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Bone mineral density directly correlates with elevated serum leptin in haemodialysis patients

Abderrahmane Ghazali[1], Franck Grados[2], Roxana Oprisiu[1], Delia Bunea[1], Philippe Morinière[1], Najeh El Esper[1], Isabelle El Esper[3], Michel Brazier[4], Jean Claude Souberbielle[5], Albert Fournier[1] and Thierry Thomas[6]

[1]Service de Néphrologie, Médecine Interne, [2]Service de Rhumatologie, [3]Service de Médecine Nucléaire, and [4]Laboratoire d'Hormonologie, CHU Amiens, France, [5]Laboratoire d'Exploration fonctionnelle, Hôpital Necker, Paris, France and [6]Equipe INSERM 9901, Service de Rhumatologie, CHU St Etienne, France

Abstract

Background. Experimentally, leptin has a positive effect on bone mass when infused intravenously, but a negative one after intracerebroventricular administration. Renal failure increases its serum level above the concentration beyond which its transport to the brain may be saturated. Thus, we tested, in a chronic haemodialysis population, the hypothesis of a positive relationship between serum leptin and bone mineral density (BMD) when serum levels are above this threshold.

Methods. Serum leptin (using a two-site RIA), and BMD at the femoral neck, midshaft, and ultradistal radius, as measured by DEXA, were assessed in 17 female and 16 male chronic dialysis patients, with comparable calcium and phosphate metabolism, age and dialysis duration.

Results. Polynomial regression analysis showed a U-shaped correlation between BMD *Z*-score, with an inflexion point, which may correspond to the concentration threshold at which leptin blood–brain carrier is saturated. Linear regression analysis showed no correlation between BMD and serum leptin levels below these points but a significant positive correlation between BMD at the two radius sites and leptin levels above these points. The correlation remained significant after adjustment for BMI, serum PTH and duration of dialysis. Leptin levels were twice as high in female patients and associated with higher BMD *Z*-scores close to zero.

Conclusions. This study suggests a bone-sparing effect of serum leptin in haemodialysis patients only when the serum levels of leptin were higher than the presumed threshold of blood–brain transport saturation. Higher leptin levels in post-menopausal female haemodialysis patients than in male patients may account for their slower bone loss with ageing.

Keywords: blood–brain barrier; bone mineral density; haemodialysis; leptin; sex dimorphism

Introduction

Leptin is a polypeptidic hormone produced by the white adipocyte under the control of the adiposity gene [1]. This hormone has been initially considered mainly as an anti-obesity hormone as by its action or the hypothalamic centre, through its OBRb receptor, it suppresses appetite and increases basal metabolism. Besides being an energy homeostasic hormone, leptin has been shown to be also involved in gonadal maturation and in somatotropic and adrenocorticotropic functions regulating the immune system and body development [1]. It is only recently that it has been found to be involved in bone metabolism as well. *In vitro* studies have shown that it promotes differentiation of mesenchymal stem cells to osteoblasts rather than adipocytes [2] and inhibits osteoclastogenesis [3,4] by increasing osteoprotegerin and decreasing RANK ligand synthesis. Furthermore, *in vivo* experiments of systemic leptin administration have been shown to stimulate bone growth [5], to increase bone strength and to prevent ovariectomy induced bone loss [3]. In contrast, its intracerebroventricular administration in wild or ob/ob leptin deficient mice resulted in bone loss [6].

Epidemiological evidence in humans for a protective effect of leptin on bone mass is still uncertain. Leptin being produced quasi exclusively by the white adipose tissue, the link between serum leptin and body fat mass

Correspondence and offprint requests to: Professor Albert Fournier, Hôpital Sud, Service de Néphrologie Médecine Interne, F-80054 Amiens Cedex1. France. Email: neph@chu-amiens.fr

and/or body mass index (BMI) is very strong so that the adjustment of serum leptin or BMD to fat mass or BMI often leads to the disappearance of the positive link between serum leptin and BMD when it is present [7]. Such a link is, however, not always present, even in women who have more than twice higher levels of serum leptin than men [8,9]. In men, no link has been found between serum leptin and BMD in the Mayo Clinic study [10], and a negative correlation was even reported in young men (< 60 years) in the NHANES III study [7] after adjustment for other bone density-related factors. Only three studies conducted in humans demonstrated a positive relationship between bone parameters and leptin independent of fat mass. The first article, by Pasco *et al*. [11], has reported in pre- and post-menopausal non-obese women a persistent link, after adjustment for age, body weight and body fat mass, between serum leptin and mainly the bone mineral content (BMC), with a less clear-cut effect on BMD. Indeed leptin correlated with BMD only at the lateral spine whereas it correlated with BMC at most other sites. The second article supporting a positive bone effect of leptin is that of Yamauchi *et al*. [12]. They found in post-menopausal women that plasma leptin was directly correlated to femoral neck and whole body BMD even after adjustment to body fat mass. Furthermore, they reported that leptin levels, but not fat mass were lower in women with fractures than in those without fractures. The third, by Blain *et al*. [13], reported that in healthy post-menopausal women whole body BMD variance, in stepwise multiple linear regression, was explained for 28% by lean mass, for 10% by age and for 7.2% by leptin, whereas femoral neck BMD variance was explained for, respectively, 13–21% and 3.7% by these parameters.

Animal and clinical studies [14,15] have demonstrated that the leptin access to the hypothalamic centre mediating its negative effect on appetite and bone mass (the OBRb receptor with long intracellular domain) is limited by the blood–brain barrier. This access implies a saturable transport system involving the OBRa receptors (with a shorter intracellular domain than that of its effective receptor OBRb) [14]. In a population of lean and obese non-uraemic patients, Caro *et al*. [15] suggested that the saturation of this transport occurred for a serum total leptin level near 25 ng/ml, such that the energy homeostasic centre would not be stimulated any more by serum leptin levels above this threshold, accounting for leptin resistance in common obesity. Renal failure leads to a retention of active leptin, whose levels are increased by a factor 2.2, whereas bound leptin concentrations remain comparable with that of the controls [16]. The link between serum leptin and BMI in uraemia is thus dissociated by this retention phenomenon, which suggests that the uraemic state could represent an optimal situation to find a close relation between serum leptin and BMD after adjustment for BMI. In such conditions the link between serum leptin and BMD adjusted to BMI might be expected to be lacking or even negative when serum leptin concentration is below that corresponding to the saturation of leptin transport to the brain, whereas it would be positive above this threshold as the hypothalamic centre responsible for the negative bone effect of leptin would be no more stimulated.

We report here a study in 33 haemodialysis patients in whom we have documented not only BMD and serum total leptin levels but also bone alkaline phosphatase as well as vitamin D, PTH, aluminum and acid–base status, dietary intake of calcium, phosphate and protein, calcium carbonate supplement as phosphate binders, 1α-hydroxylated vitamin D supplement and dialysate calcium concentration, i.e. the main factors having a potential impact on BMD.

Subjects and methods

Patients

Thirty-three patients at our dialysis centre were included in this study. They agreed to have a their bone mineral density measured in addition to serum biochemistry evaluation. There were 16 men and 17 women, 15 of the latter being post-menopausal without hormone replacement therapy. Their mean age was 64 ± 15 years. They were on dialysis for 66 ± 70 months. Nine of them were diabetics, six had had a surgical parathyroidectomy more than a year before the evaluation and four had had an unsuccessful transplantation. They were haemodialysed three times for 4 h a week and had been exclusively taking $CaCO_3$ as phosphate binders since they started maintenance dialysis. In order to maintain serum 25OH vitamin D levels > 20 ng/ml, most of them received a weekly 25OH vitamin D supplement. Only seven received 1α-hydroxylated vitamin D. Dialysate calcium concentration was 1.5 mmol/l in 22 patients, 1.75 mmol/l in six patients and 1.25 mmol/l in five patients. The dialysate was prepared with reverse osmosis-treated water, with < 0.20 μmol/l of aluminum.

Methods

During the year preceding BMD measurement, the patients had not only a determination of serum calcium, phosphate and total protein (weekly before the first dialysis of the week) but also of intact PTH, 25OHD and bone alkaline phosphatase (BAP) on a monthly basis and of aluminum on a quarterly basis. Serum leptin was measured only once within the 3 months of BMD measurement. Serum calcium was corrected for protein concentration by the Parfitt formula: corrected PCa = measured total PCa/[0.55 ± (protein g/l/160)]. Serum concentrations of aluminum were measured by inductively coupled plasma emission spectrometry (normal < 0.35 μmol/l). Serum intact PTH was measured by the CHIRON chemoluminescence method (normal range 10–55 pg/ml). Serum BAP was measured by the Ostase® radioimmunoassay of Hybritec laboratory (normal range 3–20 μg/l). Serum 25OH vitamin D was measured by radiocompetition method. The serum leptin concentration was measured by a two-site radioimmunometric assay (Active Human Leptin; Diagnostic System Laboratory, Webster, TX). The normal ranges in individuals with normal BMI (18–25) are 2–10 ng/ml in men and

2–23 ng/ml in women. The intra-assay coefficient of variation was 3.7–4.9 and 2.6% at concentrations of 2.8–13.5 and 73.9 ng/ml, respectively. The inter-assay coefficient of variation was 6.6–5.3 and 3.7% at concentrations of 2.8–14.4 and 73.9 ng/ml, respectively.

BMD was measured by DEXA with a Hologic QDR 2000 apparatus at the level of their femoral neck (mixed bone) and of the midshaft (mainly cortical bone) and ultradistal radius (mainly cancellous bone) of the non-dominant forearm. Quality control of the machine was performed by daily scanning of an anthropomorphic phantom supplied by the manufacturer. The inter-day *in vitro* variability was 0.4%. The results are given in *Z*-scores, i.e. in number of standard deviations from the normal mean of a gender-, age- and race-adjusted control group.

Evaluation of the dietary intake

It was evaluated on a week diary, twice at 6 months intervals.

Statistical methods

Statistics were computed using Stat View 4.57 and Statistica 5 software for Windows. Results have been expressed as mean ± SD. Data were analysed by unpaired *t*-test and one-way analysis of variance (ANOVA), followed by Mann–Whitney test for non-parametric data to compare two groups. Correlations between variables were assessed using simple linear regression as well as polynomial regression analysis for two variables. The formula of this curve is $y = a + bx + cx^2$ and the inflexion point of this curve is given by the formul $x = -b/2 \times c$. $P < 0.05$ was accepted as statistically significant.

Results

Table 1 summarizes the means of all the parameters measured for the whole population with a subdivision according to gender. There was no significant difference between the two genders as regards age, BMI, prevalence of diabetes, transplantation failure or prior surgical parathyroidectomy. The duration on dialysis treatment was longer in women (86 ± 85 *vs* 47 ± 48) but the difference was statistically significant. The use of different dialysate calcium concentrations as well as the prescription of alfacalcidol and $CaCO_3$ were comparable between the two gender. Dietary intake of calcium, phosphate and protein were also comparable.

There was also no significant difference in the following serum biochemical parameters: corrected calcium, phosphate, 25OH vitamin D, bicarbonate, bone alkaline phosphatase and PTH with the exception of leptin levels, which were significantly higher in women than in men (72 ± 48 *vs* 33 ± 44 ng/ml), with the significance persisting after adjustment to BMI ($P < 0.01$). BMD *Z*-scores were higher in women at all

Table 1. Clinical, serum biochemistry and bone mineral density data (mean ± SD) in all patients and inpatients according to gender

Parameters	All patients	Female (17)	Male (16)	*P* value[a]
Number of patients	33	17	16	–
Age (years)	64 ± 15	63 ± 14	65 ± 16	NS
Diabetics, *n* (%)	9 (27)	3 (19)	6 (35)	NS
Transplantation failure, *n* (%)	4 (12)	3 (19)	1 (6)	NS
Parathyroidectomy, *n* (%)	6 (18)	4 (25)	2 (12)	NS
Duration of dialysis (months)	66 ± 70	86 ± 85	47 ± 48	NS
Dialysate calcium (1.75 mmol/l/other)	6/33	3/17	3/16	NS
Alfacalcidol	7	3	4	NS
Dietary protein intake (g/day)	57 ± 13	55 ± 8	59 ± 17	NS
Dietary calcium intake (mg/day)	293 ± 100	260 ± 72	323 ± 116	NS
Dietary phosphorus intake (mg/day)	704 ± 179	663 ± 106	742 ± 226	NS
$CaCO_3$ (g/day)	8.6 ± 3.3	9.5 ± 2.7	7.8 ± 3.7	NS
Serum calcium (mmol/l)[b]	2.47 ± 0.11	2.47 ± 0.10	2.46 ± 0.13	NS
Serum phosphate (mmol/l)	1.55 ± 0.26	1.63 ± 0.25	1.48 ± 0.26	NS
Serum intact PTH (pg/ml)	210 ± 238	235 ± 298	187 ± 169	NS
Serum 25 OH vitamin D3 (ng/ml)	25 ± 18	25 ± 14	26 ± 22	NS
Serum aluminum (μmol/l)	< 0.35	< 0.35	< 0.35	NS
Serum bone alkaline phosphatase (μg/l)	11 ± 6	11 ± 6	11 ± 6	NS
Serum bicarbonate (mmol/l)	27 ± 2	27 ± 2	27 ± 2	NS
Serum leptin (ng/ml)	52 ± 49	72 ± 48	33 ± 44	0.01
Serum leptin ratio on BMI	1.87 ± 1.76	2.62 ± 1.79	1.16 ± 1.43	0.007
BMI[c]	26 ± 6	27 ± 7	25 ± 5	NS
Femoral neck *Z*-score	−0.68 ± 1.40	−0.17 ± 1.33	−1.22 ± 1.27	0.05
Femoral neck ratio on BMI	−0.03 ± 0.06	−0.01 ± 0.04	−0.06 ± 0.06	0.04
Mid shaft radius *Z*-score	−0.47 ± 2.40	0.21 ± 2.24	−1.19 ± 2.47	NS
Mid shaft radius ratio on BMI	−0.02 ± 0.10	0.01 ± 0.08	−0.05 ± 0.11	NS
Ultradistal radius *Z*-score	−0.57 ± 1.87	−0.04 ± 1.89	−1.20 ± 1.72	NS
Ultradistal radius ratio on BMI	−0.03 ± 0.07	−0.01 ± 0.06	−0.05 ± 0.08	NS

[a]Female *vs* male (Mann–Whitney or χ^2 test).
[b]Plasma measured total calcium/{0.55 + [protein (g/l)/160]}.
[c]Body mass index.

sites measured; they were ±0.20 in women and near −1.20 in men, although the difference was only significant at the femoral neck. This significance persisted after adjustment to BMI ($P = 0.04$).

Tables 2 and 3 show that serum leptin and the BMD *Z*-score were comparable in patients in the presence or absence of diabetes, previous parathyroidectomy or unsuccessful transplantation.

Table 4 shows the correlation with serum leptin while using the full range of data. Serum leptin was not correlated to serum concentrations of the PTH, bone alkaline phosphatase or 25OH vitamin D, to dietary intake of protein, calcium and phosphate, or to the dose of $CaCO_3$ supplement, be it for the whole population or each gender separately. Serum leptin levels tended to be negatively correlated for the duration on haemodialysis treatment but the correlation was significant only in women.

Serum leptin levels were positively correlated with BMI in the whole population and in both genders but the level of statistical significance, which was higher in the whole population than in the male population in spite of a lower r coefficient (0.59 *vs* 0.73), was only borderline in the female population ($r = 0.47$; $P = 0.06$) (Figure 1).

The correlation of serum leptin with BMD at the femoral neck showed a significant correlation only in the male population. The significance persisted after

Table 2. Comparison of serum leptin concentrations (ng/ml; mean ± SD) according to the presence of absence of pathological conditions related to uraemia

Parameter *P* value (Mann–Whitney)	Presence	Absence	Significance of difference
Diabetes	58 ± 51 ($n = 9$)	50 ± 49 ($n = 24$)	NS
Parathyroidectomy	30 ± 45 ($n = 6$)	30 ± 45 ($n = 27$)	NS
Unsuccessful kidney transplantation	76 ± 49 ($n = 4$)	49 ± 49 ($n = 29$)	NS

Table 3. Comparison of bone mineral density *Z*-score (mean ± p SD) according to the presence or absence of pathological conditions related to uraemia

Parameters	Diabetes			Parathyroidectomy			Unsuccessful kidney transplantation		
	Presence ($n = 9$)	Absence ($n = 24$)	P^a	Presence ($n = 6$)	Absence ($n = 27$)	P^a	Presence ($n = 4$)	Absence ($n = 29$)	P^a
Femoral neck *Z*-score	−0.79 ± 2.16	−0.62 ± 0.92	NS	−0.72 ± 1.40	−0.67 ± 1.41	NS	−1.83 ± 0.25	−0.59 ± 1.40	NS
Femoral neck ratio on BMI	−0.05 ± 0.09	−0.03 ± 0.04	NS	−0.02 ± 0.06	−0.03 ± 0.06	NS	−0.08 ± 0.02	−0.03 ± 0.06	NS
Mid shaft radius *Z*-score	−0.69 ± 2.12	−0.37 ± 2.57	NS	−1.81 ± 1.17	−0.16 ± 2.53	NS	−2.36 ± 0.38	−0.23 ± 2.46	NS
Mid shaft radius ratio on BMI	−0.03 ± 0.06	−0.02 ± 0.12	NS	−0.08 ± 0.05	−0.01 ± 0.11	NS	−0.10 ± 0.02	−0.01 ± 0.02	NS
Ultradistal radius *Z*-score	−1.39 ± 2.61	−0.21 ± 1.38	NS	−1.50 ± 1.97	−0.35 ± 1.97	NS	−1.02 ± 0.94	−0.51 ± 1.97	NS
Ultradistal radius ratio on BMI	−0.07 ± 0.09	−0.01 ± 0.05	NS	−0.06 ± 0.05	−0.02 ± 0.07	NS	−0.04 ± 0.04	−0.03 ± 0.08	NS

[a] *P* value Mann–Whitney.

Table 4. Linear regression analysis of serum leptin in the whole population and separately for male and female patients (using the full range of serum leptin)

Simple correlation parameter	All patients		Female		Male	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Duration of dialysis	−0.25	0.157	−0.56	0.02	−0.19	0.46
Serum PTH	0.005	0.97	0.032	0.09	−0.20	0.43
Serum 25OH vitamin D	0.103	0.56	−0.186	0.49	0.05	0.83
Serum bone alkaline phosphatase	−0.08	0.66	0.012	0.96	−0.211	0.41
BMI	0.58	0.0003	0.467	0.06	0.726	0.001
Protein intake	0.131	0.59	0.307	0.42	0.24	0.5
Calcium intake	−0.16	0.51	0.231	0.55	0.005	0.988
Phosphate intake	0.04	0.86	0.08	0.83	0.142	0.6
Oral calcium bicarbonate	0.014	0.94	0.056	0.836	−0.197	0.4
Femoral Neck *Z*-score	0.319	0.09	0.098	0.728	0.589	0.028
its ratio on BMI	0.311	0.10	−0.229	0.411	0.551	0.04
Mid shaft radius *Z*-score	0.387	0.046	0.178	0.54	0.468	0.107
its ratio on BMI	0.333	0.09	0.075	0.79	0.426	0.147
Ultradistal radius	0.417	0.034	0.313	0.27	0.415	0.18
its ratio on BMI	0.407	0.039	0.276	0.339	0.407	0.189

adjustment to BMI ($r = 0.55$; $P = 0.04$). At the level of the radius midshaft, the correlation was significant only for the whole population ($r = 0.387$; $P = 0.046$) but the significance was lost after adjustment to BMI. At the level of the ultradistal radius the correlation was significant and the significance persisted after adjustment to BMI ($r = 0.407$; $P = 0.039$).

To test the hypothesis of a greater bone-sparing effect of serum leptin for levels above that corresponding to the presumed threshold of its blood–brain transport saturation, we used polynomial regression to correlate BMD to serum leptin. At the three sites the regression curve had a flat U shape but was not significant ($r = 0.34$, $P = 0.19$; $r = 0.41$, $P = 0.11$; $r = 0.45$; $P = 0.07$) at, respectively, the femoral neck (Figure 2), the radius midshaft (Figure 3) and the ultradistal radius (Figure 4). The determined inflexion points of the curve were at a serum leptin concentration of 24, 17 and 24 ng/ml, respectively, at the three sites.

Linear regression analysis with the patients editing serum leptin below these values did not a show significant correlation, whereas that with the patients having serum leptin above the curve inflexion point showed significant positive correlations at both radius sites ($r = 0.53$, $P = 0.03$ at midshaft radius and $r = 0.50$, $P = 0.04$ at ultradistal radius). Adjustment of BMD

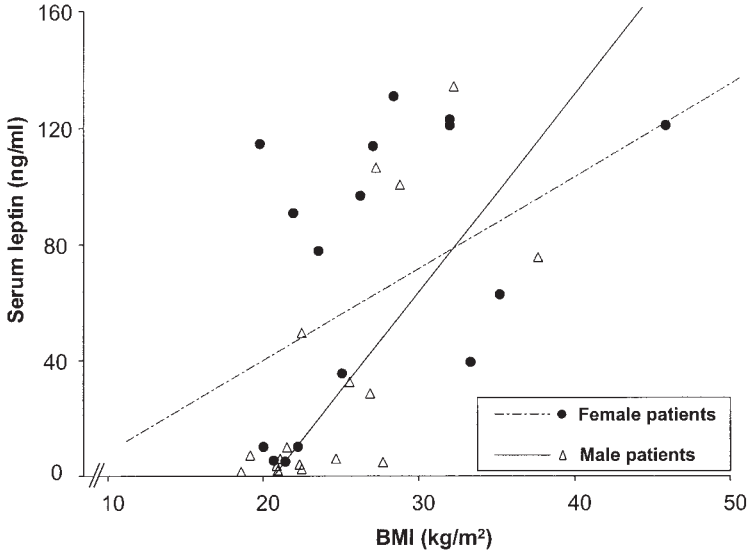

Fig. 1. Correlation between peripheral serum leptin (ng/ml) and body mass index (BMI) in haemodialysis patients, according to gender. All patients: $y = 4.7x - 71.2$, $n = 33$, $r = 0.59$, $P = 0.0003$; female patients $y = 3.2x - 13.9$, $n = 16$, $r = 0.47$, $P = 0.06$; male patients $y = 6.4x - 124.8$, $n = 17$, $r = 0.73$, $P = 0.001$.

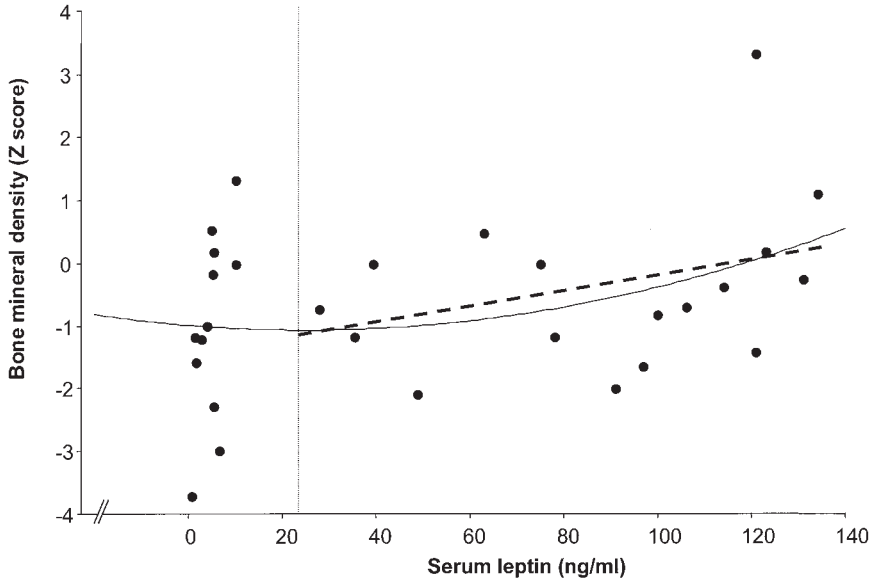

Fig. 2. Correlation between femoral neck bone density and peripheral serum leptin (ng/ml) in haemodialysis patients. Polynominal regression analysis with all leptin values: $y = 1.2 \times 10^{-4}\, x^2 - 6 \times 10^{-3}\, x - 1$, $n = 29$, $r = 0.34$, $P = 0.19$; simple regression analysis for leptin > 24.41 ng/ml: $y = 0.013x - 1.5$, $n = 17$, $r = 0.35$, $P = 0.18$.

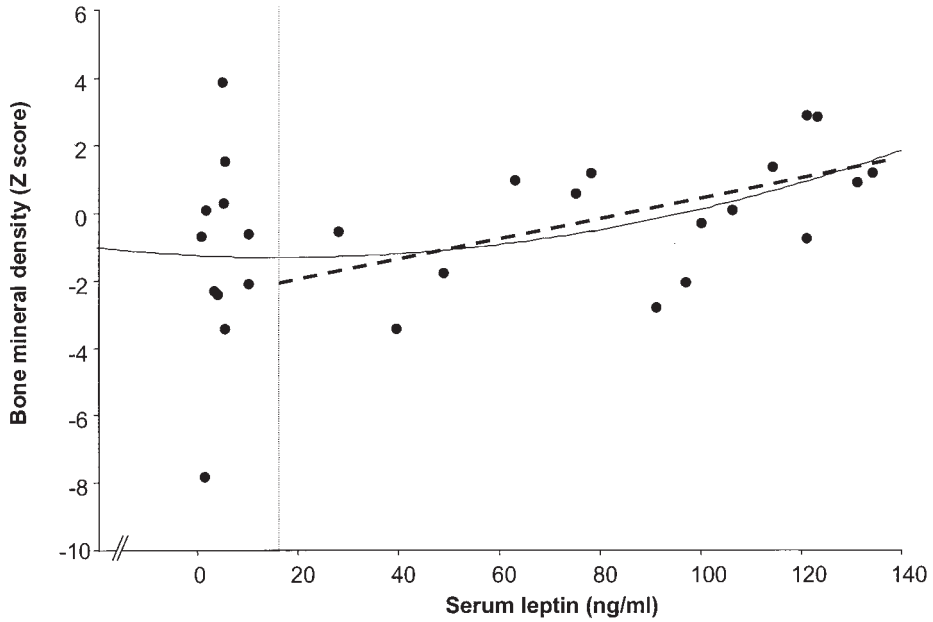

Fig. 3. Correlation between midshaft radius bone density and peripheral serum leptin in haemodialysis patients. Polynominal regression analysis with all leptin values: $y = 2.1 \times 10^{-4}\, x^2 - 7 \times 10^{-3}\, x - 1.2$, $n = 27$, $r = 0.41$, $P = 0.11$; simple regression analysis for leptin > 16.74 ng/ml: $y + 0.03\text{x} - 2.7$, $n = 16$, $r = 0.53$; $P = 0.03$.

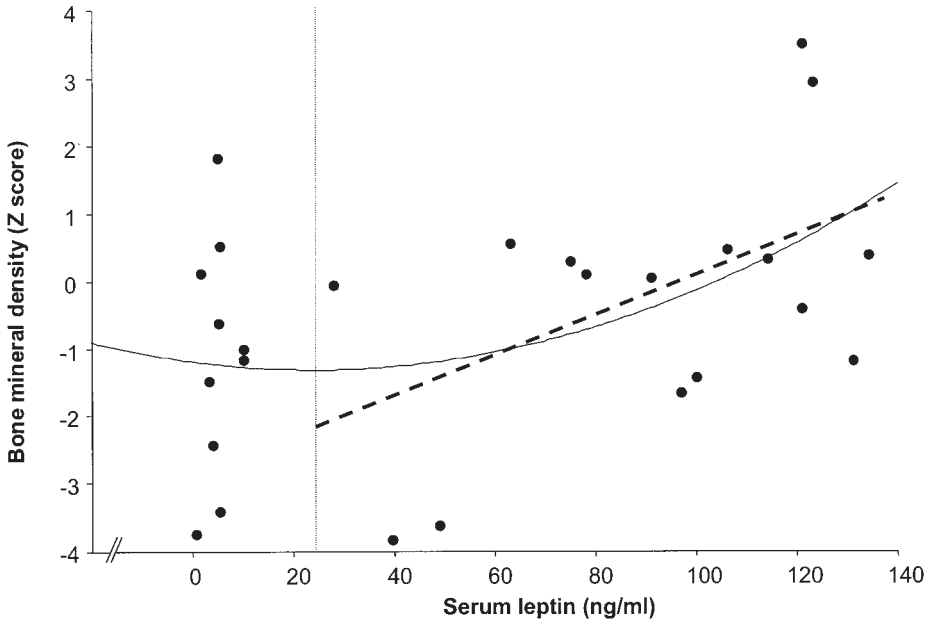

Fig. 4. Correlation between ultradistal radius bone density and peripheral serum leptin in haemodialysis patients. Polynominal regression analysis and all leptin values: $y = 2.1 \times 10^{-4}\, x^2 - 1 \times 10^{-2}\, x - 1.2$, $n = 26$, $r = 0.45$, $P = 0.07$; simple regression analysis for leptin > 24.15 ng/ml: $y = 0.03x - 2.9$, $n = 16$, $r = 0.50$, $P = 0.04$.

Z-score to BMI did not cancel the significance ($r = 0.50$; $P = 0.04$ for Figures 3 and 4). Although serum PTH and dialysis duration were not correlated to BMD in univariate analysis, they were included with leptin serum level in multiple regression analysis as independent variables correlated to BMD for the patients with leptin values above the presumed saturation threshold. The correlation was still significant at $P = 0.037$ at the midshaft radius but no more at the ultradistal radius.

To exclude the role of uraemia-related factors in the occurrence of a link between serum leptin and BMD for values above the presumed blood–brain carrier saturation threshold and in the absence of a link below this threshold, we compared all the pathophysiological factors involved in renal bone disease reported in Table 1 in the patients with leptin values < 17 ng/ml and > 24 ng/ml, as shown by Table 5. All parameters were comparable in these two groups of patients.

Table 5. Clinical data and serum biochemistry (mean ± SD) in all patients and in patients with leptin levels below or above the presumed blood-brain carrier saturation threshold

Parameters	All patients	Leptine < threshold	Leptine > threshold	*P* value[c]
Number of patients	33	14	19	–
Age (years)	64 ± 15	61 ± 17	66 ± 13	NS
Diabetics, *n* (%)	9 (27)	3 (19)	6 (35)	NS
Transplantation failure, *n* (%)	4 (12)	1 (6)	3 (19)	NS
Parathyroidectomy, *n* (%)	6 (18)	4 (25)	2 (12)	NS
Duration on dialysis (months)	66 ± 70	84 ± 93	53 ± 45	NS
Dialysate calcium (1.75/other)	6/33	1/13	5/17	NS
Alfacalcidol	7	5	2	NS
Dietary protein intake (g/day)	57 ± 13	55 ± 17	58 ± 11	NS
Dietary calcium intake (mg/day)	293 ± 100	314 ± 104	281 ± 100	NS
Dietary phosphorus intake (mg/day)	704 ± 179	705 ± 216	704 ± 165	NS
Oral $CaCO_3$ (g/day)	8.6 ± 3.3	8.9 ± 4.1	8.4 ± 2.7	NS
Serum calcium (mmol/l)[a]	2.47 ± 0.11	2.46 ± 0.13	2.47 ± 0.10	NS
Serum phosphate (mmol/l)	1.55 ± 0.26	1.50 ± 0.30	1.58 ± 0.23	NS
Serum intact PTH (pg/ml)	210 ± 238	207 ± 214	213 ± 259	NS
Serum 25 OH vitamin D3 (ng/ml)	25 ± 18	27 ± 19	24 ± 18	NS
Serum aluminum (μmol/l)	< 0.35	< 0.35	< 0.35	NS
Serum bone alkaline phosphatase (μg/l)	11 ± 6	11 ± 6	11 ± 6	NS
Serum bicarbonate (mmol/l)	27 ± 2	28 ± 2	26 ± 1	NS

[a]Measured serum total calcium/{0.55 + [protein (g/l)/160]}.
[b]Body mass index.
[c]Females *vs* males (Mann–Whitney or χ^2 test).

Discussion

Our data on total serum leptin in chronic haemodialysis patients confirm the gender dimorphism of their levels as they were more than twice as high in women as in men, whether adjusted to BMI or not. This is remarkable since in their study, Considine *et al*. [17] pointed out that women and men with equivalent percentages of body fat had comparable levels of serum leptin. Considering that free active leptin represents 12% of total leptin in haemodialysis patients and 7% in controls [16], the mean value of free leptin in our patients may be estimated at 8.64 ng/ml in women, and 3.96 ng/ml in men whereas the upper limit of normal of free leptin would be 1.61 ng/ml in women and 0.7 ng/ml in men. In parallel with higher total and free leptin concentration the BMD *Z*-score has much higher in women than in men at all sites as it was between −0.20 and +0.20 in women whereas it was around −1.20 in men, but the difference was significant only at the femoral neck. This finding is noteworthy as previous studies, reported that female dialysis patients had a lower BMD *Z*-score than normal women with a 20% prevalence of ultradistal radius BMD *Z*-score below −2 and a greater risk of low BMD in females than in males [18]. This association of higher leptin levels and higher BMD *Z*-score adjusted to BMI in post-menopausal women not receiving hormonal replacement therapy as compared with men, strongly suggests a bone-sparing effect of leptin as all the other factors known to influence BMD in dialysis patients (such as age, diabetes, transplantation, parathyroidectomy, current PTH levels, vitamin D, aluminum and acid–base status, dietary intakes of calcium, phosphate and protein, amount of $CaCO_3$ used as phosphate binder, dialysate calcium concentration and use of 1α-hydroxylated vitamin D) were comparable. Only the duration of dialysis was remarkably different (although the difference did not reach the level of significance, not statistically) but this well-known bone mass-decreasing factor [18] was actually longer in the female population. The fact that 15 of the 17 women were amenorrhoeic and that in haemodialysed women amenorrhoea usually occurs at a younger age, makes our observation all the more remarkable [18].

Polynomial regression analysis showed that the correlation curve between the BMD *Z*-score and serum leptin had a flat U-shape with an inflexion point of 24–17 and 24 ng/ml of serum leptin. Interestingly, these values are very close to the 25 ng/ml threshold value of serum leptin levels reported by Caro *et al*. in a cross-sectional study of a mixed lean and obese population, a threshold above which an increase in serum leptin concentration was not accompanied by an increase in leptin cerebrospinal fluid concentrations. The normal range of the serum leptin in the lean individuals of Caro *et al*. was comparable with that of our RIA, this coincidence is quite remarkable. The leptin concentration of 25 ng/ml has been interpreted by these authors as being the threshold value at which the blood–brain transport of leptin is saturated. As below this threshold, the CSF leptin concentration linearly increases with serum leptin, we speculate that for this lower range of leptin concentration the centrally mediated negative effects of leptin on bone may cancel or even override the positive direct effect of systemic leptin on bone mass. Hence, the net effect on bone will be null or even negative. On the contrary, above this threshold of blood–brain transport saturation, the CSF/serum leptin concentration ratio is expected to decrease so that the positive bone effects of systemic leptin would overcome the negative central

effects. Considering that free active leptin represents 7% of total leptin in non uraemic patients and 12% in haemodialysis patients [16] the saturation threshold of the blood–brain carrier of free leptin proposed by Caro *et al*. in obese non-uraemic patients is actually 1.7 ng/ml and the inflexion points of the polynomial regression curve relating BMD to leptin are 2 and 2.9 ng suggesting that uraemia only slightly shifts this saturation threshold to higher values. Whether the saturation is considered for total or free leptin, the fact that the correlation between serum leptin and BMD adjusted for BMI does not exist when serum leptin is below this threshold, whereas this correlation is significantly positive above it, fully supports the pathophysiological hypothesis that above the threshold the positive bone effect of systemic leptin overrides the negative bone effect of centrally acting leptin. The fact that the positive correlation between BMD and leptin is not significant at the femoral neck site suggests that on a weight-bearing bone site the positive effects of fat mass on bone mass are predominantly dependent upon mechanical forces rather that upon endocrine factors. Interestingly, the positive link between serum leptin and BMD is stronger in non weight-bearing bone site and persists at the mid shaft even after adjustment to PTH levels and duration on dialysis. The fact that all potential factors linked to uraemic bone disease as detailed in Table 5, are comparable in the patients with serum leptin above or below the presumed saturation threshold (17–24 ng) of blood–brain leptin carrier, further supports the concept that the bone-sparing effect of leptin levels above this threshold is not a fallacy induced by the pathophysiological complexity of uraemic bone disease.

Our data would, therefore, reconcile two apparently contradictory concepts, namely that of a centrally mediated negative effect of leptin on bone proposed by the group of Karsenty [6] and that of various other groups supporting a positive bone effect of peripheral leptin. Indeed systemic leptin administration increases bone mass and strength [5] and reduces ovariectomy induced bone loss in the rat [3]. The mechanism of this bone-sparing effect could be explained by a dual synergistic effect of leptin on bone, namely an increase in bone formation by favouring osteoblastogenesis and a decrease in bone resorption by decreasing osteoclastogenesis. The latter may be related, at least in part, to an increased expression of osteoprotegerin coupled with a suppression of RANK ligand expression in human marrow stromal cells [3,4].

Other data in uraemic patients support both positive and negative effects of leptin on BMD. A positive correlation has been reported in 25 Japanese postmenopausal dialysis patients between serum leptin and ultradistal radius BMD but this latter was not adjusted to BMI [19]. Another positive correlation between serum leptin and BMD adjusted to BMI was however also recently reported in a Turkish mixed dialysis population ($n = 33$) [20]. However, the RIAs used in these two studies were different as compared with both our study and that by Caro *et al*., as serum leptin values were much lower (i.e. ~12 and 3 ng/ml in dialysis women and men, respectively). On the contrary, in uraemic patients not yet on dialysis a weak negative correlation has been reported between BMD and serum leptin adjusted to body fat mass ($r = 0.20$; $P < 0.05$ in a 113 patient population) [21]. Interestingly the mean leptin values were lower in that study than in our population (46.4 ± 8.5 in women and 12.5 ± 1.8 ng/ml in men) and polynomial regression analysis was not used, so that any threshold research was not done.

Negative relations between serum leptin and bone turnover markers were also noted in the Mayo Clinic Cohort [10]. These relations were more consistent with bone resorption markers (i.e. urine N telopeptide of type I collagen) than with bone formation markers (i.e. osteocalcin and C-terminal propeptide of collagen-I but not bone alkaline phosphatase), suggesting a preponderant anti-resorptive effect of leptin mediated by the related increase in osteoprotegerin over RANK ligand. In the present study, only bone alkaline phosphatase was measured and no correlation with leptin was found, in accordance with the Mayo Clinic Cohort funding.

We also found no correlation with serum PTH. This is noteworthy as among cases of human leptin deficiency [22] PTH levels were increased in three patients and associated with a decrease BMD in one case. Furthermore, in haemodialysis patients Kokot *et al*. [23] suggested that PTH could have a negative effect on serum leptin concentration. Our observations are not in accord with this suggestion.

Finally the main limitation of the present study is the relative small sample size and the observational nature. Therefore, it has not the pretension to demonstrate a positive bone effect of leptin. The positivity of the link between BMD and serum leptin in all the patients and more specifically in those with a serum leptin level above the theoretical threshold of leptin brain transport is however compatible with this hypothesis.

Conclusion

Our study establishes for the first time, in a mixed population of chronic haemodialysis patients, a positive link between BMD and serum leptin levels which persists after adjustment for BMI, PTH concentration and duration on dialysis.

This link is particularly evident at bone sites where mechanical forces have the least effect on BMD, and when considering only leptin values above the presumed saturation threshold of the leptin blood–brain transporter. The lack of correlation between serum leptin and BMD for serum concentrations below this threshold is remarkable; it is independent of major uraemia-linked, which potentially influence bone density.

These findings provide support to an innovative hypothesis of a balance between a direct positive bone effect of peripherally acting leptin and an indirect the negative bone effect of centrally acting leptin. This

hypothesis clearly needs further studies to confirm our still speculative interpretation.

The bone-sparing effect of high serum leptin levels could account for the better BMD preservation with aging in women than in men on dialysis treatment as estimated free leptin levels were >2-fold higher in uraemic females, whereas all the uraemia and dialysis-related risk factors of osteopenia were comparable in the two genders.

Conflict of interest statement. None declared.

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