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An overview on the correlation between blood zinc, zinc intake, zinc supplementation and bone mineral density in humans

Una visión general sobre la correlación entre el zinc en la sangre, la ingesta de zinc, la suplementación de zinc y la densidad mineral ósea en los seres humanos

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ABSTRACT. Introduction: In case of zinc (Zn) deficiency, this mineral becomes a nutrient limiting muscle and bone synthesis. The study in humans on zinc and bone health are few and no reviews have been published on this topic. So, the aim of this narrative review was to consider the state of the art on the correlation between blood zinc, daily zinc intake, zinc supplementation and bone mineral density. **Material and methods:** A narrative review was performed. **Results:** This review included 16 eligible studies: eight studies concern Zn blood; three studies concern Zn intake and five studies concern Zn supplementation. **Conclusion:** Blood zinc levels seem to be lower in subjects with pathology related to bone metabolism. Regarding daily zinc intake, a high proportion of the population, more than 20%, seems to be at risk of having inadequate zinc intake. The literature suggests that an insufficient zinc intake (less than 3 mg/day) could be a risk factor for fractures and for development of osteopenia and osteoporosis. Zinc supplementation (40-50 g/day) could have beneficial effects on bone health in terms of maintaining bone mineral density and faster healing in the event of fractures, with even better results in situations of reduced intake zinc through food.

RESUMEN. Introducción: En caso de deficiencia de zinc, se limitará la síntesis muscular y ósea. Los estudios en humanos sobre zinc y salud ósea son pocos y no se han publicado comentarios sobre este tema. Por lo tanto, el objetivo de esta revisión narrativa es considerar el estado de la técnica sobre la correlación entre el zinc en la sangre, la ingesta diaria de zinc, la suplementación de zinc y la densidad mineral ósea. **Material y métodos:** Se realizó una revisión narrativa. **Resultados:** Esta revisión incluyó 16 estudios elegibles: ocho se refieren al zinc en sangre; tres estudios se refieren a la ingesta de Zn y cinco estudios se refieren a la suplementación de Zn. **Conclusión:** Los niveles de zinc en sangre parecen ser más bajos en sujetos con patología relacionada con el metabolismo óseo. En cuanto a la ingesta diaria de zinc, una alta proporción de la población, más de 20%, parece estar en riesgo de tener una ingesta inadecuada de zinc. La literatura sugiere que una ingesta insuficiente de zinc (menos de 3 mg/día) podría ser un factor de riesgo de fracturas y para el desarrollo de osteopenia y osteoporosis. La suplementación con zinc (40-50 g/día) podría tener efectos beneficiosos sobre la salud ósea para mantener la densidad mineral ósea y una curación más rápida en caso

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Keywords: Zinc, bone, dietary supplementation, bone mineral density, nutrients.

de fracturas, con resultados aún mejores en situaciones de reducción de la ingesta de zinc a través de los alimentos.

Palabras clave: Zinc, hueso, suplementación dietética, densidad mineral ósea, nutrientes.

Introduction

Zinc is an essential component for our body. Over 85 percent of body zinc total is found in skeletal muscles and bones,[1] while zinc contained in plasma represents only 0.1 percent of the total and its concentration, strictly regulated, varies from about 10 to 15 µmol/l. Zinc plasma concentrations are maintained without significant changes even when zinc intake has decreased or increased, unless these changes in intake are severe and prolonged.[2]

It is widely distributed in food, but the best food sources are meat, eggs, fish, cheeses and cereals.[3]

The RDA for adult men is 8 mg per day while for adult women it is 11 mg per day.[4]

Since zinc in foods is not present as a free ion, bioavailability depends on the extent of digestion. With digestion zinc has the opportunity to bind to exogenous and endogenous components in the intestinal lumen (peptides, amino acids, nucleic acids and other organic acids and inorganic anions) to be absorbed through a transcellular process in fasting, which is the site with the higher transport speed.[5,6,7]

The homeostatic regulation of zinc metabolism is mainly achieved through a balance between absorption and secretion of endogenous reserves that involve adaptive mechanisms programmed by the intake of zinc in the diet.[1]

These losses can vary from less than 1 mg/day with a low zinc diet to more than 5 mg/day with a zinc rich diet, to underline the important regulatory role that the intestinal tract plays in zinc homeostasis.[1]

Zinc concentrations decreased rapidly in humans fed a diet with a very low zinc content and containing phytates.[8]

Zinc is a fundamental constituent of various enzymes that play a role in maintaining the structural integrity of proteins and in regulating gene expression; there are almost 100 specific enzymes that depend on zinc for catalytic activity.[4]

Its biological functions can be divided into three categories: catalytic, structural and regulatory.[9]

Among the many functions performed by zinc (for example the maturation of the cells of the immune system and the prevention of lipid peroxidation by reducing the formation of free radicals) also includes the formation of bones and muscles where, in case of deficiency, it becomes a nutrient limiting this synthesis. It also has an important role in stimulating the synthesis of alkaline phosphatase in osteoblasts.[10]

Zinc and bone development: in vitro and in animal model studies

In vitro it has been shown that the proliferation of osteoblastic cells has been stimulated after zinc culture with an inhibitory effect on the formation of osteoclastic cells.[11,12,13,14,15,16] Furthermore, always *in vitro*, zinc modulates the anabolic effect of 1,25-dihydroxyvitamin D3 or of estrogens on bone metabolism *in vitro*.[17] The same anabolic effect is confirmed in animal studies.[18,19] as well as the role of osteoblastic stimulation and osteoclastic inhibition is confirmed.[20,21,22] Also in animals it has been shown that zinc deficiency seems to interfere with bone metabolism with consequent reduction of bone formation[23] and causes criticalities in bone consolidation in the spine;[24] in another study it is highlighted how zinc deficiency can lead to a reduction in serum calcium concentration and to an increase in parathyroid hormone with subsequent bone fragility.[25]

Despite this background, the study in humans on zinc and bone health are few and no reviews have been published on this topic. So, the aim of this narrative review was to consider the state of the art on the correlation between blood zinc, daily zinc intake, zinc supplementation and bone mineral density.

Material and methods

The present narrative review was performed following the steps by Egger et al.[26] as follows:

1. Configuration of a working group: three operators skilled in clinical nutrition (one acting as a methodological operator and two participating as clinical operators).
2. Formulation of the revision question on the basis of considerations made in the abstract: «the state of the art on the correlation between human blood zinc concentrations, daily zinc intake with food, zinc supplementation and bone mineral density».
3. Identification of relevant studies: a research strategy was planned on PubMed (Public MedLine run by the National Center of Biotechnology Information [NCBI] of the National Library of Medicine of Bethesda [USA]) as follows: (a) Definition of the keywords (zinc, bone health, humans, intake, supplementation, bone mineral density), allowing the definition of the interest field of the documents to be searched, grouped in quotation marks

(«…») and used separately or in combination; (b) use of: the Boolean (a data type with only two possible values: true or false) AND operator, that allows the establishments of logical relations among concepts; (c) Research modalities: advanced search; (d) Limits: time limits: papers published in the last 30 years; humans; adults; languages: English; (e) Manual search performed by the senior researchers experienced in clinical nutrition through the revision of articles on the state of the art on the correlation between human blood zinc concentrations, daily zinc intake with food, zinc supplementation and bone mineral density.

4. Published in journals qualified in the Index Medicus.
5. Analysis and presentation of the outcomes: we create paragraphs about the state of the art on the correlation between human blood zinc concentrations, daily zinc intake with food, zinc supplementation and bone mineral density, and the data extrapolated from the «revised studies» were collocated in tables; in particular, for each study we specified the author and year of publication and study characteristics.
6. The analysis was carried out in the form of a narrative review of the reports. At the beginning of each section, the keywords considered and the type of studies chosen are reported. We evaluated, as is suitable for the narrative review, studies of any design which considered the the state of the art on the correlation between human blood zinc concentrations, daily zinc intake with food, zinc supplementation and bone mineral density. *Figure 1* shows the flow chart of literature research.

Results

Blood zinc concentrations in relation to bone metabolism

This research was conducted based on the keywords: «zinc» AND «zinc blood concentrations» and «bone» and «humans». For the present review we have analyzed a total of eight studies: five cross-sectional studies, two case-control studies and one clinical trial.

The results of these eight studies have been shown in *Table 1*.

Data from three studies, from 1983 to 2007, agree on a reduction in zinc values in osteoporotic women compared to healthy controls. In 1983 Atik took into consideration 22 women aged between 48 and 86 and found significantly lower blood zinc values in women with osteoporosis compared to healthy controls.[27]

In a study from the early 2000s, the same two groups of postmenopausal women (70 with osteoporosis and 30 healthy) were compared to the same conclusion as the previous study.[28]

In 2007 Mutlu et al. added osteopenic subjects to the comparison, for a total of 120 menopausal women divided into three groups (osteoporotic, osteopenic, healthy), concluding that the blood zinc levels in women with osteoporosis were significantly lower than osteopenic and healthy women, and which in turn women with osteopenia had significantly decreased blood zinc levels compared to healthy controls.[29] In the 2013 study by Okyay, zinc

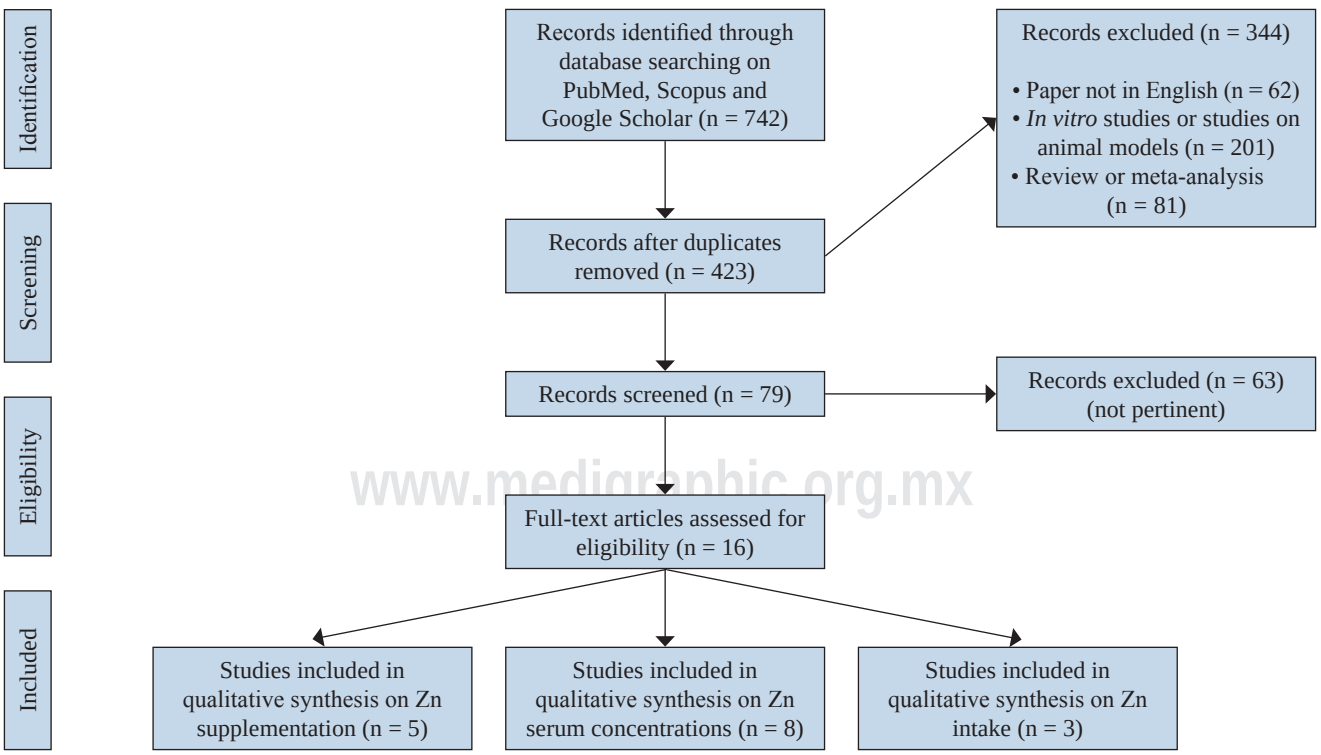

Figure 1: Flow chart of literature research.

Table 1: Studies cover blood zinc levels.

First author, year	Study design	Setting	Inclusion criteria	Exclusion criteria	Number of subjects (M-F)	Micronutrient serum concentration osteoporosis	Micronutrient serum concentration osteopenia	Micronutrient serum concentration normal	Micronutrient serum reference value	Primary outcomes	Results
Gur A, 2002	Clinical trial	Department of Physical Therapy and Rehabilitation of Dicle University Hospital	Women were eligible for our study if they were 50 years of age or older and in good general health as determined by medical history and routine clinical blood analysis (complete blood counts and differential count)	Women were excluded if they (1) had used any drug or had any disease or condition known to affect bone or calcium metabolism; (2) had taken corticosteroid medications during the previous 6 months; (3) had a history of chronic renal, hepatic, or gastrointestinal disease or lumbar compression fracture; or (4) had evidence of collapsed or focal vertebral sclerosis	100 postmenopausal women: 70 osteoporotic and 30 non-osteoporotic	Zinc serum level : 0.61 ± 0.425(test pre supplementation of calcitonin)		Zinc serum level : 1.22 ± 0.31 (test pre supplementation of calcitonin)		To determine whether the mineral profile was different between 70 osteoporotic and 30 non-osteoporotic postmenopausal women and to evaluate the efficacy of calcitonin therapy for 6 months on these trace minerals in postmenopausal osteoporotic women	Zn levels in the serum of patients with postmenopausal osteoporosis were lower than those in the control group
Okyay E, 2013	Cross-sectional study	Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gyne-cology at Dokuz Eylul University School of Medicine, Izmir, Turkey	Postmenopausal women between age 45 and 80 were included inthe study	Women with a history of drug abuse or alcohol con-sumption (to drink at least ≥ 2 days per week z), and highly intake ofcaffeinated coffee (> 2 cups per day), laboratory tests or radiogra-phy of any bone metabolism disorder were excluded. Any otherdisease or drugs that effect bone metabolism were excluded	728 postmenopausal women	Women at 45-59 years: (p value < 0.05) - L1-L4 OP Zinc (µg/ml): 82.6 ± 21.7 Women at 60-80 years: (p value < 0.05) - L1–L4 OP Zinc (µg/ml): 84.2 ± 17.7		Women at 45-59 years: (p value < 0.05) - L1-L4 non OP Zinc (µg/ml): 88.1 ± 15.8 Women at 60-80 years: (p value < 0.05)- L1-L4 non OP Zinc (µg/ml): 90.3 ± 14.9		To determine the relationship between serum main minerals and postmenopausal osteoporosis.	low serum levels of Zn were independent risk factors for development of OP especially in early menopausal period
Mutlu M, 2007	Cross-sectional study	Orthopaedics Department of the Erciyes University Medical Faculty	Women post-menopausal if they were > 55 years of age and there had been no menstruation for ≥ 6 months prior to entry into the study.		120 post-menopausal women	Zinc (mg/l) 0.47 ± 0.1	Zinc (mg/l) 0.63 ± 0.09	Zinc (mg/l) 0.82 ± 0.13		To investigate the changes of Zinc in osteoporotic, osteopenic and normal postmenopausal women	Mean concentration of zinc were significantly lower in osteoporotic women than in both osteopenic women and normal women.

Continue Table 1: Studies cover blood zinc levels.

First author, year	Study design	Setting	Inclusion criteria	Exclusion criteria	Number of subjects (M-F)	Micronutrient serum concentration osteoporosis	Micronutrient serum concentration osteopenia	Micronutrient serum concentration normal	Micronutrient serum reference value	Primary outcomes	Results
			Women 50-55 years of age were classified as postmenopausal if their plasma follicle stimulating hormone (FSH) level was > 50 IU/l and their plasma estradiol concentration was < 100 pmol/l								In addition, zinc concentration in osteopenic women were significantly lower than in normal women
Arikan DC, 2011	Case–control study	Department of Gynecology and Obstetrics of the Medical Faculty of Kahramanmaras Sutcu Imam (Kahramanmaras, Turkey)	Natural menopause for more than 6 months	Surgical menopause and secondary osteoporosis or other medical conditions that might affect bone metabolism or trace element status such as kidney disease, diabetes mellitus or drug use (e.g. diuretics). Patients who were treated with bisphosphonates, calcitonin, anabolic steroids, hormone replacement therapy, calcium or vitamin D up to six months before the investigation were also excluded	107 postmenopausal women divided into three groups according to BMD; 35 healthy 37 osteopenic and 35 osteoporotic	Zn (µg/dl) 106.25 ± 36.45	Zn (µg/dl) 116.48 ± 35.46	Zn (µg/dl) 127.53 ± 45.04		To investigate serum zinc (Zn) levels in postmenopausal women with osteoporosis, osteopenia and in healthy controls, and to determine the relationship between Zn and bone mineral density (BMD)	Plasma Zn levels were higher in the healthy group when compared to the osteopenic and osteoporotic groups but the difference was not statistically significant ($p > 0.05$)
Mahdavi-Roshan, 2015	Cross-sectional study	Rheumatology clinic in Tabriz, Islamic Republic of Iran	women > 50 years old who had been no menstruation for ≥ 6 months prior to entry into the study, having no history of hormone replacement therapy, other bone disease, kidney stones, endocrine disorders or any medical conditions that could influence on the mineral status	use of mineral supplements, having history of hormone replacement therapy, bone disease, kidney stones, endocrine disorders or any medical conditions that could influence on the mineral status.	A total of 51 post-menopausal women		Serum Zinc (µg/dl) 70.44 ± 4.5	Serum Zinc (µg/dl) 63.3 ± 4.8		Investigate and compare the mineral status between osteopenic and osteoporotic postmenopausal women in Tabriz, Islamic Republic of Iran.	No statistically significant differences were observed between the osteopenic and osteoporotic groups with respect to serum levels of zinc

Continue Table 1: Studies cover blood zinc levels.

First author, year	Study design	Setting	Inclusion criteria	Exclusion criteria	Number of subjects (M-F)	Micronutrient serum concentration osteoporosis	Micronutrient serum concentration osteopenia	Micronutrient serum concentration normal	Micronutrient serum reference value	Primary outcomes	Results
Atik OS, 1983	Case–control study	Department of Orthopedic Surgery of Hacettepe University Hospital.	Osteoporosis for cases and non osteoporosis for controls		22 patients (with senile Osteoporosis and controls)	Zinc in Serum (µg/dl) 53.5 ± 2.8		Zinc in Serum (µg/dl) 75.9 ± 4.1		to determine the zinc ion levels in serum and bone tissue of patients with senile osteoporosis.	zinc levels in serum of the patients with senile osteoporosis were lower than those of the control group
Relea P, 1995	Cross-sectional study	Clinic of the Rheumatology Unit of the University Hospital “Princepe de Asturias”, Madrid	No pharmacological treatment, haven’t any condition that might affect calcium metabolism, such as liver disease , diabetes or renal dysfunction		60 postmenopausal women (30 controls and 30 with osteoporosis)	Zinc serum: (mg/dl) 72.7 ± 9.9 [Urinary zinc (µg/g Cr): 5.5 ± 1.9]		Zinc serum: (mg/dl) 74.9 ± 18.4 [Urinary zinc (µg/g Cr): 4,0 ± 2.0]		To evaluate the correlation between the concentrations of plasmatic zinc and urinary zinc with bone mass	Plasma zinc levels did not differ between the women with postmenopausal osteoporosis and the healthy postmenopausal controls, but urinary zinc excretion was higher (p=0.002) in the woman with postmenopausal osteoporosis
LIU SZ, 2009	Cross-sectional study	Xi’an urban area	45 to 65-year-old females of Chinese Han Nationality who had lived in the Xi’an urban area more than 10 years, and had been in natural menopause for more than half a year, with no diseases which might influence bone metabolism, no other severe chronic diseases which needed long-term therapy, no gynecological diseases which could influence the secretion of female sex hormones, and no hormone drugs intake and osteoporosis treatment six months before investigation		290 women	Zn serum (mg/l): 0.9168 ± 0.2557	Zn serum (mg/l): 0.9181 ± 0.3177	Zn serum (mg/l): 0.9345 ± 0.2726		to investigate the correlation between serum macroelement and trace element contents and bone mineral density (BMD) as well as the occurrence of osteoporosis	There exist significant correlations between the contents of serum elements such as calcium, phosphonium, sodium, potassium, magnesium, zinc, iron, copper, and selenium, but no significant differences in these elements contents between the osteoporosis group, osteopenia group, and healthy group

deficiency is directly defined as an independent risk factor for developing osteoporosis and in the researh it is specified how this deficiency is related to lumbar osteoporosis both in the 45-59 years and 60-80 age groups years.[30]

At an intermediate level there are two other studies, from 2009 and 2011. Liu et al in 2009 define the existence of a difference in blood zinc levels between osteoporotic, osteopenic and healthy women, but that however this difference is not statistically significant.[31] Two years later, in 2011 Arikan reaches the same conclusion by comparing the same three groups.[32]

Unlike these studies, in 1995 Relea et al, found statistically significant differences between osteoporotic and healthy women, not in the blood zinc levels (which are not different in this study), but in the urinary zinc excretion which is increased in a way significant in women with osteoporosis compared to healthy controls.[33]

The study by Mahdavi-Roshan published in 2015 is totally opposite, where there is no difference in plasma zinc levels in the comparison between women with osteopenia and healthy women.[34]

Zinc intake in relation to bone metabolism

This research was conducted based on the keywords: «zinc» and «zinc intake» and «bone» and «humans».

For the present review we have analyzed a total of three studies: two cohort studies and one cross-sectional study.

The results of these three studies have been shown in *Table 2*.

The daily zinc intake with food in relation to bone metabolism is analyzed in three studies, two of which substantially agree in the results.

In 1998, Elmstahl and colleagues study a large male population, consisting of 6,576 Swedish men aged between 46 and 68 years, concluding that low zinc intakes are a risk factor for fractures and that about 20% of the population studied took on inadequate quantities.[35] The same results emerged from study by Hyun's 2004 conducted on 396 men, where zinc intake levels are significantly reduced in subjects with osteoporosis.[36]

Different results emerge from the 2015 Mahdavi-Roshan study on 51 menopausal women, where no difference in zinc intake was observed between women with osteoporosis, osteopenia and healthy; however, the study shows a general zinc intake lower than the values recommended by the RDA.[34]

Zinc supplementation in relation to bone metabolism

This research was conducted based on the keywords: «zinc» and «zinc supplementation» and «bone» and «humans».

For the present review we have analyzed a total of five studies: three double-blind placebo controlled trials, one clinical trial and one randomized controlled trial.

The results of these five studies have been shown in *Table 3*.

As regards zinc supplementation, both alone and in associations with other nutrients, all the studies identified are substantially in agreement in defining beneficial effects for the bone *(Table 3)*.

Already in 1974, in a study carried out on adolescents for 18 months, comparing an integration with 40 mg of zinc against placebo, there was an increase in bone age and bone development in the integrated group, with better results especially after 12 months of integration.[37]

The effect of a zinc-only supplement on 60 men and women with fractures is also evaluated in the study by Sadighi in 2008; the subjects were divided into two groups destined to receive 220 g of zinc sulphate (corresponding to 50 g of zinc) or placebo and the final results show that in the group of subjects treated with zinc there was a faster healing of the fracture and significant change in bone callus formation 60 days after fracture.[10]

In other three papers the effects of zinc in association with other nutrients are studied.

The study by Strause in 1994 involved healthy postmenopausal women divided into four groups (calcium supplement + micronutrient supplement, calcium supplement + micronutrient placebo, calcium placebo + micronutrient supplement, calcium placebo + micronutrient placebo), where the group that received both calcium and micronutrient supplementation, including zinc, maintained lumbar bone mineral density with a significant difference compared to the group that received only placebo; the remaining two groups positioned themselves at an intermediate level, without showing significant differences with the treated group or with the placebo group.[38]

Subsequently, in 2011, the study by Nielsen and colleagues compares calcium supplementation versus calcium supplementation associated with zinc and copper in a group of menopausal women; the results confirm that zinc could bring beneficial effects on bone health only if the intake of zinc with diet is reduced (< 8 mg per day), while there were no significant beneficial effects with adequate zinc intake.[39]

Always the same author, a few years earlier, wanted to check whether zinc supplementation could lead to changes in copper metabolism such as to lead to changes in bone turnover. The results did not lead to defining significant changes in copper metabolism even with high zinc supplements (53 mg per day), while this supplement led to an excessive excretion of magnesium. Low doses of zinc (3 mg per day) have instead caused unwanted changes in circulating osteocalcin and calcitonin.[40]

Conclusion

We can define that blood zinc levels seem to be lower in subjects with pathology related to bone metabolism. The literature suggests that an insufficient daily intake of

Table 2: Studies involving daily zinc intake in humans.

First author, year	Study design	Setting	Inclusion criteria	Exclusion criteria	Number of subjects (M-F)	Lowest quintile intake/RDA or EAR	% subject in lowest quintile intake/% subject < RDA or EAR	Highest Quintile intake	% subject in highest quintile intake	Primary outcomes	Results
Hyun, 2004	Cohort study	All surviving from the original Rancho Bernardo cohort who still resided in southern California	≥ 45 years		396 men					To examine the independent association between dietary zinc and plasma zinc and the association of each with bone mineral density (BMD)	Age- and BMI-adjusted dietary and total zinc intakes were significantly lower in the men with osteoporosis at the spine than in men without osteoporosis at that location
Elmstahl, 1998	population-based prospective cohort study	city of Malmo, in the southern part of Sweden	Aged 46-68 years		6576 men	The intakes in the lowest quintiles were 10 mg for zinc Low zinc intake showed a threshold with increased fracture risk in the lowest decentile, corresponding to a zinc intake of 9.5 mg daily	A high proportion of the population, more than 20%, seems to be at risk of having inadequate dietary habits with respect to zinc	A lower fracture risk was noted in men with zinc intake in the second quintile [RR = 0.58 (0.34-0.99)] and fifth quintile [RR = 0.47 (0.25-0.89)] compared with the lowest decentile		To determine dietary risk factors for fracture in men aged 46-68 years	Inadequate intakes of zinc are important risk factors for fracture
Mahdavi-Roshan, 2015	Cross-sectional study	Rheumatology clinic in Tabriz, Islamic Republic of Iran	women > 50 years old who had been no menstruation for ≥ 6 months prior to entry into the study, having no history of hormone replacement therapy, other bone disease, kidney stones, endocrine disorders or any medical conditions that could influence on the mineral status	Use of mineral supplements, having history of hormone replacement therapy, bone disease, kidney stones, endocrine disorders or any medical conditions that could influence on the mineral status	A total of 51 post-menopausal women	The mean dietary intake (and percent from RDA) of zinc in post-menopausal women with low bone density was 3.82 ± 0.19 mg/day (48 ± 2.41% RDA)				To investigate and compare the mineral status between osteopenic and osteoporotic postmenopausal women in Tabriz, Islamic Republic of Iran	The mean dietary intake of zinc was significantly lower than recommended dietary allowance (RDA). No statistically significant differences were observed between the osteopenic and osteoporotic groups with respect to dietary intake of zinc

Table 3: Studies regarding zinc supplementation and bone metabolism.

First author, year	Study design	Setting	Inclusion criteria	Eclusion criteria	Intervention	Parallel treatments	Number of subjects (M-F) If only	Duration of the intervention	Primary outcomes	Secondary outcomes	Results
Sadighi A, 2008	Randomized, double blind, placebo controlled clinical trial	Shohada Hospital of Tabriz, Iran	Men and women, aged 20-50 years old with traumatic long bone fracture	No history of osteoporosis, osteoarthritis, kidney stones, diabetes, and other endocrine disorders.taking any medication or supplementation known to influence bone metabolism or zinc status	One capsule of 220 mg zinc sulfate contain 50 mg zinc	Control group receiving placebo contain starch	60 (39 M, 21 F)	60 days	Determine the effect of zinc supplementation on fracture healing	Determine the relation between callus formation with zinc and alkaline phosphatase activity in serum	Significant change in callus formation if the group will compare to the control group after 60 days (Figure 3), and fracture healing was faster in the supplement group than control group
Nielsen FH, 2011	Double-blind, placebo-controlled design		Postmenopausal women aged 51-80 years, BMI ≤ 32 kg/ m^2, bone mineral density not more than 2·5 standard deviations below that for young adults; no collapsed/compressed vertebrae determined by using dual-energy X-ray absorptiometry (DXA); history of no menses for at least five years; and a circulating folliclestimulating hormone concentration, 40 IU/l Eligible applicants were invited to an information meeting	Use hormone replacement therapy for one year before the study, use medications that interfere with Ca absorption, have thyroid, liver and kidney disease	600 mg Ca supplement plus a 2 mg Cu (copper gluconate) and 12mg Zn (zinc gluconate) supplement	Supplement containing 600 mg Ca plus a maize starch placebo	649 M	2 years	Determine whether increased Zn intakes would reduce the risk for bone loss		The findings indicate that Zn supplementation may be beneficial to bone health in postmenopausal women with usual Zn intakes < 8,0 mg/d but not in women consuming adequate amounts of Zn
Strause L, 1994	Double-blind, placebo-controlled trial	San Diego greater Metropolitanarea	> 50 y old and in good general health	A positive Pap smear or mammogram during the previous year, any disease or condition known to affect bone or calcium metabolism, a history of chronic renal, hepatic or gastrointestinal disease, evidence of collapsed or focal vertebral sclerosis	Groups 2) placebo calcium, active trace minerals,- groups 3) active calcium, placebo trace minerals; and groups 4) active calcium, active trace minerals Subjects received placebo or 1,000 mg elemental calcium/d in the form of calcium citrate malate	Groups: 1) placebo calcium, placebo trace minerals	59 F	2 years	Evaluate the impact of supplementary calcium with and without the addition of a combination of copper, manganese and zinc on spinal bone loss in healthy older post		Older postmenopausal women supplemented with 1000 mg of calcium, 15 mg of zinc, 5 mg of manganese, and 2.5 mg of copper maintained spinal bone density and differed significantly from a placebo group that lost

Continue Table 3: Studies regarding zinc supplementation and bone metabolism.

First author, year	Study design	Setting	Inclusion criteria	Esclusion criteria	Intervention	Parallel treatments	Number of subjects (M-F)	Duration of the intervention	Primary outcomes	Secondary outcomes	Results
					Each active supplement contained 15.0 mg of zinc as sulfate salt, 2.5 mg of copper, and 5.0 mg of manganese as gluconate salts				menopausal women		bone density Bone losses in the groups supplemented with trace mineral alone and with calcium alone were intermediate, but not significantly different from loss for either the placebo group or the group receiving calcium plus trace minerals
Nielsen FH, 2004	Randomized controlled trial	The metabolic unit of the Grand Forks Human Nutrition Research Center, Grand Forks, ND, USA	No underlying disease				25 postmenopausal women	200 days	To determine whether moderately high or low intakes of zinc adversely affect the copper status of postmenopausal women to result in unfavorable changes in calcium and magnesium metabolism and other indicators of bone turnover		Low dietary zinc (45.9 mmol/day; 3 mg/day) apparently resulted in undesirable changes in circulating calcitonin and osteocalcin The moderately high intake compared to the low intake of zinc increased the excretion of magnesium in the feces and urine, which resulted in a decreased magnesium balance
Ronaghy 1974	Clinical trial	Southern Iran	13-year-old prepubertal schoolboys		Grupo C) Zinc carbonate 40 mg + supplement of egg-white protein (10 g daily), corn oil, minerals, and vitamins	Grupo A) placebo Grupo B) supplement of egg-white protein (10 g daily), corn oil, minerals, and vitamins	49 boys 13-year-old prepubertal village schoolboys	18 months	To learn whether these failures could have been in part the result of administration of insufficient quantities of zinc as a dietary supplement		Significantly increased heights, weights, and bone ages occurred in those receiving the supplementary zinc During the 2nd year, bone development of the zinc-supplemented group surpassed that of the other groups by a substantial and statistically significant margin

zinc through nutrition (less than 3 mg/day) could be a risk factor for fractures and for the development of osteopenia and osteoporosis. A high proportion of the population, more than 20%, seems to be at risk of having inadequate dietary habits with respect to zinc. The supplementation of zinc in an amount equal to 40-50 g, on the other hand, could have beneficial effects on bone health in terms of maintaining bone mineral density and faster healing in the event of fractures, with even better results in the situation of reduced zinc intake through food.

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