

Bone Mineral Density, Body Composition, and Serum Leptin in Premenopausal Egyptian Women

¹Wafaa A. Kandeel, ¹Moushira E. Zaki, ¹Azza M. Sarry El Din
and ²Mona Anwar

¹Biological Anthropology Department, National Research Centre, Cairo, Egypt.

²Medical Biochemistry Department, National Research Centre, Cairo, Egypt

Abstract: To assess whether lean mass or fat mass is a better predictor of bone mineral density (BMD) in premenopausal Egyptian women, associations between BMD and body composition were analyzed. In addition, the study aims to evaluate the relationships between serum leptin concentrations, bone mineral density and body composition. The sample consisted of 84 premenopausal women aged 25 - 50 years. BMI was used to classify overweight women (BMI > 25) and normal weight women (BMI < 25). Body composition and fasting serum leptin were measured. Dual energy x-ray absorptiometry (DXA) was used to determine BMD (g/cm²) at proximal femur (femoral neck, Ward's triangle, trochanter), lumbar spine (L2, L3, L4 and L2-L4) and ultradistal(UD) forearm sites. Regression techniques were used to detect associations. Among overweight women, significant positive association was found between lean mass and BMD at all sites except at UD-forearm. Body fat mass was positively associated with BMD at the femoral neck and Ward's triangle only. On the other hand, there was no association between body composition components and BMD among normal weight women. Serum leptin level was negatively correlated with BMD at UD-forearm among overweight women as well as L2 and L2-L4 among normal weight women. Age was consistently negatively associated with BMD. The study showed that lean mass is a better predictor than fat mass of BMD in overweight Egyptian women and that serum leptin levels are not associated with BMD. However, leptin may have some effects on BMD regionally.

Key words: Bone mineral density- body composition- serum leptin- premenopausal women.

INTRODUCTION

Bone Mineral Density (BMD) attained in early life is considered to be determinant of the risk of osteoporotic fractures (Cummings, *et al* 1998) .

Body weight is one of the strongest positive predictors of BMD in subjects of all age groups (Ngyuen *et al*,1989) . However, the nature of this relationship remains unknown (Martini *et al* 2001). There is also controversy as to whether lean mass or fat mass is a better predictor of BMD(Thomas *et al* 2001, Van Langendonk *et al* 2002 and Mizuma *et al* 2006) .

An association of lean mass with BMD may be due to mechanical load forces on bone (Takada *et al* 1997)

Fat mass is metabolically active; its effect on the skeleton may be influenced by hormonal factors, which include alterations in vitamin D metabolism (Lie *et al* 1988). Fat mass may act as a peripheral site for the conversion of androgens to estrogen (Reid *et al* 1992 and Aloia *et al* 1995) and serum estrogens are thought to be associated with BMD and the low risk of fracture in women (Ettinger *et al* 1998) . Additionally, decreased sex hormones binding globulin(SHBG)levels with increased free fraction of sex steroids is also considered as possible mechanism for the association between fat mass and BMD (Haffner 1992), as well as the direct augmentation of bone formation, by increased levels of circulating insulin (Reid *et al* 1993) The latter may be caused by direct mitogenic effects of insulin on osteoblasts (Barret- Connor and Kritz-Silverstein 1996) .

Leptin, the protein product of the obesity (*OB*) gene, is secreted mainly by adipocytes (Tartaglia *et al* 1995) and is strongly correlated with fat mass (Thomas *et al* 2000). Rarely obese individuals are leptin

Corresponding Author: Wafaa A. Kandeel, Biological Anthropology Department, National Research Centre, Cairo, Egypt.
E-mail: wafakandeel@yahoo.com

deficient and most of them have hyperleptinemia proportionate to body fat mass and are leptin resistant (Cosidine *et al* 1996).

Several lines of evidence suggest a relationship between leptin and bone health (Thomas *et al* 2004 and Weiss *et al* ,2006, but published results are contradictory. Some studies found that leptin was associated with greater BMD (Pasco *et al* 2001 and Blain *et al* 2002),reduced risk of fracture (Yamauchi *et al* 2001) and decreased bone resorption (Schett *et al* 2004 and Roux 2003) whereas others reported a negative association with BMD(Odabasi *et al* 2000, Sato 2001 and Blum *et al* 2003) and bone formation (Kontogianni *et al* 2004) ,or no association with BMD(Ruhl and Everhart 2002, Scariano e al 2003 and Abou Samara 2005) , bone loss (Dennison *et al* 2004) or bone turnover (Goulding *et al* 1998 and Martini *et al* 2001) .

In the present study, we investigated whether lean mass or fat mass is a better predictor of Bone Mineral Density (BMD) in premenopausal Egyptian women. In addition, we analyzed the relationship between serum leptin levels and (BMD) values as well as the relationship between serum leptin levels and body composition.

MATERIAL AND METHODS

Subjects:

The study comprises 84 premenopausal women living in Cairo with age range 22 - 50 years. They visited outpatient clinic in the National Research Centre for the evaluation of osteoporosis. All of them gave informed consent and entered the study. The study was approved by the ethics committee of National Research Centre.

Exclusion Criteria:

Participants with current or previous (within 6 months) following conditions were excluded from the study: type I diabetes; renal failure; chronic infection, such as tuberculosis or other diseases; gastrointestinal diseases, malignancy; rickets or other metabolic bone diseases; liver diseases; and endocrinal diseases e.g. thyrotoxicosis, current or anticipated pregnancy; use of contraceptive pills, cortisone, anti -epileptic drugs, cholesterol -lowering drugs, or binders.

Questionnaires:

All subjects were screened with a comprehensive questionnaire to collect the participants' occupational, and lifestyle information; menarche (start, cessation), reproductive history; disease history; consumption of alcohol; cigarette smoking; physical activity; history of fractures; and daily diet.

Anthropometric Measurements:

Anthropometric assessment of body weight and height were performed, using standardized equipment and following the recommendations of the International Biological Program (Hiernaux and Tanner 1969) . Three consecutive measurements were taken and the mean was recorded. Body weight to the nearest 0.01 Kg was measured using Seca scale balance adult type, with minimal clothes for which no correction was made. Body height was measured to the nearest 0.1 cm using Holtain anthropometer .The body mass index (BMI) was calculated as weight/ height² (Kg/m²).

Body Composition:

Determination of body composition including total body fat (FM), lean mass (LM), LM% FM% and total body water (TBW) were carried out for each case, using a bioelectrical impedance analyzer (BIA) -RJL.

Dual Energy x-ray Absorptiometry (DXA):

BMD (g/cm²) at the lumbar spine (L2, L3, L4 and L2-L4) ,proximal femur(femoral neck, Ward's triangle, trochanter) and ulradistal (UD) sites were determined on each subject by use of dual-energy x-ray absorptionmetry (DXA; Norland Bone Densitometer soft version : 3.9.6.17 August 01.)

Leptin Analysis:

All subjects were instructed to come after an overnight fasting on the day of the study. Venous blood samples were collected into plain tubes. The samples were allowed to clot, serum was separated and stored at -80 °C until assayed. Serum leptin concentration was measured using a commercially available quantitative ELISA kit from Diagnostics Biochem Canada Inc.

Statistical Analysis:

Serum leptin concentrations were transformed to the natural logarithm (ln) to normalize the data before analysis. Regression techniques were used to develop equations for predicting BMD at each site. Partial r^2 values of the predictors were calculated for each predictor using site- specific models. Significance was set at $P < 0.05$ and all statistical analyses were performed using SPSS (12) software package.

RESULTS AND DISCUSSIONS

Results:

Table 1 lists anthropometry, body composition, BMD and leptin characteristics for normal and overweight women. Body weight, BMI, fat mass, fat free mass and serum leptin were significantly increased in overweight women compared to normal body weight women. There were no significant differences in other clinical characteristics such as age, body composition and BMD at several sites.

Table 1: Anthropometry, body composition, BMD (g/cm^2) and serum leptin characteristics(mean and SD in normal and overweight premenopausal women.

Variable	Normal Weight Women (N=32)		Overweight Women (N=52)	
	Mean	S.D.	Mean	S.D.
Age	35.564	14.54	40.936	10.479
Weight	58.672	1.031	70.290*	0.911
Height	158.181	6.091	158.704	7.156
BMI	23.427	1.621	27.874**	1.335
Sum.SFT	57.6	14.83	83.654**	14.691
LM (g)	41,752	41,27	44,947**	44,80
FM (g)	16.919	31,40	25,290**	42,86
F. BMD	0.847	0.147	0.878	0.135
T. BMD	0.695	0.093	0.7	0.108
W.BMD	0.725	0.197	0.725	0.156
L2 BMD	0.998	0.155	1.038	0.151
L3 BMD	1.011	0.157	1.054	0.154
L4 BMD	0.981	0.135	1.013	0.146
L2-4 BMD	0.996	0.144	1.038	0.149
UD BMD	0.357	0.06	0.374	0.068
Leptin	24.803	13.801	30.927	14.214
Leptin (ln)	2.968	0.827	3.287*	0.6

* $P < 0.05$ ** $P < 0.01$

Table 2 shows multiple regression analysis with BMD (g/cm^2) as the dependent variable and age (year), fat mass (g), fat free mass (g) and leptin (ln) as independent variables in the models for the proximal femur, spine and UD –forearm sites of the overweight women. There was an independent significant positive association between lean mass and BMD at all sites except at UD-forearm. Fat mass showed significant positive association with BMD at Ward’s triangle only. The significant negative associations between age and BMD at all sites were observed, except at trochanter and UD-forearm sites. There was an independent negative association of leptin (ln) with BMD at UD-forearm site.

Among normal body weight women (table 3), there was an independent significant negative association of leptin (ln) with BMD at L2, L3 and L2-L4 sites. Age had negative significant association with BMD at all sites except at UD-forearm site. No significant associations were found between either body lean mass or body fat mass and BMD at all the studied sites.

Discussion:

Several mechanisms have been proposed regarding the association between BMD and estimates of lean mass and fat mass. Increased fat mass or lean mass implies an increased mechanical load on bone. In the present study, lean mass was significantly increased in overweight women compared to normal body weight women. The relationship between body composition and BMD was studied in overweight women (table 2) and indicated to be determined by lean mass than by fat mass.

Fox *et al.*, 2000 concluded that BMD is associated more with lean mass than with fat mass., while increased body fat and lean tissue mass are the main determinants of bone mass. It has also been reported that both BMD and lean mass were significantly lost shortly after hip fracture (Bedogni *et al* 2000). Nakaoka *et al.*, 2000 found that lean mass was positively correlated with BMD at all sites studied in postmenopausal Japanese

Table 2: Multiple regression analysis of BMD (g/cm²) as the dependent variable for the overweight premenopausal women.

Variable	Coefficient β	P Value	Partial r ²
Femoral Neck			
Age	-0.290	0.006*	-0.397
F M	0.259	0.011*	0.371
L M	0.493	0.000**	0.585
Leptin(ln)	-0.11	0.228	-0.181
Trochanter			
Age	-0.242	0.303	-0.314
F M	0.15	0.155	0.213
L M	0.530	0.000**	0.579
Leptin(ln)	-0.101	0.357	-0.139
Ward's Triangle			
Age	-0.402	0.000**	-0.517
F M	0.217	0.030*	0.320
L M	0.440	0.000**	0.544
Leptin(ln)	-0.90	0.368	-0.136
L2			
Age	-0.349	0.006*	-0.397
F M	0.107	0.368	0.136
L M	0.377	0.004**	0.417
Leptin(ln)	-0.106	0.381	-0.132
L3			
Age	-0.366	0.003**	-0.430
F M	0.094	0.405	0.126
L M	0.359	0.004**	0.417
Leptin(ln)	-0.208	0.073	-0.267
L4			
Age	-0.226	0.077	-0.263
F M	0.130	0.288	0.16
L M	0.368	0.006**	0.399
Leptin(ln)	-0.227	0.071	0.268
L2- L4			
Age	-0.342	0.007*	-0.391
F M	0.083	0.483	0.106
L M	0.362	0.005*	0.404
Leptin(ln)	-0.171	0.158	-0.212
UD forearm			
Age	0.024	0.863	0.026
F M	0.047	0.728	0.053
L M	0.261	0.075	0.265
Leptin(ln)	-0.337	0.018	-0.347

* p< 0.05 ** p< 0.01

women and reported that reduced lean mass was a useful mark which predicted the risk of reduced BMD and its cause to spinal fractures. Similar results were reported in premenopausal Turkish women (Sahin *et al* 2003) women from Estonia (Jurimae *et al* 2005) and from Sweden (Lorentzon *et al* 2006)

However, controversial findings were reported regarding the association between fat mass and BMD. Yamauchi *et al.* 2001,found no correlation between percentage of fat and BMD. Blum *et al.* 2003, and Hsu *et al.* 2006, suggested that higher proportion of fat appear to be negatively associated with BMD in their studies on premenopausal women from Boston and premenopausal and post menopausal Chinese women respectively .

No association was found between body composition component and BMD values in normal weight women in the present study. This may be due to decrease of the mechanical load effect on BMD as compared to overweight women who had higher values of body composition components. Several studies reported that increase lean mass indicate a higher level of habitual physical activity and is associated with higher bone strength as a consequence of greater forces acting on the bone (Inomoto *et al* 2008 and Hingorjo *et al* 2008) These conflicting results in the literature regarding the association of body composition with BMD may be due to difference between studies in skeletal regions measured, bone mass parameters or body composition parameters used in analysis and / or age and menopausal status of the subject studied . Alternatively, body composition may affect BMD differently according to race (Nakaoka *et al* 2000)

Regarding serum leptin this study revealed that while serum (ln)leptin was significantly increased in overweight women compared to normal weight women, there was insignificant association between serum leptin and BMD at most sites of the body. There was an independent negative association with BMD at UD-forearm site in overweight women and at L2, L3 and L2-L4 sites among normal weight women therefore, leptin is not a major regulator of BMD.

Table 3: Multiple regression analysis of BMD (g/cm³) as the dependent variable for the normal BMI premenopausal women.

Variable	Coefficientβ	P Value	Partial r ²
Femoral Neck			
Age	-0.614	0.002**	-0.578
F M	0.208	0.223	0.243
L M	0.128	0.444	0.153
Leptin(ln)	-0.198	0.226	-0.241
Trochanter			
Age	-0.575	0.004**	-0.539
F M	0.308	0.085	0.337
L M	0.139	0.421	0.161
Leptin(ln)	-0.166	0.323	-0.198
Ward's Triangle			
Age	-0.724	0.000**	-0.673
F M	0.244	0.121	0.305
L M	0.092	0.545	0.122
Leptin(ln)	-0.133	0.372	-0.179
L2			
Age	-0.444	0.015*	-0.462
F M	0.230	0.172	0.271
L M	0.096	0.557	0.118
Leptin(ln)	-0.426	0.012	-0.478
L3			
Age	-0.452	0.020	-0.447
F M	0.167	0.345	0.189
L M	0.167	0.339	0.191
Leptin(ln)	-0.167	0.054	-0.375
L4			
Age	-0.455	0.024	-0.433
F M	0.162	0.380	0.176
L M	0.252	0.174	0.270
Leptin(ln)	-0.217	0.224	-0.242
L2- L4			
Age	-0.463	0.016	-0.459
F M	0.192	0.274	0.218
L M	0.179	0.302	0.206
Leptin(ln)	-0.334	0.053	-0.376
UD forearm			
Age	-0.168	0.418	-0.150
F M	-0.128	0.518	-0.120
L M	-0.233	0.239	-0.220
Leptin(ln)	-0.105	0.580	-0.102

* p < 0.05 ** p < 0.01

The exact role of leptin on bone mass remains controversial and unclear. Yamauchi *et al.* 2001 concluded that serum leptin levels are increased in obesity and directly related to fat mass in Japanese postmenopausal women. In contrast, Goulding *et al.* 1998 speculated that leptin played insignificant role in the regulation of bone cell activity. Moreover, his results didn't support the hypothesis that leptin mediates the bone-sparing effects of obesity. Ducy *et al.* 2000, reported that leptin was a potent inhibitor of bone formation acting through the central nervous system. Blum *et al.* 2003, found that for premenopausal women in general, for any given weight, higher fat % or a higher serum leptin appears to be associated with an increased risk of low bone mass. Chanprasertyothin *et al.* 2005, concluded that circulating leptin is negatively associated with BMD in men and premenopausal not in postmenopausal women from Thailand. They cited that these associations strengthen the notion that leptin may inhibit bone formation during the accumulation of bone mass early in life. Kontogianni *et al.* 2004, concluded that leptin doesn't seem to contribute as fat mediator to increased BMD in perimenopausal women. Moreover, the negative correlation between leptin and BMD may be attributed to a more complicated circuit of events between hormones, sympathetic system, and fat distribution during this period of a woman's life rather than to only fat content. Martini *et al.* 2001, concluded that serum leptin had no direct effect on bone formation. Iwamoto *et al.* 2000, reported that leptin is not a key regulator of bone metabolism, although it may have some effects on bone metabolic markers and BMD regionally in premenopausal Japanese women. At a local level, bone marrow adipocytes produce leptin, which may enhance osteogenic activity and inhibit adipogenic activity. Thus it is possible that local production of leptin may play a partial role in bone metabolism.

Conclusion:

The study concluded that lean mass is a better predictor than fat mass of BMD in overweight Egyptian women and that serum leptin levels are not associated with BMD. However, leptin may have some effects on BMD regionally.

Recommendations:

Due to the controversy found in the different studies, it is recommended to undergo a large scale National wide study to include the different stages in the life of Egyptian women.

REFERENCES

- Abou Samara, R., Hwalla N. Baba, N. Torbay, L. Dib, G.E. Fuleihan, 2005. High plasma leptin is not associated with higher bone mineral density in insulin resistant premenopausal obese women. *J Clin Endocrinol Metab.*, 90: 2588-2594.
- Aloia, J.F., A. Vaswani, E. Flaster, 1995. To what extent is bone mass determined by fat-free or fat mass? *Am J Clin Nutr.*, 61: 1110-1114.
- Barret-Connor, E., D. Kritiz-Silverstein, 1996. Does hyperinsulinemia preserve bone? *Diabetes Care*, 19: 1388-1392.
- Bedogni, G., A. Pietrobelli, S.B. Heynsfield, 2000. Influence of body composition on bone content in elderly women. *Ann NY Acad Sci.*, 904: 489-490.
- Blain, H., A. Vuillemin, F. Guillemin, R. Durant, B. Hanesse, N. de Talance, B. Doucet, C. Jeandel, 2002. Serum leptin level is a predictor of bone mineral density in postmenopausal women. *J Clin Endocrinol Metab.*, 87: 1030-1035.
- Blum, M., S.S. Harris, A. Must, E.N. Naumova, S.M. Phillips, W.M. Rand, B. Dawson- Hughes, 2003. Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int.*, 73: 27-32.
- Chanprasertyothin, S., N. Piaseu, L. Chailurkit, R. Rajatanavin, B. Ongphiphadhanakul, 2005. Association of circulating leptin with bone mineral density in males and females. *J Med Assoc Thi.*, 88: 655-659.
- Cosidine, R.V., M.K. Sinha, M.L. Heim, A. Kriauciunas, T.W. Stephens, M.R. Nyce, J.P. Ohannesian, C.C. Marco, L.J. Mckee, T.L. Bauer, J.F. Caro, 1996. serum immunoreactive-leptin concentrations in normal-weight and obese humans. *NEngel J Med.*, 334: 292-295.
- Cummings, S.R., W.S. Browner, D. Bauer, K. Stone, K. Enstrud, S. Jamal, B. Ettinger, 1998. Endogenous hormones and the risk of hip and vertebral fractures among older women. *N Engl J Med.*, 339: 733-738.
- Dennison, E.M., H.E. Syddall, C.H. Fall, M.K. Javaid, N.K. Arden, D.I. Phillips, C. Cooper, 2004. Plasma leptin concentration and change in bone density among elderly men and women: the Hertfordshire Cohort Study. *Calcif Tissue Int.*, 74: 401-406.
- Ducy P., M. Amling, S. Takeda, M. Priemel, A.F. Schilling, F.T. Beil, J. Shen, C. Vinson, J.M. Rueger, G. Karsenty, 2000. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell*, 100: 197-207.
- Ettinger, B., A. Pressman, P. Sklarin, D.C. Bauer, J.A. Cauley, S.R. Cummings, 1998. Associations between low levels of serum estradiol, bone density, and fractures among elderly women: the study of osteoporosis fractures. *J Clin Endocrinol Metab.*, 83: 2239-2243.
- Fox, K.M., J. Magaziner, W.G. Hawken, 2000. Loss of bone density and lean body mass after hip fracture. *Osteoporosis Int.*, 11: 31-35.
- Goulding, A., 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.
- Haffner S.M., R.L. Bauer, 1992. Excess androgenicity only partially explains the relationship between obesity and bone density in premenopausal women. *Int J Obes Relat Metab Disord.*, 16: 869-874.
- Hiernaux, J. and J.M. Tanner, 1969. Growth and physical studies. In *Human Biology: A Guide to Field Methods*. Eds. Weiner JS, Lourie SA, IBP London, Blackwell Scientific Publications Oxford UK.
- Hingorjo, M.R., S. Syed, M.A. Qureshi, 2008. Role of exercise in osteoporosis prevention current concepts. *J Pak Med Assoc.*, 58: 78-81.
- Hsu, Y., S.A. Venners, H.A. Terwedow, Y. Feng, T. Niu, Z. Li, N. Laird, J.D. Brain, S.R. Cummings, M.L. Boussein, C.J. Rosen, X. Xu, 2006. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. *Am J Clin Nutr.*, 83: 146-154.

- Inomoto, T., 2008. Physical activity / sports and bone mineral density *Clin Calcium.*, 18(9): 1339-48.
- Iwamoto, I., T. Douchi, S. Kosha, M. Murakami, T. Fujino, Y. Nagata, 2000. Relationships between serum leptin level and regional bone mineral density, bone metabolic markers in healthy women. *Acta Obstet Gynecol Scand.*, 79: 1060-1064.
- Jurimae, T., T. Soot, J. Jurimae, 2005. Relationships of anthropometrical parameters and body composition with bone mineral content or density in young women with different levels of physical activity. *J Physiol Anthropol Appl Human Sci.*, 24: 579-587.
- Kontogianni, M.D., U.G. Dafni, J.G. Routsias, 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.
- Liel, Y., E. Ulmer, J. Shary, B.W. Hollis, N.H. Bell, 1988. Low circulating vitamin D in obesity. *Calcif Tissue Int.*, 43: 199-201.
- Lorentzon, M., K. Landin, D. Mellström, C. Ohlsson, 2006. Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. *J Bone Miner Res.*, 21(12): 1871-8.
- Martini, G., R. Valenti, S. Giovani, B. Franci, S. Campagna, R. Nuti, 2001. Influence of insulin-like growth factor-1 and leptin on bone mass in healthy postmenopausal women. *Bone*, 28: 113-117.
- Mizuma, N., M. Mizuma, M. Yoshinaga, I. Iwamoto, T. Matsuo, T. Douchi, M. Osame, 2006. Difference in the relative contribution of lean and fat mass components to bone mineral density with generation. *J Obstet Gynaecol Res.*, 32(2): 184-9.
- Nakaoka, D., T. Sugimoto, H. Kaji, 2000. Determinants of bone mineral density and spinal fracture risk in postmenopausal Japanese women. *Osteoporosis Int.*, 12: 548-554.
- Ngyuen, T.V., P.N. Sambrook, J.A. Eisman, 1998. Bone loss, physical activity, and weight change in elderly women: the Dubbo Osteoporosis Epidemiology Study. *J Bone Miner Res.*, 13: 1458-1467.
- Odabasi, E., M. Ozata, M. Turan, N. Bingol, A. Yonem, B. Cakir, M. Kutlu, I.C. Ozdemir, 2000. Plasma leptin concentrations in postmenopausal women with osteoporosis. *European J Endocrinol.*, 142: 170-173.
- Pasco, J.A., M.J. Henry, G.R. Collier, M.J. Ball, A.M. Ugoni, G.C. Nicholson, 2001. Serum leptin levels are associated with bone mass in non obese women. *J Clin Endocrinol Metab.*, 86: 1884-1887.
- Reid, I.R., R. Ames, M.C. Evans, S. Sharpe, G. Gamble, J.T. France, T.M. Lim, T.F. Cundy, 1992. Determinants of total body and regional bone mineral density in normal postmenopausal women—a key role for fat mass. *J Clin Endocrinol Metab.*, 75: 45-51.
- Reid, I.R., M.L. Evans, G.J. Cooper, R.W. Ames, J. Stapleton, 1993. Circulating insulin levels are related to bone density in normal postmenopausal women. *Am J Physiol.*, 265: E655-659.
- Roux, C., A. Arabi, R. Porcher, P. Garnero, 2003. Serum leptin as a determinant of bone reorption in healthy postmenopausal women. *Bone*, 33: 847-852.
- Ruhl, C.E., J.E. Everhart, 2002. Relationship of serum leptin concentration with bone mineral density in the United States population. *J Bone Miner Res.*, 17: 1896-1903.
- Sahin, G., G. Polat, S. Baois, A. Milcan, O. Baodatoolu, C. Erdoan, H. Camdeviren, 2003. Body composition, bone mineral density, and circulating leptin levels in postmenopausal Turkish women. *Rheumatol Int.*, 23: 87-91.
- Sato, M., N. Takeda, H. Sarui, R. Takami, K. Takami, M. Hayashi, A. Sasaki, S. Kawachi, K. Yoshino, K. Yasuda, 2001. Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnover in adult men. *J Clin Endocrinol Metab.*, 86: 5273-5276.
- Scariano, J.K., P.J. Garry, G.D. Montoya, A.K. Chandani, J.M. Wilson, R.N. Baumgartner, 2003. Serum leptin levels, bone mineral density and osteoblast alkaline phosphatase activity in elderly men and women. *Mech Ageing Dev.*, 124: 281-286.
- Schett, G., S. Kiechl, E. Bonora, K. Redlich, W. Woloszczuk, F. Oberhollenzer, J. Jocher, R. Dprizzi, M. Muggeo, J. Smolen, J. Willieit, 2004. Serum leptin level and risk of nontraumatic fracture. *Am J Med.*, 117: 952-956.
- Takada, H., K. Washino, H. Iwata, 1997. Risk factors for low bone mineral density among females: the effect of lean body mass. *Prev Med.*, 26: 633-638.
- Tartaglia, L.A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Davos, G.J. Richards, L.A. Campfield, F.T. Clark, J. Deeds, C. Muir, S. Sanker, A. Moriarty, K.J. Moore, J.S. Smutko, G.G. Mays, E.A. Wool, C.A. Monroe, R.I. Tepper, 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell*, 83: 1263-1271.
- Thomas, T., B. Burguera, L.J. Melton, E.J. Atkinson, W.M. O'Fallon, B.L. Riggs, S. Khosla, 2001. Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. *Bone*, 29: 114-120.

Thomas, T., B. Burguera, L.J. Melton, E.J. Atkinson, W.M. O'Fallon, B.L. Riggs, S. Khosla, 2000. Relationship of serum leptin levels with body composition and sex steroid and insulin levels in men and women. *Metabolism*, 49: 1278-1284.

Thomas, T., 2004. Leptin and fragility fracture: Evidence for a protective effect in humans. *Am J Med.*, 117: 966-968.

Van Langendonk, L., A.L. Classens, J. Lefevre, M. Thomis, R. Philippaerts, K. Delvaux, R. Lysens, B. Vanden Eynde, G. Beunen, 2002. Association between bone mineral density (DXA), body structure, and body composition in middle- aged men. *Am J Hum Biol.*, 14: 735-742.

Weiss, L.A., E. Barrett-Connor, D. von Mühlen, P. Clark, 2006. Leptin predicts BMD and bone resorption in older women but not older men: the Rancho Bernardo study. *J Bone Miner Res.*, 21(5): 758-64.

Yamauchi, M., T. Sugimoto, T. Yamaguchi, D. Nakaoka, M. Kanzawa, S. Yano, R. Ozuru, T. Sugishita, K. Chihara, 2001. Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women. *Clin Endocrinol (Oxf)*, 55: 341-347.