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# Association between bone trace elements and osteoporosis in older adults: a cross-sectional study

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***Abstract***

**Objectives:** Metal micronutrients deficiency may be one of the risk factors for the development of osteoporosis. This study aimed to measure the trace element contents in human bone tissue to analyze the relationship between micronutrients and osteoporosis.

**Design:** A cross-sectional survey was performed on data from 51 elderly patients with proximal femoral fracture.

**Methods:** The concentrations of calcium, phosphorus, manganese, iron, copper, and zinc in bone tissue samples from 51 elderly patients with proximal femoral fracture were determined by energy-dispersive X-ray fluorescence (EDX). Subjects were divided into osteoporosis and non-osteoporosis groups according to their bone mineral density (BMD) *T*-score values. The difference in metal elements concentrations in bone tissue between the two groups was compared, and the role of metal elements in osteoporosis was discussed.

**Results:** There was no statistical difference in age, sex, body mass index (BMI), serum albumin, biochemical blood indices, and bone turnover markers between the two groups. The Mann–Whitney *U* test was used to compare the difference in metal elements concentrations in bone tissue samples between the two groups. The results showed that manganese, copper, and zinc concentrations in the cancellous bone were significantly higher in the non-osteoporosis group than in the osteoporosis group. Multivariate logistic regression analysis indicated that high bone zinc concentration [odds ratio = 0.26, 95% confidence interval (CI) = 0.075–0.928, $p = 0.038$] was negatively correlated with osteoporosis.

**Conclusion:** Manganese, copper, and zinc play an essential role in bone mineralization and metabolism. Among them, zinc may be most closely related to osteoporosis and play a key role in bone development and maintenance of bone mass. Therefore, we believe that the design of zinc-rich compounds or nutrients as a new complementary factor to increase the intake of zinc for the elderly could be able to prevent and intervene in the occurrence of osteoporosis in the early stage.

***Keywords:*** BMD, EDX, osteoporosis, trace element, zinc

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## Introduction

With the increase in aged population, age-related chronic diseases have become a potential public health problem, and osteoporosis is one of the most critical diseases.[1] Osteoporosis is a systemic skeletal disease characterized by decreased bone mass and manifested by increased bone fragility, leading to osteoporosis fracture susceptibility. In Western countries, the lifetime risk of osteoporosis fracture ranges from 40% to 50% for women and 13% to 22% for men.[2] Hence, osteoporosis and its complications pose a substantial economic burden to the health insurance system.

The pathogenesis of osteoporosis is still unclear; however, it is believed to be related to genetic

factors, endocrine function, exercise, and insufficient intake of trace elements caused by malnutrition.[3] With the growth of age, the intestinal function of elderly patients is weakened, such as gastrointestinal motility and healthy digestion and absorption. Therefore, for elderly patients with osteoporosis, the incidence of malnutrition or inadequate nutritional intake is higher. Imbalance of nutrient intake, especially mineral deficiency due to reduced dietary intake and absorption of metal elements, can lead to excessive bone loss and accelerated destruction of bone microstructure in the elderly, which may be an essential factor in the pathophysiological mechanism of low bone mineral density (BMD).[4] Metal elements can affect the synthesis and crosslink of bone organic collagen fiber, which plays a vital role in the growth and development of bone. The process of new bone formation and transformation in the body is regulated and involved by metal elements, including manganese, iron, zinc, and copper, whose high or low content in the body can affect bone mineral formation and bone matrix synthesis.[5] Manganese is an important element necessary for bone growth and development. Although manganese deficiency is rare in the human body, it can lead to abnormal bone development, chondrodysplasia, epiphyseal development disorder, and osteoporosis.[6] The metabolic disorder of iron in the body promotes the differentiation of osteoclasts and apoptosis of osteoblasts and inhibits the proliferation and differentiation of osteoblasts, ultimately leading to severe bone loss and osteoporosis.[7] Zinc is an essential metal micronutrient for the healthy development of bone and the maintenance of bone homeostasis.[8] Copper is a cofactor of enzymatic reactions, participating in the body's ossein synthesis and bone mineralization process.[9]

As previous studies on the relationship between micronutrients and osteoporosis were mainly in the stage of animal experiments or only measured trace element levels in blood or hair, we directly measured the trace element contents in human bone tissue to discuss the relationship between micronutrients and osteoporosis. In this study, the contents of calcium, phosphorus, manganese, iron, copper, and zinc in bone tissue samples from 51 elderly patients with proximal femoral fractures were determined by energy-dispersive X-ray fluorescence (EDX). According to BMD measurements, subjects were divided into the osteoporosis group ($T$-score value $\leqslant -2.5$) and non-osteoporosis group ($T$-score value $> -2.5$). The differences of metal trace elements in the cortical bone area, junction area between cortical and cancellous bone area, and cancellous bone area were compared between the two groups, and the relationship between bone metal trace elements and osteoporosis was explored and analyzed.

## Materials and methods

### *Subjects*

Elderly patients who received Proximal Femoral Nail Antirotation (PFNA)-II intramedullary fixation surgery for proximal femoral fractures (including intertrochanteric fractures and subtrochanteric fractures) in the Department of Orthopedics, Huadong hospital affiliated to Fudan University from September 2015 to September 2018 were consecutively selected. Informed written consent signed by each participant included consent to publish the study results. Besides, the authors confirm that all patient identifiers have been removed, so that the patients described are not identifiable. The clinical data of 51 eligible patients with proximal femoral fracture were obtained by screening the inclusion conditions, and bone tissue samples including cortical and cancellous bone were obtained. This clinical study obtained the informed consent of all patients and was approved by the Ethics Committee of Huadong Hospital affiliated to Fudan University (no. 2014K40). According to the diagnostic criteria of osteoporosis recommended by WHO,[10] 51 elderly patients in this study were divided into two groups based on the $T$-score value of BMD: osteoporosis group ($n = 37$, $T$-score value $\leqslant -2.5$); non-osteoporosis group ($n = 14$, $T$-score value $> -2.5$). Subjects inclusion criteria were as follows: (1) elderly patients (>60 years old) with proximal femoral fracture treated with PFNA-II fixation, among which the fracture in the non-osteoporosis group was not caused by low energy injury; (2) no anti-osteoporosis treatment such as estrogen, calcitonin, and bisphosphonates was received before admission; (3) no complications of chronic liver and kidney diseases, metabolic diseases, immune diseases, tumors, and so on; (4) no history of long-term use of glucocorticoids and other drugs affecting bone metabolism. The findings are reported in accordance with the STROB (Strengthening the Reporting of Observational Studies in Epidemiology) statement (see Supplementary 1 STROBE Checklist).[11]

### *Detection of serum bone turnover and biochemical index*

All subjects received 15 ml of venous blood on an empty stomach on the morning of the second day after admission, and serum separation was completed within 2 h. Bone turnover markers – including N-terminal propeptide of type I procollagen (PINP), collagen cross-linked C-telopeptide (CTX), osteocalcin (OC), 25-hydroxy vitamin D3 (25-OH-D3), and parathyroid hormone (PTH) – were determined by automatic electrochemiluminescence immunoassay system (Roche E170, Mannheim, Germany). The enzyme kinetic method determined serum albumin, alkaline phosphatase (ALP), serum calcium, and phosphorus levels. The within-run and between-run intra-assay coefficient of variability (CV) was $<6\%$ for P1NP, $<6\%$ for CTX, $<3.4\%$ for PTH, and $<7\%$ for 25-OH-D3.

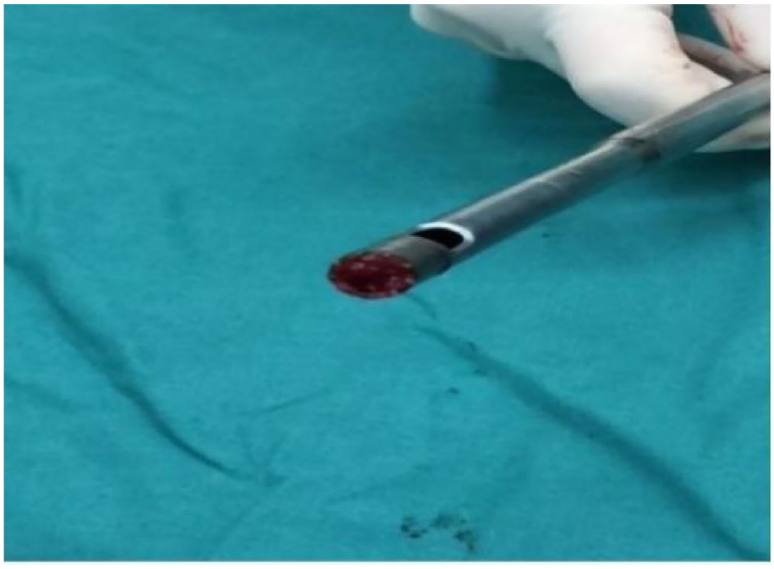

**Figure 1.** The modification of the proximal femoral screw drilling bit to hollow bit.

### *Measurement of BMD*

BMD was determined in the contralateral total hip and femoral neck by dual-energy X-ray absorptiometry (Hologic Discovery, Mississauga, ON, Canada). The instrument was operated by professionally trained staff, and the quality control of the instrument was carried out daily according to the routine. The CV was 1.86% for femoral neck and 0.95% for total hip. The patients were scored for osteoporosis as osteoporosis ($n=37$, $T$-score value $\leqslant -2.5$) and non-osteoporosis ($n=14$, $T$-score value $> -2.5$).

### *Bone sample collection and EDX measurement*

In this study, the proximal femoral screw bit was modified to a hollow bit (Figure 1) so that the cylindrical specimens containing cortical and cancellous bone could be obtained directly during surgery without any impact on the patient (Figure 2). PFNA-II nails were inserted during surgery, and samples were extracted using this modified hollow bit before the proximal spiral blade was inserted. The bone tissue samples were immediately fixed in paraformaldehyde fixative for 48 h; the specimens were rinsed with water for 24 h and dehydrated. The dehydrated bone tissue specimens were placed into plastic molds for plexiglass embedding. After embedding, the surface of the specimen was cut and polished with a diamond saw blade. The polished specimen was placed in the vacuum sample chamber of EDX, and the acceleration voltage was set to 40 kV. After the specimens were focused, the cortical area, cancellous area, and junction area were selected for quantitative analysis of six elements, including Ca, P, Fe, Mn, Zn, and Cu. Two parts of each zone were randomly selected for measurement, and the concentrations of element components were averaged. The sum concentrations of six elements were 100%. Each measurement time was 100 s.

### *Statistical analysis*

All analyses were performed using IBM SPSS software for Windows version 23.0 (SPSS, Chicago, IL, USA). First, these continuous variables, such as the clinical data and the concentrations of bone tissue elements of the two groups of 51 patients with and without osteoporosis, were checked to determine whether they were normally distributed by the Shapiro–Wilk test. As these variables were not normally distributed, the Mann–Whitney $U$ test was used to examine the association between continuous variables (age, serum calcium, phosphorus, ALP, PTH, CTX, PINP, OC, 25-OH-D3 levels, and bone trace elements contents) in patients with and without osteoporosis. We converted the continuous variables such as body mass index (BMI), serum albumin, and hemoglobin into categorical variables. Univariate logistic regression analysis was used to determine the association between osteoporosis and each categorical variable. Variables with a significance probability of $p<0.05$ were then included in the multivariate logistic regression analysis. As this was a safety and

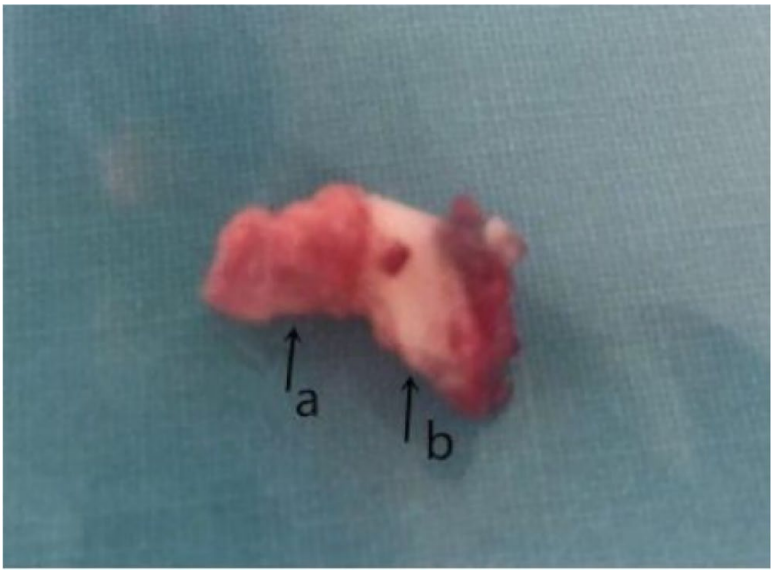


**Figure 2.** Cylindrical bone samples including cortical and cancellous bone: (a) cancellous bone zone and (b) cortical bone zone.

feasibility study, the number of samples was determined from the aspect of feasibility. Corresponding 95% confidence intervals (CIs) were reckoned with confidence interval estimation, and $p < 0.05$ was considered to be statistically significant.

## Results

### *Subject clinical characteristics*

The study included 37 patients (11 males and 26 females) in the osteoporosis group and 14 patients (3 males and 11 females) in the non-osteoporosis group, and no significant sex differences were found between the two groups ($p = 0.208$). Baseline clinical data were collected and shown in Tables 1 and 2. There was no significant difference in age and sex distribution between the two groups. In addition, there was no statistical difference between the two groups in the presence or absence of the medication history of chronic diseases. Likewise, the clinical data of the two groups, including biochemical blood indices and bone turnover markers, showed no statistical difference.

### *Comparison of bone metal elements concentrations between osteoporosis group and non-osteoporosis group*

Comparing bone metal elements concentrations in different bone tissue regions between the osteoporosis and non-osteoporosis groups, we found statistically significant differences in bone manganese, copper, and zinc concentrations in the cancellous bone areas between the two groups. There, however, was no statistical difference in the calcium, phosphorus, and metal elements concentrations in the cortical area and junction area between the two groups, as shown in Table 3.

In the cancellous bone area, bone manganese concentration in the non-osteoporosis group [median = 1.96%, interquartile range (IQR) = 1.43–3.57%] was significantly higher than that in the osteoporosis group (median = 1.96%, IQR = 1.43–3.57%), and the difference was statistically significant ($Z$ value = 3.143, $p = 0.002$). Similarly, the median concentrations of bone copper and zinc in the non-osteoporosis group were 1.75% (IQR = 0.97–3.92%) and 2.16% (IQR = 1.82–4.21%), respectively, and the median concentrations of bone copper and zinc in osteoporosis group were 0.43% (IQR = 0.15–0.98%) and 0.47% (IQR = 0.26–1.63%), respectively, with statistically significant differences between the two groups ($Z$ value = 3.652 and 4.602, $p < 0.001$). Although bone iron concentration was significantly higher in the non-osteoporosis group (median = 3.12%, IQR = 1.92–4.35%) than in the osteoporosis group (median = 1.21%, IQR = 0.48–3.85%), the difference was not statistically significant surprisingly ($p = 0.089$).

### *Evaluating the potential correlations between bone micronutrients and osteoporosis*

Based on the result of the univariate logistic regression analysis and the Mann–Whitney $U$ test, variables with a significant probability of $p < 0.05$ were put into the following multivariate logistic regression analysis. Among them, high bone zinc concentration (odds ratio 0.26, 95% CI = 0.075–0.928, $p = 0.038$) were negatively correlated with osteoporosis, which was the protective factor ($B < 0$). The specific analysis results are shown in Table 4.

## Discussion

It is well known that calcium and phosphorus elements are the main components of the bone tissue mineral hydroxyapatite, accounting for 65% of the inorganic bone matrix.[12] Calcium levels in bone tissue are significantly reduced in patients with osteoporosis; thus, calcium and active vitamin D3 are used as the basis of anti-osteoporosis treatment. Metal elements, such as zinc and copper, are involved in the composition of various catalytic

**Table 1.** Comparison of baseline characteristics of patients between osteoporosis group and non-osteoporosis group.

| Clinical parameters | Mean rank | Median | IQR | *Z* score | *p* value |
|---|---|---|---|---|---|
| Age (years) | | | | | |
| Osteoporosis group | 28.08 | 74 | 71–79 | 1.631 | 0.103 |
| Non-osteoporosis group | 20.5 | 72 | 70–73 | | |
| Serum calcium (mmol/l) | | | | | |
| Osteoporosis group | 26.09 | 2.2 | 2.1–2.3 | 0.076 | 0.939 |
| Non-osteoporosis group | 25.75 | 2.2 | 2.1–2.3 | | |
| Serum phosphorus (mmol/l) | | | | | |
| Osteoporosis group | 28.15 | 1 | 0.92–1.19 | 1.678 | 0.093 |
| Non-osteoporosis group | 20.32 | 0.92 | 0.79–1.09 | | |
| ALP (μ/l) | | | | | |
| Osteoporosis group | 27.55 | 68 | 57–97 | 1.214 | 0.225 |
| Non-osteoporosis group | 21.89 | 64 | 59–71 | | |
| CTX (pg/ml) | | | | | |
| Osteoporosis group | 27.35 | 523.3 | 453.3–687.3 | 1.055 | 0.291 |
| Non-osteoporosis group | 22.43 | 487.5 | 346.5–674.2 | | |
| OC (ng/ml) | | | | | |
| Osteoporosis group | 25.08 | 17.2 | 11.6–24.1 | 0.718 | 0.473 |
| Non-osteoporosis group | 28.43 | 18.75 | 12.6–26.9 | | |
| P1NP (ng/ml) | | | | | |
| Osteoporosis group | 26.95 | 36.6 | 30–53 | 0.739 | 0.460 |
| Non-osteoporosis group | 23.5 | 34.8 | 27.5–43.1 | | |
| PTH (pg/ml) | | | | | |
| Osteoporosis group | 26.01 | 36.4 | 30.7–52.1 | 0.011 | 0.992 |
| Non-osteoporosis group | 25.96 | 37.3 | 29.9–58.4 | | |
| 25-OH-D3 (ng/ml) | | | | | |
| Osteoporosis group | 25.91 | 15.8 | 11.3–21.4 | 0.074 | 0.941 |
| Non-osteoporosis group | 26.25 | 15.4 | 12.1–21.4 | | |

25-OH-D, 25-hydroxy vitamin D3; ALP, alkaline phosphatase; CTX, collagen cross-linked C-telopeptide; IQR, interquartile range; PTH, parathyroid hormone; OC, osteocalcin.

enzymes or coenzyme factors in the body and play an indispensable role in bone transformation and formation.[13,14] Insufficient or excessive intake of these metal elements will cause bone metabolism disorder and BMD decline. As there are few studies on the direct detection of trace elements in bone

**Table 2.** Univariate logistic regression for association of categorical variables with osteoporosis.

| Clinical parameters | Osteoporosis | | | | |
|---|---|---|---|---|---|
| | ***B* value** | **SE value** | **Wald value** | **OR (95% CI)** | ***p* value** |
| Sex | | | | | |
| Male | −1.058 | 0.841 | 1.583 | 0.347 (0.067–1.804) | 0.208 |
| Female | | | | 1 | |
| BMI | | | | | |
| Low weight | −0.201 | 0.891 | 0.051 | 0.818 (0.143–4.695) | 0.822 |
| Overweight | 0.288 | 1.384 | 0.043 | 1.333 (0.384–8.108) | 0.835 |
| Normal | | | | 1 | |
| Hemoglobin | | | | | |
| Anemia | −0.125 | 0.633 | 0.039 | 0.882 (0.255–3.050) | 0.843 |
| Normal | | | | 1 | |
| Albumin | | | | | |
| Hypoalbuminemia | 0.701 | 0.686 | 1.041 | 2.014 (0.525–7.726) | 0.307 |
| Normal | | | | 1 | |
| The use of antiplatelet drugs | | | | | |
| Yes | 0.799 | 0.849 | 0.885 | 2.222 (0.421–11.728) | 0.347 |
| No | | | | 1 | |
| The use of antiarrhythmic drugs | | | | | |
| Yes | 1.277 | 1.112 | 1.319 | 3.586 (0.406–11.704) | 0.251 |
| No | | | | 1 | |
| The use of antihypertensive drugs | | | | | |
| Yes | 0.932 | 0.844 | 1.218 | 2.538 (0.485–13.279) | 0.270 |
| No | | | | 1 | |

BMI, body mass index; CI, confidence interval; OR, odds ratio.

tissue, the sample data of trace elements in human bone tissue by EDX has not been reported. Therefore, this research used EDX to measure the concentrations of calcium, phosphorus, manganese, iron, copper, and zinc in different areas of bone tissue samples from elderly patients with proximal femoral fractures in order to explore the relationship between bone micronutrients and osteoporosis.

As we know, the accurate determination of trace elements in bone tissue mainly includes flame atomic absorption spectrometry (FAAS), inductively coupled plasma–mass spectrometry (ICP-MS), and EDX.[15–17] The bone tissue measured by the first two methods is incomplete because atomic absorption spectrometry (AAS) and ICP-MS require the removal of collagen, fat, bone marrow, and other components of bone samples through combustion or solvent dissolution, which may cause the loss of some trace elements. EDX is characterized by nondestructive sample analysis with the minimum amount of sample preparation, and samples can be detected under atmospheric pressure or in a low vacuum

**Table 3.** Comparison of bone calcium, phosphorus, and metal elements in osteoporosis and non-osteoporosis groups in different regions.

| Element | Osteoporosis group | | Non-osteoporosis group | | *Z* score | *p* value |
|---|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | | |
| Cortical zone | | | | | | |
| Ca (%) | 62.94 | 60.82–64.88 | 63.7 | 58.38–65.63 | 0.021 | 0.983 |
| P (%) | 36.87 | 34.96–39.02 | 36.16 | 34.24–41.47 | 0.022 | 0.981 |
| Mn (%) | 0.03 | 0.03–0.05 | 0.03 | 0.02–0.03 | 1.942 | 0.052 |
| Fe (%) | 0.03 | 0.02–0.05 | 0.04 | 0.035–0.045 | 1.022 | 0.307 |
| Cu (%) | 0.02 | 0.02–0.03 | 0.02 | 0.02–0.03 | 0.183 | 0.855 |
| Zn (%) | 0.06 | 0.05–0.07 | 0.055 | 0.05–0.07 | 0.577 | 0.564 |
| Cancellous zone | | | | | | |
| Ca (%) | 53.12 | 40.48–69.72 | 43.88 | 31.39–52.39 | 1.921 | 0.055 |
| P (%) | 43.16 | 27.26–53.87 | 45.97 | 34.89–58.82 | 1.144 | 0.253 |
| Mn (%) | 0.81 | 0.44–1.87 | 1.96 | 1.43–3.57 | 3.143 | 0.002* |
| Fe (%) | 1.21 | 0.48–3.89 | 3.12 | 1.92–4.35 | 1.815 | 0.069 |
| Cu (%) | 0.43 | 0.15–0.98 | 1.75 | 0.97–3.92 | 3.652 | <0.001* |
| Zn (%) | 0.47 | 0.26–1.63 | 2.16 | 1.82–4.21 | 4.602 | <0.001* |
| Interfacing zone | | | | | | |
| Ca (%) | 60.36 | 53.52–64.22 | 62.18 | 55.09–69.78 | 1.266 | 0.205 |
| P (%) | 39.21 | 35.26–45.61 | 37.63 | 28.71–44.74 | 1.203 | 0.229 |
| Mn (%) | 0.07 | 0.035–0.11 | 0.12 | 0.07–0.19 | 1.806 | 0.071 |
| Fe (%) | 0.13 | 0.05–0.275 | 0.2 | 0.05–0.42 | 0.645 | 0.519 |
| Cu (%) | 0.03 | 0.02–0.105 | 0.08 | 0.03–0.31 | 0.956 | 0.339 |
| Zn (%) | 0.09 | 0.06–0.155 | 0.17 | 0.05–0.29 | 0.867 | 0.386 |

IQR, interquartile range.
*Statistically significant with $p < 0.05$.

environment by polishing the sample surface.[18] This method of EDX not only avoids the loss of trace elements in the original bone tissue but also measures the contents of inorganic elements in the cortical bone, cancellous bone, and their junction areas, with higher precision and accuracy than the previous two methods, which meets the requirements of this study.

At present, we have a consensus that osteoporosis is a complex systemic disease affected by multiple risk factors, including age, sex, malnutrition, medications, and related diseases affecting bone metabolism (such as endocrine system diseases and rheumatic immune system diseases). Therefore, the inclusion criteria of this study were rigorous, including elderly patients of similar age and subjects without chronic diseases affecting bone metabolism. The patient characteristics that might influence the concentration of metal elements in bone tissue were sex and age. There was no statistical difference in baseline clinical data

**Table 4.** The correlations between bone micronutrients and osteoporosis following multivariate analysis.

| Parameters | *B* value | SE value | Wald value | *p* value | OR value | 95% CI |
|---|---|---|---|---|---|---|
| Mn (cancellous) | −0.805 | 0.624 | 1.665 | 0.197 | 0.447 | 0.132–1.519 |
| Cu (cancellous) | −0.301 | 0.474 | 0.402 | 0.526 | 0.74 | 0.292–1.875 |
| Zn (cancellous) | −1.345 | 0.648 | 4.305 | 0.038* | 0.26 | 0.073–0.928 |

CI, confidence interval; OR, odds ratio; SE, standard error.
*Statistically significant with $p < 0.05$.

between the two groups in this study, including age, sex, serum albumin, and serum bone turnover markers. Therefore, the difference in bone trace elements concentrations between the osteoporosis group and non-osteoporosis group could better indicate the correlation between bone metal trace elements and osteoporosis. According to the Mann–Whitney *U* test results of this study, we found that there were significant differences in the contents of manganese, zinc, and copper in the bone between osteoporosis and non-osteoporosis groups in the cancellous bone region, which might indicate that the concentrations of manganese, zinc, and copper in the body were closely related to the pathogenesis of osteoporosis.

Manganese is a cofactor or active center of many biological enzymes in the human body, which plays an essential role in metabolic activities.[19] In the skeletal system, manganese can participate in bone mineralization and cartilage mucopolysaccharide synthesis, which plays a vital role in bone formation and chondrogenesis because manganese superoxide dismutase can protect the activity of osteoblasts by removing free radicals secreted by osteoclasts.[20] In addition, manganese is an essential cofactor of many hydrolases and transferases, such as phosphohydrolase and glucosyltransferase.[21–23] This study found that the bone manganese concentration in the osteoporosis group was significantly lower than that in the non-osteoporosis group. Rahnama *et al.*[24] applied an osteoporosis model by ovariectomy in rats and found that manganese concentration in teeth and mandible of rats with osteoporosis decreased significantly compared with the control group. Similarly, another research found that Mn deficiency affected the tibia development of broilers and led to epiphyseal osteoporosis by feeding male broiler chickens with an Mn deficiency diet.[25] Bae and Kim[26] fed 20 6-week-old ovariectomized Sprague–Dawley (SD) rats with different concentrations of manganese diet for 12 weeks, and the results showed that the BMD of rats with high concentration manganese diet was significantly higher than that of rats with low concentration manganese diet, suggesting that manganese element had a strong promoting effect on bone formation and mineralization. It could be seen that manganese deficiency might be involved in the occurrence of osteoporosis.

Copper forms the active center of many biological enzymes in the body, such as lysine oxidase, peroxide dismutase, and cytochrome oxidase, which plays a vital role in the hematopoietic nervous system and skeletal system.[27] Copper can stimulate the proliferation and differentiation of bone marrow mesenchymal stem cells into osteoblasts and enhance the activity of osteoblasts to promote osteogenesis.[28] In this study, the bone copper concentration in the osteoporosis group was significantly lower than that in the non-osteoporosis group, especially in the cancellous bone area, and the difference was statistically significant. Mahdavi-Roshan *et al.*[29] found that serum copper level in patients with osteoporosis was significantly lower than that in the non-osteoporosis population of similar age, suggesting that reduced copper intake was related to the incidence of osteoporosis, and suggested that patients with osteoporosis combined with a copper deficiency should be given compensative copper therapy as well as anti-osteoporosis therapy. Zheng *et al.*[13] applied a meta-analysis approach to study the relationship between serum copper concentration and osteoporosis and found that low serum copper concentration might be an independent risk factor for osteoporosis. Fan *et al.*[30] explored the relationship between copper intake and osteoporosis and found that dietary copper intake was positively correlated with increased BMD in American adults. Therefore, we believe that increasing copper intake in the diet of the elderly should be considered to reduce the occurrence and development of osteoporosis.

The Mann–Whitney *U* test was used to compare the differences in trace elements between the osteoporosis group and non-osteoporosis group, and the results revealed significant differences in the three metal elements of bone manganese, copper, and zinc between the two groups. After putting these three statistically significant variables into the multiple logistics regression model, however, we found that only bone zinc concentration (odds ratio = 0.26, 95% CI = 0.075–0.928, $p = 0.038$) had a significantly negative correlation with osteoporosis. It might indicate that the zinc element played the most crucial role in the occurrence and development of osteoporosis and had an essential role in bone mineralization and metabolism. A recent report found that serum zinc level and dietary zinc intake played a vital role in preventing osteoporosis and suggested that zinc supplementation might improve bone turnover indices of bone formation and BMD at the neck of the femur.[4] Likewise, several clinical studies on the relationship between zinc and osteoporosis had found that serum zinc level in patients with osteoporosis was much lower than that in patients without osteoporosis, and there was a positive correlation between serum zinc level and BMD,[31–34] indicating that insufficient zinc content might be a high-risk factor for osteoporosis. Zinc is involved in about 200 kinds of biological enzymes in the human body.[35] The auxiliary group of ALP secreted by mature osteoblasts also needs zinc to participate in the composition, indicating that it plays an essential role in bone metabolism.[36] It has been reported that it could regulate the function and activity of vitamin D3 and play a particular role in bone mineralization and formation.[37] In terms of bone metabolism, zinc can stimulate the synthesis of collagen protease, promote the synthesis of the organic bone matrix, and increase bone strength and toughness.[38] Zinc can stimulate the expression of transcription factors related to the proliferation differentiation of osteoblasts, inhibit the differentiation of bone marrow progenitor cells into osteoclasts, and stimulate the initiation of gene apoptosis of mature osteoclasts to inhibit bone resorption.[39] From what has been discussed above, we believe that for elderly patients with osteoporosis, increasing dietary zinc intake is necessary for bone development and maintenance of bone mass. It might even be considered to design zinc-rich compounds or supplements as new complementary factors to increase zinc intake to prevent and treat osteoporosis.

The current research has inconsistent conclusions on the relationship between iron content in the human body and osteoporosis. More and more scholars, however, emphasized that iron metabolism disorder was one of the critical causes of bone loss and bone microstructure destruction. Iron metabolism disorders include iron overload or iron deficiency, which might promote osteoclast differentiation and osteoblast apoptosis, inhibit osteoblast proliferation, and ultimately increase the risk of osteoporosis.[7] The ability of the human body to excrete iron decreases with age, and the skeletal system is the primary source of iron deposition. More and more evidence also indicated that iron overload was one of the risk factors of osteoporosis, which could inhibit bone remodeling and damage bone microstructure, increasing fracture incidence.[40,41] Kim *et al.*[42] recruited 1729 healthy subjects to measure serum iron concentration and BMD at the hip, femoral neck, and femoral trochanter. They found that serum iron concentration was negatively correlated with BMD at these three sites, suggesting that excessive iron accumulation in the body would accelerate bone loss, which was also an independent risk factor of osteoporosis, even in healthy people. Similarly, the Korean National Health and Nutrition Examination Survey from 2008 to 2010 examined serum iron and BMD in 14,017 Korean people aged 10–80 years and found a negative correlation between serum iron content and BMD in female volunteers aged over 45 years.[43] Our results suggested that bone iron content in the cancellous bone region was significantly lower in the osteoporosis group than in the non-osteoporosis group, even though the difference was not statistically significant. Although there have been conflicting results in assessing iron status in patients with osteoporosis, considering the small sample size of this study and the variety of methods used to determine its concentration, multiple potential causes of variation in its concentration need to be considered. Therefore, it is difficult to determine the best method for assessing iron content in elderly patients with osteoporosis.

Strict and uniform inclusion criteria were established in this research, and all eligible patients were recruited continuously and randomly. First, the fracture site of the subjects in the osteoporosis group was only the hip, while patients with fractures in other sites of the spine or distal radius were excluded. Second, the female population in the research were all postmenopausal, and patients

with secondary osteoporosis were excluded due to prolonged use of drugs that may affect bone metabolism. More importantly, we did not select the lumbar spine but the hip as the measurement site to determine BMD values in this study. Because the subjects of this study were the elderly (over 60 years) who were often associated with degenerative changes of the lumbar spine, such as osteophytes, end-plate sclerosis, and disk space narrowing.

Previous studies have focused on the relationship between osteoporosis and serum trace elements. Nevertheless, few studies had been carried out on the determination of trace elements in human bone tissue using the EDX method because it was difficult to obtain human bone tissue samples, and it was even more challenging to obtain bone tissue from the elderly non-osteoporosis population in this study. This research was the first in the literature to use the EDX method to detect the concentrations of bone calcium, phosphorus, and metal elements in different areas of bone tissue samples of elderly patients with and without osteoporosis to determine the difference of bone metal elements in the two groups. Therefore, the data of this study had a particular reference value for discussing and analyzing the relationship between bone metal elements and osteoporosis. The research sample size was, however, small, especially the sample size of the non-osteoporosis group was only 14 cases. Most elderly patients with proximal femoral fractures were mainly caused by low energy injuries, while the number of patients with fractures caused by non-low energy injuries such as car accidents and high falls was relatively tiny. Besides, considering ethical issues, the Ethics Committee of Huadong Hospital did not allow a larger number of studies in the normal control group. Another limitation was that there were not many kinds of bone trace elements measured in this research. We measured only four metal elements closely related to bone development but did not measure the levels of some harmful metal elements such as mercury and lead. Therefore, we need to conduct more in-depth and comprehensive research in the long term. These long-term studies require a larger sample size and measurement of more trace elements, which may make up for some limitations of this study and reflect trace element concentration more comprehensively and accurately. In addition, it is also beneficial to analyze and discuss the relationship between trace elements concentrations in bone tissue and osteoporosis.

## Conclusion

Manganese, copper, and zinc appear to play an essential role in bone mineralization and metabolism. Among them, zinc may be most closely related to osteoporosis and play a key role in bone development and maintenance of bone mass. Therefore, we believe that the design of zinc-rich compounds or nutrients as a new complementary factor to increase the intake of zinc for the elderly may be able to prevent and intervene in the occurrence of osteoporosis in the early stage.

## Declarations

### *Ethics approval and consent to participate*

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Huadong Hospital affiliated to Fudan University (no. 2014K40). All participants in this study received oral information prior to giving written consent.

### *Consent for publication*

Not applicable.

### *Author contributions*

**Shangjin Lin:** Formal analysis; Methodology; Writing – original draft.

**Fengjian Yang:** Data curation; Funding acquisition; Investigation; Supervision.

**Ming Ling:** Project administration; Resources; Validation; Visualization.

**Yongqian Fan:** Conceptualization; Funding acquisition; Data curation; Writing – review & editing.

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### *Competing interests*

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

*Availability of data and materials*

The data sets generated during this study are available from the corresponding author on reasonable request.

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## Supplemental material

Supplemental material for this article is available online.

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