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Low Serum Levels of Zinc, Copper, and Iron as Risk Factors for Osteoporosis: a Meta-analysis

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Abstract Zinc (Zn), copper (Cu), and iron (Fe) are essential trace elements for the growth, development, and maintenance of healthy bones. However, there are conflicting reports as to the relationship between serum level of Zn, Cu, or Fe and osteoporosis (OP). The purpose of the present study is to clarify the relationship between serum Zn, Cu, or Fe and OP using a meta-analysis approach. We searched all articles indexed in PubMed published up to May 2014 concerning the association between serum level of Zn, Cu, or Fe and OP. Eight eligible articles involving 2,188 subjects were identified. Overall, pooled analysis indicated that patients with OP had a lower serum level of Zn, Cu, or Fe than the healthy controls (Zn standardized mean difference (SMD)=−1.396, 95 % confidence interval (CI)=[−2.129, −0.663]; Cu SMD=−0.386, 95 % CI=[−0.538, −0.234]; Fe SMD=−0.22, 95 % CI=[−0.30, −0.13]). Further subgroup analysis found that geographical location and gender had an influence on the serum level of Zn in OP and healthy controls, but not on the serum level of Cu or Fe. No evidence of publication bias was observed. In conclusion, this meta-analysis suggests that low serum levels of Zn, Cu, and Fe seem to be important risk factors for OP and well-designed studies with adequate control for confounding factors are required in future investigations.

Keywords Zinc · Copper · Iron · Osteoporosis · Risk factor · Meta-analysis

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Introduction

Trace elements are essential for normal growth and development of the skeleton in human and animals. Although they are minor building components in bones, they play important functional roles in bone metabolism and bone turnover [1]. Our previous study finds that low serum level of magnesium is a risk factor for osteoporosis (OP), which drives us to explore more on the association between OP and other trace elements [2].

Trace element disturbances, such as zinc (Zn), copper (Cu), or iron (Fe) deficiency, have become an increasing important risk factor for OP [3–5]. Zn is essential for the growth of human and animals and is required for the growth, development, and maintenance of healthy bones [5–7]. Zn has been demonstrated to stimulate osteoblastic bone formation and to inhibit osteoclastic bone resorption, thereby increasing bone mass [8–10]. Cu plays a pivotal role in crosslinking of collagen and elastin, and it is a necessary mineral for bone development and maintenance [11, 12]. Fe is an integral part of many enzymes and an essential element for life cycle of cells and plays a role in the synthesis of collagen and other proteins that form the structure of bones [13, 14]. Animal studies indicate that bone growth retardation is associated with Zn, Cu, or Fe deficiency and that deficiencies lead to OP [15–21]. Human studies show that there is a significant relationship between low serum level of Zn, Cu, or Fe and low bone mineral density (BMD), and supplementation of these trace elements is found to restore both skeletal growth and

maturation [22–28]. However, other human studies find no association between serum level of Zn, Cu, or Fe and OP [29–31]. Although serum Zn, Cu, or Fe deficiency is plausibly linked to an increase risk of OP, the inconsistency among the findings of previous studies precludes definitive recommendations at present.

Meta-analysis is an important tool for revealing trends that might not be apparent. Therefore, we performed a comprehensive and critical meta-analysis of the studies, in order to draw a more clear and evidence-based conclusion on the association between serum level of Zn, Cu, or Fe and OP.

Methods

Search Strategy

We searched all English-written articles indexed in PubMed published up to May 2014. Literature searches were performed using medical subject heading (MeSH) or free text words. The searching keywords were as follows: "serum zinc" or zinc or "serum copper" or copper or "serum iron" or iron and osteoporosis. Reference lists of all eligible studies were screened to identify potentially eligible studies. Emails were sent to the authors of identified studies for additional information if necessary.

Selection Criteria

Three authors (Jianmao Zheng, Jingjing Quan, Xueli Mao) conducted the search independently. Titles and abstracts were screened for subject relevance. Studies that could not be definitely excluded based on abstract information were also selected for full-text screening. Two authors (Jianmao Zheng, Jingjing Quan) independently selected eligible studies for inclusion possibility. Where there was a disagreement for study inclusion, a discussion was held (with Xueli Mao) to reach a consensus.

Eligible studies should meet the following criteria: (1) human study; (2) case-control study or cohort study or randomized clinical trial; (3) studies focusing on the association between serum level of Zn, Cu, or Fe and OP; (4) studies providing data of serum level of Zn, Cu, or Fe for both osteoporotic and non-osteoporotic subjects; and (5) subjects with no diseases and no drugs intake which might influence the serum level of Zn, Cu, or Fe.

Exclusion criteria included the following: (1) animal study; (2) in vitro or laboratory study; (3) review or case report; (4) studies not providing serum level of Zn, Cu, or Fe for both osteoporotic and non-osteoporotic subjects; (5) subjects with diseases and drugs intake which might

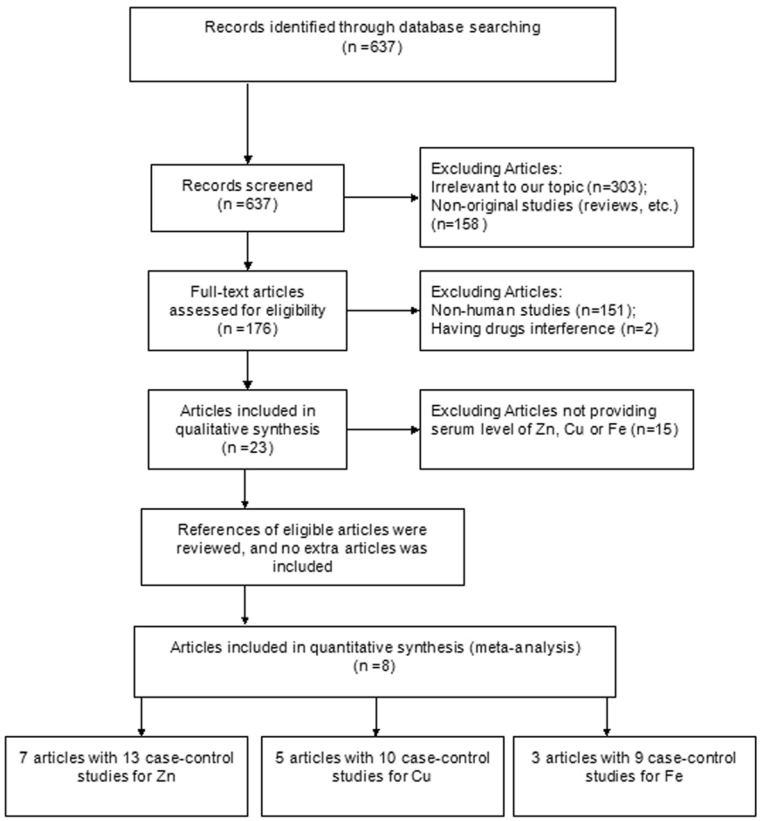

Fig. 1 Flow diagram of study selections

Table 1 Characteristics of subjects in eligible studies (serum Zn for patients with OP versus healthy controls)

Studies	Location	Population	OP			Healthy controls			Score
			Mean age	*N*	Zn (mg/l) (mean±SD)	Mean age	*N*	Zn (mg/l) (mean±SD)	
Relea 1995	Spain	Post-menopausal women	>60	30	727±99	>60	30	749±184	6
Gur 2002	Turkey	Post-menopausal women	>60	70	0.61±0.425	>60	30	1.22±0.31	7
Hyun 2004-1	USA	Men	>60	30	0.771±0.0261	>60	213	0.843±0.0065	7
Hyun 2004-2	USA	Men	>60	40	0.791±0.0196	>60	258	0.843±0.0065	7
Mutlu 2007	Turkey	Post-menopausal women	<60	40	0.47±0.1	<60	40	0.82±0.13	7
Liu 2009	China	Post-menopausal women	<60	123	0.9168±0.2557	<60	31	0.9345±0.2726	7
Arikan 2011	Turkey	Post-menopausal women	>60	35	1.0625±0.3645	<60	35	1.2753±0.4504	6
Okyay 2013-1	Turkey	Post-menopausal women	<60	142	82.6±21.7	<60	434	88.1±15.8	7
Okyay 2013-2	Turkey	Post-menopausal women	<60	71	84.6±20.9	<60	505	87±17.1	7
Okyay 2013-3	Turkey	Post-menopausal women	<60	102	86.5±17.3	<60	474	87.5±19.3	7
Okyay 2013-4	Turkey	Post-menopausal women	>60	95	84.2±17.7	>60	57	90.3±14.9	7
Okyay 2013-5	Turkey	Post-menopausal women	>60	65	85±19	>60	87	87±15.5	7
Okyay 2013-6	Turkey	Post-menopausal women	>60	87	85.4±17.7	>60	65	87.9±15.8	7

influence the serum level of Zn, Cu, or Fe; and (6) sample size less than 20.

Data Extraction and Quality Assessment

Two authors (Xueli Mao, Jianmao Zheng) independently extracted data using a standard form. The following information was extracted from each included study: first author's family name; year of publication; country; demography of subjects (number of subjects and gender); and data on serum Zn, Cu, or Fe.

The qualities of all included studies were assessed using the Newcastle-Ottawa scale (NOS). The assessment tool focused on three aspects, including participant selection, comparability, and exposure. The studies would be assigned stars of nine if all items were satisfied. Two authors (Xueli Mao, Jianmao Zheng) assessed the quality independently.

Statistical Analysis

The extracted data were used to perform meta-analysis to obtain *the standardized mean difference* (*SMD*) and *95% confidence interval* (*CI*). The SMDs were calculated using either fixed-effects models or, in the presence of heterogeneity, random-effects models. Heterogeneity between studies was tested through the *Chi-square* and *I-square* tests. If the I^2 value was less than 50 % and the *p* value was greater than 0.05, the meta-analysis was not considered as homogeneous.

Subgroup analysis was used to identify associations between the serum level of Zn, Cu, or Fe and other relevant study characteristics (such as geographical location and

Table 2 Characteristics of subjects in eligible studies (serum Cu for patients with OP versus healthy controls)

Studies	Location	Population	OP			Healthy controls			Score
			Mean age	*N*	Cu (mg/l)e (mean±SD)	Mean age	*N*	Cu (mg/l) (mean±SD)	
Gur 2002	Turkey	Post-menopausal women	>60	70	1.59±0.64	>60	30	2.09±0.75	7
Mutlu 2007	Turkey	Post-menopausal women	<60	40	1.54±0.12	<60	40	1.6±0.08	7
Liu 2009	China	Post-menopausal women	<60	123	0.8873±0.293	<60	31	0.8498±0.3106	7
Arikan 2011	Turkey	Post-menopausal women	>60	35	13.876±3.721	<60	35	14.092±3.274	6
Okyay 2013-1	Turkey	Post-menopausal women	<60	142	96.6±39	<60	434	112.6±28.2	7
Okyay 2013-2	Turkey	Post-menopausal women	<60	71	90±37.5	<60	505	111.3±30.2	7
Okyay 2013-3	Turkey	Post-menopausal women	<60	102	96.3±33.9	<60	474	111.4±30.9	7
Okyay 2013-4	Turkey	Post-menopausal women	>60	95	102.9±37	>60	57	110.3±35.6	7
Okyay 2013-5	Turkey	Post-menopausal women	>60	65	100±39.2	>60	87	109.7±34.2	7
Okyay 2013-6	Turkey	Post-menopausal women	>60	87	101.7±37.4	>60	65	111±35.1	7

Table 3 Characteristics of subjects in eligible studies (serum Fe for patients with OP versus healthy controls)

Studies	Location	Population	OP			Healthy controls			Score
			Mean age	*N*	Fe (mg/l) (mean±SD)	Mean age	*N*	Fe (mg/l) (mean±SD)	
D'Amelio 2008 (1)	Italy	Post-menopausal women	>60	180	0.9292±0.252	>60	195	0.993±0.2554	7
D'Amelio 2008 (2)	Italy	Post-menopausal women	>60	80	0.9188±0.222	>60	195	0.993±0.2554	7
Shun 2009	China	Post-menopausal women	<60	123	1.3±0.74	<60	31	1.3±0.94	7
Okyay 2013 (1)	Turkey	Post-menopausal women	<60	142	0.93±0.481	<60	434	1.013±0.435	7
Okyay 2013 (2)	Turkey	Post-menopausal women	<60	71	0.868±0.434	<60	505	1.01±0.447	7
Okyay 2013 (3)	Turkey	Post-menopausal women	<60	102	0.907±0.431	<60	474	1.011±0.45	7
Okyay 2013 (4)	Turkey	Post-menopausal women	>60	95	1.01±0.468	>60	57	1.073±0.504	7
Okyay 2013 (5)	Turkey	Post-menopausal women	>60	65	0.997±0.497	>60	87	1.06±0.469	7
Okyay 2013 (6)	Turkey	Post-menopausal women	>60	87	0.996±0.483	>60	65	1.087±0.47	7

gender) as possible sources of heterogeneity. Publication bias was measured using Begg's tests and visualization of funnel plots. The stability of the study was also detected by sensitivity analysis, through re-meta-analysis with one involved study excluded each time. All statistical analyses were performed with Stata version 11.0 (StataCorp, College Station, TX).

Results

Literature Search

The literature search yielded a total of 637 primary articles. These articles were included for full-text assessment, of which 629 were excluded for one of the following reasons: (1) irrelevant to our topic (n=303), (2) non-original studies (reviews etc.) (n=158), (3) non-human studies (n=153), and (4) articles not providing serum level of Zn or Cu or Fe for both osteoporotic and non-osteoporotic subjects (n=15). Overall, 8 eligible articles with 2,188 subjects met the inclusion criteria for meta-analysis, in which 7 articles with 13 case-control studies for Zn [23–25, 29–32], 5 articles with 10 case-control studies for Cu [23, 24, 30–32], and 3 articles with 9 case-control studies for Fe [27, 30, 32]. Of note, five articles involved more than one risk factor and were included in more than one group [23, 24, 30–32]. A flow diagram of the study selection process is presented in Fig. 1.

Study Characteristics and Quality Assessment

The detailed characteristics of the included studies and the results of the quality assessment were summarized in Tables 1,

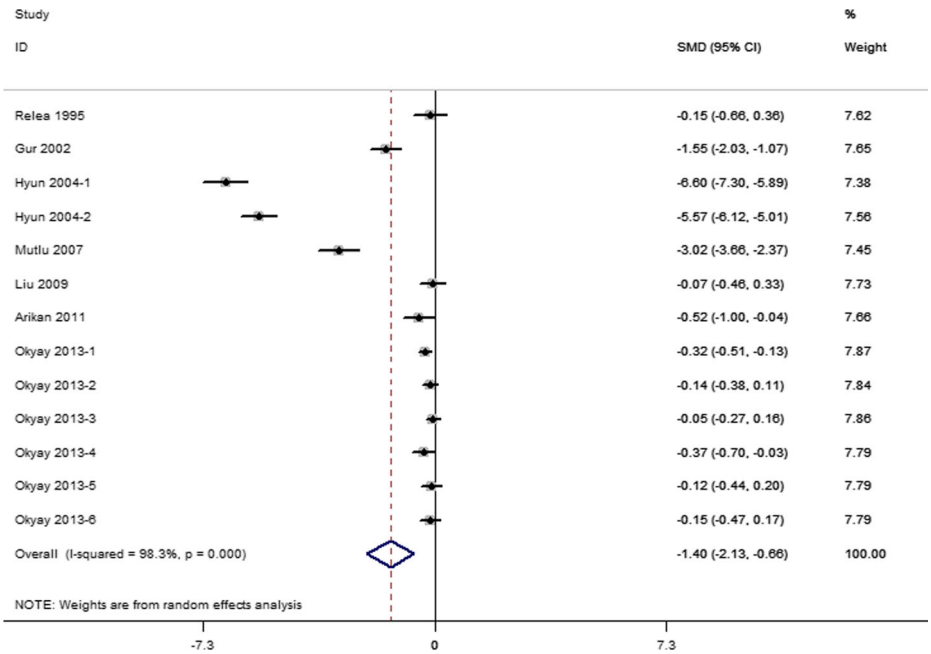

Fig. 2 Forest plot of studies in serum Zn for patients with OP versus healthy controls. The combined SMD and 95 % confidence intervals (CIs) were calculated using the random-effects model

Table 4 Effects by subgroup analysis

Subgroups	No. of studies	SMD (95 % CI)	I^2 (%)	p
Serum Zn geographical location				
Western countries	3	−4.099 (−8.187, −0.011)	99.3	<0.001
Oriental countries	10	−0.562 (−0.901, −0.222)	91.5	<0.001
Gender				
Female	11	−0.525 (−0.843, −0.207)	90.5	<0.001
Male	2	−6.058 (−7.067, −5.049)	80.3	0.024
Serum Cu geographical location				
Turkey and China	10	−0.386 (−0.538,−0.234)	57.5	0.012
Turkey	9	−0.455 (−0.552, −0.359)	39.7	0.103
Serum Fe geographical location				
Western countries	2	−0.195 (−0.295, −0.096)	0	0.888
Oriental countries	7	−0.270 (−0.431, −0.110)	0	0.767

2, and 3. The number of subjects in each study ranged from 60 [29] to 576 [32]. The earliest study was published in 1995 [29], and the latest in 2013 [32]. By geographic location, five case-control studies were conducted in western countries [25, 27, 29], and 10 case-control studies in oriental countries [23, 24, 30–32]. Thirteen case-control studies were conducted with the post-menopausal women [23, 24, 27, 29–32], and 2 case-control studies with men [25]. The overall study quality averaged 6.9 stars on a scale of 0 to 9.

Serum Zn and OP

The random-effects meta-analysis results indicated that patients with OP had a lower serum level of Zn than the healthy controls (SMD=−1.396, 95 % CI=[−2.129, −0.663]) (Fig. 2). The 13 sets of results showed a statistically significant amount of heterogeneity (I^2=98.3 %, p<0.001) (Fig. 2). The subgroup analysis showed that geographical location and gender had an influence on the serum level of Zn in OP and healthy controls. The difference of serum level of Zn between OP and healthy controls in western countries (or male) was higher than that in oriental countries (or female) (Table 4).

Serum Cu and OP

The random-effects meta-analysis results indicated that patients with OP had a lower serum level of Cu than healthy controls (SMD=−0.386, 95 % CI=[−0.538, −0.234]) (Fig. 3). The 10 sets of results showed a statistically significant amount of heterogeneity (I^2=57.5 %, p=0.012) (Fig. 3). The subgroup analysis showed that geographical location was the main sources of heterogeneity. Further analysis found that the

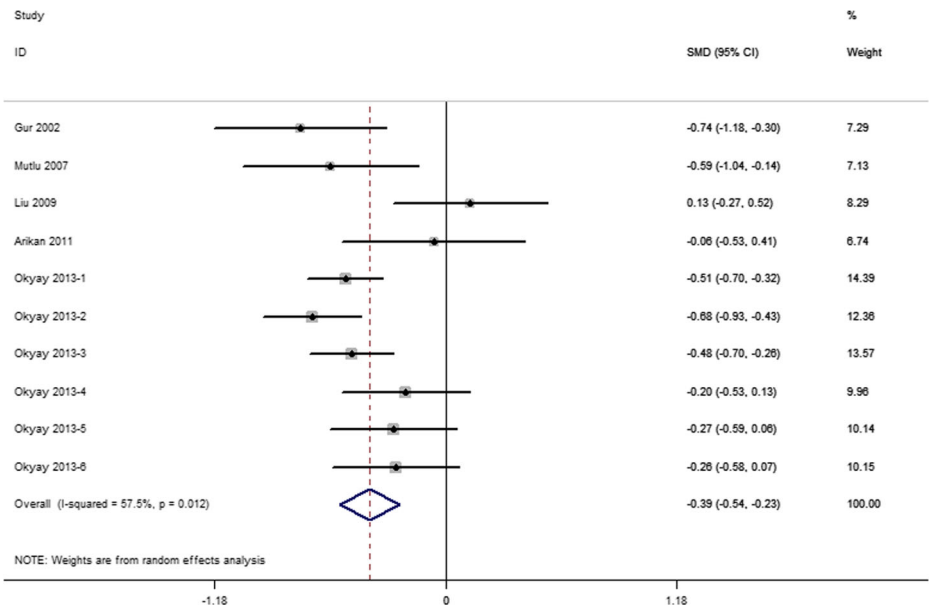

Fig. 3 Forest plot of studies in serum Cu for patients with OP versus healthy controls. The combined SMD and 95 % confidence intervals (CIs) were calculated using the random-effects model

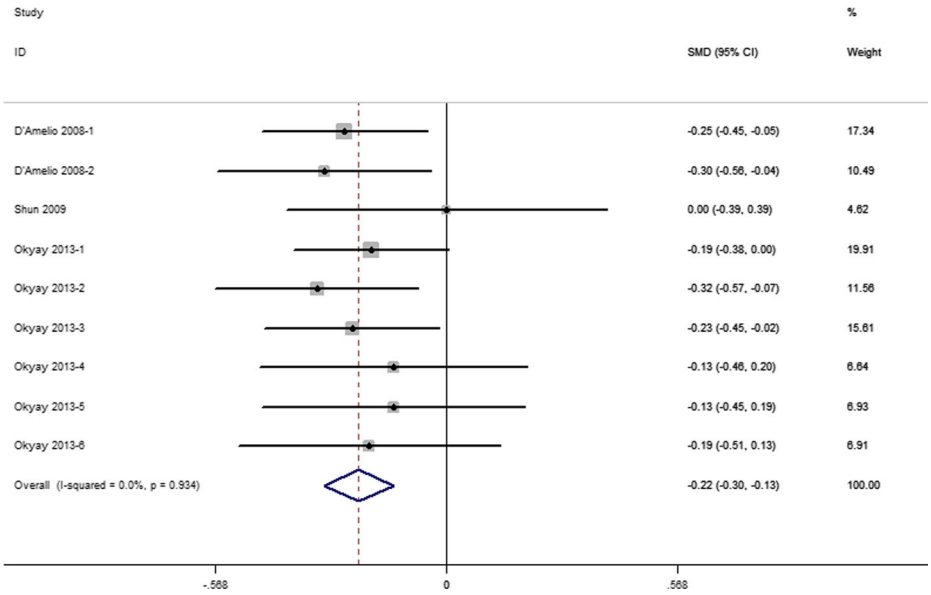

Fig. 4 Forest plot of studies in serum Fe for patients with OP versus healthy controls. The combined SMD and 95 % confidence intervals (CIs) were calculated using the fixed-effects model

subgroup which excluded the only one Chinese study (Liu in 2009) showed no significant amount of heterogeneity (I^2= 39.7 %, p=0.103) (Table 4).

Serum Fe and OP

The fixed-effects meta-analysis results indicated that patients with OP had a lower serum level of Fe than healthy controls (SMD=−0.22, 95 % CI=[−0.30, −0.13]) (Fig. 4). The nine sets of results showed no significant amount of heterogeneity (I^2=0, p=0.934) (Fig. 4).

Publication Bias and Sensitivity Analysis

Publication bias was measured using Begg's tests and visualization of funnel plots. There was no evidence of publication bias for Zn (Begg's test: p=0.300), Cu (Begg's test: p=0.999), and Fe (Begg's test: p=0.251) (Fig. 5). Sensitivity analysis showed that excluding any one involved study from the pooled analysis did not vary the results substantially, except for the study performed by Liu in 2009, which can eliminate the heterogeneity of studies for serum Cu (Table 5).

Discussion

The result of random-effects meta-analysis indicated that the low serum level of Zn was associated with OP. This result is in keeping with some data showing that Zn deficiency might have harmful effects on osseous metabolism, leading to OP. First, Zn has been shown to be concentrated in the layer of osteoid prior to calcification; the deficiency of Zn causes the deterioration of bone formation, which can be completely prevented by the supplementation of Zn [16, 33–35]. Second, Zn stimulates osteoblastic bone formation and the deficiency of Zn decreases osteoblastogenesis. The proliferation of osteoblastic cells was stimulated after culture with Zn [36]. Zn has been shown to stimulate cell differentiation of osteoblastic cells and can remarkably increase alkaline phosphatase activity and the expression of Runx2 [8, 37]. Deficiency of Zn decreases osteoblastogenesis associated with the reduced expression of Runx2 through the inhibition of Wnt/beta-catenin signaling [9]. Third, Zn has been shown to have suppressive effects on osteoclastogenesis and osteoclastic cell death [38]. Zn has an inhibitory effect on RANKL-induced osteoclast-like cell formation and TNFa-induced osteoclastogenesis [39, 40]. Finally, Zn modulates anabolic effect of 1,25-dihydroxyvitamin D3 or estrogen on bone metabolism

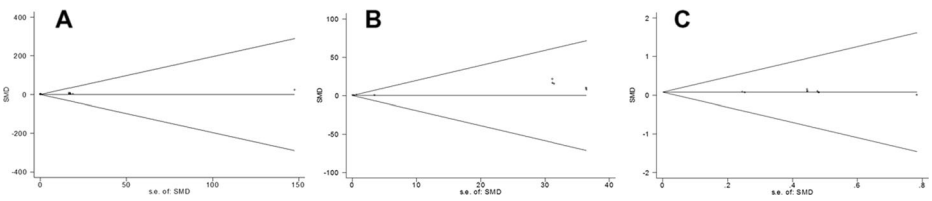

Fig. 5 Funnel plot for studies in serum Zn, Cu, or Fe for patients with OP versus healthy controls

Table 5 The heterogeneity of the included studies through sensitivity analysis

Excluded study	SMD (95 % CI)	I^2 (%)	*p* value
Serum Zn			
Before excluding	−1.396 (−2.129, −0.663)	98.3	<0.001
Relea 1995	−1.500 (−2.278, −0.723)	98.5	<0.001
Gur 2002	−1.384 (−2.156, −0.613)	98.5	<0.001
Hyun 2004 (1)	−0.971 (−1.570, −0.372)	97.5	<0.001
Hyun 2004 (2)	−1.038 (−1.618, −0.450)	97.3	<0.001
Mutlu 2007	−1.264 (−2.005, −0.522)	98.4	<0.001
Liu 2009	−1.509 (−2.296 ,−0.723)	98.5	<0.001
Arikan 2011	−1.470 (−2.252, −0.689)	98.5	<0.001
Okyay 2013 (1)	−1.496 (−2.359, −0.633)	98.5	<0.001
Okyay 2013 (2)	−1.508 (−2.330, −0.686)	98.5	<0.001
Okyay 2013 (3)	−1.516 (−2.351, −0.682)	98.4	<0.001
Okyay 2013 (4)	−1.486 (−2.286, −0.686)	98.5	<0.001
Okyay 2013 (5)	−1.507 (−2.306, −0.708)	98.5	<0.001
Okyay 2013 (6)	−1.505 (−2.304, −0.705)	98.5	<0.001
Serum Cu			
Before excluding	−0.386 (−0.538, −0.234)	57.5	0.012
Gur 2002	−0.359 (−0.515, −0.203)	58.0	0.015
Mutlu 2007	−0.369 (−0.531, −0.207)	61.2	0.008
Liu 2009	−0.455 (−0.552, −0.359)	39.7	0.103
Arikan 2011	−0.410 (−0.565, −0.256)	57.4	0.016
Okyay 2013 (1)	−0.362 (−0.540, −0.185)	60.1	0.010
Okyay 2013 (2)	−0.346 (−0.501, −0.192)	51.1	0.037
Okyay 2013 (3)	−0.368 (−0.546, −0.190)	61.6	0.008
Okyay 2013 (4)	−0.406 (−0.568, −0.244)	58.6	0.013
Okyay 2013 (5)	−0.398 (−0.564, −0.232)	60.4	0.010
Okyay 2013 (6)	−0.399 (−0.565, −0.234)	60.1	0.010
Serum Fe			
Before excluding	−0.220 (−0.300, −0.130)	0	0.934
D'Amelio 2008 (1)	−0.209 (−0.302, −0.116)	0	0.897
D'Amelio 2008 (2)	−0.206 (−0.296, −0.117)	0	0.923
Shun 2009	−0.227 (−0.313, −0.140)	0	0.970
Okyay 2013 (1)	−0.224 (−0.318, −0.129)	0	0.896
Okyay 2013 (2)	−0.203 (−0.293, −0.113)	0	0.943
Okyay 2013 (3)	−0.213 (−0.305, −0.121)	0	0.887
Okyay 2013 (4)	−0.222 (−0.310, −0.135)	0	0.909
Okyay 2013 (5)	−0.223 (−0.310, −0.135)	0	0.910
Okyay 2013 (6)	−0.218 (−0.306, −0.130)	0	0.887

in vitro and in vivo [41–43]. Thus, Zn deficiency may directly result in a decrease in bone formation.

The outcome of this meta-analysis also suggested that low serum level of Cu was an important risk factor for OP. This finding is consistent with previous studies finding that deficiency or low intake of Cu leads to OP. Michelle et al. found that serum Cu deficiency can cause bone lesions in infants, and therapeutic supplementation with Cu corrected their deficits and clinical and radiologic findings [28]. Cu plays a pivotal role in the crosslinking of collagen and elastin, which participates in the production of bony matrix and, when deficient, would result in OP [12, 44, 45]. Cu has a positive effect on the proliferation and function of osteoblast, which is responsible for bone formation deriving from mesenchymal stem cells (MSCs) present in bone marrow stroma. Previous studies find that Cu not only improves the viability of osteoblast but also stimulates MSCs differentiation towards the osteogenic lineage [46, 47]. Thus, Cu deficiency may decrease the bone formation.

The present study still found that low serum level of Fe was associated with OP. Previous studies find that the patients with OP are Fe deficient, and this reduced Fe bioavailability could influence bone metabolism, since Fe acts as a cofactor in enzymes involved in collagen bone matrix synthesis as well as in 25 OH vitamin D hydroxylase, an enzyme involved in activating vitamin D and hence in calcium absorption [27, 48]. Animal studies show that Fe deficiency decreased serum osteocalcin concentration, bone mineral content, bone mineral density, and mechanical strength of the femur [14]. Depletion of Fe in cultured osteoblast cells impaired mineralization, similar to that occurring among some human populations, and reduced bone microarchitecture [19]. Impaired mineralization with Fe deficiency appears to be a possible mechanism for OP.

To the best of our knowledge, this is the first comprehensive meta-analysis to estimate the association between serum level of Zn, Cu, or Fe and OP. However, the possible limitations of our study must be considered. First, only 2,188 subjects included in the meta-analysis might weaken the quality of the results. In addition, because of the heterogeneity which might have been introduced by geographical location and gender, the results are polemic and the conclusion should be more conservative. Despite these limitations, our findings point out new direction for future research, like what is the compound effect of multiple risk factors on OP? For instance, what is the risk of OP with Zn and Cu deficiency or Fe and Cu deficiency? To answer this question, several well-designed studies with adequate control for confounding factors should be considered.

Conclusion

In conclusion, this meta-analysis suggests that low levels of Zn, Cu, and Fe seem to be important risk factors for OP, and well-designed studies with adequate control for confounding factors are required in future investigations.

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