ORIGINAL PAPER
ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

The association of serum leptin with biochemical parameters of bone turnover in maintenance hemodialysis patients

OBJECTIVE Leptin is a small peptide hormone that is mainly, but not exclusively, produced in adipose tissue and cleared principally by the kidney. Serum leptin concentrations and bone mass are directly related. This cross-sectional study was conducted on patients with end-stage renal disease (ESRD), who were undergoing maintenance hemodialysis (HD). **METHOD** Serum calcium, phosphorus, predialysis serum creatinine, blood urea nitrogen, alkaline phosphatase (ALP), intact serum PTH (iPTH) and serum leptin were measured in 36 dialysis patients, 10 of whom were diabetic. **RESULTS** A significant difference in serum leptin between male and female diabetic patients was seen with higher values in females. In all patients a significant positive correlation of the logarithm of serum leptin with the logarithm of serum iPTH and a significant positive correlation of serum leptin with body mass index (BMI) were found. In male HD patients a near significant and inverse correlation of serum ALP with serum leptin was seen. In female HD patients a near significant inverse correlation of serum leptin with serum phosphorus and a significant inverse correlation of serum leptin with Ca×P products were found. **CONCLUSIONS** In HD patients serum leptin affects bone activity.

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ΑΡΧΕΙΑ ΕΛΛΗΝΙΚΗΣ ΙΑΤΡΙΚΗΣ 2006, 23(5):501–506

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Συσχέτιση της λεπτίνης του ορού με βιοχημικές παραμέτρους του μεταβολισμού των οστών σε ασθενείς υπό χρονία αιμοκάθαρση

Περίληψη στο τέλος του άρθρου

Key words

Alkaline phosphatase
Bone turnover
Hemodialysis
PTH
Serum leptin

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The adipose tissue cytokine leptin is a small peptide hormone that is mainly but not exclusively, produced in adipose tissue.[1] Leptin exerts several important metabolic effects on peripheral tissue, including modification of insulin action, induction of angiogenesis, and modulation of the immune system.[1,2] Leptin reaches the brain by a saturable transport mechanism, and via direct effects on the hypothalamus it decreases appetite and increases metabolism.[3] Serum leptin concentrations in normal humans have been reported to correlate with the body mass index (BMI) as well as with the body fat mass.[4] The hormone leptin is considered to play a role in the prevention of osteoporosis and it probably acts on bone tissue through inhibition of osteoclasia.[1,5] Several recent studies have demonstrated that leptin is cleared principally by the kidney. Thus serum leptin concentrations are increased in patients with chronic renal failure and those undergoing maintenance dialysis,[2] and it has been speculated that hyperleptinemia may contribute to uremic anorexia and malnutrition.[3] The recent discovery that leptin is a bone mass determinant[1,5–7] is an important new facet of the physiological repertoire of this protein. Human studies have shown that serum leptin concentrations and bone mass are directly associated.[1,5–7] Evidence for a stimulatory role of leptin on bone mineralization in humans includes direct relationships between serum leptin concentrations and bone mineral mass and BMD in lean women[6] and lean girls.[7] In this context, it is particularly intriguing that altered plasma leptin concentration has been reported in diseases which are typically associated with osteopenia, such as liver cirrhosis[8] and type 2 diabetes.[9] Studies on the relationship of serum leptin and biochemical markers of bone turnover in hemodialysis (HD) patients are scarce, and the association between serum leptin levels and bone activity in patients with chronic renal failure and on HD have not been widely investigated. Preliminary results in one study revealed that high plasma leptin in male pa-

tients on chronic HD therapy is associated with biochemical evidence of reduced bone turnover,[1] but contradictory data also have been reported. This cross-sectional study aim to examine relationships between serum leptin levels and certain biochemical parameters of bone turnover in maintenance HD patients.

MATERIAL AND METHOD

This cross-sectional study was carried out on 36 patients, 14 females and 22 males, with end-stage renal disease (ESRD), who were undergoing maintenance HD treatment with acetate basis dialysate and polysulfone membrane. According to the severity of secondary hyperparathyroidism (SHPTH), each patient being treated for SHPTH was given oral active vitamin D_3 (Rocaltrol), calcium carbonate and sevelamer (Renagel®) capsules in various doses. After 12-hour fasting, levels of serum calcium (Ca), phosphorus (P), predialysis serum creatinine, pre- and post-dialysis blood urea nitrogen (BUN) and alkaline phosphatase (ALP) were measured using standard kits. Intact serum PTH (iPTH) was measured by the radioimmunoassay (RIA) method using DSL-8000 of USA (normal range of values is 10–65 pg/mL). Serum leptin (normal range, for males 3.84±1.79 and for females 7.36±3.73 ng/mL) was measured by enzyme-linked immunosorbent assay (ELISA) method using DRG of Germany. For the efficacy of HD the urea reduction rate (URR) was calculated from pre- and post-dialysis BUN data.[10] The body mass index (BMI) was calculated using postdialysis weight and height (kg/m^2).[11] Duration and dosages during HD treatment were calculated from the patients' records. The duration of each HD session was 4 hours. For statistical analysis, the data were expressed as the mean±SD. Comparison between the groups was made using Student's t-test. Statistical correlations were assessed using a partial correlation test. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at a P value <0.05.

RESULTS

Of the 36 patients, 26 were non-diabetic (F=10, M=16) and 10 (F=4, M=6) were diabetic HD patients. Tables 1, 2 and 3 show the patients' mean±SD age, the length of time they had been on HD, the dialysis dosage, and the results of laboratory tests of the total, the non-diabetic and the diabetic HD patients. The patients' mean age was 46±18 years. The mean length of the time patients had received HD was 30±36 months (median: 17.5 months). The mean serum leptin was 7±9.2 ng/mL (median: 4.2 ng/mL). The mean serum leptin values in the diabetic and non-diabetic groups were 7.63±4.63 ng/mL (median: 7.8 ng/mL) and 6.85±10.47 ng/mL (median: 3.45 ng/mL), respectively. The mean serum iPTH was 435±454 (median: 309) pg/mL. The mean iPTH values in the diabetic and non-diabetic groups were 218±287 (median: 43) pg/mL and 519±482 (median: 335) pg/mL, respectively. In this study, no significant differences between age, BMI, duration of HD treat-

Table 1. Mean±SD, minimum and maximum of age, duration and dosage of hemodialysis and laboratory results of hemodialyzed patients.

Total patients n=36	Minimum	Maximum	Mean±SD	Median
Leptin (ng/mL)	0.10	51.9	7±9.2	4.2
BMI (kg/m^2)	16	33	21.33±3.98	20.5
Age (years)	16	80	45.7±16.5	43
DH* (months)	2	156	30±36	17.5
Dosages sessions	18	1584	285±396	144
PTH (pg/mL)	16	1980	435±454	309
Ca (mg/dL)	5	10	7.7±0.93	8
P (mg/dL)	3.4	10	6.4±1.8	6.2
ALP (IU/L)	150	5487	633±891	444
CA×P (mg^2/dL^2)	25	80	51±15	50
URR (%)	39	76	59±9.2	57.5
Creatinine (mg/dL)	3	18	9.4±3.6	9.5
BUN (mg/dL)	30	180	82±34	78

*Duration of hemodialysis

Table 2. Mean±SD, minimum and maximum of age, duration and dosage of hemodialysis and laboratory results of non-diabetic hemodialysis patients.

Non diabetic patients n=26	Minimum	Maximum	Mean±SD	Median
Leptin (ng/mL)	0.10	51.9	6.85±10.5	3.45
BMI (kg/m^2)	16	80	43.6±16.5	19
Age (years)	16	33	21±4.5	41.5
DH* (months)	2	156	36.8±40.9	20.5
Dosages sessions	18	1584	348.8±451.3	154
PTH (pg/mL)	22	1980	519±483	335
Ca (mg/dL)	6	9	7.8±0.7	8
P (mg/dL)	3.4	10	6.9±1.8	6.5
ALP (IU/L)	150	5487	749±10	479
CA×P (mg^2/dL^2)	25	80	52.31±16	53
URR (%)	50	76	61±7.9	60
Creatinine (mg/dL)	3	15	9.54±3	10
BUN (mg/dL)	30	180	79±33	74

*Duration of hemodialysis

Table 3. Mean±SD, minimum and maximum of age, duration and dosage of hemodialysis and laboratory results of diabetic hemodialysis patients.

Diabetic patients n=10	Minimum	Maximum	Mean±SD	Median
Leptin (ng/mL)	0.20	15.2	7.6±4.63	7.8
BMI (kg/m^2)	27	75	51±15.9	22.5
Age (years)	20	24	22±1.6	55
DH* (months)	6	24	14±6.3	12
Dosages sessions	54	216	119±55.5	99
PTH (pg/mL)	16	860	218±287	43
Ca (mg/dL)	4	10	6±2	7.5
P (mg/dL)	175	584	330±155	6
ALP (IU/L)	32	70	48±12.8	289
CA×P (mg^2/dL^2)	39	75	53.6±10.36	49
URR (%)	5	10	7.5±1.3	54
Creatinine (mg/dL)	3	18	9.5±4.8	9
BUN (mg/dL)	30	140	90±37	98

*Duration of hemodialysis

ment, dialysis dosage, Ca×P products, URR, serum iPTH, serum leptin, Ca, P, ALP, creatinine or BUN were found between males and females of the total group of patients (P=NS). However, there was a near significant difference of serum iPTH levels between diabetic and non-diabetic HD patients (P=0.075). No significant difference in serum leptin between males and females of the non diabetic group of HD patients was seen, however a significant difference in serum leptin was seen between males and females of the diabetic HD population (r=0.035; fig. 1). In the total group of patients a significant positive correlation of BMI with serum leptin (r=0.45, P=0.008; fig. 2) (adjusted for age) was observed, and a significant positive correlation of the logarithm of serum leptin with the logarithm of serum iPTH (r=0.42, P=0.045) [adjusted for age, duration and dosage of HD, serum Ca, P, URR, gender, presence of diabetes mellitus (DM) and BMI] was found. In male HD patients a significant inverse correlation of dialysis efficacy (as determined by URR) with serum leptin (r=-0.46, P=0.036; fig. 3), and also a near significant inverse correlation of serum ALP and serum leptin (r=-0.39, P=0.079) were seen after adjustment for duration of HD treatment. Moreover in this group a significant positive correlation between age and serum leptin (r=0.44, P=0.046; fig. 4), adjusted for HD dosage, and a significant positive correlation between HD dosage and serum leptin (r=0.44, P=0.046; fig. 5), with adjustment for age, were seen. In this group also a

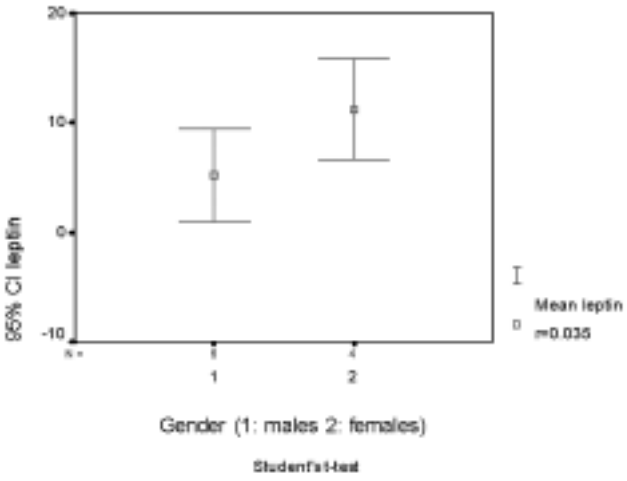

Figure 1. Significant difference in serum leptin between male and female diabetic hemodialysis (HD) patients.

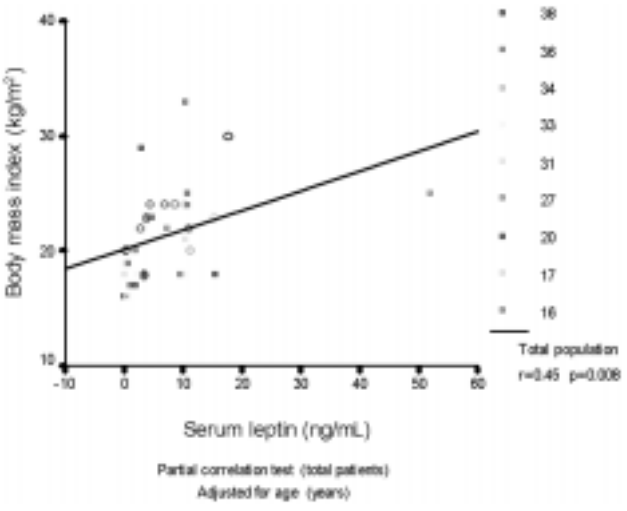

Figure 2. Significant positive correlation of BMI and serum leptin.

near significant positive correlation between duration of HD treatment and serum leptin (r=0.42, P=0.052), after adjustment for age, was seen too. In the female HD patients, a near significant and inverse correlation between serum leptin and P (r=-0.54, P=0.057) was observed, and also a significant inverse correlation between serum leptin and Ca×P products was found (r=-0.62, P=0.025), after adjustment for age.

DISCUSSION

The principal findings of this study were near significant differences in serum iPTH levels between diabetic and non-diabetic HD patients and a significant differ-

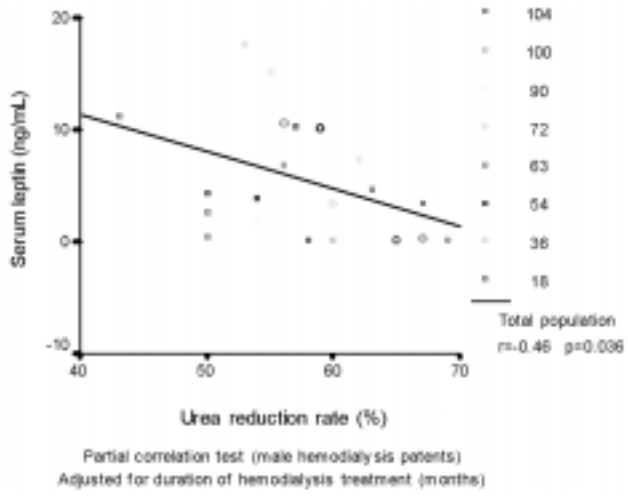

Figure 3. Significant inverse correlation of dialysis efficacy (as determined by URR) and serum leptin.

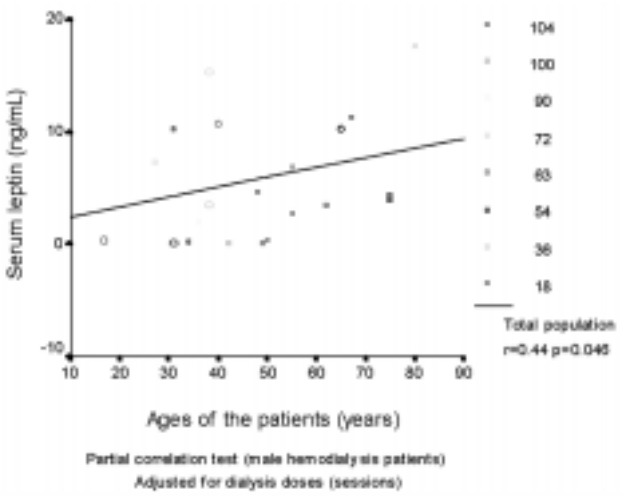

Figure 4. Significant positive correlation of age and serum leptin.

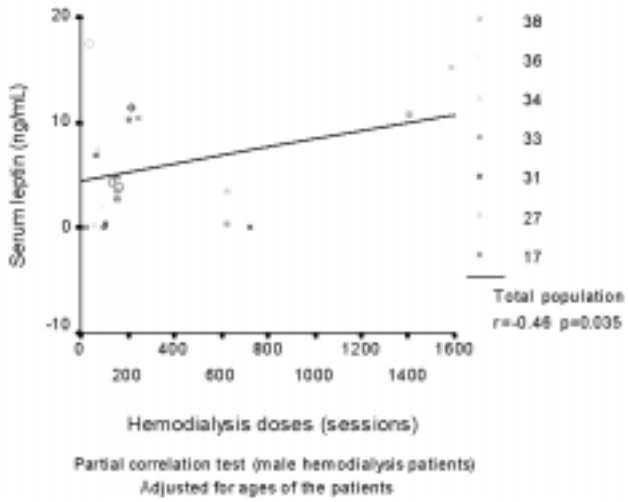

Figure 5. Significant positive correlation of hemodialysis dosage and serum leptin.

ence in serum leptin levels between male and female diabetic HD patients, with higher values in females. In the total group of patients a significant positive correlation of the logarithm of serum leptin with the logarithm of serum iPTH, and a significant positive correlation between BMI and serum leptin were seen. In the male HD patients a significant inverse correlation between dialysis efficacy and serum leptin and with a near significant inverse correlation of serum ALP with serum leptin were observed, and also significant positive correlation between age and serum leptin and between HD dosage and serum leptin. Moreover in this group a near significant positive correlation between duration of HD treatment and serum leptin was seen. In the female HD patients a near significant inverse correlation of serum leptin with serum P and a significant inverse correlation of serum leptin with Ca×P products were found. Leptin is a hormone produced by adipose tissue and its serum levels correlate with total fat mass.[12] Several direct effects on bone have recently been attributed to leptin. It was shown *in vitro* that leptin acts on marrow stromal cells by inhibiting differentiation into adipocytes, favoring osteoblastic differentiation.[13] In experimental animals leptin inhibited bone resorption,[14] and also it has been shown that leptin deficient mice have increased bone mass associated with an increased rate of bone formation.[15] With the exception of the effects of age, aluminum intoxication, DM, Ca and magnesium (Mg) overload, advanced glycation end product accumulation and beta-blocker use, the factors reducing serum PTH and/or bone turnover in patients with ESRD remain incompletely understood.[1] As mentioned earlier, plasma leptin has been found raised in HD patients.[16-19] Studies concerning the relationship between plasma leptin concentration and bone biochemical parameters have shown interesting results. In the study conducted by Ghazali on 17 female and 16 male chronic HD patients, leptin levels were twice as high in the female patients. Ghazali suggested that serum leptin in HD patients may have a bone-sparing effect only when the serum levels of leptin were higher than the presumed threshold of blood-brain transport saturation and he concluded that the higher leptin levels in post-menopausal female HD patients than in male patients may account for their slower bone loss with ageing.[10] A study conducted by Coen et al on 46 HD patients (32 men, 14 women; aged 57.2±11.4 years)

found firstly a positive relation between serum leptin level and BMI, and higher serum levels of leptin in women than in men, and secondly in total patients an inverse significant correlation of serum leptin with serum PTH; this correlation was more significant in the male group. Coen's study results are in support of there being an inverse association between serum leptin levels and some histomorphometric and histodynamic indices of bone turnover, which would be in accordance with experimental data showing decreased osteoblastic activity related to the administration of leptin *in vivo,*[13,14] or to the addition of leptin to preosteoclastic culture *in vitro.*[20] Previously, in a study conducted by Kokot et al the inverse relationship between serum leptin and PTH1–84 had also been noted.[21] Zoccali et al in a study conducted on 161 HD patients to investigate the association between plasma leptin and biochemical bone turnover indicators, showed that plasma leptin was sex-dependent, being significantly higher in female HD than in male patients, and related directly to BMI.[1] The present study, in agreement with the studies of Coen and Zoccali, also found a significant positive relationship between serum leptin and BMI. Concerning sex-dependency this study showed higher levels of serum leptin only in the females of the diabetic HD population. The other findings of the study conducted by Zoccali et al were: in male patients, plasma leptin, after adjustment for BMI was related inversely to serum iPTH, serum PTH1–84, C-PTH fragment and serum PTH1–84/C-PTH fragment ratio, while no such relationships were found in female patients. Zoccali et al also found that the link between plasma leptin and bone turnover markers was independent of other factors. Also plasma leptin was related inversely to skeletal ALP in male but not in female patients. In an agreement with earlier studies the present study showed a significant positive correlation of the logarithm of serum leptin with the logarithm of serum iPTH, and also a very near significant inverse correlation of serum leptin with serum ALP in the male HD population. In this group other findings were significant positive correlation between age and serum leptin, and significant inverse correlation between dialysis efficacy and serum leptin, and a significant positive correlation between HD dosage and serum leptin, with a near significant positive correlation between duration of HD treatment and serum leptin. In female HD patients, a near significant inverse correlation between serum leptin and serum P and a significant inverse correlation between serum leptin and Ca×P products were found.

In conclusion, the inverse correlation demonstrated in this study between leptin and P or Ca×P products could reflect the negative association of bone activity with serum leptin. Increased serum P or Ca×P products observed in cases of uncontrolled secondary hyperparathyroidism, could support the negative association between bone activity and serum leptin in HD patients. These findings underline the need for more attention to investigation be given to larger HD population to clarify further the role of leptin in HD patients.

ΠΕΡΙΛΗΨΗ

Συσχέτιση της λεπτίνης του ορού με βιοχημικές παραμέτρους του μεταβολισμού των οστών σε ασθενείς υπό χρονία αιμοκάθαρση

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ΣΚΟΠΟΣ Η λεπτίνη είναι μια πεπτιδική ορμόνη που παράγεται, κυρίως αλλά όχι μόνο, από το λιπώδη ιστό και καθαίρεται κυρίως από τους νεφρούς. Η συγκέντρωση της λεπτίνης στον ορό συσχετίζεται με την οστική μάζα. Η μελέτη αυτή έγινε σε ασθενείς με χρονία νεφρική ανεπάρκεια τελικού σταδίου, που βρίσκονταν σε χρονία-αιμοκάθαρση. **ΥΛΙΚΟ-ΜΕΘΟΔΟΣ** Σε 36 ασθενείς υπό χρονία αιμοκάθαρση, 10 από τους οποίους ήταν διαβητικοί, μετρήθηκαν στον ορό το ασβέστιο, ο φωσφόρος, η προ της αιμοκάθαρσης κρεατινίνη, η ουρία, η αλκαλική φωσφατάση (ALP), η παραθορμόνη (iPTH) και η λεπτίνη. **ΑΠΟΤΕΛΕΣΜΑΤΑ** Οι γυναίκες διαβητικές ασθενείς παρατηρήθηκε ότι είχαν σημαντικά μεγαλύτερη συγκέντρωση λεπτίνης. Στο σύνολο των ασθενών, παρατηρήθηκε ισχυρή θετική συσχέτιση μεταξύ του λογαρίθμου των συγκεντρώσεων της λεπτίνης του ορού με το λογάριθμο των συγκεντρώσεων της iPTH, καθώς και ισχυρή θετική συσχέτιση μεταξύ των συγκε-

ντρώσεων της λεπτίνης του ορού και του δείκτη μάζας σώματος. Στους άνδρες ασθενείς, βρέθηκε ασθενής αρνητική συσχέτιση μεταξύ της ALP και της λεπτίνης του ορού. Στις γυναίκες ασθενείς, παρατηρήθηκε ασθενής αρνητική συσχέτιση μεταξύ της λεπτίνης και του φωσφόρου του ορού, ενώ βρέθηκε ισχυρή αρνητική συσχέτιση μεταξύ της λεπτίνης του ορού και του γινομένου CaxP. **ΣΥΜΠΕΡΑΣΜΑΤΑ** Η συγκέντρωση της λεπτίνης του ορού φαίνεται να επηρεάζει το μεταβολισμό των οστών στους ασθενείς με χρονία νεφρική ανεπάρκεια τελικού σταδίου, που βρίσκονται σε χρονία αιμοκάθαρση.

Λέξεις ευρετηρίου: Αλκαλική φωσφατάση, Λεπτίνη ορού, Παραθορμόνη, Ρυθμός οστικού μεταβολισμού

References

1. ZOCCALI C, PANUCCIO V, TRIPEPI G, CUTRUPI S, PIZZINI P, MALLAMACI F. Leptin and biochemical markers of bone turnover in dialysis patients. *J Nephrol* 2004, 17:253–260
2. WOLF G, CHEN S, HAN DC, ZIYADEH FN. Leptin and renal disease. *Am J Kidney Dis* 2002, 39:1–11
3. STENVINKEL P. Leptin and its clinical implications in chronic renal failure. *Min Electrol Metab* 1999, 25:298–302
4. NAKAZONO H, NAGAKEY, ICHIKAWA H, MAKINO H. Serum leptin concentrations in patients on hemodialysis. *Nephron* 1998, 80:35–40
5. IBANEZ L, POTAU N, ONG K, DUNGER DB, De ZEGHER F. Increased bone mineral density and serum leptin in non-obese girls with precocious pubarche: Relation to low birth weight and hyperinsulinism. *Horm Res* 2000, 54:192–197
6. PASCO JA, HENRY MJ, KOTOWICZ MA, COLLIER GR, BALL MJ, UGONI AM ET AL. Serum leptin levels are associated with bone mass in non-obese women. *J Clin Endocrinol Metab* 2001, 86:1884–1887
7. MATKOVIC V, ILICH JZ, SKUGOR M, BADENHOP NE, GOEL P, CLAIRMONT A ET AL. Leptin is inversely related to age at menarche in human females. *J Clin Endocrinol Metab* 1997, 82:3239–3245
8. DUCY P, AMLING M, TAKEDA S, PRIEMEL M, SCHILLING AF, BEIL FT. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. *Cell* 2000, 100:197–207
9. GORDELADZE JO, RESELAND JE. A unified model for the action of leptin on bone turnover. *J Cell Biochem* 2003, 88:706–712
10. GHAZALI A, GRADOS F, OPRISIU R, BUNEA D, MORINIΘRE P, ESPER NE ET AL. Bone mineral density directly correlates with elevated serum leptin in haemodialysis patients. *Nephrol Dial Transplant* 2003, 18:1882–1890
11. http://www.halls.md/body-mass-index/av.htm
12. CONSIDINE RV, SINHA MK, HEIMAN ML, KRIAUCIUNAS A, STEPHENS TW, NYCE MR ET AL. Serum leptin in normal weight and obese humans. *N Engl J Med* 1996, 334:292–295
13. COEN G, BALLANTI P, FISCHER MS, BALDUCCI A, CALABRIA S, COLAMARCO L ET AL. Serum leptin in dialysis renal osteodystrophy. *Am J Kidney Dis* 2003, 42:1036–1042
14. BURGUERA B, HOFBAUER LC, THOMAS T, GORI F, EVANS GL, KHOSLA S ET AL. Leptin reduces ovariectomy-induced bone loss in rats. *Endocrinology* 2001, 142:3546–3553
15. 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
16. MERABET E, DAGOGO-JACK S, COYNE DW, KLEIN S, SANTIAGO JV, HMIEL SP ET AL. Increased plasma leptin concentration in end-stage renal disease. *J Clin Endocrinol Metab* 1997, 82:847–850
17. HEIMBURGER O, LONNQVIST F, DANIELSSON A, NORDENSTROM J, STENVINKEL P. Serum immunoreactive leptin concentration and its relation to the body fat content in chronic renal failure. *J Am Soc Nephrol* 1997, 8:1423–1430
18. NISHIZAWA Y, SHOJI T, TANAKA S, YAMASHITA M, MORITA A, EMOTO M. Plasma leptin level and its relationship with body composition in hemodialysis patients. *Am J Kidney Dis* 1998, 31:655–661
19. JOHANSEN KL, MULLIGAN K, TAI V, SCHAMBELAN M. Leptin, body composition, and indices of malnutrition in patients on dialysis. *J Am Soc Nephrol* 1998, 9:1080–1084
20. HOLLOWAY WR, COLLIER FM, AITKEN CJ, MYERS DE, HODGE JM, MALAKELLI S. Leptin inhibits osteoclast generation. *J Bone Miner Res* 2002, 17:200–209
21. KOKOT F, WIECEK A, MESJASZ J, ADAMCZAK M, SPIECHOWICZ U. Influence of long-term recombinant human erythropoietin (rHuEpo) therapy on plasma leptin and neuropeptide Y concentration in haemodialysed uraemic patients. *Nephrol Dial Transplant* 1998, 13:1200–1205

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