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MACRO- AND MICROELEMENTS AND THEIR PHYSIOLOGICAL IMPORTANCE FOR THE BONE MINERAL DENSITY

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Abstract: The form, the interorgan distribution, and the functions of macro- and microelements in a number of diseases of the human body have not yet been completely established. Studies are still being conducted in order to define their concentration, as well as to diagnose the level of oxidative stress. The aim is for them to be used for the prophylaxis and for the identification of new aspects of the aetiology and pathogenesis of osteoporosis.
The aim of this study is to establish the changes in the concentrations of the macro- and microelements that participate in bone homeostasis, and their impact on bone density. A concise review was done on the factors affecting the calcium-phosphorus metabolism, and some micronutrient reactions.. The physiological functions of certain essential microelements related to the onset of osteoporosis, their optimal serum levels in healthy patients, and their necessary daily intake were also reviewed. Contradictory results of studies on these microelements in patients with reduced bone density were highlighted. The severe deficit of macroelements Ca and P and the impaired metabolism of bioelements Zn, Cu, Mg and Fe have and unfavourable effect on bone health and can lead to an array of diseases and chronic conditions. Establishing the toxicity, role and mechanism of these metals is a necessary step for the diagnosing, treatment and prophylaxis of osteoporosis. The contradictory results from the experimental trials on serum levels of microelements and element reactions require many more trials to be conducted in order to establish their physiological benefits, the neutralising of oxidative stress, and the reduction of bone resorption.
This study presents the results from the atom-absorption analysis of the concentration of certain bone density-influencing microelements Zn and Cu in menopausal women over the age of 35 years from the district of the town of Pleven, with proven osteopenia or osteoporosis, and in a control group menopausal women with normal bone density. The vitamin D_3 levels were used as a biochemical were patients with secondary osteoporosis due to other diseases or medications. The onset and severity of osteoporosis depended solely on the patients' lifestyles.
Keywords: Macro- and microelements, physiological functions, bone

1. INTRODUCTION

Osteoporosis is a topmost socio-economic disease with severe psychological and economic consequences not only for the affected individuals, but also for their families and society as a whole. In the European Union, annual expenditures for osteoporotic fractures aggregate 37 billion euro. As defined by the World Health Organisation, osteoporosis is a generalised skeletal disorder of low bone mass (thinning of the bone) and deterioration in its architecture, causing susceptibility to fracture. The impaired bone strength, which is at the core of osteoporosis, too often is asymptomatic until a fracture occurs. Most commonly, fractures affect the thoracic and lumbar vertebrae (1.4 mln people per annum), and the femoral neck (1.6 mln people per annum) [1]. The former condition

deteriorates the quality of life; while recovery from the latter is a prolonged and difficult process – regaining of independence and mobility is only possible after a minimum of two months, given that the patient survives the surgical procedure. Too often, however, femoral neck fractures have fatal outcomes. This is due to the high rates of postoperative mortality, especially in elderly patients, due to the changes in their myocardial vessels regardless of their satisfactory pre-operative health status [2].

The loss of bone mass is more commonly observed in women. Typically, the onset is at the age of 40, with the beginning of menopause. It also affects men over the age of 50 years old, due to the oncoming testosterone deficit. Every fifth man over 50 suffers an osteoporotic fracture [3].

To date, there are a number of known risk factors for the development of osteoporosis. Its pathogenesis is well-studied, and there are a number of medications available for its pathogenetic treatment. Yet, the frequency of this disease and of its complications is still high – there are over 200 mln affected individuals worldwide, and 9 mln osteoporotic fractures per annum [4]. One of the main reasons for these statistics is probably the late diagnosing of this disease, due to its asymptomatic course. One cannot, however, ignore the presence of yet-unknown pathogenetic and lifestyle risk factors which contribute to the difficulty in controlling this disease and its complications.

There is strong evidence for the link between the daily intake of certain macroelements such as calcium and phosphorus and the risk of osteoporosis development. A number of trials have indicated that this risk is increased when stably low serum levels of calcium and phosphorus are maintained. This is not the case with the serum levels of certain microelements. In recent years, a number of researcher teams have directed their research towards studying these links.

The mechanism for achieving a better bone metabolism and for improving physical impairments is yet unclear. Trace minerals such as Ca, Mn, Cu, and Zn are important precursors for the biological provess of bone health [5].

The serum levels of copper, magnesium, zinc and calcium was studied in postmenopausal women with osteoporosis (n = 23) and osteopenia (n = 28). Considerably lower levels of zinc (P = 0.001) and copper (P = 0.05) were detected, and in 40.4% of them lower than normal serum magnesium levels were detected. No statistically significant differences in serum levels were detected among osteoporotic patients. Due to the significant role of these minerals for the bone health and their insufficiency in postmenopausal women with low bone density, the addition of magnesium, calcium, zinc and possibly copper to their diet is recommended [6].

Another trial included 50 participants (men and women with mean age 47.5 ± 5 years) with severe dental wear. Enamel biopsies showed reduced copper contents, which the authors associated with reduced mineral density of the spine. Copper deficit was seen as a potential factor for bone demineralization and dental wear [7].

Conflicting results were obtained from the measuring of serum levels of zinc and copper in 135 Iranian women. The mean levels of copper and zinc were 1.168 ± 0.115 and 1.097 ± 0.091 μg/mL in the control group (n = 51, T-score ≥ -1); 1.237 ± 0.182 and 1.127 ± 0,176 μg/mL in patients with 1> T-score > -1.7; 1.463 ± 0, 174 and 1,327 ± 0.147 μg/mL in patients with T-score < -1.7. In this trial, higher serum levels of zinc and copper were measured in patients with reduced bone density than in the control group participants, even though the differences were insignificant [8].

The ratios between the levels of biogenic elements which are of metabolic importance are not yet fully understood. There are trials indicating a negative effect of high intakes of calcium and phosphorus (even from milk) on zinc absorption has been demonstrated in adults and premature infants as well as in animal models of human nutrition. The potential mechanisms by which calcium interferes with zinc absorption include competition for a divalent cation channel across the brush border membrane or calcium-stimulated excess loss of endogenous zinc. This type of interaction can be achieved in calcium to zinc ratio of 20:1 (in weight) to ≥ 50:1. The serum level of zinc is an important factor for bone health, as 29% of the zinc in the body is part of the bone mineral and is a cofactor for a number of enzymes that participate in bone metabolism and collagen degradation.

To date, there are no reliable guidelines for defining the necessary amounts of magnesium in patients who are taking calcium supplements; however, it is generally accepted that a lack of magnesium is one of the most important factors for bone demineralization. A ratio of 4:1 or lower (in weight) of calcium: magnesium is recommended. Although the risks and benefits of this way of supplementing are yet to be appraised, it is unlikely that it can lead to side effects. Consuming excessive levels of magnesium supplements can undoubtedly lead to diarrhea, and in highly excessive levels it can lead to neurological and cardiac toxicity [9].

In a one-year continuous measuring of serum zinc, magnesium, iron, and copper in 21 patients with primary osteoporosis treated with the anti-resorption drug denosumab it was found that denosumab can improve the metabolism of Zn and Fe, but not of Mg. The authors recommended taking magnesium supplements. The serum levels of Cu did not change until the 8th month of the trial, but it decreased in the 10th and 12th months [10].

Hence, during continuous treatment with medications affecting bone resorption, it is not enough to do a single measurement of the serum microelements, but it is necessary to follow the inter-mineral interactions and to establish the longterm effects of the medication.

Trials to this date were focused primarily onto the intake of calcium, vit. D, medications, several microelements, and certain types of foods. Few trials have examined oxidative stress as a key factor for osteoporosis.

Oxidative stress is a major determinant of ageing and life expectancy. It results from the increasing of intracellular reactive oxygen species (ROS). Real-time measurements of H_2O_2 in mesenchymal stem cells (MSCs) in bone marrow indicate that oxidative stress is linked to age degeneration. MSCs are precursors for osteoblasts and their reductionist status depends on the complex antioxidant mechanisms. The authors identified functional differences between healthy and osteoporotic MSCs. When comparing the reaction of MSCs with the oxidant, an improved antioxidant activity is observed in the stem cells of osteoporotic women [11].

Other researchers directed their research towards finding a powerful antioxidant. There is experimental evidence for the antioxidant properties of pyrroloquinoline quinone (PQQ), added to mice feed. The results obtained show that PQQ can decrease oxidative stress and inhibit the osteoclast bone resorption, and thus prevent osteoporosis in testosterone-deficit mice [12].

In order to prevent impairments in bone metabolism, the provision of the necessary macro- and microelements via supplements or via a nutritionist-prepared diet should be considered. This should be tailored to the individual's deficits and to the optimal serum levels, showcased in Table 1 and Table 2.

Table 1. Recommended daily intake of some macro- and microelements, mg/day for men and women over 19 years old [13].

Daily intake mg/day	Men	Women	Pregnant women	Breastfeeding women
Fe	8	18 (19-50 years) 8 (50+ years)	27	10
Cu	0,90	0,90	1,00	1,30
Zn	11	8	11	12
Mn	2,3	1,8	2,0	2,6
Mg	400 (19-30 years) 420 (30+ years)	310 (19-30 years) 320 (30+ years)	350 (19-30 years) 360 (30+ years)	310 (19-30 years) 320 (30+ years)
P	700	700	700	700
Ca	1000 (19-50 years)	1000 (19- years) 1200 (50+ years)	1200	1200

Table 2. Optimal serum levels of some macro- and microelements in adults [14]

Optimal serum level of calcium 2,12÷2,62 µmol/L
Optimal serum level of iCa^{++} 1,1÷1,3 µmol/L
Optimal serum level of phosphorus 0,84÷1,45 µmol/L
Optimal serum level of zinc 12÷2,4 µmol/L
Optimal serum level of magnesium 0,7÷1,2 µmol/L
Optimal serum level of iMg^{++} 0,33÷0,57 µmol/L
Optimal serum level of iron (ferosin), women 10,7÷23,4 µmol/L
Optimal serum level of copper, women 13,2÷24,3 µmol/L
Optimal serum level of manganese 0÷10 µmol/L

A number of trials are directed towards researching **correlational micronutrient dependences**. According to some authors, high levels of copper decrease iron resorption and negatively affect hematologic parameters. While measuring the continuous intake of zinc (50mg/day or more), a copper deficit was found. The higher intake of zinc increases the synthesis of intestinal cell proteins known as metallothionines. They bind the metals and disallow their absorption by the intestinal cells. Metallothionines have a higher affinity to copper than zinc, hence higher levels of metallothionines obtained due to an increase in zinc levels cause a decrease in copper absorption. On the other hand,

higher doses of copper have not been found to affect the food status of zinc. The effect of copper supplements in humans has not been established. The link between the serum levels of copper, zinc, iron, and oil-soluble vitamins A and E has also been studied. The increased intake of vitamin C also lowers the level of copper in the body. It has been found that the serum levels of zinc are strongly dependent on serum copper and iron [15].

Copper and zinc are essential microelements that play an important role in the human body as cofactors in a number of enzyme systems in the brain, muscles, bones, kidneys and liver. Copper participates in the synthesis of collagen and elastin. Cuproenzymes in the body act as antioxidants and prevent damage to the cells from free radicals. The daily intake of zinc is essential, as the body does not possess a system for its storage. Zinc is necessary for the catalyst activity of nearly 100 enzymes and for maintaining the immune system, protein syntheses, DNA-syntheses, and cell division. Zinc is responsible for the maintenance of taste and smell. Vegetarians typically have significantly lower doses of zinc intake. The zinc found in plant foods is in lower concentrations and is harder to assimilate. Zinc insufficiency presents itself with weakened immunity, loss of appetite, and a slower growth rate in children [13].

The important role of microelements copper and zinc and the non-equivocal trial results motivated us to conduct our own research, with the participation of patients from the region of Pleven.

2. AIM OF THE PUBLICATION

The aim of our research is to contribute to the clarification of the aforementioned correlation, to analyze the existence of any regional particularities, and ultimately to achieve a better control over the disease and its complications via the enriching of the diet with the corresponding microelements of high-risk patients for osteoporosis development.

3. EXPERIMENTAL

Materials and Methods

Included in the trial were 50 female patients settled into menopause (confirmed <5 years ago), ages between 52 and 77 years (mean age 59 years). In order to exclude the effect from any correlating risk factors such as tobacco smoking, coffee intake, motor activity, dieting, decreased levels of serum calcium and vitamin D, 24 out of these patients were specifically selected due to having this specific risk profile. Based on the T-score of measuring their bone density via the method of Dual X-ray Absorption (DEXA), the patients were separated into three groups: osteoporotic (T-score < -2.5) – 12 patients; osteopenia (T-score between -1 and -2.5) – 6 patients, and a control group with patients of normal bone density (T-score > -1/-6).

Serum Cu and Zn concentrations are measured by flame atomic absorption spectrometry, Perkin-Elmer AAnalyst 300 spectrophotometer. The serum samples are preliminary diluted (1:3 for Cu and 1:5 for Zn) with distilled water with acceptable purity. The instrumental parameters for the analyses of both elements are present in Table 1. The quality of the results is guaranteed by application of ICQ schemes and participation of the lab in EQAS programmes.

Table 3 . Instrumental parameters for analyses of Cu and Zn in serum

Instrumental parameters	Analyses of serum Cu	Analyses of serum Zn
Model of the spectrophotometer	AAnalyst 300/Perkin –Elmer	AAnalyst 300/Perkin –Elmer
Wavelength nm	324.8	213.9
Slit nm	0.7	0.7
Light Source	HCL	EDL
Current mA	15	250/modulated on

RESULTS

The results obtained from absorbent photometry were analyzed with the ANOVA statistical method. The results were as follows:

Serum zinc levels: The lowest mean level was found in the control group: 12.6 ± 2.7 μmol/L vs 13.6 ± 3.3 μmol/L in the osteopenia group and 13 ± 1.1 μmol/L in the osteoporotic group. However, there were no statistically significant differences between the measurements in the different groups /p=0.73/.
Serum copper levels: Again, the lowest mean level was found in the control group: 17.6 ± 2.6 μmol/L vs 19.5 ± 3.4 μmol/L in the osteopenia group and 20.2 ± 4.7 μmol/L in the osteoporotic group. However, once again there were no statistically significant differences between the measurements in the different groups /p=0.25/.

4. CONCLUSION

Visible from the results is that in the control group, the mean level of serum zinc and copper was significantly lower than that of the patients with osteoporosis and osteopenia, even if the difference was not statistically significant. This can be explained by the fact that copper and zinc participate as co-factors in a number of enzyme systems in bone metabolism. This might be the reason why the control group patients with normal bone metabolism did not possess these elements in high quantities in their serum, as they were engaged on an intracellular level. In order to find statistically significant results and to study this hypothesis, it will be necessary to increase the number of patients in all groups tested.
This is preliminary investigation which will continue with extended number of patients. In case the found correlation is confirmed in future trials with a statistical significance of the results, the levels of copper and zinc will be able to be used as biomarkers for the efficiency of the bone metabolism.

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