Associations between Serum Leptin Level and Bone Turnover in Kidney Transplant Recipients

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Background and objectives: Obesity is associated with increased parathyroid hormone (PTH) in the general population and in patients with chronic kidney disease (CKD). A direct effect of adipose tissue on bone turnover through leptin production has been suggested, but such an association has not been explored in kidney transplant recipients.

Design, setting, participants, & measurements: This study examined associations of serum leptin with PTH and with biomarkers of bone turnover (serum beta crosslaps [CTX, a marker of bone resorption] and osteocalcin [OC, a marker of bone formation]) in 978 kidney transplant recipients. Associations were examined in multivariable regression models. Path analyses were used to determine if the association of leptin with bone turnover is independent of PTH.

Results: Higher leptin levels were associated with higher PTH and lower vitamin D levels, and adjustment for vitamin D attenuated the association between leptin and PTH. However, higher leptin was also significantly associated with lower levels of the bone turnover markers: 1 SD higher leptin was associated with 0.13 lower log-OC (−0.17, −0.08, $P < 0.001$) and 0.030 lower log-CTX (−0.045, −0.016, $P < 0.001$) after multivariable adjustments. Path analysis indicated that the association of leptin with PTH was mostly mediated through vitamin D, and that the association between leptin and bone turnover was independent of PTH and vitamin D.

Conclusions: Elevated leptin level is associated with lower bone turnover independent of its effects on serum PTH in kidney transplant recipients.

Clin J Am Soc Nephrol 5: 2297–2304, 2010. doi: 10.2215/CJN.03520410

Secondary hyperparathyroidism (SHPT) develops early in the course of chronic kidney disease (CKD) (1), and it has been associated with higher cardiovascular morbidity (2) and mortality (3) in hemodialysis patients and with higher mortality in patients with nondialysis-dependent CKD (4). In addition to factors directly related to worsening kidney function (*e.g.*, abnormalities in calcium, phosphorus, vitamin D, and FGF23 metabolism) (1,5–8), PTH levels are also affected by demographic (9,10) and co-morbidity characteristics (11) in CKD. There is mounting evidence that obesity is also associated with higher PTH levels in the general population (12–16) and in patients with CKD (17,18). Furthermore, measurements of body composition suggest that the higher PTH associated with elevated body mass index (BMI) is directly related to the higher adiposity of these individuals (16). There have been speculations that obesity and adiposity indirectly cause elevated PTH levels by affecting vitamin D metabolism (15,19). This would logically imply a consequent increase in bone turnover mediated by PTH. More recently it has been suggested that adipose tissue may also exert a direct effect on bone tissue, possibly mediated through leptin secretion (20), providing an explanation for the decrease in bone turnover reported by some studies in obese individuals, despite relatively higher PTH levels (12). Earlier studies in dialysis patients reported an inverse association between leptin level and bone turnover (21,22). It is unclear if similar associations are present in kidney transplant recipients, a population that is also characterized by distinct changes in bone metabolism (23–26).

The gold standard of determining bone turnover is bone histology, but this method is not feasible for application in large groups of patients. Possible alternatives to bone histology are

Received April 22, 2010. Accepted July 8, 2010.

Published online ahead of print. Publication date available at www.cjasn.org.

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ISSN: 1555-9041/512–2297

biochemical markers of bone turnover such as serum beta crosslaps (CTX)—the C-terminal telopeptide fragments of type I collagen, a marker of bone resorption (27), or serum osteocalcin (OC) and serum alkaline phosphatase (ALP), markers of bone formation (28,29). To test the hypothesis that leptin may be directly associated with bone metabolism rather than through its effects on PTH, we examined the association of serum leptin with serum PTH level and with biochemical markers of bone resorption and formation in a large prevalent cohort of kidney transplant recipients.

Materials and Methods

All stable adult outpatient renal allograft recipients ($n = 1214$) who were followed at the Department of Transplantation and Surgery at Semmelweis University, Budapest, Hungary on December 31, 2006 were evaluated for inclusion. Patients with a current hospitalization or an acute rejection within the preceding 4 weeks, those who underwent transplantation in the previous 3 months, and those with acute infection or bleeding were excluded. Two hundred and five patients (17%) refused to participate in the study and 16 (1%) patients satisfied exclusion criteria. Fifteen patients (1%) were excluded because of missing relevant data points; the final study population consisted of 978 patients.

Assessments were conducted between February 2007 and August 2007 (Malnutrition-Inflammation in Transplant—Hungary Study [MINIT-HU Study]) and included the recording of demographics, comorbidities, medication use, and anthropometric measurements including BMI and abdominal circumference (AC) in a single session. Routinely available laboratory data were extracted from the patients' charts and from the hospital's electronic laboratory database. GFR was estimated using the abbreviated equation developed for the Modification of Diet in Renal Disease study (30) and categorized according to the staging system introduced by the Kidney Disease Outcomes Quality Initiative (K/DOQI) Clinical Practice Guidelines for CKD: Evaluation, Classification, and Stratification (31).

Serum parathyroid hormone (PTH), 25-hydroxy vitamin D (25OHD), CTX, OC, ALP, C-reactive protein (CRP), and IL-6 levels were measured at the time of enrollment from a single morning fasting blood collection. Intact PTH was measured by the Elecsys PTH STAT Assay (Elecsys System; Roche, Mannheim, Germany; reference range 15 to 65 pg/ml) using a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1–37) and a monoclonal antibody labeled with a ruthenium complex reacts with the C-terminal fragment (38–84) (32). Serum CTX were measured on a Roche Elecsys 1010 analyzer (Elecsys System; Roche, Mannheim, Germany; reference range 0 to 0.32 ng/ml) by an electrochemiluminescence immunoassay method. Serum leptin was measured using a solid-phase sandwich ELISA method (R&D Systems, Minneapolis, MN), serum OC was measured by a sandwich ELISA method (N-MID Osteocalcin assay, Elecsys System; Roche, Mannheim, Germany), and serum total 25OHD was measured with a direct competitive chemiluminescence immunoassay ("LIAISON 25 OH Vitamin D Total," DiaSorin, Inc., Stillwater, MN).

After descriptive statistics skewed variables (serum PTH, 25OHD, CTX, OC, and leptin) were natural log-transformed, obesity was assessed by measuring BMI and AC. Correlation coefficients were used to determine the association among BMI, AC, and serum leptin levels and among PTH, 25OHD, CTX, OC, and ALP levels. The association of leptin with PTH, CTX, OC, and ALP levels was assessed in separate linear regression models, with adjustments for covariates believed to be important confounders (age; gender; diabetes mellitus; use of beta blockers; dose of steroids, cyclosporine A, and tacrolimus; estimated GFR; and serum calcium, phosphorus, 25OHD, CRP, and IL-6) (17). PTH, CTX, OC, and ALP were treated as dependent variables in all models. The multivariable-adjusted geometric means or means of PTH, CTX, OC, and ALP by quartiles of serum leptin were estimated from the multivariable regression models using STATA postestimation commands (ADJUST). To determine if the PTH-vitamin D axis mediates the associations between leptin and bone turnover, we created separate regression models assessing associations of leptin with markers of bone turnover after adjustment for PTH and vitamin D levels (33). To better describe the complex interrelationship between leptin, 25OHD, PTH, and bone turnover, we performed path analyses using the maximum likelihood method for parameter estimation (34). Various models were tested using assumptions of different directions of effect between leptin, PTH, 25OHD, CTX, and OC. More complex models including age, BMI, GFR, ALP (as an additional marker of bone formation along with OC), calcium, and phosphorus were also tested, but effect estimates did not change substantially and hence the most parsimonious model was selected. A range of goodness-of-fit statistics were computed to assess model fits, including model χ^2, goodness-of-fit index (GFI), adjusted goodness-of-fit index (AGFI), Bentler comparative fit index (CFI), root mean square error of approximation (RMSEA), and the standardized root mean square residual (SRMR) (34).

We performed secondary analyses to incorporate blood levels of cyclosporine and tacrolimus in multivariable models. Interactions with gender and with estimated GFR were explored by examining associations separately in men and women and in subgroups of patients divided by their level of estimated GFR and by including interaction terms in regression models. P values of <0.05 were considered significant. Statistical analyses were performed using STATA version 10 (STATA Corporation, College Station, TX) and Amos version 16.0 (SPSS, Inc., Chicago, IL). The Institutional Review Board of Semmelweis University approved the study protocol.

Results

Baseline characteristics of all patients and of patients grouped according to their leptin levels are shown in Table 1. Overall, 65% of patients were classified as having CKD stages 3 or 4. Patients with higher serum leptin level were older, were more likely to be female, had higher BMI and AC, used calcitriol more frequently, and were administered lower doses of cyclosporine A. Doses of steroids and tacrolimus and blood levels of cyclosporine A and tacrolimus were not different in patients with higher and lower leptin levels. Patient with higher serum leptin also had shorter dialysis vintage; lower estimated GFR, vitamin D, CTX, and OC levels; and higher PTH, CRP, and IL6 levels.

Tables 2 and 3 show correlation coefficients for markers of obesity and inflammation (Table 2) and for biochemical markers of CKD-mineral and bone disorder (Table 3). Markers of obesity and adiposity (BMI, AC, and leptin) correlated significantly with each other ($P < 0.0001$ for all, Table 2). CRP and IL-6 also correlated significantly with each other, and CRP showed a modest but significant correlation with BMI and AC; a similar correlation was seen between IL-6 and AC (Table 2). Biochemical markers of CKD-mineral and bone disorder all showed significant correlations, with the exception of calcium and phosphorus *versus* ALP and calcium *versus* OC (Table 3). Figure 1 shows unadjusted and adjusted estimated PTH levels

Table 1. Baseline characteristics of the entire cohort and of patients divided by their serum leptin level

	All (n = 978)	Serum Leptin <15 μg/L (n = 485)	Serum Leptin ≥15 μg/L (n = 493)
Age (years)	50.9 ± 12.8	49.8 ± 13.7	52.0 ± 11.7[b]
Gender (female)	414 (42)	122 (25)	292 (59)[c]
Diabetes mellitus	204 (21)	97 (20)	107 (22)
BMI (kg/m^2)	27.0 ± 4.8	24.7 ± 4.0	29.3 ± 4.5[c]
AC (cm)	98.9 ± 14.3	94.1 ± 12.9	103.6 ± 14.1[c]
Calcitriol use	325 (33)	144 (30)	181 (37)[a]
Calcium-containing binder use	58 (6)	28 (6)	30 (6)
Steroid use	797 (81)	388 (80)	409 (83)
Steroid dose in users (mg/day)	5 (5 to 5)	5 (5 to 5)	5 (5 to 5)
Cyclosporine A use	472 (48)	233 (48)	239 (48)
Cyclosporine A dose in users (mg/day)	175 (150 to 200)	200 (150 to 225)	150 (125 to 200)[c]
Serum cyclosporine A level (ng/ml)	117 ± 93	113 ± 87	122 ± 99
Tacrolimus use	422 (43)	212 (44)	210 (43)
Tacrolimus dose in users (mg/day)	3 (2 to 5)	4 (2 to 5)	3 (2 to 5)
Serum tacrolimus level (ng/ml)	6.9 ± 3.7	6.7 ± 3.9	7.0 ± 3.5
Transplant vintage (months)	78 (42 to 121)	78 (43 to 120)	77 (41 to 121)
Time on dialysis (months)	20 (9 to 38)	22 (10 to 42)	18 (9 to 36)[a]
eGFR (ml/min per 1.73 m^2)	50.8 ± 21.0	53.2 ± 21.0	48.5 ± 20.3[c]
K/DOQI stages 1/2/3/4/5	36 (4)/275 (28)/508 (51)/142 (14)/30 (3)	19 (4)/156 (32)/236 (49)/55 (11)/18 (4)	15 (3)/117 (24)/266 (54)/84 (17)/10 (2)[b]
Serum calcium (mg/dl)	9.5 ± 0.7	9.4 ± 0.6	9.5 ± 0.6
Serum phosphorus (mg/dl)	3.2 ± 0.6	3.3 ± 0.9	3.4 ± 0.7
Serum 25OHD (ng/ml)	9.6 (5.9 to 14.6)	10.6 (6.3 to 15.8)	9.1 (5.7 to 13.6)[b]
Serum PTH (pg/ml)	67 (46 to 102)	64 (45 to 96)	70 (49 to 110)[a]
Serum CTX (ng/ml)	0.47 (0.28 to 0.73)	0.49 (0.31 to 0.76)	0.46 (0.27 to 0.68)[b]
Serum OC (ng/ml)	35.6 (34.0 to 37.2)	36.9 (23.7 to 60.3)	33.7 (20.8 to 50.8)[b]
Serum ALP (U/L)	88 ± 40	89 ± 45	87 ± 35
Serum leptin (μg/L)	15.06 (6.81 to 32.10)	6.73 (3.64 to 10.54)	31.81 (22.26 to 52.90)[c]
Serum CRP (mg/L)	3.1 (1.4 to 6.8)	2.6 (1.2 to 5.3)	3.9 (1.9 to 8.0)[c]
Serum IL-6 (pg/ml)	2.1 (1.2 to 3.6)	1.9 (1.2 to 3.3)	2.3 (1.3 to 4.0)[b]

Data are presented as mean ± SD, number (% of total), or median (interquartile range). CNI, calcineurin inhibitor; eGFR, estimated GFR. Comparisons were made by t test, rank sum test, or χ^2 test.

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ compared with the group with serum leptin <15 μg/L.

Table 2. Pairwise correlation coefficients and significance levels for various markers of obesity, adiposity, and inflammation

	BMI	AC	Log-Leptin	CRP	IL-6
BMI	1.00				
AC	0.81, $P < 0.0001$	1.00			
Log-leptin	0.54, $P < 0.0001$	0.39, $P < 0.0001$	1.00		
CRP	0.09, $P = 0.004$	0.12, $P = 0.0003$	0.05, $P = 0.1$	1.00	
IL-6	0.05, $P = 0.09$	0.07, $P = 0.03$	0.05, $P = 0.11$	0.44, $P < 0.0001$	1.00

in groups divided along quartiles of serum leptin. Higher leptin showed an association with higher PTH, which was attenuated after adjustments, especially for serum 25OHD levels. Figures 2 and 3 show unadjusted and adjusted estimated serum CTX (Figure 2) and OC (Figure 3) levels in groups divided by quartiles of serum leptin. Higher leptin levels were associated with significantly lower serum CTX and OC, even after adjustment for PTH and vitamin D. Associations between leptin and ALP were similar to those seen for OC (results not shown). The association between leptin and PTH was more pronounced in men (1 μg/L higher leptin was associated with 0.006 log-unit higher PTH in men [95% confidence interval {CI}: 0.003 to 0.01, $P = 0.001$] and 0.0009 log-units higher PTH in women [95% CI: −0.001 to 0.003, $P = 0.4$]), but the interaction term was NS ($P = 0.2$). No significant gender interaction was detected for the association between leptin and markers of bone turnover. There was also no interaction with GFR for any of the studied associations (data not shown). In path analyses, the best fit was seen for the model assuming a positive effect of leptin on PTH that was almost entirely mediated by 25OHD and a direct negative effect independent of PTH and 25OHD on bone turnover (Figure 4). Goodness-of-fit statistics for this model suggested a reasonably good fit (model χ^2: 58.765 [$P < 0.001$], RMSEA: 0.098, GFI: 0.979, AGFI: 0.928, CFI: 0.957, and SRMR: 0.0451).

Discussion

We describe robust associations of serum leptin with markers of bone resorption (CTX) and formation (OC and ALP) independent of PTH and vitamin D levels in a large transplant sample with various levels of kidney function. We also detected a direct association between leptin and PTH and an attenuation of this association with adjustment for serum 25OHD levels. Importantly, our analyses suggest that leptin has a direct negative effect on bone turnover independent of its concomitant positive association with PTH.

Previous studies in patients with normal kidney function showed that higher BMI was associated with elevated PTH and lower 25OHD levels (13–16), suggesting that the mechanism behind the higher PTH level seen in obese individuals may have been via a direct effect of adiposity on vitamin D metabolism. More recently it was suggested that by virtue of its endocrine functions, adipose tissue may also exert a direct effect on bone metabolism (20). Indeed, recent studies of patients who underwent weight-reducing surgery for obesity reported a decrease in leptin levels and increased bone turnover after significant weight loss (35,36). PTH levels remained unchanged in one of these studies (35) and decreased significantly in the other (36). Leptin, which is a hormone secreted by fat cells and is involved in energy homeostasis (37), was shown to affect bone turnover, possibly through its effects on hypothalamic β-adrenergic activation and the upregulation of β-adrenergic receptors in bone cells (38), but also directly impacting bone turnover by modulating osteoblast differentiation (39). Extrapolating such findings from the general population to patients with CKD is difficult given the complex nature of CKD-associated changes in bone metabolism. Two studies in hemodialysis-dependent patients reported an inverse association between leptin and biochemically (22) and histologically (21) defined bone turnover, with the findings limited to men only in one these studies (22). To the best of our knowledge, before our study, the effects of leptin on PTH and on bone turnover have not been examined in patients with nondialysis-dependent CKD or in kidney transplant recipients. Our results support and extend the findings of these earlier studies in dialysis patients, including a quantitatively more pronounced association between leptin and PTH (but not between leptin and bone turnover markers) in men. Further studies are needed to clarify a potential gender-specific effect of leptin on PTH secretion. Clarifying the complex associations between adiposity, PTH, and bone turnover in individuals with CKD is especially important because elevated PTH is widely regarded as a marker of high bone turnover and remains an important therapeutic target in this patient population. Because it appears that the effects of adiposity on bone metabolism dissociate from its effects on PTH level, the effect of weight loss or weight gain on bone turnover should not be assessed based on the changes in PTH that occur in this context.

The effects of the above detailed mechanisms on clinical end points are less well defined. General population studies examining the effect of leptin on bone density did not yield a conclusive answer (40–44), possibly because the net effects on bone tissue may depend on other concomitantly acting factors (45). Similar studies have not yet been performed in patients with CKD or in kidney transplant recipients, and no studies have examined the effect of the complex interrelationship between obesity and bone turnover on patient survival. The latter is an especially important question given the opposite association of obesity (46) and SHPT (4) with survival in CKD patients. Future

Table 3. Pairwise correlation coefficients and significance levels for serum PTH, ALP, and CTX

	PTH	25OHD	CTX	ALP	OC	Calcium	Phosphorus
PTH	1.00						
25OHD	-0.25, $P < 0.0001$	1.00					
CTX	0.52, $P < 0.0001$	-0.14, $P < 0.0001$	1.00				
ALP	0.15, $P < 0.0001$	-0.12, $P = 0.0003$	0.37, $P < 0.0001$	1.00			
OC	0.40, $P < 0.0001$	-0.11, $P = 0.0005$	0.70, $P < 0.0001$	0.34, $P < 0.0001$	1.00		
Calcium	-0.17, $P < 0.0001$	0.10, $P = 0.02$	-0.10, $P = 0.003$	0.04, $P = 0.16$	-0.004, $P = 0.9$	1.00	
Phosphorus	0.16, $P < 0.0001$	-0.10, $P = 0.003$	0.28, $P < 0.0001$	0.004, $P = 0.90$	0.21, $P < 0.0001$	-0.32, $P < 0.0001$	1.00

Figure 1. Unadjusted (model 1) and estimated multivariable adjusted (models 2 and 3) intact PTH levels (95% CI) in patients with different quartiles of serum leptin. Intact PTH levels were adjusted for age, gender, diabetes mellitus, transplant vintage, estimated GFR, corrected calcium, phosphorus, CRP, IL-6, use of beta blockers, doses of administered corticosteroids, cyclosporine A, and tacrolimus (model 2), and serum vitamin D level (model 3). Comparisons between the PTH levels in the different quartiles of serum leptin were performed in regression models with patients in the first quartile serving as referent, with adjustment for the listed variables in the respective models. *$P < 0.05$.

Figure 2. Unadjusted (model 1) and estimated multivariable adjusted (models 2 and 3) serum CTX levels (95% CI) in patients with different quartiles of serum leptin. Serum CTX levels were adjusted for age, gender, diabetes mellitus, transplant vintage, estimated GFR, corrected calcium, phosphorus, CRP, IL-6, use of beta blockers, doses of administered corticosteroids, cyclosporine A, and tacrolimus (model 2), and serum PTH and vitamin D level (model 3). Comparisons between the serum CTX levels in the different quartiles of serum leptin were performed in regression models with patients in the first quartile serving as referent, with adjustment for the listed variables in the respective models. **$P < 0.01$, ***$P < 0.001$.

studies could explore if the direct effects of adiposity on bone turnover could serve as an explanation for such discrepancies.

Our study has several limitations. Its cross-sectional design does not allow us to conclude a causal role for leptin in the observed lower bone turnover. However, such a causal relationship can be postulated based on experimental results of animal studies (38) and based on the directionality of the associations seen in our path analyses. We examined Caucasian kidney transplant recipients from a single medical institution; thus, our results may not apply to the transplant population at

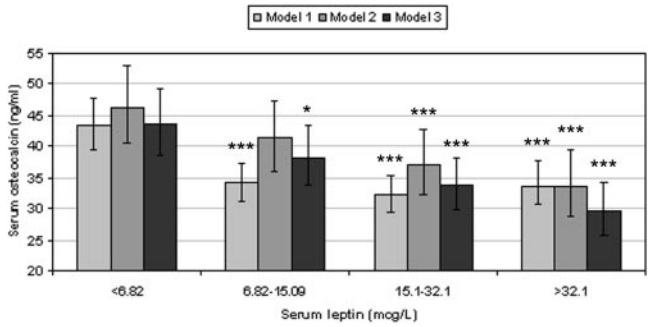

Figure 3. Unadjusted (model 1) and estimated multivariable adjusted (models 2 and 3) serum OC levels (95% CI) in patients with different quartiles of serum leptin. Serum OC levels were adjusted for age, gender, diabetes mellitus, transplant vintage, estimated GFR, corrected calcium, phosphorus, CRP, IL-6, use of beta blockers, doses of administered corticosteroids, cyclosporine A, and tacrolimus (model 2), and serum PTH and vitamin D level (model 3). Comparisons between the OC levels in the different quartiles of serum leptin were performed in regression models with patients in the first quartile serving as referent, with adjustment for the listed variables in the respective models. $*P < 0.05$, $***P < 0.001$.

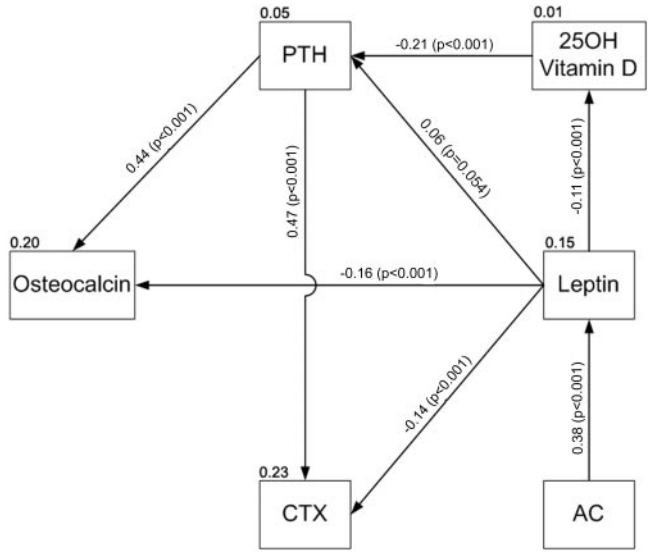

Figure 4. Path model describing the effect of leptin on serum PTH, 25OHD, OC, and CTX. Arrows represent direct effects. Numbers on the arrows represent standardized regression coefficients with *P* values, and the numbers on the boxes represent squared multiple correlations.

large or to nontransplant recipients with CKD because bone metabolism and SHPT in transplant recipients is also influenced by factors such as their underlying pretransplant renal bone disease and the various medications received while on dialysis and later as part of their immune suppressant regimen (47). Ideally bone turnover should be assessed using the gold standard of bone biopsy, but such studies are unlikely to be performed in large numbers of patients with various stages of CKD and would thus be less likely to detect smaller effects. We used instead biochemical markers of bone turnover, but it is unclear to what extent these abnormalities correlate with bone histologic patterns in kidney transplant recipients with or without CKD. Serum CTX is accepted as a marker of bone resorption in patients with normal kidney function (27), but it is unknown to what extent it is diagnostic of histologically defined high turnover bone disease in kidney transplant recipients with or without CKD, especially because levels of CTX are also influenced by kidney function (48). However, we have found similar results when examining other serum markers of bone turnover, such as serum OC levels, an accepted marker of bone formation (28). Because of the uncertain meaning of elevated biochemical bone turnover marker levels on bone health and structure, it remains unclear to what extent leptin levels are associated with histologic abnormalities of the bone in kidney transplant recipients. Finally, we did not have information about the participants' physical activity to explore whether its changes could explain the mechanism of the associations between obesity and bone turnover.

In conclusion, higher serum leptin levels are associated with lower levels of biomarkers of bone resorption and formation and with higher serum PTH levels in kidney transplant recipients. These findings suggest that leptin may exert a direct suppressive effect on bone turnover independent of its effects on PTH. Further research is needed to examine if adiposity can affect bone metabolism and PTH secretion independent of leptin and to determine the practical effect of these novel regulatory pathways.

Acknowledgments

Part of this material was presented in abstract form at the annual meeting of the American Society of Nephrology; October 27 through November 1, 2009; San Diego, CA. We thank the patients and the staff in the Department of Transplantation and Surgery, Semmelweis University Budapest. This study was supported by grants from the National Research Fund (OTKA; F-68841; HUMAN-MB08-A-81231), ETT (206/09), the Hungarian Kidney Foundation, Hungarian Society of Hypertension, Hungarian Society of Nephrology, and the Foundation for Prevention in Medicine. This paper was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (Drs. Novak and Molnar). Dr. Molnar was recipient of the Hungarian Eötvös Scholarship (MÖB/66-2/2010). The research of Dr. Novak has been supported by an unrestricted research grant from Canadian Home Healthcare, Inc., and the Center for Integrative Mood Research, Toronto, Canada.

Disclosures

Dr. Kovesdy has received grants without salary support from Abbott, Genzyme, and Shire and honoraria from Genzyme.

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