beckman et al., 2001; Oteiza et al., 2000; Virgili et al., 1999).

Although lacking redox capacity, Zn is proposed to be a physiological component of the oxidant defense system. Zn could have antioxidant actions through different

mechanisms (Bray and Bettger, 1990; Powell, 2000) including sulfhydryl protection against oxidation, the induction of metallothionein, a cysteine rich protein with capacity to scavenge oxidants and bind redox active metals, and as a constituent of the intracellular and extracellular antioxidant enzyme Cu–Zn superoxide dismutase. Furthermore, Zn can replace redox active metals (copper and iron) from membrane binding sites. In this regard, Zn inhibited lipid oxidation in liposomes by preventing iron and copper binding to negatively charged phospholipids (Zago and Oteiza, 2001). Importantly, Zn acted jointly with other lipid-soluble (α-tocopherol) and water-soluble (epicatechin) antioxidants in the prevention of Fe^{2+}-induced lipid oxidation (Zago and Oteiza, 2001). These combined effects can be explained by the selective environment of action of the corresponding antioxidants. Epicatechin, with antioxidant capacity mostly in the hydrophilic milieu, α-tocopherol, basically a chain-breaking antioxidant acting in hydrophobic domains, and Zn, bound mainly to negatively charged phospholipids at the membrane surfaces, would prevent iron or copper binding to the membrane and the subsequent initiation of oxidative events.

5. Zinc, oxidants and cell signaling

5.1. Zinc finger transcription factors

The Zn finger motif, which characterizes the Zn finger transcription factors, is one of the most common motifs in eukaryotic cells, and is mainly a DNA binding domain. It utilizes Zn^{2+} to fold structural domains that participate in intermolecular interactions. This structural function for Zn was first proposed in Xenopus TFIIIA (Hanas et al., 1983), and several classes of Zn finger motifs, depending on the amino acids that coordinate the Zn ion, have been described. The most common types of Zn finger motifs contain two cysteine and two histidine residues (C2H2) or four cysteine residues (C2C2) in the Zn coordination domain.

The C2H2 motif is a stretch of 30 amino acids, consisting of the sequence Y/F-X-C-X2-4-C-X3-L-X2-H-X3-5-H, where X represents any variable amino acid residue. The conserved cysteines and histidines that coordinate the Zn ion allow the finger to fold into a structure containing two β-sheets followed by an α-helix (Berg, 1988; Lee et al., 1989). C2H2 Zn fingers occur typically in tandem arrays, and depending on the number of Zn finger motifs present within the protein sequence, they can be divided into two classes. The first class, the C2H2 proteins, includes the early growth response factor-1 (Egr1) and the specificity protein-1 (Sp1) family, which have less than five C2H2 motifs. For example, the general transcription factor Sp1 has a DNA-binding domain that consists of three Zn fingers and is known to stimulate the SV40 early promoter (Dynan and Tjian, 1983). In general, proteins in this group have been typically identified as transcriptional activators or repressors involved in the regulation of cell proliferation and differentiation. The second class of C2H2 Zn finger proteins contains five or more Zn finger motifs. One protein that fits in this group is TFIIIA, which has nine repeats in its sequence, binds to the 5S RNA gene

and to 5S RNA (Theunissen et al., 1992). This second class can be subdivided into the proteins without or with the KRAB domains.

In contrast to C2H2 motifs, proteins with C2C2 fingers often have non-repetitive fingers. Binding sites on DNA are usually short and palindromic. Generally, it follows the sequence C-X2-C-X13-C-X2-C, where X represents any variable amino acid. Nuclear receptors (glucocorticoid, estrogen, thyroid hormone, retinoic acid) have this type of Zn fingers. The glucocorticoid and estrogen receptors each have two fingers motif that form α-helices that fold together to form a large globular domain.

Among the Zn finger proteins that control a variety of cellular processes, a number of them are known to participate in bone and cartilage development (Ganss and Jheon, 2004). Several members of the KRAB-Zn finger protein family act as transcriptional repressors. For example, ZFP60 delays chondrocyte differentiation (Ganss and Kobayashi, 2002), while NT2 inhibits type XI collagen transcription, as cartilage differentiation progresses (Tanaka et al., 2002). On the other hand, the Sp family of Zn transcription factors have been implicated in skeletal development. For example Sp3 is crucial for enamel matrix development since Sp3 knockout studies have shown defects in tooth and bone formation (Bouwman et al., 2000; Gollner et al., 2001). In addition, Sp7 was identified as an early induced gene in bone formation and appears to regulate the expression of osteocalcin and type XI collagen, and the absence of Sp7 led to no bone formation (Nakashima et al., 2002). Therefore, regarding the role of Zn in skeletal development, adequate Zn concentrations can prevent the bone growth retardation observed in Zn deficiency. Based on the above, Zn deficiency is identified as a risk factor in the outcome of osteoporosis. Zn is also crucial for normal cardiac development. In this regard, Zn deficiency affected Zn finger transcription factor GATA-4 and the dependent expression of alpha myosin heavy chain and cardiac troponin I, two critical genes involved in early stages of cardiac development (Duffy et al., 2004).

The cysteines that coordinate Zn must be in the reduced form. The oxidation of the thiol group present in cysteine residues eliminates Zn coordination, disassembles the secondary structure, affecting the DNA binding properties. Therefore, oxidative stress could lead to a reduced transcription of genes regulated by Zn finger transcription factors. DNA binding and transcriptional activities of Erg1, Sp1 and glucocorticoid receptor are sensitive to oxidizing agents and are reactivated by reducing agents such as dithiothreitol (DTT) or β-mercaptoethanol. For example, nitric oxide (NO) can inhibit the DNA-binding activity of transcription factors containing at least one cysteine residue (Kroncke, 2001). The inhibitory effect of NO on the DNA binding activity of Zn finger transcription factors is reversible. Thus, DTT reverses the NO induced inhibition of the vitamin D3 receptor-retinoid X receptor heterodimer binding to its DNA consensus sequence (Kroncke, 2001).

Oxidation of the estrogen receptor provides additional relevant evidence for the physiological role of Zn finger transcription factors. Estrogen receptor has been shown to be essential for breast cancer treatment (Webster et al., 2001). Estrogen receptor isolated from untreated breast cancer patients was not capable of binding to its cognate DNA. Moreover, there is a lower responsiveness to estrogen and

tamoxifen therapy in the tumors that have an abnormal estrogen receptor (Webster et al., 2001). The finding that the abnormal DNA-binding activity of the estrogen receptor can be reversed by DTT indicates that this transcription factor is highly susceptible to oxidation. Other example of physiologically relevant Zn finger oxidation occurs in age-related Sp1 oxidation (Ammendola et al., 1992). Sp1 DNA binding activity is dramatically reduced in brain and liver of old rats compared to young rats, while other non Zn finger transcription factors, such as nuclear factor 1, are not affected (Ammendola et al., 1992). The incubation of nuclear extracts of old rat brain or liver with DTT restored Sp1 DNA-binding capacity, suggesting an increase of oxidants in the old rats. The reduced Sp1 DNA-binding activity of old rats was correlated with a decrease in Sp1-dependent gene transcription, including transferrin (Adrian et al., 1996).

In conclusion, oxidative stress can impair the DNA-binding activity of Zn finger transcription factors, by oxidizing the cysteine residues and therefore altering the secondary structure of the protein. An adequate Zn status and subsequently the prevention of oxidative stress can be of particular relevance in the modulation of Zn finger transcription factor-regulated gene expression.

5.2. Other oxidant-sensitive transcription factors

Oxidants and/or alterations in the cell thiol redox status can affect cell signaling at different steps, such as in the triggering of early activation events or later, due to the requirement of reduced thiol groups for the binding of transcription factors to promoter/enhancer regions in the DNA. A deficit of Zn influences the activity of transcription factors AP-1 and NF-κB, which are sensitive to oxidants and conditions of oxidative stress. Thus, Zn deficiency is in general associated with the activation of AP-1 and with alterations in NF-κB activation and NF-κB-dependent gene transcription in animal and cell models.

AP-1 is activated by multiple physiological stimuli and stress conditions, including oxidative stress. Upstream activation of AP-1 is mediated by the mitogen-activated protein kinases (MAPKs) JNK, p38 and ERK. H_2O_2 triggers AP-1 activation primarily through the activation of the stress-responsive MAPKs p38 and JNK. We recently demonstrated in neuronal cells (IMR-32), that Zn deficiency triggers the activation of the MAPKs JNK and p38, leading to high nuclear AP-1-DNA binding activity and increased AP-1-dependent gene expression (Zago et al., in press). The signal mediating the activation of JNK, p38 and AP-1 in Zn deficient IMR-32 cells was identified to be mainly H_2O_2 since the addition of catalase to the incubation media prevented its occurrence (Zago et al., in press). On the contrary, Zn deficiency led to decreased activation of the MAPK ERK1/2 that was independent of H_2O_2 increased production and that was associated with decreased cell proliferation (Fig. 1).

Although transcription factor NF-κB is activated by oxidants, in general Zn deficiency has been found to be associated with a decreased NF-κB-dependent gene expression. In Zn deficiency, a decreased NF-κB-DNA binding activity has been described in nuclear extracts from rat testes (Oteiza et al., 2001), 3T3 fibroblasts

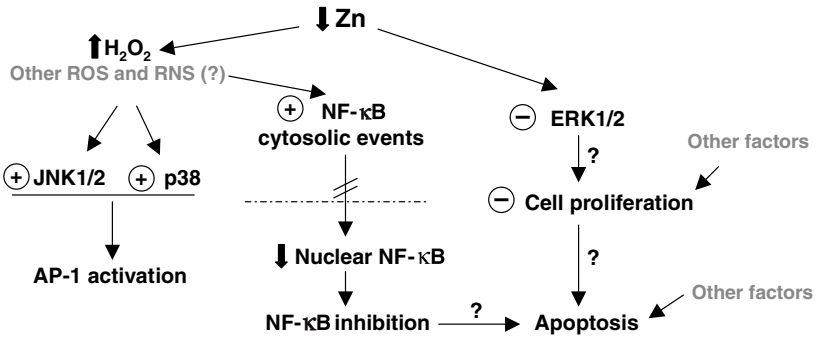

Fig. 1. Proposed scheme for the regulation of MAPKs, AP-1 and NF-κB signaling by Zn deficiency in neuronal cells. Zn deficiency leads to a rapid cellular decrease of labile Zn pools and increased ROS (H_2O_2) cellular levels. The increase in intracellular H_2O_2 activates p38 and JNK MAPKs, triggering the activation of transcription factor AP-1 and an increased transactivation of AP-1-driven genes (Zago et al., in press). Although the increase in oxidant levels is also associated with the activation of NF-κB cytosolic events, NF-κB nuclear translocation is impaired (Mackenzie et al., 2002). This leads to a decrease in transactivation of NF-κB-driven genes (Mackenzie et al., 2002). Through H_2O_2-independent pathways, a decrease in cellular Zn causes a reduction in the activity of ERK1/2 MAPK (Zago et al., in press) that could lead to a reduction in cell proliferation. Both, NF-κB inhibition and reduced cell proliferation, in addition to other factors, could contribute to the triggering of neuronal apoptosis associated with Zn deficiency (Verstraeten et al., 2004).

(Oteiza et al., 2000), C6 rat glioma cells (Ho and Ames, 2002), a T-lymphoblastoid cell line (HUT-78) (Prasad et al., 2002), and human neuroblastoma IMR-32 cells (Mackenzie et al., 2002). Although, NF-κB-DNA binding activity in nuclear extracts was low in the Zn deficient IMR-32 cells, the NF-κB-DNA binding activity in total cell extracts was higher in the Zn deficient cells than in controls (Mackenzie et al., 2002). We have presented evidence that the cytosolic events in the NF-κB cascade (IκBα phosphorylation and degradation) are activated in Zn deficient cells (Mackenzie et al., 2002) and that this activation is triggered by a cellular increase in oxidants (Mackenzie et al., 2004). However, alterations in tubulin polymerization occurring secondary to Zn deficiency (Mackenzie et al., 2002; Oteiza et al., 1990) can impair the translocation of the active NF-κB into the nuclei, inhibiting NF-κB-dependent gene expression (Mackenzie et al., 2002).

The observed alterations in NF-κB and AP-1 activation (Fig. 1) can be involved in the pathology associated with Zn deficiency. AP-1 is mainly involved in processes of proliferation, differentiation and apoptotic cell death and NF-κB regulates multiple genes (i.e. genes involved in cell proliferation and survival, in the immune response, in early embryonic development, in inflammation, etc.). In animal and cell models, Zn deficiency induces apoptotic cell death that can affect critical processes (reviewed in Fraker, 2005), such as embryogenesis (Jankowski-Hennig et al., 2000) and immune function. Apoptosis also accompanies Zn deficiency-induced atrophy of the testes and thymus.

Although Zn deficiency induced cell death by apoptosis in IMR-32 neuroblastoma cells (Verstraeten et al., 2004), this was not found to be associated with an in-

crease in cell oxidants and subsequent AP-1 activation (Zago et al., in press). The consequences of AP-1 activation in Zn deficiency remains to be established. A decreased NF-κB-dependent gene expression in Zn deficiency could participate in the increased apoptotic cell death, due to the general antiapoptotic function of this transcription factor. Alterations in cell proliferation, apoptosis, and in the regulation of other NF-κB-dependent genes can in part explain Zn deficiency-induced alterations in immune function as well as the impaired development of the nervous system and other organs, and other aspects of Zn deficiency-related pathology.

6. Concluding remarks

In summary, Zn deficiency has been demonstrated to trigger oxidative stress and oxidant-mediated damage to cell components. The increase in cellular ROS or RNS and/or alterations in the thiol redox status associated with Zn deficiency can activate/inhibit select transcription factors such as those containing Zn finger motifs and the oxidant-sensitive AP-1 and NF-κB. Alterations in the modulation of these transcription factors could be involved in Zn deficiency-induced alterations of cell functions, cell proliferation and apoptotic death rates. However, further research is required to establish the role of ROS and RNS in the pathology associated with Zn deficiency.

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References

Adrian, G.S., Seto, E., Fischbach, K.S., Rivera, E.V., Adrian, E.K., Herbert, D.C., Walter, C.A., Weaker, F.J., Bowman, B.H., 1996. YY1 and Sp1 transcription factors bind the human transferrin gene in an age-related manner. J. Gerontol. A. Biol. Sci. Med. Sci. 51, B66–B75.

Ammendola, R., Mesuraca, M., Russo, T., Cimino, F., 1992. Sp1 DNA binding efficiency is highly reduced in nuclear extracts from aged rat tissues. J. Biol. Chem. 267, 17944–17948.

Bagchi, D., Vuchetich, P.J., Bagchi, M., Tran, M.X., Krohn, R.L., Ray, S.D., Stohs, S.J., 1998. Protective effects of zinc salts on TPA-induced hepatic and brain lipid peroxidation, glutathione depletion, DNA damage and peritoneal macrophage activation in mice. Gen. Pharmacol. 30, 43–50.

Beckman, J.S., Estevez, A.G., Crow, J.P., Barbeito, L., 2001. Superoxide dismutase and the death of motoneurons in ALS. Trends Neurosci. 24, S15–S20.

Berg, J.M., 1988. Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins. Proc. Natl. Acad. Sci. USA. 85, 99–102.

Black, R.E., 2003. Zinc deficiency, infectious disease and mortality in the developing world. J. Nutr. 133, 1485S–1489S.

Bouwman, P., Gollner, H., Elsasser, H.P., Eckhoff, G., Karis, A., Grosveld, F., Philipsen, S., Suske, G., 2000. Transcription factor Sp3 is essential for post-natal survival and late tooth development. Embo J. 19, 655–661.

Bray, T.M., Bettger, W.J., 1990. The physiological role of zinc as an antioxidant. Free Radic. Biol. Med. 8, 281–291.

Burke, J.P., Fenton, M.R., 1985. Effect of a zinc-deficient diet on lipid peroxidation in liver and tumor subcellular membranes. Proc. Soc. Exp. Biol. Med. 179, 187–191.

Caulfield, L.E., Zavaleta, N., Shankar, A.H., Merialdi, M., 1998. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am. J. Clin. Nutr. 68, 499S–508S.

Duffy, J.Y., Overmann, G.J., Keen, C.L., Clegg, M.S., Daston, G.P., 2004. Cardiac abnormalities induced by zinc deficiency are associated with alterations in the expression of genes regulated by the zinc-finger transcription factor GATA-4. Birth Defects Res. B. Dev. Reprod. Toxicol. 71, 102–109.

Dynan, W.S., Tjian, R., 1983. The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. Cell 35, 79–87.

Fraker, P.J., 2005. Roles for cell death in zinc deficiency. J. Nutr. 135, 359–362.

Ganss, B., Jheon, A., 2004. Zinc finger transcription factors in skeletal development. Crit. Rev. Oral Biol. Med. 15, 282–297.

Ganss, B., Kobayashi, H., 2002. The zinc finger transcription factor Zfp60 is a negative regulator of cartilage differentiation. J. Bone Miner. Res. 17, 2151–2160.

Gollner, H., Dani, C., Phillips, B., Philipsen, S., Suske, G., 2001. Impaired ossification in mice lacking the transcription factor Sp3. Mech. Dev. 106, 77–83.

Hanas, J.S., Hazuda, D.J., Bogenhagen, D.F., Wu, F.Y., Wu, C.W., 1983. Xenopus transcription factor A requires zinc for binding to the 5 S RNA gene. J. Biol. Chem. 258, 14120–14125.

Ho, E., Ames, B.N., 2002. Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. Proc. Natl. Acad. Sci. USA. 99, 16770–16775.

Ho, E., Courtemanche, C., Ames, B.N., 2003. Zinc deficiency induces oxidative DNA damage and increases p53 expression in human lung fibroblasts. J. Nutr. 133, 2543–2548.

Jankowski-Hennig, M.A., Clegg, M.S., Daston, G.P., Rogers, J.M., Keen, C.L., 2000. Zinc-deficient rat embryos have increased caspase 3-like activity and apoptosis. Biochem. Biophys. Res. Commun. 271, 250–256.

King, J.C., Keen, C.L., 1999. Zinc. In: Shils, M., Olson, J., Shike, M., Ross, A. (Eds.), Modern Nutrition in Health and Disease. Williams and Wilkins, Baltimore, pp. 223–239.

Kraus, A., Roth, H.P., Kirchgessner, M., 1997. Supplementation with vitamin C, vitamin E or beta-carotene influences osmotic fragility and oxidative damage of erythrocytes of zinc-deficient rats. J. Nutr. 127, 1290–1296.

Kroncke, K.D., 2001. Zinc finger proteins as molecular targets for nitric oxide-mediated gene regulation. Antioxid. Redox Signal. 3, 565–575.

Lee, M.S., Gippert, G.P., Soman, K.V., Case, D.A., Wright, P.E., 1989. Three-dimensional solution structure of a single zinc finger DNA-binding domain. Science 245, 635–637.

Mackenzie, G.G., Zago, M.P., Erlejman, A.G., Aimo, L., Keen, C.L., Oteiza, P.I., 2004. A decrease in neuronal zinc triggers the oxidant-mediated activation of NF-κB and AP-1 in neuroblastoma cells. Free Rad. Biol. Med. 36, S87.

Mackenzie, G.G., Zago, M.P., Keen, C.L., Oteiza, P.I., 2002. Low intracellular zinc impairs the translocation of activated NF-kappa B to the nuclei in human neuroblastoma IMR-32 cells. J. Biol. Chem. 277, 34610–34617.

Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J.M., Behringer, R.R., de Crombrugghe, B., 2002. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108, 17–29.

Olin, K.L., Shigenaga, M.K., Ames, B.N., Golub, M.S., Gershwin, M.E., Hendrickx, A.G., Keen, C.L., 1993. Maternal dietary zinc influences DNA strand break and 8-hydroxy-2′-deoxyguanosine levels in infant rhesus monkey liver. Proc. Soc. Exp. Biol. Med. 203, 461–466.

Osendarp, S.J., West, C.E., Black, R.E., 2003. The need for maternal zinc supplementation in developing countries: an unresolved issue. J. Nutr. 133, 817S–827S.

Oteiza, P.I., Clegg, M.S., Keen, C.L., 2001. Short-term zinc deficiency affects nuclear factor-kappab nuclear binding activity in rat testes. J. Nutr. 131, 21–26.

Oteiza, P.I., Clegg, M.S., Zago, M.P., Keen, C.L., 2000. Zinc deficiency induces oxidative stress and AP-1 activation in 3T3 cells. Free Radic. Biol. Med. 28, 1091–1099.

Oteiza, P.I., Cuellar, S., Lonnerdal, B., Hurley, L.S., Keen, C.L., 1990. Influence of maternal dietary zinc intake on in vitro tubulin polymerization in fetal rat brain. Teratology 41, 97–104.

Oteiza, P.I., Mackenzie, G.G., Verstraeten, S.V., 2004. Metals in neurodegeneration: involvement of oxidants and oxidant-sensitive transcription factors. Mol. Aspects Med. 25, 103–115.

Oteiza, P.I., Olin, K.L., Fraga, C.G., Keen, C.L., 1995. Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes. J. Nutr. 125, 823–829.

Oteiza, P.L., Olin, K.L., Fraga, C.G., Keen, C.L., 1996. Oxidant defense systems in testes from zinc-deficient rats. Proc. Soc. Exp. Biol. Med. 213, 85–91.

Powell, S.R., 2000. The antioxidant properties of zinc. J. Nutr. 130, 1447S–1454S.

Prasad, A.S., Bao, B., Beck, F.W., Sarkar, F.H., 2002. Zinc enhances the expression of interleukin-2 and interleukin-2 receptors in HUT-78 cells by way of NF-kappaB activation. J. Lab. Clin. Med. 140, 272–289.

Sullivan, J.F., Jetton, M.M., Hahn, H.K., Burch, R.E., 1980. Enhanced lipid peroxidation in liver microsomes of zinc-deficient rats. Am. J. Clin. Nutr. 33, 51–56.

Tanaka, K., Tsumaki, N., Kozak, C.A., Matsumoto, Y., Nakatani, F., Iwamoto, Y., Yamada, Y., 2002. A Kruppel-associated box-zinc finger protein, NT2, represses cell-type-specific promoter activity of the alpha 2(XI) collagen gene. Mol. Cell. Biol. 22, 4256–4267.

Theunissen, O., Rudt, F., Guddat, U., Mentzel, H., Pieler, T., 1992. RNA and DNA binding zinc fingers in Xenopus TFIIIA. Cell 71, 679–690.

Tupler, R., Perini, G., Green, M.R., 2001. Expressing the human genome. Nature 409, 832–833.

Verstraeten, S.V., Zago, M.P., Mackenzie, G.G., Keen, C.L., Oteiza, P.I., 2004. Influence of zinc deficiency on cell-membrane fluidity in Jurkat, 3T3 and IMR-32 cells. Biochem. J. 378, 579–587.

Virgili, F., Canali, R., Figus, E., Vignolini, F., Nobili, F., Mengheri, E., 1999. Intestinal damage induced by zinc deficiency is associated with enhanced CuZn superoxide dismutase activity in rats: effect of dexamethasone or thyroxine treatment. Free Radic. Biol. Med. 26, 1194–1201.

Webster, K.A., Prentice, H., Bishopric, N.H., 2001. Oxidation of zinc finger transcription factors: physiological consequences. Antioxid. Redox Signal. 3, 535–548.

Zago, M.P., Mackenzie, G.G., Adamo, A.M., Keen, C.L., Oteiza, P.I., in press. Differential modulation of MAP kinases by zinc deficiency in IMR-32 cells: role of H_2O_2. Antioxid. Redox Signal.

Zago, M.P., Oteiza, P.I., 2001. The antioxidant properties of zinc: interactions with iron and antioxidants. Free Radic. Biol. Med. 31, 266–274.