THE ESSENTIAL ROLE OF ZINC IN GROWTH

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ABSTRACT

Zinc is known to play a relevant role in growth and development. The basic mechanisms of action of this trace element are intimately linked to the structure and action of countless enzymes involved in many different metabolic processes. In this respect, when zinc specifically acts on cartilage growth it is involved in multiple enzymatic reactions which make this a multifactorial event. Thus, we may divide the actions of zinc into three distinct types: 1) action on taste and smell acuity, appetite regulation, and food consumption and regulation; 2) action on DNA and RNA synthesis stimulating a) cell replication and differentiation of chondrocytes, osteoblasts and fibroblasts; b) cell transcription culminating in the synthesis of somatomedin-C (liver), alkaline phosphatase, collagen and osteocalcin (bone), and c) protein, carbohydrate and lipid metabolism, that is intimately related to the mechanisms of smell, taste, appetite, and food consumption and utilization; 3) action on hormonal mediation by participating in a) GH synthesis and secretion in somatomammotroph cells, b) the action of GH on liver somatomedin-C production, and c) somatomedin-C activation in bone cartilage. In addition to these multiple functions, zinc also interacts with other hormones somehow related to bone growth such as testosterone, thyroid hormones, insulin, and vitamin D.

On the basis of the above considerations, we conclude that the integration of these mechanisms contributes to the perfect physiological functioning of bone. In the presence of zinc deficiency, this homeostasis is impaired, causing the weight-height deficiency detected in several species studied, the human species in particular.

KEYWORDS: Zinc, Growth, Enzymatic mechanisms, Bone metabolism, Hormonal mediation.

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INTRODUCTION

Zinc is fundamental for cell growth, development and differentiation, but the biochemical mechanisms involved are not totally known. We will review in this paper the zinc action over the several mechanisms related to growth (Fig. 1). Zinc interacts with general metabolism of protein, carbohydrate and lipid, as well as on taste, smell, appetite regulation and food consumption. On the other hand, it is closely involved in the synthesis and action of growth hormone (GH), somatomedin-C, alkaline phosphatase, collagen, and osteocalcin. In addition to GH, the interrelationship between zinc and other hormones such as testosterone, thyroid hormones, insulin, and vitamin D, will be considered. This micronutrient participates both in the synthesis and actions of these hormones, which are intimately linked to bone metabolism. Thus, in all these events zinc acts positively on growth and development.

I. ENZYMATIC MECHANISMS

More than 300 different zinc-dependent enzymes have been identified in all phyla. Zinc has a recognized action on these metalloenzymes since it participates in their structure, catalytic and regulatory actions. Many of these enzymes are relevant to the human species (1). Zinc enzymes encompass all enzyme classes and many of them participate in a variety of metabolic processes such as synthesis and/or degradation of lipids, carbohydrates, proteins and nucleic acids (Fig. 1). The utilization of amino acids in protein synthesis, for example, is impaired in zinc deficiency (2,3). Zinc is known to be present in appreciable amounts in cell nuclei, nucleoli, chromosomes, ribosomes, and secretory granules (4-6).

Zinc is intimately linked to DNA and RNA structure, synthesis and degradation, thus playing an important role in the control of cellular replication and transcription (7) (Fig. 1). The activity of DNA and RNA polymerases may be affected by zinc deficiency in a variety of tissues or cell cultures since they are considered to be metalloenzymes (8).

II. TASTE AND SMELL ACUITY

APPETITE REGULATION

FOOD CONSUMPTION AND EFFICIENCY

Zinc deficiency is invariably accompanied by alterations in smell and taste and by anorexia and weight loss. A metalloenzyme responsible for taste sensation has been discovered and is related to human hypogeusia or dysgeusia, disorders that can be fully corrected with zinc therapy (9-11). The alterations may occur even in individuals consuming a diet with marginal amounts of zinc. They were first confirmed in Denver (CO, USA) where children with poor growth and appetite with hypogeusia associated with subnormal hair zinc levels were detected. When supplemented with small zinc doses the children showed improvement in taste acuity and
FIG. 1 - The role of zinc on linear growth, through its activity in (a) smell, taste, appetite, food consumption and utilization; (b) intermediary metabolism; (c) chondrocytes, osteoblasts and fibroblasts; (d) and hormonal mediation by growth and thyroid hormones, somatomedin-C, testosterone, insulin, vitamin D₃.
growth (12). Zinc may also affect appetite control by acting directly on the central nervous system, altering the responsiveness of receptors of norepinephrine, dopamine, serotonin, gamma-aminobutyric acid, and opiates (13-15).

The growth inhibition caused by zinc deficiency may be partially responsible for the changes in smell, taste and appetite, which in turn reduce food consumption and utilization (11,16-18). Zn-deficient rats presented a reduction of food ingestion of approximately 70% (19,20) and increased urinary N, urea and uric acid excretion, as well as increased liver tryptophan pyrrolase and arginase activity, confirming a state of protein catabolism (21). Zinc deficiency in malnourished children was also found to increase energy expenditures for growth (22).

III - DNA REPLICATION: CHONDROCYTES, OSTEOBLASTS AND FIBROBLASTS

Zinc plays a relevant role in gene expression, and one of the most fascinating examples of this is the induction of enzymes linked to DNA synthesis before cells enter the S phase of the cell cycle, as well as the induction of new proteins during cell differentiation (23). Growth can be shared by the increase in number (hyperplasia) and size (hypertrophy) of cells. All experimental animals submitted to a specifically zinc-deficient diet present a reduction in growth long before the decrease in total body zinc concentration. This phenomenon may be explained by the greater susceptibility of DNA synthesis and consequent cell division to the fall in free ionic zinc, than in protein synthesis and cell size (24,25). A confirmation of this report was later observed in zinc-deficient lymphocytes, i.e., only a third of the total amount of zinc for DNA synthesis was required for RNA synthesis (26). Zinc deficiency in experimental animals caused considerable changes in the differentiation of chondrocytes, osteoblasts and fibroblasts, thus impairing bone maturation. For example, any interference with DNA synthesis and osteoblast (27-29) or fibroblast proliferation (30,31) will deeply affect calcium and fibrous collagen deposition in bone connective tissue. The same phenomenon will occur with chondrocyte cell division (27) and immediate zinc administration will increase DNA concentration, resulting in chondrocyte proliferation in diaphysis and epiphysis of rats (32).

IV - GENE TRANSCRIPTION: PROTEIN SYNTHESIS RELATED TO BONE SOMATOMEDIN

Somatomedin-C (SM-C) is a polypeptide hormone, also called insulin-like growth factor I (IGF-I), which shows homology to proinsulin and is synthesized in the liver under the stimulus of growth hormone (GH) (33). SM-C carries out negative feedback with the hypothalamus and pituitary (34) and acts as an intermediary in the effect of GH on cartilage proliferation and the consequent linear growth of the skeleton (35) (Fig.
2). SM-C stimulates DNA and RNA synthesis, thus promoting cell multiplication (chondrocytes, fibroblasts and osteoblasts), and also increases sulfate incorporation into proteoglycans and proline in collagen (36). All of these actions are actually mediated by zinc (Fig. 2). Protein malnutrition is intimately linked to zinc deficiency, and both are intimately related to depressed SM-C synthesis and activity (37,38). In rats, for example, SM-C synthesis is affected by dietary protein restriction. However, when the same animals receiving a zinc-deficient diet were supplemented with protein, plasma SM-C levels did not increase. Conversely, when a group receiving a low-protein diet was supplemented with zinc, plasma SM-C levels were increased (38). A high correlation between SM-C activity and weight gain and increased zinc concentrations in the tibia was found in the same experiment. Other experiments have also demonstrated that dietary zinc deficiency causes a reduction of serum SM-C levels (39,40) as well as reduced tibial epiphyseal width in rats (39). The administration of bovine growth hormone (bGH) to these animals did not lead to normal SM-C levels, which were only obtained by zinc addition (39). A high correlation has also been observed between tibial epiphyseal widths and zinc levels in both serum and femur. This lack of response to bGH administration by zinc-deprived rats has also been

![Zinc-dependent hormonal mechanisms on linear growth.](image)

FIG. 2. Zinc-dependent hormonal mechanisms on linear growth. Zinc stimulates (Zn⁺⁺ ⇒ [+]) GH synthesis, secretion and action on the liver and cartilage. Besides this ion stimulates the activity of SM-C, testosterone, thyroid hormones, insulin, and vitamin D₃ on chondroblasts, osteoblasts and fibroblasts. Zinc positively increments the linear growth.
observed by other investigators (41). The conclusion reached by the former investigators (39) was that these facts speak against the assumption of decrease GH secretion or action and support the idea that SM-C synthesis depends on the presence of zinc as a cofactor in addition to GH stimulation. The improved growth in response to zinc supplementation has also been detected in humans (42). However, the relationship between zinc and SM-C has been verified in only a few reports. We did not observe changes in SM-C levels after GH and zinc therapy (43,44), but zinc supplementation increased plasma SM-C levels in 100% of children with retarded growth (45-48).

ALKALINE PHOSPHATASE

Zinc concentration is considered to be very high in bone (49,50) and zinc concentrations in the tibia are a good indicator of zinc status in rats (51). Indeed, retarded growth and changes in bone tissue calcification have been detected in many conditions associated with zinc deficiency (52,53) (Fig. 2).

Alkaline phosphatase (AP) is an enzyme produced mainly by osteoblasts whose major function is to provide calcium deposition in bone diaphyses. Since zinc is related to RNA synthesis and the consequent protein synthesis, zinc-deprived experimental animals also present decreased bone and serum AP levels (54-56), with a return of normal activity after a zinc-adequate diet (29,55,56). Although these investigators observed that acid phosphatase activity was also increased, zinc accumulated in diaphyseal tissue stimulated osteoblasts more intensely than osteoclasts. A decline in blood AP levels was also detected in patients with zinc and growth deficiency (57,58), with elevation occurring after zinc supplementation (48,59,60). In addition to acting on AP synthesis, zinc is also a component of AP structure, which have about 4 atoms of zinc per molecule, two of which are essential for enzyme activity and are less firmly bound (61). Indeed, zinc-deficient monkeys present radiographic findings quite similar to those detected in patients with hypophosphatasia (62,63).

OSTEOCALCIN

Osteocalcin is a bone gla-protein synthesized by osteoblasts and is the most abundant noncollagenous protein (64,65). This protein is considered to be a sensitive marker of bone formation (66,67) since a large part of it binds to osseous hydroxyapatite, whereas a small fraction is detected in the peripheral circulation (68). There is a strong relationship between osteocalcin and 1,25(OH)_{2}D, since this metabolite is the most important regulator of osteocalcin synthesis (69), although insulin may also act synergistically in this direction (70) (fig.2). Osteocalcin measurements are being used as an additional biochemical parameter for the evaluation of growth deficiency. Blood osteocalcin levels may be affected by age, sex, rapid growth during adolescence (71), nocturnal GH secretion (72), and malnutrition (73). It has not
yet been fully established whether the synthesis of this protein also depends on zinc. We know, for example, that children with growth deficiency accompanied or not by hormonal changes have lower serum osteocalcin levels than controls (72,74), whereas children with precocious puberty have higher osteocalcin levels (71). Ten short children without endocrinological problems with mild to moderate zinc deficiency had high serum osteocalcin levels after zinc supplementation (48). In another study, short children with normo- or hypozincemia did not show any significant differences with respect to this parameter (75).

**COLLAGEN AND CHONDROITIN**

Fibroblasts are responsible for the production of collagen and chondroitin-4-sulfate. These substances act as structural elements of bone tissue and their synthesis is stimulated by zinc (Fig.2). Collagen is the most important extracellular structural protein of bone and, by virtue of its constitution similar to a chemical fiber, it provides the solid property of bone connective tissue. The activity of deoxythymidine kinase was reduced in the implanted sponge connective tissue, and this reduced activity was accompanied by decreased RNA/DNA and collagen synthesis in zinc-deficient humans and rats (76,77). On the other hand, oral zinc administration was sufficient to increase calcium and total collagen contents in the femoral diaphyses of weanling rats (32) and in implanted human sponge connective tissue (77). Decreased [3H]-proline uptake and decreased transformation of proline to hydroxyproline in the epiphyseal plates have also been observed in zinc-deficient rats (78,79). With respect to chondroitin, $^{35}$SO$_4$ incorporation into epiphyseal plates was lower in zinc-deficient chicks and rats than in their controls (78,80) and this reduced sulfate incorporation into the cartilaginous matrix contributed to inadequate bone calcification. In the light of current information, it is not known exactly how zinc acts in the synthesis of collagen and chondroitin. All indications are that this effect is related to the generalized effect on nucleic acids and protein synthesis, rather than to a defect on inter- and intramolecular crosslinking.

**GENERAL PROTEIN, LIPID, AND CARBOHYDRATE METABOLISM**

As previously mentioned, zinc controls the synthesis and activity of countless enzymes, many of them participating in a variety of metabolic processes (Fig.1). It is the integrity of these processes that permits good food utilization. The utilization of amino acids for protein synthesis, for example, is impaired in zinc deficiency (2). One of the enzymes affected is gustin, which is responsible for taste function. This protein was isolated from human parotid saliva (9), has a molecular weight of 37,000, is composed of 8% histidine residues and has 2 moles of zinc per mole of protein (81). Gustin appears to be involved in the differentiation, growth,
and nutrition of taste buds, and it has been considered to be a type of nerve growth factor (82). There is a high correlation between parotid saliva gustin and parotid saliva zinc, and mean levels of gustin in parotid saliva in patients with hypogeusia were significantly lower than in normal controls (83). With respect to carbohydrates, zinc is important for the synthesis, crystallization and peripheral action of insulin (84). Zinc deficiency reduces glucose utilization in experimental animals and humans (85-87). For example, in zinc-deficient animals [14C]-glucose incorporation into fatty acids was reduced by 75% in comparison to the control animals (88). Furthermore, this reduction in the utilization of glucose increased lipid catabolism since zinc-deficient animals rapidly consumed their triglyceride reserves, with consequent elevation in plasma free fatty acids (88,89).

IV - HORMONAL MEDIATION

GH SYNTHESIS AND SECRETION

GH is a peptide synthesized and secreted by the pituitary that reaches target tissues through the peripheral blood stream. Its regulation is mediated by somatostatin (an inhibitor) and by GH-RH (a stimulator). In turn, SM-C inhibits GH release, stimulating somatostatin synthesis and inhibiting GH-RH synthesis in the hypothalamus (34) (Fig.2).

The hypothalamic and pituitary concentrations of zinc are very high (90,91). The relationship between zinc, GH and animal growth supports the idea that zinc may be directly involved in the synthesis and action of GH. The first report published on this topic was that of LaBella et al. (91), who demonstrated that zinc at the concentration of 6 x 10^{-5} M stimulated GH secretion when bovine pituitary extracts were used. Later reports have confirmed this result. For instance, Palmiter et al. (92), working with transgenic mice, reported that zinc treatment increased the amount of MThGH mRNA about 170-fold, resulting in an increase in circulating hGH and SM-C as well. Acute or chronic zinc administration was later found to be able to elevate basal plasma GH levels in normal individuals (93), as well as basal or stimulated GH levels in children with zinc and growth deficiency (45,94). Conversely, there are reports in the literature demonstrating that zinc deficiency reduces blood GH levels both in animals and humans. For example, in Egypt, only 4 of 18 "retarded" boys had an appropriate rise in plasma GH during insulin hypoglycemia (87). In sexually mature and immature zinc-deficient rats, serum concentrations of GH were significantly more depressed than in ad libitum-fed control rats (95). The same findings were later obtained by other investigators using a zinc marginal diet, when food intake was only slightly or not reduced (96). Our GH-deficient patients responded satisfactorily to hGH administration (0.1 U/kg body weight given three times weekly) and their response was much greater when hGH was combined with zinc (70 mg ZnSO_{4}.7 H_{2}O, two times daily) (43,44). The patients were checked at 6-month intervals
and those who received zinc also presented improved appetite and elevation of serum zinc levels. The group treated with zinc alone did not show an improved growth rate, with mean values (± SD) equal to those observed during the pretreatment period. The results obtained are illustrated in Fig. 3. In addition to affecting the growth of newborns, infants and adolescents, zinc deficiency plays an important role in intrauterine growth. Studies have emphasized the nutritional importance of adequate maternal zinc nutrition for normal growth and development of the fetus. Hypotrophy has been found in different animal species, including humans (97). Many publications are available about maternal zinc status and human neonate birth weight, and some authors have found a significant correlation between these parameters (98).

**GH ACTION ON THE LIVER**

The effects of zinc on GH receptors in the liver have been previously investigated. Zinc potentiates the action of GH on SM-C synthesis, as well as the effects of SM-C on bone cartilage (Fig. 2). Zinc can also increase GH binding to other receptors such as those present in adipocytes (99).
OTHER HORMONES: ANDROGENS, THYROID HORMONES, INSULIN, AND VITAMIN D,

Zinc can influence hormones at several levels, including hormone synthesis, secretion, and peripheral activity. Conversely, hormones have been shown to influence zinc metabolism at several levels as well (100). Linear growth does not depend exclusively on the action of GH, but other hormones are also involved in the mechanisms of proliferation and maturation of bone epiphysis. The major anabolic or catabolic hormones involved in this process have a strong relationship with zinc (Fig.2).

ANDROGENS

Testosterone is a potent anabolic agent that increases weight and muscle mass and accelerates linear growth, stimulating chondrocyte proliferation (101). Testosterone enhances pituitary GH secretion (102) but its action on cartilage fully depends on the presence of GH (103), to such an extent that children with GH deficiency do not benefit from androgen treatment alone (104). On the other hand, when testosterone is administered together with GH, growth gain is superimposed to that obtained with GH alone (105). However, the clinical application of testosterone has the great disadvantage of accelerating epiphysis maturation, leading to a decrease of predicted adult stature (106). From a biochemical viewpoint, there is a direct relationship between blood levels of zinc and testosterone. The first reports on humans were characterized by retarded genital and secondary sexual development in all zinc-deficient male dwarfs (52,57), a clinical picture that was surprisingly reversed by zinc supplementation (86). This linear relationship between serum zinc and testosterone levels in boys with constitutional growth delay and familial short stature has also been observed by other authors (107). Healthy short children with retarded bone age and low hair zinc concentration increased the growth rate, GH, SM-C, and testosterone after oral zinc supplementation (45). The same results were obtained with patients with sickle-cell anemia (60,108). The changes in steroidogenesis provoked by zinc deficiency and causing primary hypogonadism may be explained by the inactivation of adenylate cyclase and gonadotropin receptors (109,110). This phenomenon was observed in females too. Halstead et al. (111), reported in the literature two women aged 19 and 20 years with growth deficiency and hypogonadism who became normal after zinc supplementation.

THYROID HORMONES

Thyroid hormone insufficiency in humans leads to impaired linear growth and delayed ossification of the skeleton. The role of thyroid hormones in cartilage growth and osseous maturation seems to be intimately related to growth hormone. GH synthesis and secretion by the pituitary is known to be
affected by thyroid hormones (112), so that hypothyroid patients may present a decreased GH secretion response and a decline in blood SM-C levels, with consequent changes in the cartilage growth plate (113,114). The importance of zinc for thyroid hormones resides in the fact that zinc reduces plasma TSH, T₃, T₄, and hypothalamic TRH levels in zinc-deficient rats (95,115,116) and impairs the extrathyroid conversion of T₄ to T₃ (115). In experimental human zinc deficiency, serum TSH, total T₃, and free T₄ declined significantly in humans (117). Furthermore, zinc may play a role in T₄ binding to nuclear receptors (118). Hypothyroid patients present reduced zinc absorption, hypozincemia and hypozincuria (119).

INSULIN

Insulin may function as a stimulator of growth, principally in the fetus. In postnatal life, hyper- and hypoinsulinism are frequently associated with greater or lesser growth in children (120,121). In vitro studies have shown that insulin can stimulate protein phosphorylation, protein and DNA synthesis in osteoblastic cells, including collagen, alkaline phosphatase, and osteocalcin (70,122,123). These cell growth mechanisms may be mediated by SM-C receptors (124,125). Linear growth potential has been reported to be limited by diabetic state. The most expressive example of this relationship between growth deficiency and insulin is seen in children and adolescents with insulin-dependent diabetes mellitus (126-128), most of whom have been reported to present hypozincemia and hyperzincuria (129). Total-body zinc clearance was significantly higher in diabetic patients than in the control subjects, and this parameter was inversely correlated with height velocity in the diabetic children (130). It should also be emphasized that zinc is fundamental for the synthesis, crystallization and peripheral action of insulin (84). Zinc has been found in high concentrations in the endocrine pancreas of many species (131) as well as in secretory granules (132), and in the insulin molecule (131), and has been shown to participate in the stabilization of proinsulin and insulin hexamers by forming complexes with them (133). In zinc-deficient rats, it has also been demonstrated that zinc affects insulin synthesis, secretion, and action since it can increase glucose tolerance (134), reduce beta cell degranulation (135), decrease histochemically detectable insulin (135), decrease serum insulin (136), and increase insulin degradation by the liver (137), as well as reduce tissue sensitivity to the action of insulin (134). Thus, zinc deficiency in association with disorders of carbohydrate, lipid and protein metabolism, certainly contributes to the installation of the delayed growth observed in these patients.

VITAMIN D,

Zinc concentration in bone is very high compared to other tissues, and for this reason zinc is considered to be an
essential component of the calcified matrix (138). Calcium metabolism is abnormal in zinc deficiency (139). Zinc-deficient infant monkeys present changes in endochondral bone mineralization very similar to human ricketts (62, 140). There are reports showing that zinc can act with vitamin D, for normal bone formation. For instance, zinc synergistically enhances 1,25(OH)\(_2\)D\(_3\)-stimulated bone metabolism, and this effect is based on stimulation of DNA synthesis in bone cells (141). The combination with zinc and PTH did not cause a synergistic increase (142). On the other hand, 1,25(OH)\(_2\)D\(_3\) enhances the accumulation of zinc and calcium in bone (143). Dipicolinate, a chelator of zinc, induces zinc deficiency in vitro and is being used as an important reagent in the study of bone metabolism (79). Conversely, a new zinc compound, β-alanyl-L-histidinate zinc, can stimulate bone formation and mineralization in weanling rats (144), and in the model of hind-limb hang (145). However, the effects of cholecalciferol and its most active metabolite on intestinal zinc absorption are contradictory. There are reports showing increased absorption (146), decreased absorption (147), or no effect (148). On the other hand, excessive amount of vitamin D, caused bone breakdown and increased the levels of zinc in blood (149).

ACKNOWLEDGMENTS

This work was supported in part by funding from FAPESP (86/2511-3). We thank Luciana Cristina Montes for her assistance in the preparation of the manuscript.

REFERENCES


6. Lorenson MY, Jacobs LS. Pituitary secretory granules contain glutathione and divalent cations. 7th International Congress of Endocrinology, Quebec City, Canada, 1984, Excerpta Medica Series, 652:1050 (Abstract


33. Salmon WD Jr, Daughaday WH. A hormonally controlled serum factor which stimulates sulfate incorporation by


58. Rothbaum RJ, Maur PK, Farrell MK. Serum alkaline phosphatase and zinc undernutrition in infants with


82. Henkin RI. Zinc dependent control of food intake, taste, and smell function. In Kirchgessner M, ed. Trace Elements in Man and Animals - III. Weihenstephan: Arbeitskresis


Hill DE. The effect of insulin on fetal growth. Semin


123. Craig RG, Rowe DW, Petersen DN, Kream BE. Insulin increases the steady state level of α-1(I)procollagen mRNA in the osteoblast-rich segment of fetal rat calvaria. Endocrinology 1989; 125: 1430-1437.


Accepted for publication September 26, 1994.