Association of Circulating Leptin with Bone Mineral Density in Males and Females

Suwannee Chanprasertyothin, MSc*,
Noppawan Piaseu, PhD**, Laor Chailurkit, PhD*
Rajata Rajatanavin, MD*, Boonsong Ongphiphadhanakul, MD**

** Department of Research Center, Ramathibodi Hospital, Mahidol Unversity*
*** Department of Medicine, Ramathibodi Hospital, Mahidol Unversity*

Leptin, a hormone produced by fat cells, possesses several physiological functions not limited to energy balance. Recently, leptin has been shown to inhibit bone formation through its action at the hypothalamus. In the present study, the authors explored the asssocation of leptin with bone mineral density (BMD) in men and women according to menopausal status. Subjects consisted of 75 men, 51 premenopausal women and 63 postmenopausal women aged 20-80 years. All were healthy and did not take medication which may affect bone metabolism. BMD was measured at L2-4 and femoral neck by DEXA. Serum leptin concentrations were measured by radioimmunoassay.

Serum leptin in males was independently related to BMD at L2-4 ($r = -0.36$, $p < 0.05$) and the femoral neck ($r = -0.32$, $p < 0.05$) in a multiple linear regression model with age, body mass index (BMI), serum free testosterone, estradiol and leptin as independent variables. In premenopausal women, serum leptin correlated negatively to L2-4 ($r = -0.29$, $p < 0.01$) and femoral neck BMD ($r = -0.29$, $p < 0.05$) independently of age and BMI. However, in postmenopausal women, no association of leptin with BMD was found after controlling for age and BMI. The authors concluded that circulating leptin is negatively associated with BMD in men and premenopausal women, but not in postmenopausal women. The negative associations found in both premenopausal women and men in the present study strengthen the notion that leptin may inhibit bone formation during the accumulation of bone mass early in life

Key words: *Leptin,bone mass,osteoporosis*

J Med Assoc Thai 2005; 88(5): 655-9
Full text. e-Journal: *http://www.medassocthai.org/journal*

Bone mass is under the control of numerous factors. Most notably is the effect of sex steroids on the modulation of bone mass throug their effects on bone turnover. Leptin, a hormone tproduced by adipose tissue, has recently been unexpectedly shown to affect bone metabolism. Mice lacking leptin or leptin receptors have high bone mass despite hypogonadism, a well-established factors adversely influencing bone mass[1]. Furthermore, infusion of leptin into the third ventricles of ob/ob mice decreased bone mass and it has been suggested that leptin inhibits bone formation primarily through its effect on the hypothalamus and an unknown secondary peripheral pathway[1]. The effect of leptin on bone metabolism in humans is, however, not clearly defined. It has been reported that mice and men with leptin deficiency have different skeletal presentation[2]. Most of the studies in humans found positive rather than negative association between serum leptin concentrations and bone mineral density (BMD)[3-5] although not without dispute[6-9]. It was, therefore, the purpose of the present study to explore the role of leptin in the determination of bone mass in both men and women by investigating the association of leptin with bone mineral density (BMD) in both genders.

Correspondence to : Chanprasertyothin S, Department of Medicine Ramathibodi Hospital Rama 6 Rd, Rajthevi Bangkok 10400, Thailand E-mail: rascy@mahidol.ac.th

Material and Method

Seventy five men, 51 premenopausal women and 63 postmenopausal women aged between 20 and

Table 1. Clinical characteristics of subjects in the study

	Premenopausal women (n = 51)	Postmenopausal women (n = 74)	Men (n = 63)
Age (year) ± SD*	36.7±8.6	59.9±7.9	52.0±15.8
BMI (kg/m^2)	20.5±2.0	23.2±2.6	23.6±2.9
L2-4 BMD (g/cm^2)	1.11±0.11	0.95±0.15	1.10±0.17
Femoral neck BMD (g/cm^2)	0.82±0.12	0.73±0.10	0.86±0.14
FT (pmol/l)	-	-	85.7±26.7
E_2 (pmol/l)	-	-	149.0±43.7
Leptin (ng/ml)	8.0±3.1	10.1±3.6	4.1±2.0

*SD = Standard duiation

80 years living in Bangkok Metropolitan area in Thailand were recruited by flyers and direct contact. All were healthy and ambulatory. None of the subjects were smokers or consumed a significant amount of alcohol. Medical history-taking and complete physical examination were performed on the volunteers to assess their health status.

BMD was measured by dual-energy X-ray absorptiometry (DEXA) (Lunar DPX-L, Lunar Corp., USA). Daily calibration and quality control were done regularly according to the manufacturer's recommendation. The in vitro precision using the spine phantom provided by the manufacturer was 0.6 %. In vivo coefficients of variation for anteroposterior spine, and femoral neck measurements was 2.0% and 2.2%, respectively. BMD at anteroposterior L2-L4 and femoral neck were measured in each subject.

Fasting blood samples were obtained from subjects between 8.00 and 10.00 am. Samples were allowed to clot and after centrifugation serum was frozen at -20 C until measurement. Radioimmunoassay kits were used to determine serum free testosterone (FT) (Diagnostic Product Corp, U.S.A.), estradiol (E_2) (CIS Bio International, France) and leptin (Linco, USA).

Data were expressed as mean ± SD. The correlations among variables were determine by linear regression analyses. Stepwise multiple regression was used to determine the relative importance of various independent variables on BMD.

Results

Table 1 demonstrates the characteristic of the subjects in the present study.

By univariate analyses, it was found that there were statistically significant positive correlations between body mass index (BMI) and serum leptin concentrations in premenopausal women (Table 2A), postmenopausal women (Table 2B) and men (Table 2C). In contrast, BMD was not related to serum leptin in any group of subjects.

Table 2A. Correlation coefficients among serum leptin, L2-4 BMD, femoral neck BMD and BMI in premenopausal women

	Leptin	L2-4 BMD	Femoral neck BMD	BMI
Leptin				
L2-4 BMD	-0.23			
Femoral neck BMD	-0.21	0.63***		
BMI	0.30*	0.22	0.23	

Table 2B. Correlation coefficients among serum leptin, L2-4 BMD, femoral neck BMD and BMI in postmenopausal women

	Leptin	L2-4 BMD	Femoral neck BMD	BMI
Leptin				
L2-4 BMD	0.10			
Femoral neck BMD	0.06	0.63***		
BMI	0.49***	0.38**	0.29*	

Table 2C. Correlation coefficients among serum leptin, L2-4 BMD, femoral neck BMD and BMI in men

	Leptin	L2-4 BMD	Femoral neck BMD	BMI
Leptin				
L2-4 BMD	-0.05			
Femoral neck BMD	-0.19	0.63***		
BMI	0.66***	0.20	-0.001	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Since it is well established that adiposity and serum leptin concentrations are related, the relation between BMD and serum leptin may actually be

indirect through the association of serum leptin and adiposity. To evaluate the independent effect of serum leptin on BMD after controlling for relevant confounders, multivariate analyses were performed in each group of subjects. As shown in Table 3, serum leptin in males was independently related to BMD at L2-4 and the femoral neck in a multiple linear regression model with age, BMI, serum FT, E_2 and leptin as independent variables. Other variables independently related to BMD included age, BMD, serum E_2 at both skeletal sites and serum FT only at L2-4 which just achieved statistical significance. Similarly, in premenopausal women, serum leptin correlated negatively to L2-4 and femoral neck BMD independently of the effects of age and BMI (Table 4). However, in postmenopausal women, only age and BMI were significantly associated with BMD at L2-4 and the femoral neck. No association of serum leptin with BMD was found after controlling for age and BMI at either skeletal sites.

Discussion

It is well known that body weight is positively associated with BMD in both women and men[10]. The underlying mechanism for the relationship between body weight and bone mass is unclear but may be related to the adaptation of connecting osteocytes to mechanical load[11,12]. It is also possible that leptin secreting from adipocytes may directly influence bone mass. In that case, leptin receptor should be present in bone cells and a positive correlation between bone mass and serum leptin levels should be demonstrated. During early development in mice, leptin binding have been localized in a number of tissues including bone[13-16] although the presence of leptin receptors in bone cells still remains to be demonstrated. However, the findings in the present study do not support the direct action of leptin on bone as the basis of the positive relation between body weight and bone mass. In contrast, the negative association betweeen circulating leptin and BMD suggests an inhibitory role, either directly or indirectly, of leptin on bone mas

The present findings are in keeping with the recently described indirect role of leptin in the modulation of bone mass through its effect at the pituitary gland[1]. Animals which lack leptin or leptin receptor have a massive increase in bone mass despite the coexisting hypogonidism. The high bone mass is not due to obesity since it is present in young ob/ob mice before obesity develops. Moreover, leptin also causes a massive decrease in bone mass when infused into the third ventricle of ob/ob mice[1]. A number of previous reports have demonstrated the association between bone mass and serum leptin. Leptin has a positive relationship with BMD in women[3-5]. Women with fractures also had lower serum leptin[17,18]. In contrast to the aforementioned studies, the present study demonstrated a negative correlation between bone mass and leptin levels in premenopausal but not postmenopausal women which is in keeping with the evidence of the inhibitory effect of leptin on bone formation. However, although the inhibitory effect of leptin on bone formation has been suggested, leptin also stimulates the proliferation of human osteoblasts[19]. Moreover, leptin receptors are present in bone marrow stromal cells and mediates the differentiation of the stromal cells into osteoblasts[20]. Recently, leptin has also been demonstated to inhibit osteoclast generation[21]. Therefore, it appears that the skeletal effects of leptin are complex and both

Table 3. Muttivariate analyses of the relationship between leptin and BMD after controlling for the effect of other confounders in men

	L2-4 BMD		Femoral neck BMD	
	Beta	P	Beta	P
Age	-0.35	<0.05	-0.62	<0.001
BMI	0.48	<0.01	0.36	<0.01
Free T	-0.32	0.05	-0.21	NS
E2	0.39	<0.01	0.36	<0.01
Leptin	-0.36	<0.05	-0.32	<0.05

Table 4. Multivariate analyses of the relationship between leptin and BMD after controlling for the effect of other confounders in pre- and postmenopausal women

	Premenopausal				Postmenopausal			
	L2-4 BMD		Femoral neck BMD		L2-4 BMD		Femoral neck BMD	
	Beta	P	Beta	P	Beta	P	Beta	P
Age	-0.36	<0.01	-0.36	<0.05	-0.39	<0.001	-0.50	<0.001
BMI	0.43	<0.01	0.43	<0.01	0.40	<0.01	0.29	<0.05
Leptin	-0.29	<0.01	-0.29	<0.05	-0.07	NS	-0.05	NS

inhibitory and stimulatory effects can be discerned depending on the net effect on bone formation and resorption.

In addition to the negative association found in premenopausal women, a negative association was also found between circulating leptin and bone mass in men independently of the effect of FT and E2 in the present study. The finding is in keeping with a study in Japanese males which found that leptin has negative association with BMD[6]. However, Thomas et al, demonstrated that serum leptin is positively associated with BMD independent of lean body mass in women but not in men[22] while another study in the US population found that circulating leptin is negatively associated with bone mass in men but not in pre- or postmenopausal women[23]. It is of note that most studies were performed in Caucasians with different results from the present and the other study in an Asian population. Given the conflicting results regarding the association between circulating leptin and bone mass in populations with different gender, age and ethnic backgrounds, it is therefore possible the effect of leptin is likely to be pleotropic depending on both genetic and environmental backgrounds. Nevertheless, the negative associations found in both premenopausal women and men in the present study strengthen the notion that leptin may inhibit bone formation during the accumulation of bone mass early in life.

References

1. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, et al. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 2000; 100: 197-207.
2. Ozata M. Different presentation of bone mass in mice and humans with congenital leptin deficiency. J Clin Endocrinol Metab 2002; 87: 951.
3. Pasco JA, Henry MJ, Kotowicz MA, Collier GR, Ball MJ, Ugoni AM, et al. Serum leptin levels are associated with bone mass in nonobese women. J Clin Endocrinol Metab 2001; 86: 1884-7.
4. Blain H, Vuillemin A, Guillemin F, Durant R, Hanesse B, de Talance N, et al. Serum leptin level is a predictor of bone mineral density in post-menopausal women. J Clin Endocrinol Metab 2002; 87: 1030-5.
5. Zoico E, Zamboni M, Adami S, Vettor R, Mazzali G, Tosoni P, et al. Relationship between leptin and bone mineral density in the elderly. Clin Endocrinol (Oxf) 2003; 59: 97-103.
6. Sato M, Takeda N, Sarui H, Takami R, Takami K, Hayashi M, et al. Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnove in adult men. J Clin Endocrinol Metab 2001; 86: 5273-6.
7. Huang KC,Cheng WC,Yen RF, Tsai KS, Tai TY, Yang WS. Lack of independent relationship between plasma adiponectin,leptin and bone density in nondiabetic female adolescents. Clin Endocrinol (Oxf) 2004; 61: 204-8.
8. Wosje KS, Binkley TL, Kalkwarf HJ, Specker BL. Relationships between bone mass and circulating leptin concentrations in Hutterites. Bone 2004; 34: 1017-22.
9. Dennison EM, Syddall HE, Fall CH, Javaid MK, Arden NK, Phillips DI, et al. Plasma leptin concentration and change in bone density among elderly men and women: the Hertfordshire Cohort Study. Calcif Tissue Int 2004; 74: 401-6.
10. Felson DT, Zhang Y, Hannan MT, Anderson JJ. Effects of weight and body mass index on bone mineral density in men and women: The Framingham Study. J Bone Miner Res 1993; 8: 567-73.
11. Noble BS, Reeve J. Osteocyte function, osteocyte death and bone fracture resistance. Mol Cell Endocrinol 2000; 159: 7-13.
12. Mikuni-Takagaki Y. Mechanical responses and signal transduction pathways in stretched osteocytes. J Bone Miner Metab 1999; 17: 57-60.
13. Chen SC, Cunningham JJ, Smeyne RJ. Expression of OB receptor splice variants during prenatal development of the mouse. J Recept Signal Transduct Res 2000; 20: 87-103.
14. Camand O, Turban S, Abitbol M, Guerre-Millo M. Embryonic expression of the leptin receptor gene in mesoderm-derived tissues. C R Biol 2002; 325: 77-87.
15. Dal Farra C, Zsurger N, Vincent JP, Cupo A. Binding of a pure 125I-monoiodoleptin analog to mouse tissues: a developmental study. Peptides 2000; 21: 577-87.
16. Morroni M, De Matteis R, Palumbo C, Ferretti M, Villa I, Rubinacci A, et al. In vivo letin expression in cartilage and bone cells of growing rats and adult humans. J Anat 2004; 205: 291-6.
17. Yamauchi M, Sugimoto T, Yamaguchi T, Nakaoka D, Kanzawa M, Yano S, et al. Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women. Clin Endocrinol (Oxf) 2001; 55: 341-7.
18. Di Monnaco M, Vallero F, Di Monaco R, Mautino F, Cavanna A. Fat body mass, leptin and femur bone mineral density in hip-fractured women. J Endocrinol Invest 2003; 26: 1180-5.
19. Gordeladze JO, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. J Cell Biochem 2002; 85: 825-36.
20. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells

to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 1999; 140: 1630-8.
21. Holloway WR, Collier FM, Aitken CJ, Myers DE, Hodge JM, Malakellis M, et al. Leptin inhibits osteoclast generation. J Bone Miner Res 2002; 17: 200-9.
22. Thomas T, Burguera B, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Riggs BL, et al. 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 2001; 29: 114-20.
23. Ruhl CE, Everhart JE. Relationship of serum leptin concentration with bone mineral density in the United States population. J Bone Miner Res 2002; 17: 1896-903.

ความสัมพันธ์ระหว่างระดับเล็ปตินในเลือดกับความหนาแน่นกระดูกในชายและหญิง

สุวรรณี ชั้นประเสริฐโยธิน, นพวรรณ เปียซื่อ, ละออ ชัยลือกิจ, รัชตะ รัชตะนาวิน, บุญส่ง องค์พิพัฒนกุล

เล็ปตินเป็นฮอร์โมนที่ผลิตโดยเซลล์ไขมันซึ่งควบคุมการทำงานหลายอย่างในร่างกายรวมทั้งความสมดุลของพลังงาน เล็ปตินสามารถยับยั้งการสร้างกระดูก โดยผ่านทางไฮโปธาลามัส ในการศึกษาครั้งนี้ เพื่อดูความสัมพันธ์ของเล็ปตินกับความหนาแน่นของกระดูกในผู้ชาย และหญิงแยกตามภาวะการหมดประจำเดือน โดยประกอบด้วย ผู้ชาย 75 คน ผู้หญิงวัยก่อนหมดประจำเดือน 51 คน และผู้หญิงวัยหมดประจำเดือน 63 คน อายุระหว่าง 20-80 ปี ทั้งหมดมีสุขภาพสมบูรณ์ และไม่ได้รับยาใด ๆ ที่มีผลต่อกระดูก ความหนาแน่นของกระดูกวัดโดย dual-energy X-ray absorbtiometry (DEXA) ระดับเล็ปตินในเลือดวัดโดยวิธี radioimmunoassay

ผลการศึกษาพบว่าระดับเล็ปตินในผู้ชายมีความสัมพันธ์เชิงลบกับความหนาแน่นของกระดูกที่ L2-4 ($r = -0.36$, $p < 0.05$) และที่ femoral neck ($r = -0.32$, $p < 0.05$) ในผู้หญิงวัยก่อนหมดประจำเดือนระดับเล็ปตินมีความสัมพันธ์เชิงลบกับ L2-4 ($r = -0.29$, $p < 0.01$) และที่ femoral neck ($r = -0.29$, $p < 0.05$) ส่วนในผู้หญิงวัยหมดประจำเดือนไม่พบความสัมพันธ์นี้

จากผลการศึกษานี้สรุปได้ว่าความสัมพันธ์เชิงลบทั้งที่พบในผู้ชายและผู้หญิงวัยก่อนหมดประจำเดือน แต่ไม่พบในหญิงวัยหมดประจำเดือนช่วยเพิ่มน้ำหนักการค้นพบที่ว่าเล็ปตินอาจจะยับยั้งการสร้างกระดูกในช่วงที่มีการสะสมกระดูกระยะแรกของชีวิต