Original Article

Bone Densitometry in the Elderly Female and Possible Relation to Serum Copper.

Mohamed A Mehanna, Noha M Elsabbagh, Mohamed M Elsawy, Ahmed H A fify, Asmaa M Nooh.

Department of Internal Medicine, Alexandria Faculty of Medicine

Abstract:

Background: Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. It is a major public health problem throughout the world. The social and economic burden of osteoporosis is increasing steadily because of the aging of the world population. Osteop orosis is frequently only diagnosed after the first clinical fracture has occurred. Consequently, therapy is often aimed at preventing further fractures. It is therefore important to assess individual osteoporosis risk early enough to prevent the first fracture. Trace elements, including copper, are essential for normal growth and development of the skeleton in humans and animals. The role of copper in bone metabolism can be linked primarily to the Cu-dependent enzyme lysyl oxidase, for which copper acts as a cofactor. Lysyl oxidase is required for the formation of lysine-derived cross-links in collagen and elastin. The aim of this study was to estimate the plasma copper level in a group of healthy elderly female, and to compare it with bone mineral density BMD of the same group, using dual energy X-ray absorptiometry DEXA to assess if serum copper could potentially be developed into a simple, cheap, and radiation-free screening method for osteoporosis. **Subjects & Methods:** This study was conducted on thirty female subjects divided into two groups; the first consisted of ten healthy control female subjects below the age of 60 years. The second group consisted of twenty healthy female subjects 60 years old and older. Serum copper, serum albumin and total serum calcium levels were assessed in all subjects. Bone mineral density using DEXA scan was done for all subjects. **Results:** The serum copper showed significant increased level in the elderly female subjects compared with the younger subjects ($p = 0.035$). This study also showed no statistically significant correlation between BMD and serum copper ($p = 0.072$), while, a statistically significant positive correlation between BMD and serum albumin was found ($p = 0.017$). **Conclusion:** In this study serum copper did not seem to correlate with BMD and so cannot be used as a screening method for osteoporosis. We also can conclude that, serum albumin may have a beneficial role in bone health.

Keyword: Bone Densitometry, Elderly Female, Serum Copper.

Introduction:

Osteoporosis is a major health problem that significantly affects the aging population. Catastrophic effects on disability and mortality accompany the increase in the incidence of osteoporotic fractures in patients aged 65 and older.[1] A combination of factors, including genetics, nutrition, physical activity, and bone turnover, determine bone mass and ultimately bone strength.[2] After the acquisition of peak bone mass during the third decade of life, there is a progressive decline of approximately 0.5% a year, which is considered a physiological age-related change.[3]

Age-related bone loss is the consequence of changes in bone cellularity as well as hormones. Increasing osteoclast formation and activity and diminished osteoclast apoptosis, follow the declining estrogen levels in women although more significantly in women during peri-menopausal years cellular changes in aging bone reduce the number of osteoblasts available for bone remodeling and formation.[4] A proportion of subjects lose their bone mass only at a physiological basis, whereas a proportion of subjects will suffer pathological bone loss leading to osteoporosis. Although diet, physical activity, and genetics play a role

in accelerated bone loss, it is likely that there are additional hormonal and molecular factors that remain to be elucidated.[5]

Osteoporosis is a silent disease until it is complicated by fractures that can occur following minimal trauma. These fractures are common and place an enormous medical and personal burden on aging individuals and a major economic toll on the nation. Osteoporosis can be prevented and can be diagnosed and treated before any fracture occurs. Prevention, detection and treatment of osteoporosis should be a mandate of primary care providers.

The National Osteoporosis Foundation NOF recommends a comprehensive approach to the diagnosis and management of osteoporosis. A detailed history and physical examination together with BMD assessment and the WHO 10-year estimated fracture probability are utilized to establish the individual patient's fracture risk.[6]

Dual-energy x-ray absorptiometry (DXA) measurement of the hip and spine is the technology now used to establish or confirm a diagnosis of osteoporosis, predict future fracture risk and monitor patients by performing serial assessments.[7]

Nutrients such as calcium, phosphorus, zinc, copper, protein, and vitamin D have a relationship with bone mass[8,9]. Proper nutrient intake has a crucial role in both prevention and treatment of osteoporosis [10].

Copper (Cu) is an essential trace element for humans and animals. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reactions and in scavenging free radicals[11]. Copper is essential in collagen-crosslink formation as a component of the metalloenzyme, lysyl oxidase. The action of lysyl oxidase helps maintain the integrity of connective tissue in the heart, blood vessels and also plays a role in bone formation [12]. Collagen cross links provide tensile strength to bone and copper deficiency in several animal species produces skeletal abnormalities [13, 14].

Subjects and Methods:

After approval of local ethics committee, this study was conducted on thirty female subjects divided into two groups, the first consisted of ten healthy control female subjects below the age of 60 years. The second group consisted of twenty healthy female subjects 60 years old and older. Subjects under hormonal replacement therapy or drugs affecting bone density, smokers, those with chronic liver disease, diabetic patients, and patients with metabolic bone disease or connective tissue disease were all excluded from the study.

All subjects were subjected to thorough history taking and clinical examination, height and weight measurement for BMI assessment, routine laboratory investigations including: CBC, fasting blood sugar, blood urea, serum creatinine, serum transaminases (ALT, AST), and assessment of serum albumin and total serum calcium levels.

For serum copper; blood samples were collected in serum separator tubes, centrifuged separating serum samples which were frozen at - 20°C. Serum copper concentrations were measured by using an induction coupled serum atomic emission spectrometer.

A standard questionnaires concerning smoking, physical activity, sun exposure, diet, medications use and disease history was done.

The diagnosis of osteoporosis was based on BMD measurements. BMD was measured at the lumbar spine (L1–L4) by dual energy X-ray absorptiometry (Hologic QDR-4500, USA). The diagnosis of osteoporosis was based on the WHO criteria[15] in which a loss of bone mass ≤1 standard deviation (SD) was considered as normal, loss of bone mass >1 SD , ≤2.5 SD was diagnosed as osteopenia and loss of bone mass >2.5 SD was diagnosed as osteoporosis.

Results:

T-score results in the studied groups showed that, in group I, 5 subjects (50%) were normal, 4 subjects (40%) were osteopenic and only one subject (10%) was osteoporotic. While in group II, 7 subjects (35%) were normal, 6 subjects (30%) were osteopenic and 7 subjects (35%) were osteoporotic.(table I)

Regarding serum copper level, in group I it ranged between 7 and 199 ug/dl with a mean of 62.70 ± 68.88 ug/dl. While in group II, it ranged between 11 and 199 ug/dl with a mean of 120.74 ± 65.83 ug/dl. The serum copper showed

significant increased level in the elderly female subjects compared with the younger subjects (p = 0.035).(table II)

Regarding serum calcium level, in group I, it ranged between 8.5 and 9.9 mg\dl with a mean of 9.11 ± 0.5 mg\dl. In group II, it ranged between 7.2 and 9.4 mg\dl with a mean of 8.82 ± 0.5 mg\dl.(table II)

Serum albumin level in group I ranged between 3.5 and 5.2 g\dl with mean of 4.27 ± 0.53 g\dl in group I. While in group II, it ranged between 3.6 and 4.9 g\dl with a mean of 4.0 ± 0.42 g\dl. There was no significant statistical difference between the studied groups regarding serum calcium or serum albumin levels.(table II)

The current study found no statistically significant correlation between T-score and serum copper (p = 0.072), while a statistically significant positive correlation between T-score and serum albumin (p = 0.017) was found. (table III, figures 1,2)

There was no statistically significant correlation between T-score and age, BMI or serum calcium level. (Table III)

Table (I): Comparison between the two groups according to T-score

	Age (years)				Total (n = 30)	
	<60 (n = 10)		≥60 (n = 20)			
	No.	%	No.	%	No.	%
Total BMD T-score						
Normal (≥-1)	5	50.0	7	35.0	12	40.0
Osteopenia (-2.5 - -1)	4	40.0	6	30.0	10	33.3
Osteoporosis (≤ -2.5)	1	10.0	7	35.0	8	26.7
χ^2(^{MC}p)	2.101 (0.386)					

χ^2: value for Chi square MC: Monte Carlo test

Table (II): Serum copper, calcium, and albumin levels in the studied groups:

	Age (years)		Total (n = 30)
	Group I (n = 10)	Group II (n = 20)	
Serum copper (ug\dl)			
Min. – Max.	7.0 – 199.0	11.0 – 199.0	7.0 – 199.0
Mean ± SD.	62.70 ± 68.88	120.74 ± 65.83	101.39 ± 71.32
Median	28.0	142.50	97.30
Z (p)	2.112* (0.035*)		
Serum calcium (mg\dl)			
Min. – Max.	8.50 – 9.90	7.20 – 9.40	7.20 – 9.90
Mean ± SD.	9.11 ± 0.50	8.82 ± 0.50	8.91 ± 0.51
Median	9.05	8.90	8.90
t (p)	1.526 (0.138)		
Serum albumin (g\dl)			
Min. – Max.	3.50 – 5.20	3.60 – 4.90	3.50 – 5.20
Mean ± SD.	4.27 ± 0.53	4.0 ± 0.42	4.09 ± 0.47
Median	4.15	3.88	3.90
t (p)	1.528 (0.138)		

t: Student t-test

Z: Z for Mann Whitney test

*: Statistically significant at p ≤ 0.05

Table (III): Correlation between T-score with age, BMI, serum copper, serum calcium and serum albumin.

	T-score	
	r_s	p
Age	-0.269	0.151
BMI	0.058	0.762
Serum copper (ug\dl)	-0.333	0.072
Serum calcium (mg\dl)	-0.032	0.868
Serum albumin (g\dl)	0.431*	0.017*

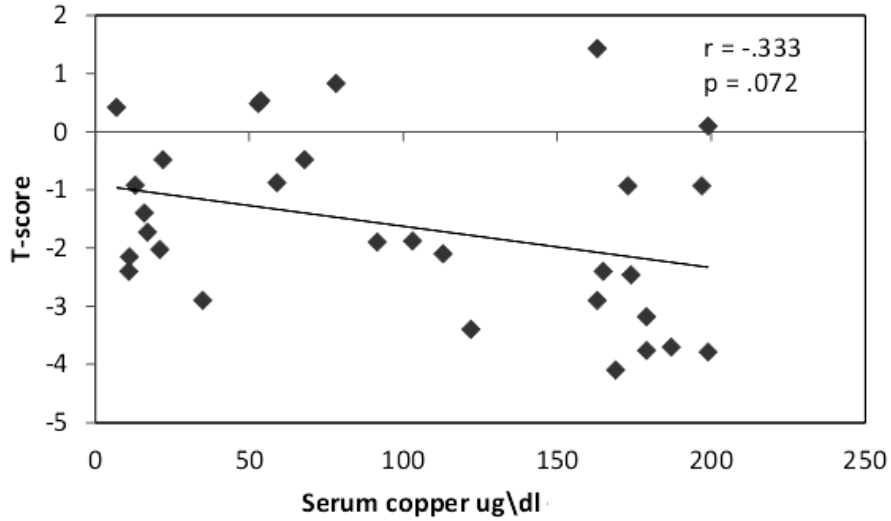

Figure (1): Correlations between serum copper and T-score.

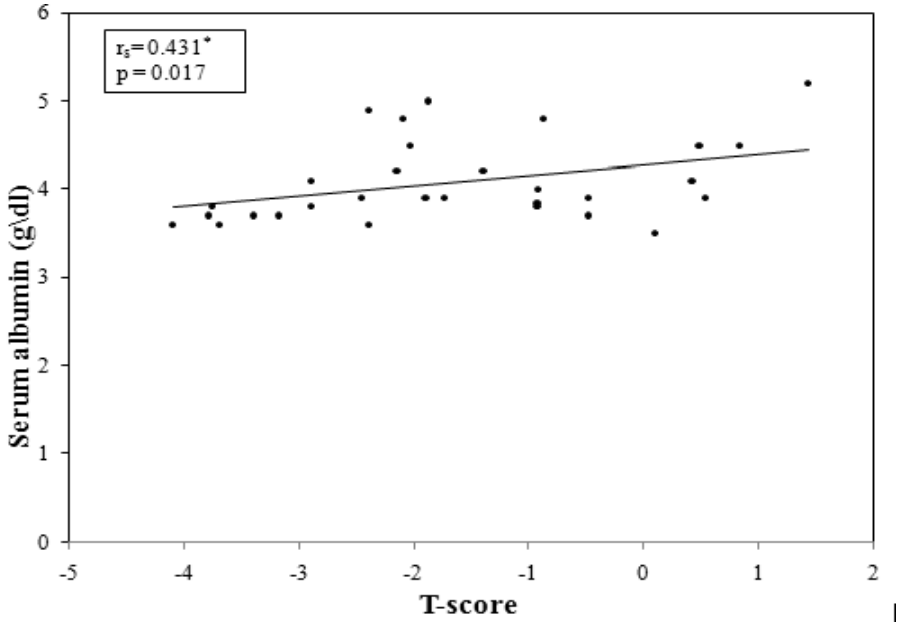

Figure (2): Correlation between T-score with Serum albumin (g\dl)

Discussion:

In the current study, serum copper level was found to be significantly higher in elderly women compared to younger healthy women (p = 0.035) (table 2). This agree with Madaric et al.[16] who found that serum copper concentrations and the copper/zinc ratio correlated positively with age (p< 0.0001). Tietz et al[17] and Bunker et al.[18] also found that serum copper values increase with age.

Similar results were found in a study conducted in the internal Medicine Department, Alexandria University on 50 subjects, aged 65 years old or older on 2012 which showed that plasma copper level was higher than normal in 40% of the cases suggesting an age related increase in plasma copper concentration .[19]

This increase of copper level with age in our population may be related to environmental factor, pollution or dietary habits.

On the other hand, Gamez et al,[20] Mir et al.[21] and Lowe et al,[22] found that there was no significant difference in plasma Cu concentration between elderly and young healthy women.

In the current study there was no significant statistical correlation between serum copper and T-score (BMD), with (p = 0.072). (Table 3).

Similarly, Odabasi et al. observed that there was no significant difference between healthy and osteoporotic elderly women, both in plasma and in red blood concentrations, for copper.[23] Similar results were found by Mutlu et al[24] and Arikan et al.[25], who reported that there was no significant difference in serum Cu levels between elderly women with osteoporosis and the non-osteoporotic control subjects. Razmandeh et al.[26] in a case–control study published on 2014 reported that there was no significant difference in serum copper level between osteoporotic and normal control female subjects.

Other studies showed that high copper level was associated with osteoporosis. Massie et al. have suggested that excess copper was associated with decreasing bone size and density.[27] Koulourides observed an inhibition of enamel remineralization by Cu.[28] Menerey described that the excessive toxic copper in the skeletal system may mediate oxygen free radical release and thus damage tissue.[29]

On the other hand, Gur et al. reported that Zn and Cu levels in serum were lower among patients with osteoporosis than the controls. [30] Sierpinska et al. reported that severe tooth wear is associated with reduced spinal BMD and that enamel in adult individuals with severe tooth wear is low in copper content.[31]

Many previous clinical studies provide evidence of beneficial role of copper and zinc in improvement of bone density and quality in both osteoporotic and healthy individuals, particularly found in cancellous bone, i.e., lumbar spine vertebrae [32-34].

Regarding serum calcium, no significant statistical difference between serum calcium levels with age was found (Table 2), also, no statistically significant correlation between BMD and serum calcium level was found. (Table 3).

Nieves et al.[35,36], Arikan et al.[25]and liu et al.[37] in agreement with our results did not find differences in serum calcium by age group or when compared between osteoporotic, osteopenic and normal subjects.

The current study showed a statistically significant positive correlation between T-score and serum albumin (p=0.017). (Table 3) Similarly, serum albumin has been reported to be positively associated with bone mineral content[38,39] and negatively associated with the risk of hip fracture, both in a prospective study[40] and in a case–control study[41,42], supporting the view that severe protein depletion plays a role in causing hip fracture[43]. Underweight hypoalbuminemic subjects had a lower T-score than underweight

cases with normal serum albumin levels[44] findings consistent with the positive correlation demonstrated by other authors in both genders[40] and in women alone [43].

Coin et al. studied the relationship between serum albumin and hip bone mineral density (BMD) in 352 elderly outpatients (216 women aged 73.57±5.3 years and 136 men aged 73.97±5.6 years) and found that albumin was significantly associated with BMD in both genders.[45]

However two large cross-sectional studies failed to confirm this correlation between albumin and BMD[46,47]. Lunde et al. in a cross-sectional study, examined the relation between serum albumin and bone mineral density (BMD) in 1593 white, community dwelling men and women aged 50–95 years. In both sexes there was positive correlation between serum albumin and BMD in the unadjusted model ($p<0.005$). After age adjustment, however, the relationship was no longer significant ($p >0.18$).[48]

Conclusion:

From the previous results and from the opposing studies in the literature we can conclude that; although the beneficial role of copper in improvement of bone density and quality, it does not seem to be correlated with BMD and so cannot be used as a screening method for osteoporosis. Serum albumin has been found to be positively correlated with bone mineral content and may have a beneficial role in bone health that needs more studying.

References:

1- **Cooper A, Cooper BB.** A treatise on dislocations, and on fractures of the joints. London, UK: Churchill livingstone; 1822.

2- **Brown LB, Streeten EA, Shapiro JR, et al.** Genetic and environmental influences on bone mineral density in pre- and post-menopausal women. Osteoporos Int 2005;16(12):1849–56.

3- **Tenenhouse A, Joseph L, Kreiger N, et al.** Estimation of the prevalence of low bone density in Canadian women and men using a population-specific DXA reference standard: The Canadian Multicentre Osteoporosis Study (CaMos). Osteoporos Int 2000;11:897–904.

4- **Manolagas SC, Kousteni S, Jilka RL.** Sex steroids and bone. Recent Prog Horm Res 2002;57:385–409.

5- **Duque G, Troen BR.** Understanding the mechanisms of senile osteoporosis: new facts for a major geriatric syndrome. J Am Geriatr Soc 2008; 56(5):935-41.

6. **Kanis JA.** Assessment of osteoporosis at the primary health care level. Technical Report. UK: WHO Collaborating Centre, University of Sheffield; 2008.

7. **Kanis JA, Melton LJ III, Christiansen C, et al.** The diagnosis of osteoporosis. J Bone Miner Res 1994;9(8):1137-41.

8. **Reid D, New S.** Nutritional influences on bone mass. Proc Nutr Soc 1997; 56:977-87.

9. **Feskanich D, Willett W, Colditz G.** Calcium, vitamin D, milk consumption, and hip fracture: a prospective study among postmenopausal women. Am J Clin Nutr 2003; 77:504-11.

10. **Kitchin B, Morgan S.** Nutritional considerations in osteoporosis. Curr Opin Rheumatol 2003; 15:476-80.

11. **Linder MC, Hazegh-Azam M.** Copper biochemistry and molecular biology. Am J Clin Nutr 1996; 63(5):797S-811S.

12. **Turnlund JR. Copper. In:** Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ (eds). Modern Nutrition in Health and Disease. 10th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.286-99.

13. **Park SJ, Joo SE, Min H, et al,** Dietary Patterns and Osteoporosis Risk in Postmenopausal Korean Women. Osong Public Health Res Perspect 2012; 3(4):199-205.

14. **Okubo H, Sasaki S, Horiguchi H, et al.** Dietary patterns associated with bone mineral density in premenopausal Japanese farm- women. Am J Clin Nutr 2006; 83:1185-92.

15. Miller PD. Guidelines for the diagnosis of osteoporosis: T-scores vs fractures. Rev Endocr Metab Disord 2006; 7:75–89.

16- Madaric A, Ginter E, Kadrabova J. Serum copper, zinc and copper/ zinc ratio in males: influence of aging. Physiol Res 1994; 43:107–11.

17- Tietz N, Shuey D, Wekstein D. Laboratory values in fit aging individuals sexagenarians through centenarians. Clinical Chemistry 1992; 38(6):1167-85.

18- Bunker VW, Hinks LJ, Lawson MS, et al. Assessment of zinc and copper status of healthy elderly people using metabolic balance studies and measurement of leucocyte concentrations. Am J Clin Nutr 1984;40(5):1096-102.

19- El-lakkany IA, Osman GA, Elsawy MM, et al. Study of serum zinc and copper level in elderly population: relation to cognitive function.MS thesis. faculty of medicine: Alex university; 2012.

20- Gamez C, Artacho R, Ruiz-Lopez MD, et al. Serum copper in institutionalized elderly subjects: relations with dietary intake of energy, specific nutrients and haematological parameters. The Science of the total environment 1997; 201(1):31-8.

21- Mir E, Hossein-nezhad A, Bahrami A, et al. Adequate serum copper concentration could improve bone density, postpone bone loss and protect osteoporosis in women. Iranian J Publ Health 2007;0: 24-9.

22- Lowe NM, Fraser WD, Jackson MJ. Is there a potential therapeutic value of copper and zinc for osteoporosis? Proc Nutr Soc 2002; 61(2):181-5.

23- Odabasi E, Turan M, Aydin A, et al. Magnesium, zinc, copper, manganese, and selenium levels in postmenopausal women with osteoporosis. Can magnesium play a key role in osteoporosis? Ann Acad Med Singapore 2008; 37:564–7.

24- Mutlu M, Argun M, Kilic E, et al. Magnesium, zinc and copper status in osteoporotic, osteopenic and normal post-menopausal women. J Int Med Res 2007; 35(5):692-5.

25- Arikan DC, Coskun A, Ozer A, et al. Plasma selenium, zinc, copper and lipid levels in postmenopausal turkish women and their relation with osteoporosis. Biol Trace Elem Res 2011; 144:407–17.

26- Razmandeh R, Nasli-Esfahani E, Heydarpour R, et al. Association of Zinc, Copper and Magnesium with bone mineral density in Iranian postmenopausal women – a case control study. Journal of Diabetes & Metabolic Disorders 2014; 13:43.

27- Massie HR, Aiello VR, Shumway ME, et al. Calcium, iron, copper, boron, collagen and density changes in bone with aging C57BL/6J male mice. Exp Gerontol 1990; 25(5):469–81.

28- Koulourides T, Feagin F, Pigman W. Effect of pH, ionic strength, and cupric ions on the rehardening rate of buffer-softened human enamel. Arch Oral Biol 1968; 13:335–4.

29- Menery KA, Eider W, Brewer GJ, et al. The arthropathy of Wilson's disease. J Rheumatol 1988; 15: 331–7.

30- Gur A, Colpan L, Nas K, et al. The role of trace minerals in the pathogenesis of postmenopausal osteoporosis and new effect of calcitonin. J Bone Miner Metab 2002; 20:39-43.

31- Sierpinska T, Konstantynowicz J, Orywal K, et al. Copper deficit as a potential pathogenic factor of reduced bone mineral density and severe tooth wear. Osteoporos Int 2014; 25(2):447-54.

32- Strause L, Saltman P, Smith KT, et al. Spinal bone loss in postmenopausal women supplemented with calcium and trace minerals. J Nutr 1994; 124(7):1060–4.

33- Saltman PD, Strause LG. The role of trace minerals in osteoporosis. J Am Coll Nutr 1993; 12:384–9.

34- Gür A, Colpan L, Nas K, et al. The role of trace minerals in the pathogenesis of postmenopausal osteoporosis and a new effect of calcitonin. J Bone Miner Metab 2002; 20(1):39-43.

35- Nieves JW. Nutrition and osteoporosis. In: Cummings S, Cosman F, Jamal S, (eds). Osteoporosis: an evidence based approach to the prevention and management. Philadelphia: American College of Physicians; 2002.85-107.

36- Nieves JW. Calcium, vitamin D, and nutrition in elderly adults. Clin Geriatr Med 2003; 19:321–35.

37- Liu SZ, Yan H, Xu P, et al. Correlation analysis between bone mineral density and serum element contents of postmenopausal women in Xi'an urban area. Biol Trace Elem Res 2009; 131:205–14.

38. Orwoll ES, Weigel RM, Oviatt SK. Serum protein concentrations and bone mineral content in normal aging men. Am J Clin Nutr 1987; 46:614–21.

39- Nakamura K, Saito T, Nishiwaki T, et al. Correlations between bone mineral density and demographic, lifestyle, and biochemical variables in community-dwelling Japanese women 69 years of age and over. Osteoporos Int 2006; 17: 1202–7.

40- Thiebaud D, Burckhardt P, Costanza M, et al. Importance of albumin, 25(OH) vitamin D and IGFBP-3 as risk factors in elderly women and men with hip fracture. Osteoporosis Int 1997; 7: 457–62.

41- Huang Z, Himes JH, McGovern PG. Nutrition and subsequent hip fracture risk among a national cohort of white women. Am J Epidemiol 1996; 144: 124–34.

42. Rico H, Revilla M, Villa LF, et al. Crush fracture syndrome in senile osteoporosis: a nutritional consequence? J Bone Miner Res 1992; 7(3):317–9.

43- Di Monaco M, Vallero F, Di Monaco R, et al. Biochemical markers of nutrition and bone mineral density in the elderly. Gerontology 2003; 49: 50–4.

44- Coin A, Sergi G, Beninca P, et al. Bone mineral density and body composition in underweight and normal elderly subjects. Osteoporos Int 2000;11: 1043–50.

45- Coin A, Perissinotto E, Enzi G, et al. Predictors of low bone mineral density in the elderly: the role of dietary intake, nutritional status and sarcopenia. Eur J Clin Nutr 2008; 62(6):802-9.

46- D'Erasmo E, Pisani D, Ragno A, et al. Relationship between serum albumin and bone mineral density in postmenopausal women and in patients with hypoalbuminemia. Horm Metab Res J 1999; 3: 385–8.

47- Lunde AV, Barrett-Connor E, Morton DJ. Serum Albumin and Bone Mineral Density in Healthy Older Men and Women: The Rancho Bernardo Study. Osteoporos Int 1998; 8:547–51.

48- LaFleur J, McAdam-Marx C, Kirkness C, et al. Clinical risk factors for fracture in postmenopausal osteoporotic women: a review of the recent literature. Ann Pharmacother 2008;42(3):375-86.