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Is Zinc an Important Trace Element on Bone-Related Diseases and Complications? A Meta-analysis and Systematic Review from Serum Level, Dietary Intake, and Supplementation Aspects

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Abstract

Bone-related diseases are very common problems, especially in the elderly population. Zinc takes part in the growth and maintenance of healthy bones. This meta-analysis aims to evaluate the effects of zinc supplementation or dietary zinc intake on serum zinc levels and bone turnover markers. A systematical research was performed with 2899 articles in PubMed, WoS, and Scopus for relevant articles in English which have mean/standard deviation values of serum zinc levels, dietary zinc intake/zinc supplementation (mg/day), and bone turnover markers up to February 2020. In the overall analysis, serum zinc level was significantly lower in patients with osteoporosis compared with controls (p 0.0002). Dietary zinc intake decreased in the fracture group compared with controls according to subgroup analysis patients with fracture (p 0.02). Zinc supplementation was effective on the femoral neck ($p < 0.0001$) and lumbar spine (p 0.05) bone mineral density (BMD). In the correlation analysis of the data obtained from all of the included studies, serum osteocalcin (p 0.0106, r − 0.9148) correlated with serum zinc level. In conclusion, serum zinc level and dietary zinc intake could have an essential role in preventing osteoporosis. Zinc supplementation might improve bone turnover markers for bone formation such as serum osteocalcin and serum alkaline phosphatase and also, BMD at the site of the femoral neck.

Keywords Bone · Diet · Fracture · Osteopenia · Osteoporosis · Zinc

Introduction

Bone remodeling is a lifelong process in which bone resorption followed by bone formation. However, an imbalance in the homeostasis between resorption and formation processes leads to a change in bone mass in the case of aging, menopause, fracture, and other bone metabolism problems such as osteoporosis [1].

Osteoporosis, a major public health problem, is characterized by low bone mass and micro-architectural deterioration of bone tissue, resulting in an increased risk of bone fragility [2] and fractures of the hip, spine, and other skeletal sites [3]. The World Health Organization has defined osteoporosis criteria based on bone mineral density (BMD) [4] or bone mineral content (BMC), i.e., normal (within 1 SD of the young adult reference mean for the population) osteopenia (between − 1 and − 2.5 SD of the young adult reference mean), osteoporosis (more than − 2.5 SD below the young adult reference mean), and established osteoporosis as the same mass definition but associated with a fragility fracture [2]. Osteoporotic fractures are a serious health concern in populations aged 50 years or older. Malnutrition and low intake of nutrients have been found more prevalent and severe among hip fracture patients as compared with the general elderly populations. In elderly people, nutrient deficiency may accelerate bone loss, micro-architectural deterioration, and increase the risk for subsequent fractures [5].

Nutrition has an important influence on the maintenance of bone mass. Besides macronutrients, minerals such as calcium (Ca), magnesium (Mg), fluoride (Fl), zinc (Zn), copper (Cu), iron (Fe), selenium (Se), and vitamins D, A, C, K, B2, B6, folate, and B12 are required for normal bone metabolism [6]. Imbalances of nutritional intake, especially mineral deficiencies as a result of reduced intake and absorption of these

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nutrients, could be an important factor in the pathophysiological mechanisms of osteopenia or osteoporosis [7].

Especially, zinc is well known as an essential trace element for the growth, development, and maintenance of bone health [8]. The adult human body contains closely 2–3 g of zinc. Approximately 60% of the total body, Zn content was found in the skeletal muscle, 0% in the bone, 5% in the liver and skin, and the remaining 2–3% in other tissues [9]. Zn affects bone metabolism via its role in RANKL/RANK/OPG and Wnt signaling pathways [10] and its action on gene expression of the Runx2/Cbfa1 transcription factor, type I collagen, alkaline phosphatase, and osteocalcin within the cells [11]. RANKL is secreted from osteoblasts, and it is a member of the tumor necrosis factor (TNF) superfamily. RANKL/RANK pathway is essential for osteoclast differentiation. RANKL expression is induced in osteoblastic cells and bone marrow stromal cells in response to osteotropic factors such as PTH, 1,25-dihydroxyvitaminD3, and PGE2. The effect of RANKL was completely abolished by adding a natural antagonist of RANKL, osteoprotegerin (OPG), which is produced in osteoblastic cells. Zinc has a suppressive effect on the receptor activator of nuclear factor (NF)-κB ligand (RANKL) – induced osteoclastogenesis, indicating that the metal inhibits RANKL signaling in pre-osteoclasts [12].

Zinc is a cofactor in bone-related enzymes such as alkaline phosphatase, collagenase, and it affects protein synthesis through activation of DNA polymerase, RNA polymerase, and tRNA polymerase synthetase [13]. In vitro and in vivo studies demonstrated that zinc stimulates bone growth and mineralization, osteoblasts proliferation, differentiation, and increases IgG-1 activity [14].

It was found that zinc intake is lower in osteoporotic or fracture patients, and this might be important for etiopathogenesis and be related to disease prognosis [15, 16]. However, it was also established that zinc supplementation with or without calcium had no significant effect on the bone health of postmenopausal women [3]. It can be seen from different studies that zinc effects on bone turnover and related complications are still unclear.

As osteoporosis, a major public health problem is becoming increasingly prevalent with the aging of the world population [3], the preventive and therapeutic factors are gaining importance. Besides medication, nutritional intervention would be beneficial to maintain bone health throughout life. The reasons mentioned above have taken the attention to dietary zinc intake, zinc supplementation, or serum zinc status in bone metabolism. However, the effects of zinc on bone homeostasis in related diseases remain unclear. The aim of this meta-analysis is to investigate for the first time the effects of zinc supplementation and dietary zinc intake on bone turnover markers and serum zinc status in bone-related comorbidities with data given in 40 studies after systematical search.

Methods

Eligibility Criteria

To show the relationship between zinc and bone metabolism in the case of complications such as osteoporosis, fracture, and fragility, all human studies including serum zinc status/dietary zinc intake/zinc intervention were searched and reviewed. The mean and standard deviation data were collected and assessed to meta-analysis. There were no restrictions imposed on age, gender, or on any other population characteristic such as race or body mass index (BMI).

The inclusion criteria were determined as studies determining the serum zinc levels/zinc intake/zinc supplementation, including the mean and the standard deviation values, reporting the zinc values having a suitable parameter that can be converted with each other between studies, giving the sample size of the groups, making the diagnosis of the disease according to the criteria accepted in the literature, and indicating that appropriate conditions were met for the collection of samples.

Sources and Search

PRISMA procedures were followed for searching and evaluating the data. PubMed, Web of Science, and Scopus databases were searched without any date restrictions, and relevant articles were detected. Searching was being performed until March 2020. Searching keywords were “zinc” OR ”zinc intake” OR ”zinc supplementation” AND “osteoporosis” OR “bone (clinical trial)” OR “fragility (clinical trial)” OR “fracture (clinical trial)” for all databases.

Statistical Analysis

Pooled data were calculated to assess the relationship of serum zinc level/zinc intake/zinc supplementation with bone metabolism in osteoporosis. The I2 was used for measuring of heterogeneity as described before (I2% values of 0–25, 25–50, 50–75, and 75–100 represent no, low, moderate, and high heterogeneity) [17]. The fixed and effect models were used according to heterogeneity chi-square value to combine the results [18]. Meta-analysis was performed with RevMan 5.3. (Cochrane Collaboration, Copenhagen, 2014). GraphPad Prism 6 was used for correlation analyses and figures.

Risk of Bias Assessment of Studies

The risk of bias for each study was assessed either as low, unclear, or high risk for each of the following criteria: selection bias, performance bias, detection bias, attrition bias, and reporting bias, and other as described in the Cochrane Handbook [19].

Results

As a result of the systematical search on PubMed, Scopus, and Web of Science, a total of 2899 publications were screened. Seven hundred forty-four publications were review, book chapters, conference papers, etc. Among 2155 articles, 2115 articles were not related/did not include the inclusion criteria according to the information obtained from the titles, abstracts, or full texts, and a totally 40 articles [6, 7, 13–16, 20–53] met with the inclusion criteria which mentioned above (Fig. 1). Publishing dates of articles were included in the meta-analysis ranged from 1994 to 2020. Characteristics of the included studies were given in Table 1.

Cumulative Meta-analysis

In all bone health complications related to osteoporosis, serum zinc level and dietary zinc intake were evaluated with the random effect model. The case groups include osteoporosis, osteopenia, postmenopausal, and fracture patients' data. The heterogeneity was found to be a high level in serum zinc meta-analysis (99%) and dietary zinc intake meta-analysis (96%). The random effect model performed on sixteen studies for serum zinc level and twelve studies for dietary zinc intake. Serum zinc level did not show significant difference between cases and controls (p 0.10, mean difference − 3.24 [− 7.05, 0.57]) (Fig. 2a). Similarly, dietary zinc intake was not significantly different in cases compared with control groups (p 0.14, mean difference − 0.33 [− 0.77, 0.11]) (Fig. 2b).

Subgroup Analysis

The random effect model performed on eight studies for osteoporosis, four studies for osteopenia, and ten studies for postmenopausal women subgroups to analyze serum zinc level. In order to analyze dietary zinc intake status, the random effect model was performed on four studies for osteoporosis, two studies for osteopenia, five studies for postmenopausal women, and three studies for fracture subgroups.

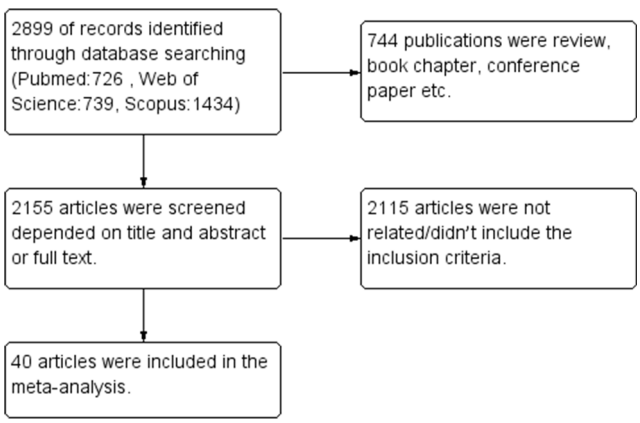

Fig. 1 Flow diagram of study selection

Osteoporosis and Osteopenia Subgroups

Following the cumulative analysis of overall cases of serum zinc and dietary zinc intake status, each complication was analyzed. Serum zinc level was significantly lower in osteoporosis subgroup compared with controls (REM p 0.0002, mean difference − 12.68 [− 19.31, − 6.05] (Fig. 3a). However, there was no any difference between osteoporosis and healthy controls in dietary zinc intake (REM p 0.99, mean difference − 0.01, [− 1.60, 1.57]). It should be mentioned that heterogeneity among included studies was low (42%) in serum zinc evaluation, but moderate heterogeneity was seen in dietary zinc intake analysis (70%) (Fig. 4a).

In osteopenia subgroup, both serum zinc level (REM p 0.14, mean difference − 8.32 [− 19.34, 2.70]) and dietary zinc intake status (REM p 0.88, mean difference − 0.25 [− 2.88, 3.37]) did not show significant difference with control groups (Figs. 3b and 4b).

Postmenopausal Subgroup

The effect of menopause on bone health and its relation with serum zinc levels in postmenopausal cases were evaluated in cumulative analysis and subgroup analysis. There was no significant difference in serum zinc level between postmenopausal women and healthy controls (REM p 0.22, mean difference − 6.77 [− 17.56, 4.01]) with high heterogeneity (97%) (Fig. 3c). Similarly, dietary zinc intake did not show the difference between groups (REM p 0.49, mean difference − 0.40 [− 1.55, 0.70]), and there was high heterogeneity among studies (87%) (Fig. 4c).

Fracture Subgroup

In fracture studies, we could not obtain enough serum zinc level data for subgroup analysis; however, dietary zinc intake status was evaluated. There was no/low heterogeneity between included studies (25%). Dietary zinc intake decreased in fracture group compared with controls according to subgroup analysis result (REM p 0.02, mean difference − 0.50 [− 0.90, 0.09]) (Fig. 4d). In addition to zinc intake, the dietary protein decreased in the fracture group compared with controls according to subgroup analysis result (REM p 0.02, mean difference − 0.41 [− 7.47, − 0.56]).

Zinc Supplementation and Bone Markers

Several common bone markers detected that studies include such as BMD of the femoral neck and lumbar spine BMD, and also serum levels of alanine phosphatase (ALP), bone alkaline

Table 1 Characteristics of included studies

Study (author, year)	Study type		Case (mean ± SD)	Case (*n*)	Control (mean ± SD)	Control (*n*)	Duration of zinc supplementation/ ingredients of supplementation	Zinc determination
Arikan 2011 (osteopenia)		Serum zinc (μg/dL)	116.48 ± 35.46	37	127.53 ± 45.04	35		Atomic absorption spectrophotometer
Arikan 2011 (osteoporosis)		Serum zinc (μg/dL)	106.25 ± 36.45	35	127.53 ± 45.04	35		Atomic absorption spectrophotometer
Braam 2003	Case-control	Zinc supplementation (mg/day)	10	46	-	60	3 years/500 mg calcium, 10 mg zinc, 150 mg magnesium, and 8 mg vitamin D	-
Candan 2020	Case-control	Zinc supplementation (mg/day	20	15	-	9	3 months/250 mg vitamin C, 20 mg zinc	-
		Serum zinc (μg/dL)	122.76 ± 19.59	15	79.01 ± 9.14	9		Atomic absorption spectrophotometer
Canhao 2007 (men)	Cross-sectional study	Serum zinc (μg/dL)	87.58 ± 18.69	10	105.1 ± 15.01	19		-
Canhao 2007 (women)		Serum zinc (μg/dL)	81.6 ± 13.07	24	87.58 ± 18.69	40		-
Elmstahl 1998	Prospective cohort study	Dietary zinc intake (mg/day)	15 ± 6	160	15 ± 5	6416		Combined 7-day menu book, quantitative food frequency questionnaire
Gunn 2013 (osteopenia)	Case-control	Dietary zinc intake (mg/day)	13 ± 6	53	11 ± 4	51		3-day diet diaries
Gunn 2013 (osteoporosis)		Dietary zinc intake (mg/day)	10 ± 4	17	11 ± 4	17		
Gür 2002 (osteoporosis)	Case-control	Serum zinc (μg/dL)	61 ± 42	70	122 ± 31	30		Atomic absorption spectrophotometry
Farrel 2009		Dietary zinc intake (mg/day)	10 ± 3	244				3 days of dietary record
Hyun 2004 (osteopenia)	Case-control	Dietary zinc intake (mg/day)	10.5 ± 0.4	153	11.7 ± 0.3	213		Induction coupled plasma atomic emission spectrometer
		Serum zinc (μg/dL)	82.38 ± 1.3	153	83.34 ± 0.65	213		
Hyun 2004 (osteoporosis)	Case-control	Dietary zinc intake (mg/day)	10.8 ± 0.8	30	11.7 ± 0.3	213		Food frequency questionnaire
		Serum zinc (μg/dL)	77.15 ± 2.62	30	83.34 ± 0.65	213		
Ilich 2002	Cross-sectional	Dietary zinc intake (mg/day)	19.1 ± 13.5	136	-	-		3-day dietary record
Jasminka 2009	Case-control	Dietary zinc intake (mg/day)	9.4 ± 4.3	22	9.7 ± 3.6	56		3-day records
Jensen 2002	Case-control	Dietary zinc intake (mg/day)	8.3 ± 1.8	25	9.7 ± 2.8	21		-
		Zinc supplementation (mg/day)	15	25	-	21	3 years/1450 mg Ca, 400 IU vitamin D, 600 mg Mg, 15 mg Zn, 3.5 mg Mn, 2 mg Cu	-
Kadam 2010	Case-control	Serum zinc (μg/dL)	71 ± 16	92	71 ± 20	80		Atomic absorption spectrometer
Kamp 2008	Case-control	Serum zinc (μg/dL)	113.6 ± 17.6	18	102.5 ± 12.4	18		Inductively coupled plasma atomic emission spectrometry
		Zinc supplementation (mg/day)	25	18	-	18	8 weeks/50 mg Fe, 25 mg Zn	-
Kim 2007	Case-control	Dietary zinc intake (mg/day)	5.42 ± 2.95	76	7.12 ± 1.71	76		24-h recall method for 3 days.
		Serum zinc (μg/dL)	76.2 ± 9.72	76	93.05 ± 17.7	76		Induced coupled plasma spectrometer

Table 1 (continued)

Study (author, year)	Study type		Case (mean ± SD)	Case (*n*)	Control (mean ± SD)	Control (*n*)	Duration of zinc supplementation/ ingredients of supplementation	Zinc determination
Kim 2016	Cross-sectional study	Dietary zinc intake (mg/day)	10.37 ± 3.85	18	-	-		Semi-quantitative food frequency questionnaire
Krebs 1998	Case-control	Dietary zinc intake (mg/day)	10.82 ± 0.49	6	-	-		Weighed conventional foods on a constant 7-day rotation menu.
		Serum zinc (μg/dL)	128.6 ± 16.97	6	-	-		Atomic absorption spectrophotometry flame
Kruger 2009	Case-control	Zinc supplementation (mg/day)	2.4	30	0.2	30	4 months/1200 mg Ca,	-
			2.4	30	0.2	30	96 mg Mg, 2.4 mg Zn, 9.6 μg vitamin D	-
Kruger 2015	Case-control	Zinc supplementation (mg/day)	2.4	67	-	69	4 months/1200 mg Ca,	-
			2.4	61	-	60	96 mg Mg, 2.4 mg Zn, 15 μg vitamin D	-
Li 2017	Cross-sectional study	Dietary zinc intake (mg/day)	5.4 ± 2.3	95				3-day food record within a single week
Lim 2014	Case-control	Dietary zinc intake (mg/day)	6.8 ± 2.3	20	9.3 ± 3.1	21		24-h recall method for 3 days
Liu 2009 (osteopenia)	Case-control	Serum zinc (μg/dL)	91 ± 3	127	93 ± 27	21		Atomic absorption spectrometry
Liu 2009 (osteoporosis)			91 ± 25	123	93 ± 27	31		Atomic absorption spectrometry
Mahdaviroshan 2013	Case-control	Serum zinc (μg/dL)	120.5 ± 7.7	30	70.5 ± 4.6	30		Atomic absorption spectrophotometer
Mutlu 2007 (osteopenia)	Case-control	Serum zinc (μg/dL)	63 ± 9	40	82 ± 13	40		Zeeman atomic absorption spectrometry
Osteoporosis	Case-control	Serum zinc (μg/dL)	47 ± 1	40	82 ± 13	40		Zeeman atomic absorption spectrometry
New 1997	Cross-sectional study	Dietary zinc intake (mg/day)	10.0 ± 2.9	994	-	-		Food frequency questionnaire (FFQ)
Nielsen 2004	Case-control	Zinc supplementation (mg/day)	53	12	3	12	2 years/3 mg Cu, 53 mg Zn	-
Nielsen 2011	Case-control	Dietary zinc intake (mg/day)	10.38 ± 0.47	112	10 ± 0.34	112		5-day food diaries
		Zinc supplementation (mg/day	12	112	-	112	2 years/600 mg Ca, 2 mg Cu, and 12 mg Zn	-
Okyay 2013-1 (osteoporosis)	Case-control	Serum zinc (μg/dL)	82.6 ± 21.7	142	88.1 ± 15.8	434		Auto-analyzer
Okyay 2013-2 (osteoporosis)			86.5 ± 17.3	102	87.5 ± 19.3	474		
Okyay 2013-3 (osteoporosis)			84.2 ± 27.7	45	90.3 ± 14.9	57		
Okyay 2013-4 (osteoporosis)			85.4 ± 17.7	87	87.9 ± 15.8	65		
Peretz 2011	Case-control	Serum zinc (μg/dL)	108.5 ± 9.8	10	116.25 ± 18.2	10		Flame atomic absorption spectroscopy
		Zinc supplementation (mg/day)	50	10	-	10	12 weeks/50 mg Zn	-
Relea 1995	Case-control	Serum zinc (μg/dL)	72.7 ± 9.9	30	74.9 ± 18.4	30		Atomic absorption spectrophotometry

Table 1 (continued)

Study (author, year)	Study type		Case (mean ± SD)	Case (*n*)	Control (mean ± SD)	Control (*n*)	Duration of zinc supplementation/ ingredients of supplementation	Zinc determination
Rodondi 2009	Case-control	Serum zinc (μg/dL)	16 ± 0.9	30	14.4 ± 3.2	31		The Randox colorimetric method
		Zinc supplementation (mg/day)	30	30	-	31	4 weeks/essential amino acids-whey protein supplements + 30 mg Zn	-
Roman 2004	Cross-sectional	Dietary zinc intake (mg/day)	13.3 ± 6.9	20	9.23 ± 3.5	72		3-day written food record
Roshan 2015 (osteopenia)		Dietary zinc intake (mg/day)	3.87 ± 0.3	28	-	-		Atomic absorption spectrophotometer
		Serum zinc (μg/dL)	70.44 ± 4.5	28	-	-		
Roshan 2015 (osteoporosis)	Case-control	Dietary zinc intake (mg/day)	3.75 ± 0.22	23	-	-		Atomic absorption spectrophotometer
		Serum zinc (μg/dL)	63.3 ± 4.8	23				
Sadighi 2008	Case-control	Dietary zinc intake (mg/day)	5.83 ± 1.08	30	5.02 ± 0.36	30		3-day food records
		Serum zinc (μg/dL)	133 ± 60	30	77 ± 21	30		The atomic absorption spectrophotometer
Samleri 2012	Case-control	Dietary zinc intake (mg/day)	9.3 ± 3.1	155	7.53 ± 6.76	1327		24-h dietary recall and a food frequency questionnaire
Sanchez 2005 (55–70 years)	Case-control	Serum zinc (μg/dL)	84.7 ± 9.7	188	-	-		Flame atomic absorption spectrometry
		Dietary zinc intake (mg/day)	10.99 ± 3.98	188	-	-		4-day recall-record method
Sanchez 2005 (> 70 years)	Case-control	Serum zinc (μg/dL)	86.26 ± 11.23	199	-	-		Flame atomic absorption spectrometry
		Dietary zinc İntake (mg/day)	11.32 ± 5.16	199	-	-		4-day recall-record method
Shiota 2004	Case-control	Serum zinc (μg/dL)	82 ± 16.7	9				Absorption spectrophotometer
Strause 1994	Case-control	Zinc supplementation (mg/day)	15	14	-	18	2 years/1000 mg elemental Ca with an addition of 15 mg zinc, 5 mg manganese, and 2.5 mg copper/day	-
Sugiyama 2000	Prospective case-control	Serum zinc (μg/dL)	125.8 ± 40	5				Atomic absorption spectrophotometry
		Zinc supplementation (mg/day)	68	5			6-month AHZ (300 mg/day)/contains 68 mg zinc per tablet	-
Sun 2012 (men)	Case-control	Dietary zinc intake (mg/day)	10.65 ± 3.38	177	11.46 ± 3.08	177		Modified semi-quantitative FFQ
Sun 2012 (women)			10.65 ± 3.38	549	11.46 ± 3.08	549		
Zhou 2011	Case-control	Serum zinc (μg/dL)	12 ± 2.7	31	-	-		-

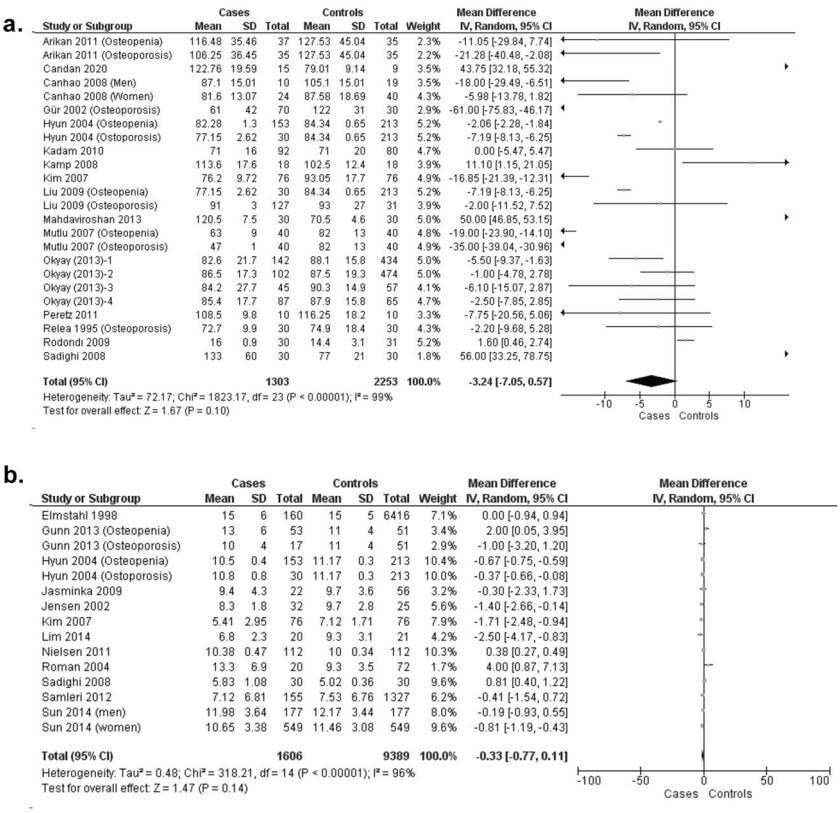

Fig. 2 **a** Cumulative meta-analysis of serum zinc status in overall bone health complications. **b** Cumulative meta-analysis of dietary zinc intake status in overall bone health complications

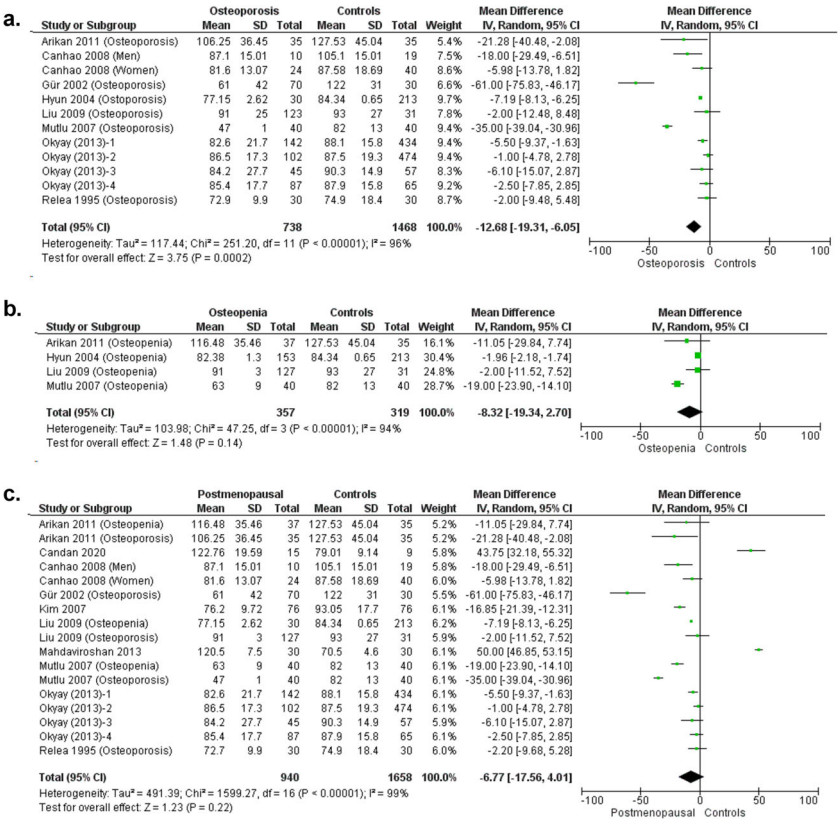

Fig. 3 Subgroup analysis of serum zinc level. **a** Random effect model of osteoporosis subgroup analysis. **b** Random effect model of osteopenia subgroup analysis. **c** Random effect model of postmenopausal cases subgroup analysis

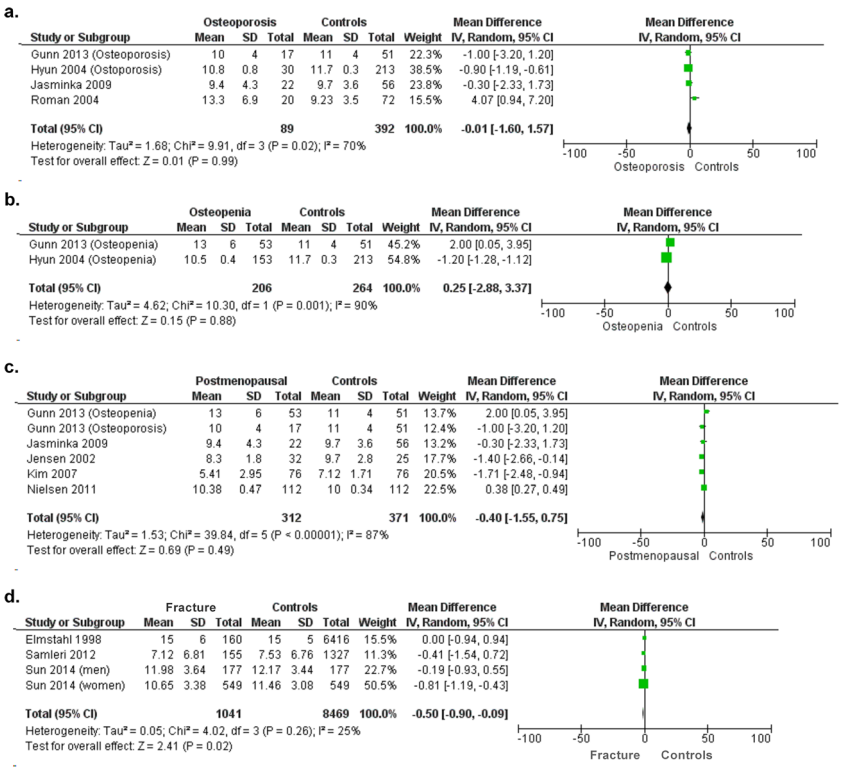

Fig. 4 Subgroup analysis of dietary zinc intake status. **a** Random effect model of osteoporosis subgroup analysis. **b** Random effect model of osteopenia subgroup analysis. **c** Random effect model of postmenopausal subgroup analysis. **d** Random effect model of fracture subgroup analysis

phosphatase (BAP), osteocalcin and parathyroid hormone (PTH). Respectively, heterogeneity i2 levels were high (85%), low (40%), no (0%), high (77%), high (94%), and high (100%) of each bone marker analysis. According to heterogeneity chi-square value, fixed and random effect models were used. The number of studies included for each bone marker analyses was mentioned at the end of the analysis results. Zinc supplementation was effective on the femoral neck (FEM $p < 0.0001$, mean difference 0.02 [0.01, 0.02])($n = 3$) and lumbar BMD (FEM p 0.05, mean difference − 0.01 [− 0.01, 0.00]) ($n = 4$). However, it showed a different effect on these different areas that while femoral neck BMD was higher in zinc supplementation groups, lumbar BMD was affected negatively. These results might be depended on differences in study protocols, supplementation duration, and characteristics among studies.

While serum ALP levels were found to be higher with zinc supplementation groups compared with control groups (FEM $p < 0.0001$, mean difference 33.70 [22.79, 44.61]) ($n = 12$), serum BAP levels did not show the difference between groups (REM p 0.73, mean difference 0.84 [− 3.96, 5.64]) ($n = 2$), notably serum osteocalcin levels were lower in supplementation groups compared with controls in the random effect model (p 0.003, mean difference − 4.14 [− 6.92, − 1.36])($n = 4$); at last, there was no any significant difference in serum PTH levels between zinc supplementation (+) and (−) groups (REM p 0.76, mean difference 3.55 [− 18.88, 25.98]) ($n = 5$) (Fig. 5).

Correlation Analysis

Correlation analysis was done to consolidate the relationship between serum, dietary, or supplementary zinc and bone health. The number of studies included for each correlation analysis was mentioned at the end of the result. As an important result, it was showed that both dietary zinc intake (p 0.8678) ($n = 11$) and zinc supplementation (p 0.35) ($n = 7$) did not affect the serum zinc levels (Fig. 6a, b). There was a correlation between dietary energy intake and serum zinc status (p 0.0215, r 0.6063) ($n = 27$) (Fig. 6c). When bone markers and serum zinc level status relation was investigated, serum osteocalcin (p 0.0106, r − 0.9148) ($n = 6$) were correlated with serum zinc level, however serum ALP level was correlated (p 0,1453, r 0.4468) ($n = 12$) (Fig. 6d, e). Neither lumbar (p 0.4102, r 0.2167) ($n = 16$) nor femoral BMD (p 0.1537, r 0.4608) ($n = 11$) was correlated with serum zinc status (Fig. 6f, g).

In addition, to understand zinc sources of individuals, dietary macronutrient intakes in relation with dietary zinc intake were examined. There were correlations with dietary protein (p 0.0129, r 0.4995) ($n = 24$) and dietary fat (p 0.0010, r 0.8216) ($n = 12$) intakes, however dietary carbohydrate intake did not show significant correlation with dietary zinc status but it was tend to have positive correlation too (p 0.0543, r 0.5446) ($n = 13$). Also, other dietary micronutrients which might have an effect on zinc absorption were evaluated, and it was seen that dietary zinc intake was correlated with dietary

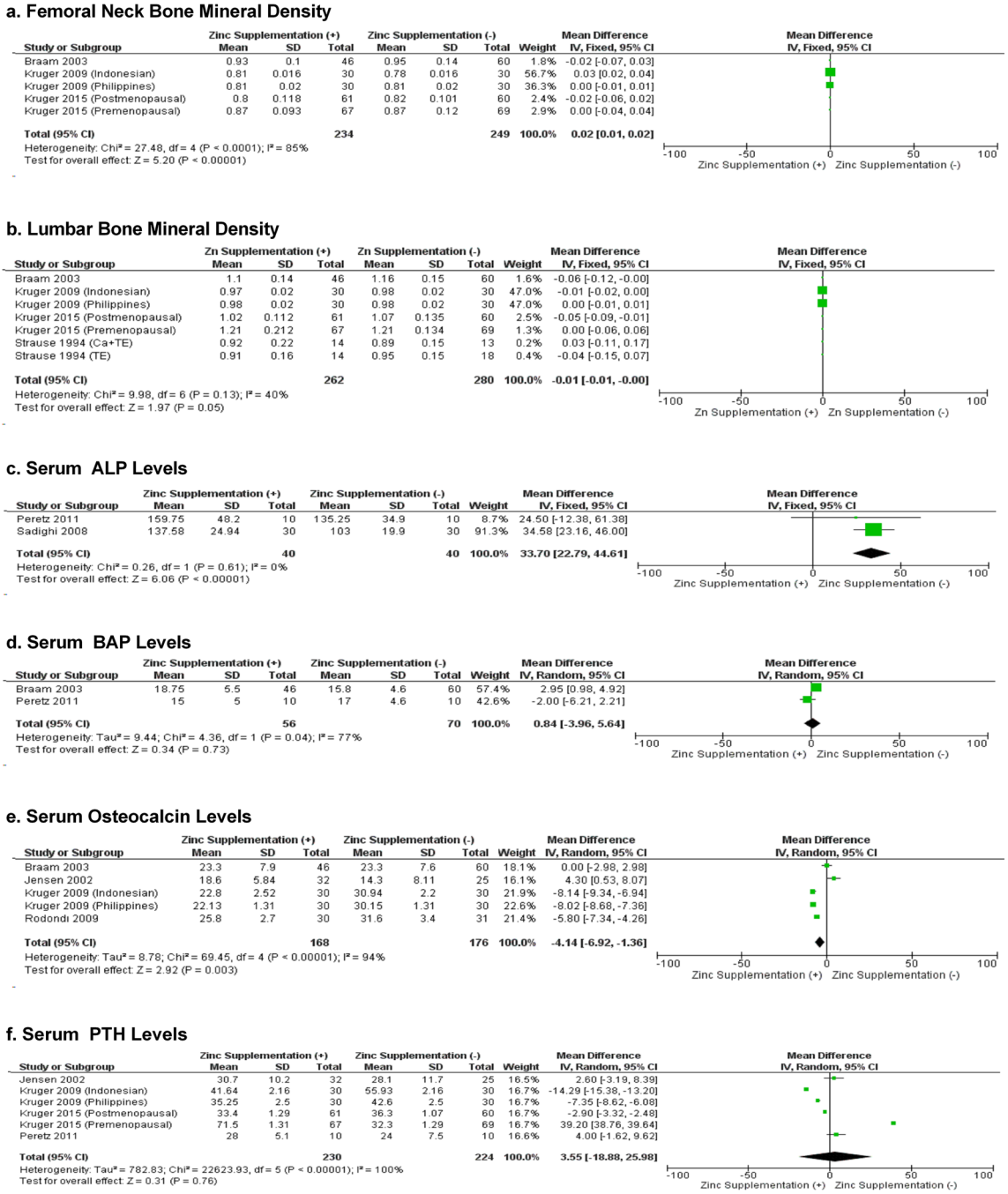

Fig. 5 Zinc supplementation effects on several bone markers. **a** The fixed effect model of femoral neck BMD data. **b** The fixed effect model of lumbar BMD data. **c** The fixed effect model of serum ALP status data. **d** The random effect model of serum BAP status data. **e** The random effect model of serum osteocalcin data. **f** The random effect model of serum PTH status data obtained from different studies

phosphorus (p 0.0118, r 0.6505) ($n = 14$), calcium (p 0.0117, r 0.4215) ($n = 35$), magnesium (p 0.0003, 0.7896) ($n = 16$), iron (Fe) (p 0.002, r 0.6937) ($n = 17$), potassium (p 0.007, r 0.891) ($n = 6$), and folate (p 0.03, r 0.681) ($n = 8$) intakes, however, there was no any correlation with dietary copper (p 0.479, r −0.266) ($n = 8$) and sodium (p 0.659, r −0.186) ($n = 7$) status (Fig. 7).

Risk of Bias Assessments

The funnel plot shown in Fig. 8a and b do not suggest evidence of publication bias in the studies included in this meta-analysis. The assessment of the bias status of each study is shown in Fig. 8c and d. There was a low risk of bias in studies included in this meta-analysis. The high risk of bias was

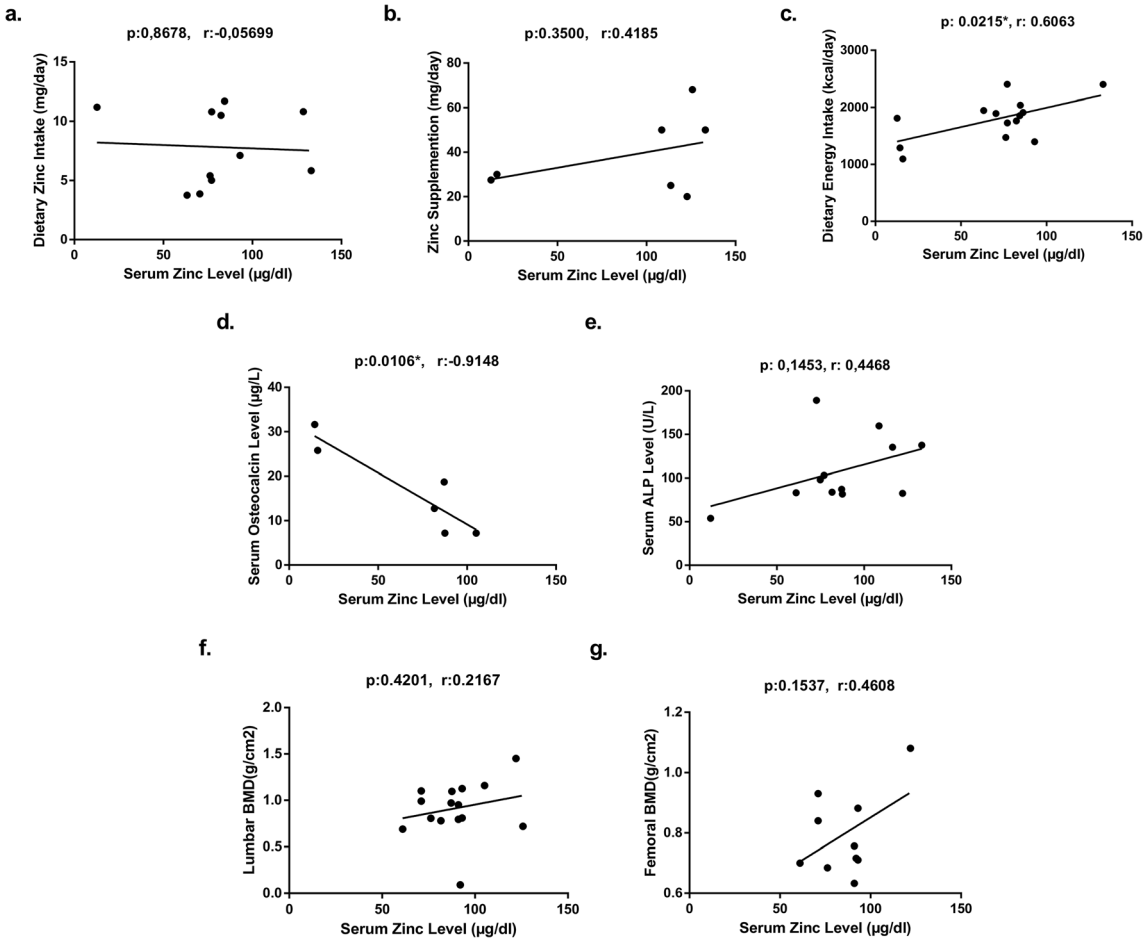

Fig. 6 Correlations between serum zinc level (µg/dL) and (**a**) dietary zinc intake (mg/day), (**b**) zinc supplementation (mg/day), (**c**) dietary energy intake (kcal/day), (**d**) serum ALP level (U/L), (**e**) serum osteocalcin level (µg/L), (**f**) lumbar bone mineral density (BMD) (g/cm^2), (**g**) femoral bone mineral density (BMD) (g/cm^2)

detected among the studies that had not given clear study group selection, exclusion, and inclusion criteria, and also had not given clear statistical analysis methodology in the study paper.

Discussion

This meta-analysis established that serum zinc level did not show a significant difference in overall bone turnover–related complications, such as osteoporosis, osteopenia, fracture, or postmenopause from control groups (Fig. 2a), however the subgroup analysis of osteoporosis patients' data showed that serum zinc level was lower in osteoporosis (Fig. 3a). The dietary zinc intake status has not differed between groups, but it was found to be lower in fracture subgroup analysis (Fig. 4). The correlation analysis also showed that both dietary zinc intake and zinc supplementation did not affect the serum zinc levels**.** Also, serum zinc levels and BMD were not found to be correlated (Fig. 6). To better interpret these results, zinc homeostasis, changing bone metabolism, and dietary factors are examined.

In a meta-analysis of Zheng et al. which examine serum zinc, iron, and copper status in osteoporotic patients, the random effect meta-analysis results show that patients with osteoporosis had a lower serum level of Zn than the healthy controls (SMD = − 1.396, 95% CI = [− 2.129, − 0.663]). The 13 sets of results showed a statistically significant amount of heterogeneity ($I2 = 98.3\%$, $p < 0.001$) [54]. In our meta-analysis, there was no significant difference in the overall analysis of bone turnover–related complications. However, osteoporotic patients' data analysis result was similar to the meta-analysis of Zheng et al. The different results may arise due to the data obtained from different patients' groups between these meta-analyses. Zheng et al. examined serum zinc levels in osteoporotic postmenopausal women; however, our data was obtained from both men and women with osteoporosis, osteopenia, fracture, and also postmenopausal women.

The subgroup analysis of osteoporotic patients showed that there was a significant difference in serum zinc level and

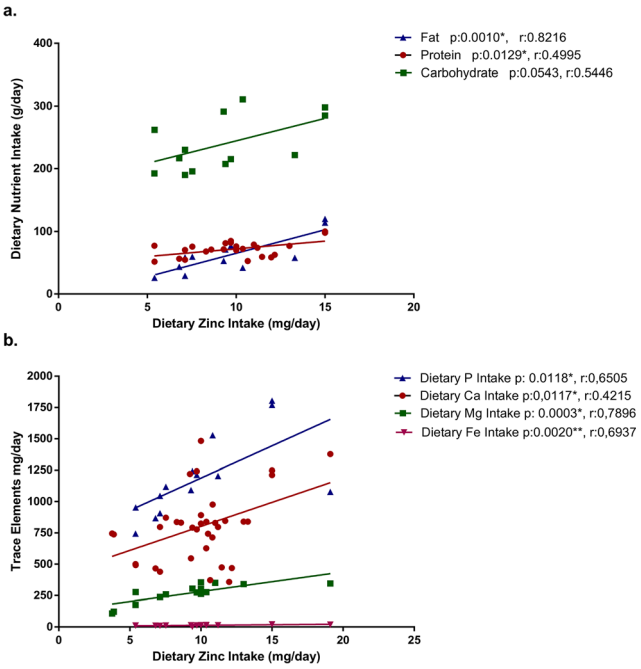

Fig. 7 Correlations of dietary zinc intake (mg/day) with (**a**) dietary macronutrients (g/day), (**b**) dietary trace elements (mg/day) intake

dietary zinc intake than their non-osteoporotic controls (Figs. 3a and 4). In the study of Hyun et al., which is also included in our meta-analysis, when the zinc intakes and plasma concentrations were examined in men with osteoporosis, plasma zinc was found to be correlated with total zinc intake, including intake from supplements, but not with dietary zinc intake alone. Also, zinc intake and measured plasma zinc levels were significantly lower in men with osteoporosis than in men without osteoporosis [27].

In another subgroup analysis, postmenopausal women were investigated. Neither serum zinc level nor dietary zinc intake showed a significant difference between postmenopausal and control groups (Figs. 3c and 4c). These results might indicate that despite postmenopausal women having a high risk for osteoporosis, all postmenopausal women should not be thought of as osteoporotic patients, and their dietary pattern and serum status should be well examined before any supplement intervention.

As it is known, total body zinc has two metabolic pools that named as the rapid and slow pool. The rapid pool includes zinc in plasma, extracellular fluid, and in the liver, pancreatic, kidney, and intestinal tissue; in contrast, a slow pool consists of the skeletal muscle and bone, which has almost 90% of the whole-body zinc. Severe dietary zinc restriction (< 1 mg/day for 4 to 5 weeks) causes a decrease (approximately 35%) of zinc in the rapid pool but has little or no measurable effect on the slow pool [55]. The homeostatic mechanisms were insufficient to maintain body zinc in case of extreme intake and that can lead to loss or accumulation of zinc in the body [56]. The similar Zn intakes in the two groups could explain why we did not observe significant differences in serum Zn levels in this meta-analysis. Also, in a previous study in the literature, it was shown that bone zinc status was significantly lower in patients with fractures compared with healthy controls [57]. Thus, it is thought that in the case of bone turnover and fracture, the zinc needs of the body might be increased.

In this meta-analysis, we examine the effects of zinc supplementation on bone mineral density at the site of the femoral neck and lumbar spine, and some biochemical markers related to bone metabolism such as serum PTH, ALP, BAP, and osteocalcin levels (Fig. 5). However, it was established that the femoral neck BMD of the zinc-supplemented group were significantly higher than controls. Interestingly, lumbar spine BMD tended to be decreased in the zinc-supplemented group compared with lean controls. Differences between study groups or measurements might cause these results. It should be well examined in further studies the underlying mechanism of zinc supplementation on BMD. Also, it should be considered that supplementation interventions did not include only zinc; in some studies, multi-supplements were used, such as vitamins and other trace elements. In the study of Braam et al., which is meta-analyzed in our study, the effects of complex mineral supplementation (contains 10 mg Zn) on BMD were

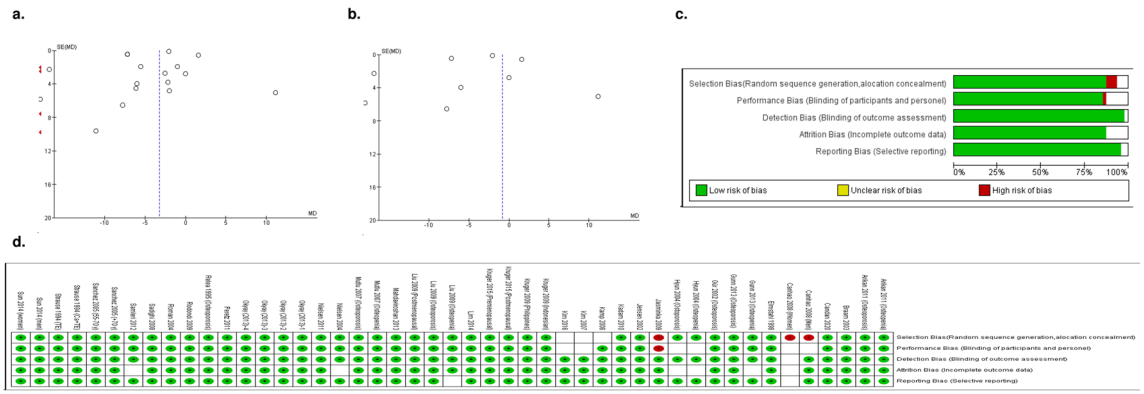

Fig. 8 Funnel plots of overall (**a**) serum zinc and (**b**) dietary zinc intake status meta-analysis. (**c**) Risk of bias summary. (**d**) Risk of bias examination of the included studies

examined in postmenopausal women during 3-year trail. They demonstrated that the femoral neck BMD had declined significantly in the treatment group, although the rate of bone loss was lower in the supplemented group than the placebo. In the study of Nielsen et al. that is meta-analyzed in our study, the supplement contains 600 mg Ca, 2 mg Cu, and 12 mg Zn, or placebo plus 600 mg Ca supplement were daily given to healthy women aged 51–80 years during 2-year trail. It was found that neither Ca + placebo nor Ca + Cu + Zn supplementation has a preventive effect on whole-body bone mineral content, density, or T score from decreasing from baseline during the supplementation period [43]. In the study of Rodondi et al. that is meta-analyzed in our study, the influence of additional 30-mg zinc on IGF-I and bone turnover responses to 4 weeks of essential amino acids-whey (EAA-W) protein supplements in frail elderly was demonstrated. The results showed that in the elderly, zinc supplementation accelerated the serum IGF-I response to EAA-W protein by 1 week and decreased a biochemical marker of bone resorption [46].

The limitation of zinc supplementation analysis is that in clinical studies, other trace elements or vitamins accompany zinc application, which limits us to see zinc effect on bone metabolism directly. To eliminate other supplement effects, animal studies might be examined. In an animal study, diabetes depended on osteoporotic bone loss in rats was investigated in the case of zinc supplementation with 0.25 mg/kg/day of zinc sulfate administration. It was observed that zinc application increased the BMD, decreased serum ALP, and RANKL increased serum OPG and RUNX 2 levels, as well as OPG/RANKL ratio [58]. Also, there are several studies that zinc supplementation have protective effects on bone structure in ovariectomized rats [59–61] and on potential promotions on bone formation [62, 63].

Our meta-analysis results showed that serum ALP and BAP levels of zinc-supplemented groups were higher than controls, however significant difference was found only in serum ALP levels between groups (Fig. 5c and d) This result may be related to limited data on serum BAP levels. Besides, serum zinc level and serum ALP levels were not found to be correlated (Fig. 6). ALP is a well-known biochemical marker used in the diagnosis and follow-up of the liver and metabolic bone disease. BAP is one of the several different isoenzymes of ALP [64]. BAP is synthesized by the osteoblasts and is presumed to be involved in the calcification of bone matrix. It is considered to be a highly specific marker of the bone-forming activity of osteoblasts [65]. BAP catalyzes the hydrolysis of pyrophosphate and provides the extracellular phosphate pool, which determines the rate of hydroxyapatite crystal formation in the bone. In vitro, Zn has been shown to stimulate osteoblastic bone formation by activation of ALP, while Zn deficiency reduced bone mineralization by decreasing the synthesis of ALP [13]. The results of the study of Peretz et al. showed that zinc supplementation results with a significant increase in serum total ALP as well as in bone-specific ALP. [14]. In the study of Cho et al., thirty rats were grouped as Zn-adequate (ZA, 35 mg/kg), pair-fed (PF, 35 mg/kg), Zn-deficient (ZD, 1 mg/kg) diet, and fed for 10 weeks. It was showed that ALP activity was decreased in plasma ($p < 0.05$) in Zn-deficient rats compared with ZA or PF controls [66].

Another important outcome of our meta-analysis is that serum osteocalcin levels of zinc-supplemented groups were significantly lower than controls (Fig. 5e). Also, we found a significant correlation between serum osteocalcin and serum zinc status, and that was a negative relationship (Fig. 6e). Osteocalcin is synthesized during the bone formation, and it exhibits a compact, calcium-dependent, alpha-helical confirmation, in which the gamma-carboxyglutamic acid (GLA) residues bind and promote absorption to hydroxyapatite in the bone matrix. In this way, bone mineralization takes place [67]. It has been hypothesized that lower female sex hormone levels cause changes in the osteocalcin homeostasis, leading to a decrease of osteocalcin and, in consequence, an increase in uncarboxylated osteocalcin levels in blood serum. Fluctuations in osteocalcin levels in the course of postmenopausal osteoporosis were presented in the study carried out by Gurban et al. Levels of osteocalcin among women who had not been menstruating for at least 15 years reached values of 20.12 ± 0.87 ng/mL, whereas, in the group where this period was less than 15 years, concentrations of osteocalcin were significantly lower (15.12 ± 1.55 ng/mL). These results allow for putting forward a thesis that sustained a decrease in the function of osteoblasts after the last menstrual period is reflected by increased levels of uncarboxylated osteocalcin in the blood serum [68, 69].

In the study of Singh et al., the results showed that serum osteocalcin levels were significantly higher in postmenopausal osteopenic ($p < 0.005$) and osteoporotic women ($p < 0.001$) compared with healthy ones. BMD at the femoral neck and lumbar spine was significantly lower ($p < 0.001$) than in women with normal BMD ($p < 0.001$) [70]. In the study with cells derived from a bone mesenchymal stem cell (BMSC) and ovariectomized rats (OVX), it was shown that zinc supplementation resulted in a modest increase in BMD and a significant increase in serum osteocalcin and ALP activity in BMSC. Serum levels of RANKL and TRAP were lower in OVX + Zn (vs OVX) rats. Osteocalcin level was significantly upregulated ex vivo in cultured OVX − Zn (vs OVX) cells [71]. In a different animal study, zinc effects on diabetic osteoclast bone resorption were examined, and it was found that zinc might prevent the diabetes-induced increase in osteoclastogenesis and decrease in osteoblastogenesis by inhibiting the RANK expression and stimulating IGF-1/IGF-1R/Akt/GSK3β/β-catenin signaling [72]. Nagata et al. showed the cellular zinc trafficking effect on osteoblastic cell lines in their study that resulted in a correlation between osteocalcin

mRNA levels and zinc exposure. They mentioned that zinc might have an important role in osteoblast mineralization through zinc storage proteins and zinc transporters [73].

In the subgroup analysis of fracture, groups showed that patients with fractures had significantly lower dietary Zn than their controls (Fig. 4d). In addition, we examined dietary protein intake between patients with fracture and control groups. Patients with fractures had significantly lower protein intake compared with control groups. It was shown with the correlation analysis that dietary zinc intake was correlated with dietary protein, fat, and total energy intakes (Fig. 7a). In the study of Kim et al. that is meta-analyzed in our study, the bone mineral density of vegetarian and non-vegetarian postmenopausal women was investigated. The results showed that the vegetarian group had significantly lower dietary zinc intake and serum zinc levels than non-vegetarian controls [32]. These results indicated that a healthy and balanced diet might be beneficial to prevent fracture and osteoporosis. Especially, adequate dietary protein intake may provide sufficient micronutrients such as zinc, iron, and copper. However, it should be considered that zinc sources and bioavailability studies are still unclear and should be examined with further studies. There were also correlations between zinc intake and dietary protein, dietary phosphorus (P), Ca, Mg, Fe, potassium (K), and folate intake. Similarly, our results also demonstrated that dietary zinc intake is correlated with protein, fat, magnesium, iron, phosphorus, potassium, folate, and calcium. There was also a significant correlation between serum zinc status and dietary energy intake (Fig. 7). The study of New et al. that is meta-analyzed in our study, suggest that high current intakes of the nutrients, potassium, magnesium, vitamin C, fiber, and zinc, were associated with a higher bone mass, and that a high past consumption of fruit had a positive effect on adult bone mass. These findings appear to indicate that high long-term intakes of nutrients found in abundance in fruit and vegetables may be important to bone health, possibly because of their beneficial effect on acid-base balance [41].

It should be mentioned that we accept the limits of our meta-analysis study based on the quality of the studies and data in the literature. Some of the limitations of our current meta-analysis are due to differences in cases or patients, supplementation procedures, dietary factors, and heterogeneity. The statistical heterogeneity of the data was high in some of our analyses. However, clinical heterogeneity can be observed, and is a natural result for meta-analysis and should be considered when interpreting the results of this study. It was performed by evaluating the PRISMA checklist. On the other hand, to the best of our knowledge, this study is the first meta-analysis in the literature evaluating the relationship between dietary zinc intake, zinc supplementation, serum zinc levels, and bone turnover–related diseases or outcomes.

Conclusion

In conclusion, as a result of the meta-analysis, it was found that serum zinc level and dietary zinc intake could have an essential role in preventing osteoporosis. Also, it was seen that zinc supplementation might improve bone turnover markers for bone formation such as serum osteocalcin and serum alkaline phosphatase, and also BMD, especially on the femoral neck. This paper is the first meta-analysis that investigated the effects of zinc supplementation and dietary zinc status on serum zinc levels and bone turnover markers on osteoporosis, osteopenia, postmenopause, and fracture. For further understanding of the underlying mechanism, different clinical studies are needed to enlighten the role of zinc supplementation or dietary zinc intake on bone turnover.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval None

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