

Obesity Modulates Bone Mineral Density and Hip Fracture Risk in Egyptian Women Around Menopause

¹*Moetazza M. Alshafei, ²Emtenan M. Hanafî, ³Maha M. Saber,
⁴Lobna M. Saber, ³Eitedal M. Dawood and ¹Seham A. Kassem*

¹Department of Food Science & Nutrition, National Research Center, Giza, Egypt

²Department of Animal Reproduction & A I, National Research Center, Giza, Egypt

³Department of Complementary Medicine, National Research Center, Giza, Egypt

⁴Department of Biochemistry, Faculty of Medicine AlAzhar University, cairo, Egypt

Abstract: Osteoporosis is prevalent multifactorial bone problem in the world and Egypt. It is the major cause of fracture in elderly. Interrelation between osteoporosis and obesity is still not clear. This study aimed to clear this relationship in Egyptian women..Eighty patients were collected from complementary medicine clinic in NRC Egypt to be enrolled in the study (60 post- and 20 pre-menopausal women above 40 years). Clinical examination, anthropometric measures were performed. Subjects were investigated for bone health by both DEXA and serum Human procollagen 1 N terminal peptide bone formation marker (PINP). DEXA was performed on left femur and lumbar vertebrae L1-L2. Frax10 calculation for probability of hip fracture (HF) and major osteoporosis event (MO) in10 years was used. Patients were categorized to four groups. First three were postmenopausal, the fourth were premenopausal. (G1) were obese patient with age 58 ± 2 y, (G2) were Obese patients with age 52 ± 0.4 y, (G3) was non obese patients of age 51.2 ± 1.04 y and (G4) were obese premenopausal, age 39.7 ± 1.2 y. Results showed positive significant correlation between body mass index (BMI) and both bone mineral density (BMD) and level of PINP while negative one between BMI and HF in all groups. Significant decrease of BMD, PINP and increase of HF and MO in older obese postmenopausal patients compared to younger obese ones (G1versusG2).The same was true in non obese postmenopausal patient, with significant marked increase of HF when compared to obese patients with same younger age group (G3versusG2). G4 premenopausal obese women were protected from HF by high BMD if compared to postmenopausal women. We concluded that obesity may protect from marked loss of BMD and increase HF which occurs at menopause and aging. There is debate about this concept. Egypt needs a wider scale study of this relation.

Key words: Obesity • Osteoporosis • Frax10 • Bone Formation Marker

INTRODUCTION

Osteoporosis and obesity are the most common diseases with increase in both morbidity and mortality worldwide [1-3]. Energy imbalance between consumed and burned calories, fast food and sedentary life with the use of automated machines are main etiology of weight gain. It was reported by World Health Organization (WHO) that more than 1.9 billion adults were overweight in 2014 and about 13% of the world's adult population (11and15% of men and women respectively) were obese [4]. Different studies mentioned that obesity has a protective role against bone fragility but recent evidence

suggests the opposite [5-7]. This means that fat and lean components of soft tissue influence bone metabolism and have relative contribution in bone health.

Osteoporosis is the silent disease leading to fractures. In the U.S., about 1.5 million fractures each year occurred due to osteoporosis [8, 9]. According to WHO criteria, osteopenia (T score of -1 to -2.5) and osteoporosis (T score ≤ -2.5) were reported [10].

Many studies were carried out on several populations and concluded that obesity is positively correlated with increased bone strength and lower fracture risk [11]. However, recent evidence suggests that this relation between body mass index (BMI) and BMD is not

always true because many different factors can play a role in this interaction such as, lower serum levels of Sex Hormone Binding Globulin, conversion of androgens-to-estrogens in adipose tissue, increased serum leptin or insulin growth factor, which may be stimulating factor for bone formation [12, 13].

The relationship between adiposity, fracture risk and BMI is controversial. Data from previous studies on the fracture risk in osteoporotic patients is inversely proportional to total bodyweight, fat mass, body fat percentage, hip girth and BMI before correction for BMD. When adjustment for BMD was performed, the relationship appeared nonlinear but U-shaped [14].

Regarding premenopausal women, it was reported that trunk fat, was inversely associated with bone formation rate and trabecular bone volume [15, 16]. Also, a linear association between BMI and BMD is recorded but an inverse one between BMI and compensation for increased impact forces during falls and risk of fracture [17].

Human procollagen 1 N terminal peptide, (PINP) is a sensitive bone formation marker and can be an indicator of bone health in serum of patients [18].

FRAZ is a web-based tool that assesses the 10-year risk of osteoporosis fracture in women and men. Individual risk factors (age, sex, weight, height and femoral neck BMD) and clinical risk factors (parental history of hip fracture, current tobacco use, long-term glucocorticoids use, rheumatoid arthritis, daily alcohol consumption, etc..) filled into the web page to calculate the probability of next 10-year fracture probability (as a percentage) of absolute, rather than relative, risk as occurs on the output of DEXA equipment [10, 19].

There is scarce data dealing with the relationship between excess weight, bone health and probability of fracture risk in menopausal and premenopausal women in Egypt.

So, we conducted the study to highlight this relation in Egyptian women around menopause, depending on (dual energy X ray densitometry) DEXA and bone formation marker PINP as tools for diagnosis of bone health and FRAZ tool to predict fracture risk in 10 years.

MATERIALS AND METHODS

The study was carried out in outpatient clinic of complementary medicine In NRC Egypt. Eighty patients above 40 years, from those visiting the clinic, were enrolled in the study. Clinical history and examination were performed. Anthropometric measures (weight and

height) were taken to calculate BMI (body weight divided by height square) of patient. Patients with BMI >30 were considered obese. Post menopausal women reported cessation of menstrual period at least 12 months ago was included. Patients with medical conditions that affect frax.10 were excluded.

Subjects were investigated for bone health by both DEXA (performed by Lunar DPX DXA system manufactured by GE Health care) and serum bone formation marker (PINP) as a marker that measures collagen bone formation using ELISA kit Glory Science Co., Ltd USA. DEXA was performed on left femur and lower lumbar vertebrae (L₁-L₄). According to DEXA results, T score -1 to -2.5 indicate osteopenia, T-score ≤ -2.5 indicates osteoporosis and that above -1 considered normal according to WHO [20]. PINP lower reference values were 16 and 19 µg / l for post and pre menopausal women respectively [21].

Patients were divided into four groups, twenty patients each. The first three groups were postmenopausal women and the fourth group was pre menopausal.

First group (G1) were obese patient with average age 58±2 and BMI was 37.3± 1.38.

Second group (G2) were obese patients with average age 52±0.4 and BMI 33± 0.42.

Third group (G3) were non obese patients (BMI <30) with average age 51.2± 1.04, BMI 26.1 ± 0.54, same age group as G2

Fourth group (G4) were obese patients, average age was 39.7±1.2, BMI 36.4± 0.99, same BMI as G1.

In this study FRAZ 10 (WHO, fracture risk assessment tool available at <http://wwwshef.ac.uk/FRAZ/20>) [22] to calculate the probability of the risk of hip fracture and major osteoporotic event in ten years, were estimated for each patient. Results were expressed as mean and standard error, one way ANOVA was done, Pearson test was used for correlation between BMI and either BMD or PINP level. Also, Correlation between BMI and fracture risk was performed, P≤0.05 was considered significant. All statistics was done using SPSS 16 and Microsoft excel.

RESULTS AND DISCUSSIONS

Analysis of data (Table 1) showed significant (P ≤ 0.05) decrease in BMD in both neck of femur and lumbar vertebrae in G1 if compared to G2 and G4 despite the three groups of patients were obese. So that, G1

Table 1: Interrelationship between Age, obesity and bone fragility in menopausal women

Group	Age	BMI	BMDL	BMDF	HF	MO	PINP
G1	58.0 ±2 ^d	37.03±1.38 ^a	0.92±0.34 ^a	0.83±0.11 ^a	0.33±0.17 ^a	2.30 ±0.7 ^a	25.90± 0.6 ^{ac}
G2	52±0.4 ^{ad}	33.00±0.4 ^b	1.12±0.06 ^b	0.99±0.11 ^b	0.08±0.04 ^b	1.30 ±0.29 ^b	27.00± 1.2 ^b
G3	51± 1.04 ^{ac}	26.1±0.54 ^c	0.76± 0.02 ^c	0.48 ±0.31 ^c	0.48± 0.4 ^a	2.28 ±0.33 ^a	23.70 ± 0.2 ^c
G4	39.7±1.2 ^b	36.4±0.99 ^a	1.18±0.02 ^{ab}	1.09± 0.09 ^{ab}	0.00± 0.00 ^b	0.33±0.04 ^c	29.50 ± 0.95 ^{ab}

Different superscript means significance $P \leq 0.05$. BMI= body mass index, BMDL = bone mineral density of lumbar vertebrae, BMDF = bone mineral density in left femur neck, HF= hip fracture risk at hip, MO= major osteoporotic event. PINP= bone formation marker.

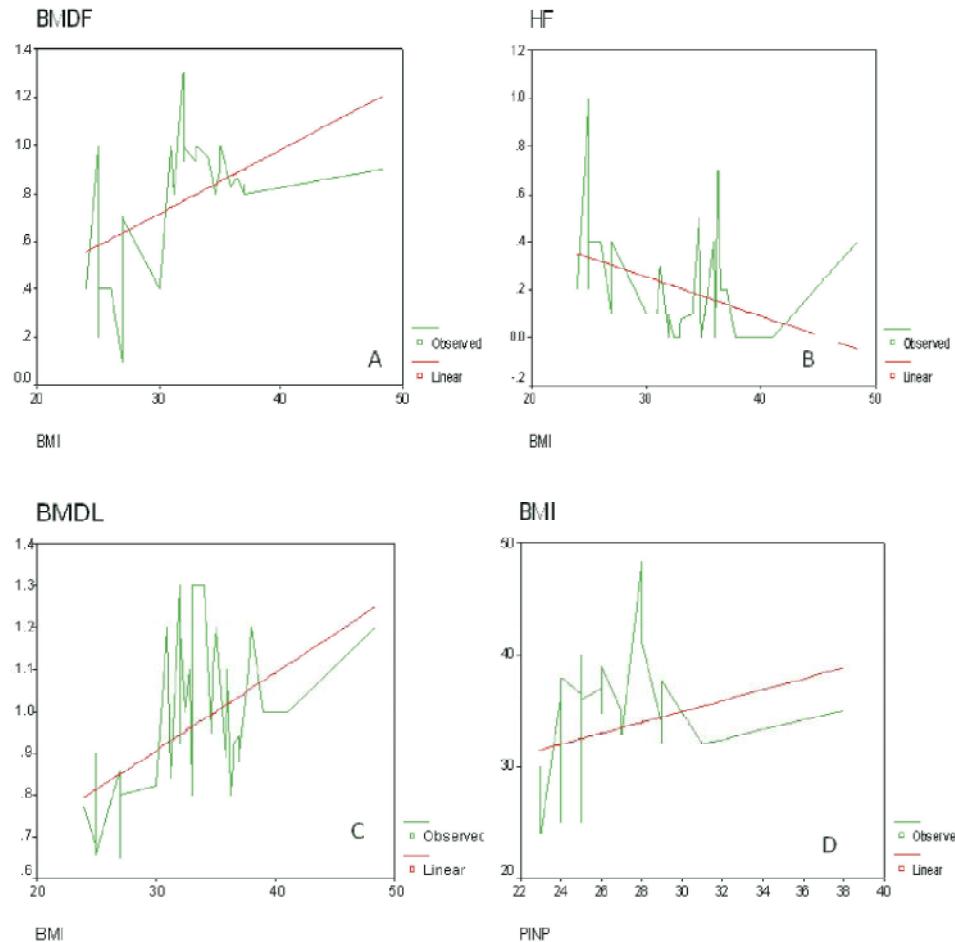


Fig. 1: (A) Positive correlation between BMI/B. (BMDF) Negative correlation between BMI/HF (BMDF=bone mineral density in neck of left femur, HF= hip fracture probability). (C) Positive correlation between BMI/BMDL and (D) Positive correlation BMI/PINP in all groups. (BMDL= bone mass density in lumbar spine L1-L4, PINP bone formation marker, BMI= body mass index).

showed significant ($p \leq 0.05$) higher probability of hip fracture risk (HF) and Major Osteoporotic event (MO) versus G2 and G4 (Table 1). Levels of PINP in serum were significantly less ($p \leq 0.05$) in G1 versus G2 and G4 pointing to more bone formation in G2 and G4. This may be explained in the light of age difference (58, 51 and 39 years old in G1, G2 and G4 respectively) (Table 1).

Despite G1 and G4 have nearly the same BMI average, patients in G4 showed better BMD and very low HF as they were younger in age (58 versus 39 in G1 and G4 respectively) and still premenopausal and have estrogen hormone secretion. Estrogen is the main influence of bone formation in premenopausal women, while, adipocyte is still relatively minor source of estrogen [16].

It was previously mentioned that after forty of age, cortical bone is lost at a rate 0.3 to 0.5 % per year accelerated to 5 to 7 % per year in postmenopausal women leading to cumulative bone loss up to 40 to 50% in some women [23]. More increase in some osteoclast cell markers activity was recorded as consequence of age-related osteoblasts apoptosis. It was concluded that those markers can be an indicator of a higher risk of bone fracture [24].

Concerning G2 and G3 who were menopausal women within the same age group, results showed significant ($P \leq 0.05$) decrease in BMD in G3 versus G2 associated with significant lower BMI. The neck of femur showed dangerous low BMD levels that add to the fracture risk (Table1).

On the other side, higher BMI in G1 protected patients (older in age) from great and rapid decrease of BMD when compared to non obese patients (younger age) in G3. Also, G3 had higher HF than G1 for the same reason.

G1 showed Positive significant correlation between BMI and BMD of left femur and lumbar vertebrae ($r = +0.71$, $P=0.01$, $r = +0.68$, $P= 0.03$ respectively). Also, negative correlations between BMI and hip fracture risk was found ($r = -0.68$, $P < 0.03$). PINP correlated positively with BMI in patients ($r = +0.95$ $P \leq 0.001$).

All groups showed (Fig. 1) Positive correlation between BMI and BMD of left femur ($r^2= 0.238$, $F=0.238$, $P=.003$) (A) and negative one between BMI and HF ($r^2=0.119$, $F=5.14$ and $P \leq 0.029$) (B). Also, Positive significant correlation between BMI and BMD of lumbar vertebrae ($r^2=0.273$, $F=14.28$ and $P \leq 0.001$) was shown (C) and between BMI and PINP ($r^2=0.089$, $F=3.73$ and $P \leq 0.061$) (D). These results are in coincidence with the findings of earlier studies where, BMD was better in obese persons. While, negative correlations between BMI and hip fracture risk were reported in patients [17, 25 and 26]. The same results were recorded in other types of bone fracture in different body sites [27, 28].

Previous studies reported that women with $BMI > 37$ kg/m^2 had 70% lower rate of hip fractures when compared with those with $BMI < 28.7$ kg/m^2 [29]. Others reported that weight loss is associated with 1–2% bone loss at the hip and at highly trabecular sites, such as the trochanter and radius [30-33].

Obesity/osteoporosis interaction had great clinical significance to evaluate the changes in bone metabolism. Adipocyte had been recognized as an estrogen-producing cell. Thus, early postmenopausal women who lost bone rapidly had lower levels of both estrone and oestradiol

than “slow losers” and this was referred to their lower fat mass [26].

Also in obese women, leptin hormone is produced in bone marrow adipocytes [34] and its receptors were found on osteoblasts [35-38]. Leptin plays an important role in the maintenance of bone mass [39] where it increases osteoblast proliferation, collagen synthesis and mineral uptake [13]. Increased leptin level is correlated with decreased bone resorption marker and decrease bone loss both in pre and post menopausal obese women [40, 41]. Leptin has a potent effect on insulin secretion, insulin stimulates differentiated osteoblast function in skeletal tissue and increases bone matrix synthesis [42]. Also, insulin increases 1, 25(OH)₂D₃ synthesis necessary for bone formation [11, 12].

Glucose-dependent insulinotropic peptide, IGF-1 and IGF-2(insulin growth factor 1, 2), have receptors on osteoblasts, whose secretion in obese is related to anabolic action on bone by stimulation of osteoblasts proliferation [43].

Fig. 1.D. showed positive correlation of P1NP and BMI in patients, similar result was mentioned by other authors [44-46]

In the current study using PINP, as bone formation marker in serum as diagnostic tool to BMD showed as high accuracy as DEXA results and should be considered as alternative easy diagnostic tool. Some authors recorded contradictory reports in this respect where many authors consider it as an accurate diagnostic tool in research only but not in routine use due to its biological variability [47].

CONCLUSIONS

It is concluded that obesity protects from rapid loss of BMD and risk of hip fracture that commonly take place in menopause and by aging. Because of the contradictory debates in this point of research, Egypt needs a wide scale study on obese persons having low BMD. Egypt needs to be member of Frax10.

REFERENCES

1. Greco, E.A., A. Lenzi and S. Migliaccio, 2015. The obesity of bone. *Ther. Adv. Endocrinol. Metab.*, 6: 273-286.
2. Kado, D.M., M.H. Huang, A.S. Karlamangla, E. Barrett-Connor and G.A. Greendale, 2004. Hyperkyphotic posture predicts mortality in older community-dwelling men and women: A prospective study. *J. Am. Geriatr. Soc.*, 52: 1662-1667.

3. WHO, 2000. Study Group. Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ. Tech. Rep. Ser., 894 (i-xii): 1-253.
4. Factsheet, N., 2016. _311obesity fact sheet from WHO. Available online: <http://www.who.int/mediacentre/factsheets/fs311/en/> (accessed on 12 January 2016).
5. Greco, E.A., R. Fornari, F. Rossi, V. Santiemma, G. Prossomariti, C. Annoscia, A. Aversa, M. Brama, M. Marini and L.M. Donini, 2010. Is obesity protective for osteoporosis? Evaluation of bone mineral density in individuals with high body mass index. *Int. J. Clin. Pract.*, 64: 817-820.
6. Kim, K.C., D.H. Shin, S.Y. Lee, J.A. Im and L. Duk-Chul, 2010. Relation between obesity and bone mineral density and vertebral fractures in Korean postmenopausal women. *Yonsei Med. J.*, 51: 857-863.
7. Compston, J.E., J. Flahive, D.W. Hosmer, N.B. Watts, E.S. Siris, S. Silverman, K.G. Saaq, C. Roux, M. Rossini and J. Pfeilschifter, 2014. Relationship of weight, height and body mass index with fracture risk at different sites in postmenopausal women: The Global Longitudinal Study of Osteoporosis in Women (GLOW). *J. Bone Miner. Res.*, 29: 487-493.
8. WHO, 1994. Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ. Tech. Rep. Ser., 843: 1-129.
9. Cooper, C., G. Campion and L.J. Melton, 1992. Hip fractures in the elderly: A world-wide projection. *Osteoporos. Int.*, 2: 285-289.
10. Shuler, F.D., J. Conjeski, D. Kendall and J. Salava, 2012. Understanding the burden of osteoporosis and use of the World Health Organization FRAX. *Orthopedics*, 35: 798-805.
11. Michel, B.A., D.A. Bloch and J.F. Fries, 1989. Weight-bearing exercise, overexercise and lumbar bone density over age 50 years. *Arch. Intern. Med.*, 149: 2325-2329.
12. Albala, C., M. Yáñez, E. Devoto, C. Sostin, L. Zeballos and J.L. Santos, 1996. Obesity as a protective factor for postmenopausal osteoporosis. *Int. J. Obes. Relat. Metab. Disord.*, 20: 1027-1032.
13. Haffner, S.M. and R.L. Bauer, 1993. The association of obesity and glucose and insulin concentrations with bone density in premenopausal and postmenopausal women. *Metabolism*, 42: 735-738.
14. De Laet, C., J.A. Kanis, A. Odén, H. Johansson, O. Johnell, P. Delmas, J.A. Eisman, H. Kroger, S. Fujiwara and P. Garnero, 2005. Body mass index as a predictor of fracture risk: A meta-analysis. *Osteoporos. Int.*, 16: 1330-1338.
15. Cohen, A., D.W. Dempster, R.R. Recker, J.M. Lappe, H. Zhou, A. Zwahlen, R. Müller, B. Zhao, X. Guo and T. Lang, 2013. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: A transiliac bone biopsy study. *J. Clin. Endocrinol. Metab.*, 98: 2562-2572.
16. Bredella, M.A., M. Torriani, R.H. Ghomi, B.J. Thomas, D.J. Brick, A.V. Gerweck, L.M. Harrington, A. Breggia, C.J. Rosen and K.K. Miller, 2011. Determinants of bone mineral density in obese premenopausal women. *Bone*, 48: 748-754.
17. Ishii, S., J.A. Cauley, G.A. Greendale, C. Nielsen, C. Karvonen-Gutierrez, K. Ruppert and A.S. Karlamangla, 2014. Pleiotropic effects of obesity on fracture risk: The Study of Women's Health Across the Nation. *J. Bone Miner. Res.*, 29: 2561-2570.
18. Morris, H.A., R. Eastell, N.R. Jorgesen, E. Cavaliere, S. Vasikaran, S.A.P. Chubb, J.A. Kanis, C. Cooper and K. Makrissi, 2016. On behalf of the IFCC-IOF Working Group for Standardisation of Bone Marker Assays (WG-BMA). Clinical usefulness of bone turnover marker concentrations in osteoporosis. *Clin Chim Acta* (2016) doi:10.1016/j.cca.06.036
19. International Osteoporosis Foundation: FRAX WHO fracture risk assessment tool: information and resources. Available at: http://www.iofbonehealth.org/health_professionals. [Accessed 11 September 2009]
20. Kanis, J.A., E.V. McCloskey, H. Johansson, A. Oden, L.J. Melton and N. Khaltaev, 2008. A reference standard for the description of osteoporosis. *Bone* 42: 467-475.
21. PINP-Clinical, 2016. Procollagen I Intact N-Terminal, Serum www.mayomedicallaboratories.com/test...and .../61695 Document generated November 20, at 5:18am CST
22. Frax site (WHO, fracture risk assessment tool available at <http://www.shef.ac.uk/FRAX/20>)
23. NIH consensus, 2001. Development panel on osteoporosis prevention, diagnosis and therapy. *J.A.M.A.M.Assoc.*, 285: 785-795.

24. Doventas, A., I.M. Bolayirli b, S. Incir b, H. Yavuzer a, S. Civelek b, D.S. Erdinclar a, D. Konukoglu b, T. Beger a and A. Seven b, 2015. Interrelationships between obesity and bone markers in postmenopausal women with either obesity or osteoporosis European Geriatric Medicine, 6: 15-20.
25. Paganini-Hill, A., A. Chao, R.K. Ross and B.E. Henderson, 1991. Exercise and other factors in the prevention of hip fracture: The Leisure World study. *Epidemiology*, 2: 16-25.
26. Cummings, S.R., M.C. Nevitt, W.S. Browner, K. Stone, K.M. Fox, K.E. Ensrud, J. Cauley, D. Black and T.M. Vogt, 1995. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N. Engl. J. Med.*, 332: 767-773.
27. Joakimsen, R.M., V. Fønnebø, J.H. Magnus, A. Tollan and A.J. Søgaard, 1998. The Tromsø Study: Body height, body mass index and fractures. *Osteoporos. Int.*, 8: 436-442.
28. Van der Voort, D.J., P.P. Geusens and G.J. Dinant, 2001. Risk factors for osteoporosis related to their outcome: Fractures. *Osteoporos. Int.*, 12: 630-638.
29. DiPietro, L., G.A. Welch, D.R. Davis, J.W. Drane and C.A. Macera, 1993. Body mass and risk of hip fracture among a national cohort of postmenopausal white women: A reanalysis. *Obes. Res.*, 1: 357-363.
30. Salamone, L.M., J.A. Cauley, D.M. Black, L. Simkin-Silverman, W. Lang, E. Gregg, L. Palermo, R.S. Epstein, L.H. Kuller and R. Wing, 1999. Effect of a lifestyle intervention on bone mineral density in premenopausal women: A randomized trial. *Am. J. Clin. Nutr.*, 70: 97-103.
31. Langlois, J.A., M.E. Mussolini, M. Visser, A.C. Looker, T. Harris and J. Madans, 2001. Weight loss from maximum body weight among middle-aged and older white women and the risk of hip fracture: The NHANES I epidemiologic follow-up study. *Osteoporos. Int.*, 12: 763-768.
32. Ensrud, K.E., R.L. Fullman, E. Barrett-Connor, J.A. Cauley, M.L. Stefanick, H.A. Fink, C.E. Lewis and E. Orwoll, 2005. Voluntary weight reduction in older men increases hip bone loss: The osteoporotic fractures in men study. *J. Clin. Endocrinol. Metab.*, 90: 1998-2004.
33. Bleicher, K., R.G. Cumming, V. Naganathan, T.G. Travison, P.N. Sambrook, F.M. Blyth, D.J. Handelsman, D.G. Le Couteur, L.M. Waite and H.M. Creasey, 2011. The role of fat and lean mass in bone loss in older men: Findings from the CHAMP study. *Bone*, 49: 1299-1305.
34. Kanis, J., O. Johnell, B. Gullberg, E. Allander, L. Elfors, J. Ranstam, J. Dequeker, G. Dilsen, C. Gennari and A.L. Vaz, 1999. Risk factors for hip fracture in men from southern Europe: The MEDOS study. *Mediterranean Osteoporosis Study. Osteoporos. Int.*, 9: 45-54.
35. Finkelstein, E.A., J.G. Trodron, J.W. Cohen and W. Dietz, 2009. Annual medical spending attributable to obesity: Payer-and service-specific estimates. *Health Aff. (Millwood)*, 28(5): w822-31.
36. Watts, N.B., 2014. Insights from the Global Longitudinal Study of Osteoporosis in Women (GLOW). *Nat. Rev. Endocrinol.*, 10: 412-422.
37. Takada, I., M. Suzawa and S. Kato, 2005. Nuclear receptors as targets for drug development: Crosstalk between peroxisome proliferator-activated receptor gamma and cytokines in bone marrow-derived mesenchymal stem cells. *J. Pharmacol. Sci.*, 97: 184-189.
38. Pei, L. and P. Tontonoz, 2004. Fat's loss is bone's gain. *J. Clin. Investig.*, 113: 805-806.
39. Goulding, A. and R.W. Taylor, 1998. Plasma leptin values in relation to bone mass and density and to dynamic biochemical markers of bone resorption and formation in postmenopausal women. *Calcif. Tissue Int.*, 63: 456-458.
40. Kontogianni, M.D., U.G. Dafni, J.G. Routsias and F.N. Skopouli, 2004. Blood leptin and adiponectin as possible mediators of the relation between fat mass and BMD in perimenopausal women. *J. Bone Miner. Res.*, 19: 546-551.
41. Holecki, M. and A. Wiecek, 2010. Relationship between body fat mass and bone metabolism. *Pol. Arch. Med. Wewn.*, 120: 361-367.
42. Liberati, A., D.G. Altman, J. Tetzlaff, C. Mulrow, P.C. Gøtzsche, J.P.A. Ioannidis, M. Clarke, P.J. Devereaux, J. Kleijnen and D. Moher, 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *J. Clin. Epidemiol.*, 62.
43. Davidson, P.L., A. Goulding and D.J. Chalmers, 2003. Biomechanical analysis of arm fracture in obese boys. *J. Paediatr. Child Health*, 39: 657-664.
44. Ni Zhonga, Xian-Ping Wu, Zhang-Rong Xu, Ai-Hong Wang, Xiang-Hang Luoa, Xing-Zhi Caoa, Hui Xiea, Peng-Fei Shana and Er-Yuan Liao, 2005. Relationship of serum leptin with age, body weight, body mass index and bone mineral density in healthy mainland Chinese women. *Clinica Chimica Acta*, 351: 161-168.

45. Kanis, J.A., N. Burlet, C. Cooper, P.D. Delmas, J.Y. Reginster, F. Borgstrom and R. Rizzoli, 2008. on behalf of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int.*, 19: 399-428.
46. Vasikaran, S., R.. Eastell and O. Bruyere, 2011. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int.*, 22: 391.
47. Hannon, R. and R. Eastell, 2000. Preanalytical variability of biochemical markers of bone turnover. *Osteoporos Int.*, 11: S30-S44.