# Relationship between Lipid Profile and Bone Turnover in Pre and Postmenopausal Women

*H Saghafi, A Hossein-nezhad, M Rahmani, \*B Larijani*

*Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences, Iran*

## Abstract

**Background:** To determine the relationship between lipid profile and bone turnover in pre and postmenopausal women.
**Methods:** In a cross-sectional study, 279 women referred to Bone Mineral Densitometry (BMD) center of endocrinology and Metabolism research center for premenopausal evaluation were assessed for serum osteoporotegerin, receptor activator of nuclear factor kB (NF-kB) ligand (RANKL) and lipid profile in biochemistry and hormone laboratory.
**Results:** Serum Total cholesterol had significant inverse correlation with spine L2-L4 BMD (r=-0.152, $P$= 0.02) and L2-L4 $t$-score (r=-0.151, $P$= 0.02). Low density lipoprotein (LDL) cholesterol also related negatively to spine L2-L4 BMD (r=-0.184, $P$= 0.007), L2-L4 T score (r=-0.184, $P$= 0.007) and L2-L4 Z score (r=-0.134, $P$= 0.04). However no relation was found between triglyceride and high density lipoprotein and lumbar spine BMD values. Whereas 35.5 % of women with LDL >130 had serum RANKL upper than percentile 75, this value was 18.7% among women with LDL< 130($P$=0.01, Odds Ratio= 2.39, CI: 1.24-4.6). Osteoprotegerin had no such a relation with LDL. In univariate analysis LDL had a significant relationship with RANKL independent of age ($P$= 0.02).
**Conclusion:** As RANKL is a bone marker that show bone loss, our finding may contribute to demonstrate a negative effect of LDL on bone metabolism.

**Keywords:** *Low density lipoprotein, Osteoporosis, Osteoprotegerin, RANKL.*

## Introduction

Osteoporotic fractures and acute cardiovascular events due to atherosclerosis remain the predominant contributors to morbidity and mortality among the elderly. Emergent epidemiological and biological evidence puts forwards a possible link between these two diseases (1, 2). In both cross-sectional and longitudinal epidemiologic studies, increased cardiovascular mortality (3-5), cardiovascular morbidity (6, 7), and sub clinical measures of atherosclerosis (8, 9) has been related to low bone mass.

Common mechanisms have been concerned in the possible relationship between bone loss and atherosclerosis. Immunological and inflammatory factors play an important role in pathophisiology of both diseases. One of these factors is Osteoprotegerin (OPG). OPG is a soluble glycoprotein that belongs to the tumor necrosis factor (TNF) receptor super family. OPG acts as a decoy receptor of the receptor activator of nuclear factor kB (NF-kB) ligand (RANKL), which is an important regulator of osteoclastogenesis OPG is known to inhibit osteoclastogenesis by binding to RANKL, to preventing it from binding to the receptor activator of NF-kB on osteoclasts (10,11).

It has been reported that OPG is highly expressed in the bones, heart, and major arteries (12). Recently, OPG has shown not only as an inhibitor of osteoclastogenesis, but also as a preventive mediator of cardiovascular diseases, such as arterial calcification and atherogenesis. Also OPG is present in atherosclerotic arteries as an inflammatory cytokines (13).

Another common culprit acting in parallel on both vascular and bone cells (14,15) are serum lipids, which in addition to their established role in atherogenesis, were also shown to act on osteoblasts (16–18) and osteoclasts (19). Products of lipoprotein oxidation and an atherogenic diet inhibit preosteoblast differentiation (20) and result in reduced bone mineralization. These ob-

servations suggest a relationship between lipid and bone metabolism.

The aim of this study was to determine relationship between lipid profile and bone turnover in pre and postmenopausal women.

## Material and Methods

The study population consisted of 279 healthy women, who selected from outpatient clinic of Endocrinology and metabolism research center (EMRC) of Tehran University of Medical Sciences. Women with diabetes mellitus, ischemic heart disease, cerebrovascular disease, thyroid dysfunction, chronic liver, and renal diseases were excluded. We also excluded the patients taking medicine that can affect bone metabolism.

The menopause was defined as the absence of menstruation for at least 12 months. The protocol used was approved by the Institutional Review Board of EMRC, and informed consent was obtained from all participants.

We measured height, weight, and systolic and diastolic blood pressures. Body mass index was calculated as weight (kg) divided by height (m) squared and was used as an index of overall adiposity (kg/m2).

Venus blood sampling was performed after 12 h fasting. Total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol and Osteoprotegerin was measured by immunoassay (ELISA) using an Immunodiagnostic kit. The intra- and inter-assay CV were 6.6% and 5.7%, respectively. Serum sRANKL was measured by immunoassay (ELISA) using a Biomedica kit, with intra- and inter-assay CV of 4.1% and 5.1%, respectively.

BMD was measured by DXA using Lunar DPX-MD device (Lunar Corporation, Madison, USA). The DXA device was calibrated daily and weekly by using appropriated phantoms methods. To assess BMD, second to fourth lumbar spine and from the femur bone (neck, trochanter and the whole femur), bone density was calculated based on gr/cm2.

## Results

Totally 279 women pre and postmenopausal women participated in this study. The study population's main clinical and laboratory features are shown in Table 1. The subjects included 177 postmenopausal women (age, 58.78±7.53 yr) and 102 premenopausal women (age, 48.36± 7.25 yr).

Serum Total cholesterol had significant inverse correlation with spine L2-L4 BMD (r= -0.152, *P*= 0.02) and L2-L4 *t* score (r= -0.151, *P*= 0.02). LDL cholesterol also related negatively to spine L2-L4 BMD (r= -0.184, *P*= 0.007), L2-L4 T score (r= -0.184, *P*= 0.007) and L2-L4 Z score (r= -0.134, *P*= 0.04). However no relation was found between TG and HDL and lumbar spine BMD values. Measured lipids had shown no significant relation with BMD values of hip. Serum OPG levels were correlated positively with age(r= 0.225, *P*= 0.001).

Serum OPG levels had negative correlation with hip BMD (r= -0.160, *P*= 0.02) and hip t score (r=-0.158, *P*= 0.02). No significant correlation was found between serum OPG level and BMD values of lumbar spine. Serum TG and HDL-C level were found to have no relationship with serum OPG level. Serum RANKL showed no significant relation with measured lumbar spine and hip BMD.

Whereas 35.5 % of women with LDL >130 had serum RANKL upper than percentile 75, this value was 18.7% among women with LDL< 130 (*P*=.01, Odds Ratio= 2.39, CI: 1.24-4.6). OPG had no such a relation with LDL. In univariate analysis LDL had a significant relationship with RANKL independent of age (*P*= 0.02).

**Table 1:** Clinical characteristics and laboratory finding of participants

| values | Mean | Std. Deviation |
|---|---|---|
| Age (yr) | 55.34 | 8.70 |
| Height (cm) | 157.60 | 5.95 |
| Weight (kg) | 69.09 | 13.69 |
| BMI (kg/m2) | 27.73 | 5.00 |
| BMD of femur (g/cm2) | 0.91 | 0.14 |
| T score of femur | -0.71 | 1.22 |
| Z score of femur | -0.16 | 1.01 |
| BMD of L2_L4 (g/cm2) | 1.03 | 0.18 |
| T score of L2_L4 | -1.37 | 1.52 |
| Z score of L2_L4 | -0.57 | 1.28 |
| Triglyceride (mg/dl) | 162.68 | 85.12 |
| Total cholesterol(mg/dl) | 222.71 | 42.28 |
| HDL(mg/dl) | 50.16 | 12.69 |
| LDL(mg/dl) | 123.21 | 27.19 |
| Osteoprotegerin(pmol/l) | 5.87 | 1.99 |
| RANKL(pmol/l) | 0.09 | 0.16 |

**Table 2:** Comparison of laboratory finding in three groups based on bone mineral status

| variables | Bone mineral status | | | *P* value |
|---|---|---|---|---|
| | Normal (72) | Osteopenia(94) | Osteoporosis(50) | |
| TC(mg/dl) | 211.88±39.54 | 227.36±46.83 | 230.36±35.34 | 0.024 |
| LDL(mg/dl) | 115.83±27.24 | 125.08±27.79 | 130.62±24.75 | 0.009 |
| HDL(mg/dl) | 49.27±11.93 | 50.51±13.25 | 50.40±12.18 | 0.805 |
| TG(mg/dl) | 153.87±83.38 | 166.77±86.22 | 170.84±88.44 | 0.496 |
| OPG (pmol/l) | 5.47±1.70 | 6.05±2.19 | 6.06±2.07 | 0.156 |
| RANKL(pmol/l) | 0.07±0.13 | 0.10±0.20 | 0.10±0.13 | 0.640 |

Mean ±SD

## Discussion

This study demonstrates that the lipid profile is moderately related to BMD. The levels of serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were inversely associated with BMD in both pre- and post-menopausal women.

Our finding is consistent with the results of Cui et al. (21) studied pre- and post-menopausal rural women in South Korea. However Zabaglia et al. (22) found no association between total cholesterol, LDL-C and BMD in meno-pausal women.

A number of studies have suggested a positive relationship between BMD and triglyceride levels. Cui et al. (21) reported that the levels of triglyceride (TG) had a significant positive correlation with BMD values at the trochanter site in the post-menopausal women. Adami et al. (23) also found that total body and hip BMD were positively related to serum triglycerides in women. However Zabaglia et al. (23) found no association between serum triglycerides and BMD in menopausal women. We also did not find relation between TG and BMD.

Literature concerning relationships between HDL cholesterol levels and BMD is contradictory (24-28). Some reports showing a negative association between BMD and HDL cholesterol even though in a limited number of postmenopausal osteoporotic women (24). Zabaglia (23) found no association between lipid profile variables and BMD, except for high density lipoprotein (HDL), which showed an inverse correlation in postmenopausal women. However Cui et al. (21) report was consistent with our finding that the levels of high-density lipoprotein cholesterol (HDL-C) were not associated with BMD values at any of the sites in the pre- and post-menopausal subjects.

In spite of that several studies show the association between different components of lipid profile and BMD, a constant relation has not been found. BMD decreases by advancing age and has a positive relation by BMI. Since both of these factors have close relation with lipid profile, age and BMI adjustment should be considered.

We found that serum level of total cholesterol and LDL significantly different in various bone conditions. These measured lipids were significantly higher in osteopenic patients than normal individuals and also higher in osteoporotic than osteopenic women. This finding was remained after adjustment for age and BMD. This upward trend is in agreement with our other finding that showed negative relationship between total cholesterol and LDL and BMD.

Despite many studies conducted to reveal the relation between lipid profile and BMD, pathophisiology and molecular mechanisms of this relation have not been fully investigated. Recent studies emphasize on important role of Osteoprotegerin (OPG) and receptor activator of nuclear factor kB (NF-kB) ligand (RANKL) in bone metabolism. OPG is a soluble glycoprotein that belongs to the tumor necrosis factor (TNF) receptor super family (12, 29, 30). OPG acts as a decoy receptor of the RANKL, which is a critical regulator of osteoclastogenesis and is known to restrain osteoclastogenesis by binding to RANKL and preventing RANKL from binding to the receptor activator of NF-kB on osteoclasts (10, 11).

We found a significant positive relation between OPG and age. Previous reports have presented similar results about the association between serum OPG levels and the age in women (31-33). The increasing serum levels of OPG with aging could be interpreted as a compensatory mechanism to neutralize the acceleration of bone resorption (31). Further studies on the changes of production and clearance of OPG with aging are required.

We found no relation between OPG and lipid profile. However Oh et al. (34) studying healthy Korean women reported that the mean serum OPG levels tended to be higher in those with high serum TC and LDL-C levels. But serum TG and HDL-C level were found to have no relationship to the mean serum OPG level. They also showed by bivariate correlation analysis that serum OPG levels were positively correlated with serum TC and LDL-C levels.

Our study showed that LDL had a significant relationship with RANKL independent of age. Through a search in the literature we found no previous report about the relation between RANKL and lipid profile. However, there are some records that hyperlipidemia and minimally oxidized low-density lipoprotein exert negative effects on bone metabolism (18, 35). As RANKL is a bone marker that show bone loss, our finding may contribute to demonstrate the adverse role of LDL playing in bone metabolism.

There were some limitations in our study. Due to its cross-sectional nature, we are not able to clarify whether lipid profile is associated with peak bone mass acquisition or to age-related bone loss. So understanding the exact effects of lipids on bone metabolism required cohort studies. In addition, the coloration of serum and tissue levels of bone markers is suspicious. So for confirmation of findings, further studies should be undertaken on target tissue to investigate the precise relation between bone and serum biomarkers.

In conclusion, our data show that lipid profile is partially associated with BMD and osteoporosis in pre and postmenopausal women. Evaluation of lipid profile in relation with bone markers may suggest LDL negative effect on bone metabolism. Regarding Crucial role of LDL-C in Pathogenesis of atherosclerosis, the common pathopysiological metabolic pathways result in atherosclerosis and osteoporosis should be investigated.

## Acknowledgements

## References

1. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW (2001). Bone loss and the progression of abdominal aortic calcification over a 25-year period: the Framingham Heart Study. *Calcif Tissue Int*, 68: 271-76.

2. Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, et al. (2001). Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*, 21:1998-2003.

3. Browner WS, Seeley DG, Vogt TM, Cummings SR (1991). Nontrauma mortality in elderly women with low bone mineral density. Study of Osteoporotic Fractures Research Group. *Lancet*, 338: 355-58.

4. Mussolino ME, Madans JH, Gillum RF (2003). Bone mineral density and mortality in women and men: the NHANES I epidemiologic follow-up study. *Ann Epidemiol*, 13: 692-697.

5. Trivedi DP, Khaw KT (2001). Bone mineral density at the hip predicts mortality in elderly men. *Osteoporos Int*, 12: 259-65.

6. Farhat GN, Strotmeyer ES, Newman AB, Sutton-Tyrrell K, Bauer DC, Harris TB, et al. (2006). Volumetric and areal bone mineral density measures are associated with cardiovascular disease in older men and women: the health, aging, and body composition study. *Calcif Tissue Int*, 79: 102-111.

7. Tanko L, Christiansen C, Cox DA, Geiger MJ, McNabb MA, Cummings SR (2005). Relationship between osteoporosis and cardiovascular disease in postmenopausal women. *J Bone Miner Res*, 20:1912-20.

8. Tanko LB, Bagger YZ, Christiansen C (2003). Low bone mineral density in the hip as a marker of advanced atherosclerosis in elderly women. *Calcif Tissue Int*, 73: 15-20

9. Barengolts EI, Berman M, Kukreja SC, Kouznetsova T, Lin C, Chomka EV (1998). Osteoporosis and coronary atherosclerosis in asymptomatic postmenopausal women. *Calcif Tissue Int*, 62: 209-13.

10. Schoppet M, Preissner KT, Hofbauer LC (2002). RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. *Arterioscler Thromb Vasc Biol*, 22: 549-53.

11. Khosla S (2001). Minireview: The OPG/ RANKL/RANK system. *Endocrinology*, 142:5050-55.

12. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*, 89:309-19.

13. McFarlane SI, Muniyappa R, Shin JJ, Bahtiyar G, Sowers JR (2004). Osteoporosis and cardiovascular disease: brittle bones and boned arteries, is there a link? *Endocrine*, 23: 1-10.

14. Mody N, Tintut Y, Radcliff K, Demer LL (2003). Vascular calcification and its relation to bone calcification: possible underlying mechanisms. *J Nucl Cardiol*, 10(2):177-83.

15. Baldini V, Mastropasqua M, Francucci CM, D'Erasmo E (2005). Cardiovascular disease and osteoporosis. *J Endocrinol Invest*, 28 (10 Suppl): 69–72.

16. Parhami F, Basseri B, Hwang J, Tintut Y, Demer LL (2002). High density lipoprotein regulates calcification of vascular cells. *Circ Res*, 91(7):570–76.

17. Parhami F, Garfinkel A, Demer LL (2000). Role of lipids in osteoporosis. *Arterioscler Thromb Vasc Biol*, 20(11): 2346-48.

18. Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, Demer LL (1997). Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. *Arterioscler Thromb Vasc Biol*, 17(4): 680-87.

19. Tintut Y, Morony S, Demer LL (2004). Hyperlipidemia promotes osteoclastic potential of bone marrow cells ex vivo. *Arterioscler Thromb Vasc Biol,* 24(2): e6-10.

20. Diascro Jr DD, Vogel RL, Johnson TE, Witherup KM, Pitzenberger SM, et al. (1998). High fatty acid content in rabbit serum is responsible for the differentiation of osteoblasts into adipocyte-like cells. *J Bone Miner Res*, 13: 96-106.

21. Cui LH, Shin MH, Chung EK, Lee YH, Kweon SS, Park KS, Choi JS (2005). Association between bone mineral densities and serum lipid profiles of pre- and post-menopausal rural women in South Korea. *Osteoporosis Int*, 16: 1975-81.

22. Zabaglia SF, Pedro AO, Pinto Neto AM, Guarisi T, Paiva LH, Lane E (1998).An exploratory study of the association between lipid profile and bone mineral density in menopausal women in a Campinas reference hospital. *Cad Saude Pub*, 14:779-86.

23. Adami S, Braga V, Zamboni M, Gatti D, Rossini M, Bakri J, Battaglia E (2004). Relationship between Lipids and Bone Mass in 2 Cohorts of Healthy Women and Men. *Calcif Tissue Int*. 74(2): 136-42.

24. D'Amelio P, Pescarmona GP, Gariboldi A, Isaia GC (2001). High density lipoproteins (HDL) in women with postmenopausal osteoporosis: a preliminary study. *Menopause*, 8: 429-32.

25. Yamaguchi T, Sugimoto T, Yano S, Yamauchi M, Sowa H, Chen Q, Chihara K (2002). Plasma lipids and osteoporosis in postmenopausal women. *Endocr J*, 49: 211–17.

26. Poli A, Bruschi F, Cesana B, Rossi M, Paoletti R, Crosignani PG (2003). Plasma low-density lipoprotein cholesterol and bone mass densitometry in postmenopausal women. *Obstet Gynecol*, 102: 922-6.

27. Brownbill RA, Ilich JZ (2006). Lipid profile and bone paradox: higher serum lipids are associated with higher bone mineral density in postmenopausal women. *J Womens Health*, 15: 261–70.

28. Solomon DH, Avorn J, Canning CF, Wang PS (2005). Lipid levels and bone mineral density. *Am J Med*, 118: 1414.e1–1414.e5.

29. Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, et al. (1997). Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochem Biophys Res Commun*, 234:137- 42.

30. Kwon BS, Wang S, Udagawa N, Haridas V, Lee ZH, Kim KK, et al. (1998). TR1, a new member of the tumor necrosis factor receptor superfamily, induces fibroblast proliferation and inhibits osteoclastogenesis and bone resorption. *FASEB J*, 12: 845- 54.

31. Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, et al. (1999). Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentra-

tions in postmenopausal women with osteoporosis. *J Bone Miner Res*, 14: 518-27.

32. Khosla S, Arrighi HM, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Dunstan C, et al. (2002). Correlates of osteoprotegerin levels in women and men. *Osteoporos Int*, 13: 394-99.

33. Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD(2001). Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. *J Clin Endocrinol Metab*, 86: 3162-65.

34. Oh ES, Rhee EJ, Oh KW, Lee WY, Baek KH, Yoon KH, Kang MI, Yun EJ, Park CY, Choi MG, Yoo HJ, Park SW (2005). Circulating osteoprotegerin levels are associated with age, waist-to-hip ratio, serum total cholesterol, and low-density lipoprotein cholesterol levels in healthy Korean women. *Metabolism*, 54(1):49-4.

35. Parhami F, Jackson SM, Tintut Y, Le V, Balucan JP, Territo M, Demer LL (1999). Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. *J Bone Miner Res*, 14: 2067–78.