

## Adequate Serum Copper Concentration Could Improve Bone Density, Postpone Bone Loss and Protect Osteoporosis in Women

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### Abstract

**Background:** To determine the protective impact of efficient serum copper concentration on BMD in women and to assess its impression on bone.

**Methods:** six hundred healthy women through a national project in Iran, IMOS, were selected via a cluster random sampling and enrolled the study. They were divided to pre menopausal (404/600, 67.7% of total) and post menopausal (190/600, 32% of total) groups. BMD was measured by biphotonic absorptimetry DEXA for hip and lumbar spine. Morning serum copper concentration was determined by atomic absorption spectrometry.

**Results:** Mean age was 40.92±14.8yr. Mean serum copper concentration= 105.85±40.15µg/dl and mean BMI= 27.13±4.81 kg/m<sup>2</sup>. Totally 2.4% was smoking, 5% had regular physical activities three times a week and 17.5% was copper deficient.

Prevalence of Osteoporosis in postmenopausal women was 12.9% in copper deficient persons vs. 11.3% in normal serum copper ones. No significant difference found according to serum copper concentration associated with BMI, age and vitamin D. Spine BMD revealed a significant correlation with serum copper content. ( $P = 0.001$ ). This correlation also existed for Total hip ( $P < 0.05$ ).

Premenopausal women with serum copper level above 105 µg/dl revealed a significant difference in hip BMD compared to whom with less copper concentration. 1.02± 0.13kg/m<sup>2</sup> vs. 0.97± 0.13kg/m<sup>2</sup> ( $P = 0.001$ ). Copper had an independent role on determining hip BMD in pre menopausal women ( $P = 0.001$ ).

**Conclusion:** copper has an independent role on bone density in all healthy women. It could have an adjourning factor for bone loss as well as a protective agent for osteoporosis.

**Keywords:** Serum copper, Bone mineral density, Osteoporosis, Iran

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### Introduction

Bone is a specialized connective tissue, composed of a collagen (protein) framework permeated with mineral salts composed of mostly calcium and phosphate, together with trace amounts of other minerals and ions. Just like the muscles, the heart and any other organ in the body, the skeleton needs a constant supply of energy and nutrients.

Comparisons between parents and their children, or between twins, suggest that genetics accounts for 60 to 80% of the variability in bone mineral density (BMD) between individu-

als. However, although genetic factors are very important in determining whether an individual is at heightened risk of osteoporosis, lifestyle factors such as good nutrition and exercise play a key role in building bone during youth, maintaining bone mass in younger adults, and helping to slow down bone loss in adults and the elderly. The importance of these lifestyle factors is that they are amenable to change (unlike your genes) individuals can take positive steps to strengthen their bones and reduce their risk of osteoporosis. The amount of skeletal mass acquired during adolescence is one of the most im-

portant factors in determining the risk of osteoporosis and fractures later in life (1). In healthy females, about 40% of peak bone mass is accumulated during the adolescent years (2). Trace elements are essential for normal growth and development of skeletons in humans and animals. Although they are minor building components in teeth and bone, they play important functional roles in bone metabolism and bone turnover.

The need for the trace element copper in the biosynthesis of bone and connective tissues and their maintenance is well established (3). There is also much evidence from human and animal studies that a lack of this mineral will lead to improper bone formation and bone fractures in adults. (4-8). This is because the enzyme lysyl oxidase, which mediates the final step in the synthesis of collagen, a constituent of bone and connective tissue, is a copper-dependent enzyme (9, 10). Thus the role of the trace element copper in the metabolism of bone and connective tissue is profound and inadequate copper nutrition may be an important factor in the etiology of osteoporosis.

The aim of this study is to investigate the beyond effect of copper as a trace element has been known for maintaining bone tissue strength.

## **Materials and Methods**

IMOS (Iranian Multicenter Osteoporosis Study) is a large scale study begun in 2000 in five large cities of Iran.

This project was performed by Endocrinology and Metabolism Research Center (EMRC) of Tehran University of Medical Sciences, Center for Disease Control of Iranian Ministry of health and Deputy for Health of involved provinces and Universities of Medical Sciences (Tehran, Shiraz, Boushehr, Mashad, Tabriz). The original subjects (n= 6000, healthy, aged 20-69 yr) were selected via a cluster sampling in the five cities with the main aim of determining the normal value of osteodensitometric variables as reference value to analyze of BMD report for evaluating of osteoporosis in Iran, to determine

the prevalence of osteoporosis and osteopenia in normal population and as a brief to measure and evaluate all aspects of osteoporosis and risk factors prone people to this debilitating disease. Six hundred healthy surviving selected women aged 20-69 yr from the original IMOS study invited to participate. The subjects completed standard questionnaires concerning smoking, alcohol use, physical activity, medication use and disease history. The height and weight of the participants were measured while they wore light clothing but no shoes and body mass index (BMI; in  $\text{kg}/\text{m}^2$ ) was calculated. BMD was measured at the trochanter, intertrochanter, femoral neck, Ward's triangle and lumbar spine by using dual X-ray absorptiometry (Lunar Corp, Madison, WI). Instruments were calibrated daily and had measurement precisions of  $\leq 1\%$  for the spine,  $\leq 1.5\%$  for the hip. Total hip BMD was obtained by summing the bone mineral content values at the femoral neck, intertrochanter and greater trochanter and dividing this value by the composite area of the three sites. Spine BMD was defined as the average BMD of lumbar vertebrae L<sub>2</sub>-L<sub>4</sub>. Osteoporosis for all axial sites was defined as  $T_{\text{score}} \leq -2.5$  and osteopenia was defined as a  $T_{\text{score}}$  between -1 and -2.5 (6).

After the subjects had fasted overnight, blood samples were drawn into trace mineral-free plastic tubes each containing 2 drops of 2% sodium oxalate and were placed on ice. The blood was centrifuged within 2 h at 3000 x g for 10 min to obtain serum. Serum samples frozen at -70 °C were shipped on dry ice to the Mineral Analysis Laboratory at the Tarbiat Modares University Biochemistry Lab. Serum copper concentrations were measured by using an induction coupled serum atomic emission spectrometer. Normal range of serum copper concentration considered 70-140  $\mu\text{g}/\text{dl}$  according to the second National Health and Nutrition Examination Survey (NHANES) of the United States (1976-80). They were divided to premenopausal (404/600, 67.7% of total) and postmenopausal (190/600, 32% of total) groups to predict

the independent effect of serum copper on bone. Moreover to unravel the specific impact of copper in postmenopausal osteoporosis we assessed its correlation in early postmenopausal (45-55 yr) to avoid BMD confounding with osteosclerosis or similar conditions. All process has been confirmed by Tehran University of Medical Sciences Medical Ethics Committee. SPSS version 11.5 was used for data analysis. Student *t*-test was used to compare means. Pearson's coefficients were calculated to evaluate the correlation of serum copper concentrations with age, BMI and BMD. Mean age, BMI and serum copper concentration was compared between two groups of women with osteoporosis, osteopenia, or normal BMD. Correlation between intermittent and constant low back pain were also assessed with serum copper concentration in postmenopausal. The strength of association between BMD and serum copper concentration was measured by linear regression model. As BMD was significantly associated with age and BMI, serum copper concentrations were compared after adjustment for these two major confounders.

## Results

The baseline characteristics of the subjects are shown in Table 1. Mean age was 40.92±14.8 yr. Mean serum copper concentration= 105.85±40.15 µg/dl and mean BMI= 27.13±4.81 kg/m<sup>2</sup>. Totally 2.4% was smoking, 5% had regular physical activities three times a week and 17.5% was copper deficient. There was no significant difference in serum copper concentration in pre and post menopausal women. There was no significant variation concerning copper depletion among two groups, 17.3% of pre menopausal women were copper deficient vs. 16.8% of post menopausal.

**Postmenopausal women findings** Prevalence of osteoporosis was 12.9% in copper deficient persons vs. 11.3% in normal serum copper ones. No significant difference was found according to serum copper concentration associated with

BMI, age and vitamin D. Spine BMD revealed a significant correlation with serum copper content in whom reported intermittent low back pain (*P*= 0.001). This correlation also existed for total hip (*P*< 0.05) (Table 2).

In Linear regression model serum copper concentration had an independent role on bone content of hip and spine in postmenopausal women.

**Premenopausal women findings** Women with serum copper level above 105 µg/dl revealed a significant difference in hip BMD compared to whom with less copper concentration. 1.02± 0.13kg/m<sup>2</sup> vs 0.97± 0.13kg/m<sup>2</sup> (*P*= 0.001). Actually BMD was about 5% greater in women with more serum copper concentration. These relations were not significant in spine 1.21± 0.13 vs. 1.19±0.19.

Copper had an independent effect on determining hip BMD in pre menopausal women (*P*= 0.001) (Table 3). The comparing diagram has been shown (Fig. 1).

**Table 1:** Baseline characteristics of the subject (n=600)

Characteristic	Value
Age (yr)	40.92 ±14.80
BMI (kg/ m <sup>2</sup> )	27.13±4.81
WHR	0.85±0.07
BMD Total (gr/cm <sup>2</sup> )	0.96±0.14
BMD Spine (gr/cm <sup>2</sup> )	1.12±0.17
Current smoker %	2.4
Exercise 3 times/week %	5
Renal stone%	6.5
Serum zinc concentration (n=600) (µg/dl)	105.85±40.15

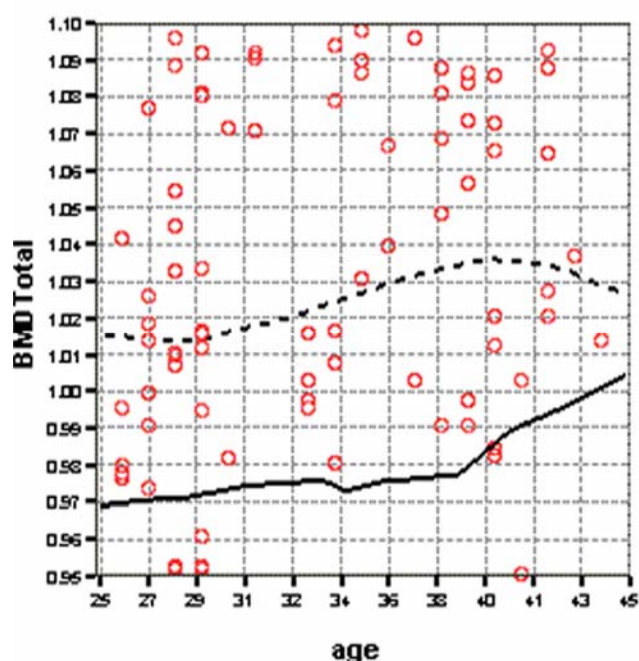
<sup>†</sup>±SD (all such values)

**Table 2:** Correlations of serum copper concentration with age, BMI, Total hip and Spine BMD in postmenopausal women

	Correlation coefficient	<i>p</i>
Age	-0.027	0.8
BMI	0.2	0.06
Hip BMD	0.22	0.04
Spine BMD	-0.002	0.005

**Table 3:** Cross sectional characteristics depending on serum copper status in premenopausal women

	NL copper concentration	copper deficient	P
BMD gr/cm <sup>2</sup> (Total Hip)	1.02± 0.13	0.92±0.14	0.00
BMD (Spine)	1.21± 0.13	1.9±0.19	0.15



**Fig. 1:** The diagram illustrates the independent effect of sufficient serum copper concentration on total hip density in younger women

### Discussion

A number of macro- and microelements are involved in maintaining skeletal health. Among the trace elements, copper is essential in collagen-crosslink formation as a component of the metalloenzyme, lysyl oxidase (11). Collagen crosslinks provide tensile strength to bone (12), and copper deficiency in several animal species produces skeletal abnormalities (13-14). In humans, there is some evidence of a relationship between copper deficiency and senile osteopo-

rosis. Conlan et al. (15) found reduced serum copper in elderly patients with fractures of the femoral neck compared with age and sex matched controls. Furthermore, copper status was reported to be a very important predictor of bone health, even more so than calcium status, in individuals with osteoporotic changes related to immobilization (16, 17).

Our study is the first ever in Iran investigating the impact of serum copper concentration on bone density in women. We hypothesized that impaired serum copper content would lead to improper bone content or osteoporosis and inspected that. Our result showed that the mean serum copper concentration is 105.85±40.15. According to NHNAES II normal range of this trace element is 70-145 which is similar to our study. The prevalence of copper deficiency was 17.5% with no significant difference between pre and post menopausal.

The results has also illustrated that there was a positive correlation between serum copper content and bone density at total hip and spine in postmenopausal women. Serum copper concentration has been correlated to spine(L<sub>2</sub>-L<sub>4</sub>) density in whom reported intermittent low back pain. We found out proper serum copper content would lead to better bone mineral density at hip in premenopausal ones. There were no associations between serum copper concentration, age and BMI in either group.

Similar studies for determining copper effect on bone are not numerous. A supplementation trial with trace elements (including copper) produced beneficial effects on bone density (18). Decreased levels of copper have been found in people with osteophytic lipping of the thoracic spine (19), ischemic necrosis of the femoral head (20), fractures of femoral neck (21) and decreased lumbar bone density (22). Three studies have shown that a combination of several minerals (zinc, manganese and copper) with calcium was able to reduce spinal bone loss in postmenopausal women (22-25).

We concluded that copper had an independent role on bone density in all women. It seems that

sufficient amount of this trace element in serum would decelerate bone loss or protect osteoporosis in healthy women. However, the effects of copper depletion on bone metabolism and bone mechanical properties warrant further cohorts and clinical trials with nutritional data. This study could be a gate way for more research in this field which tried to highlight the critical importance of copper in maintaining strong bones.

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