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Bone mass does not correlate with the serum fibroblast growth factor 23 in hemodialysis patients

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Circulating fibroblast growth factor 23 (FGF23) increases renal phosphate excretion, decreases bone mineralization and is markedly increased in hemodialysis patients. Bone cells express fibroblast growth receptor 1, suggesting that FGF23 could alter bone mineralization by means of a direct effect on the skeleton and/or secondarily due to hypophosphatemia. To distinguish between these possibilities we measured serum concentrations of FGF23, parathyroid hormone, phosphate, calcium, and markers of bone remodeling, and assessed bone mineral density in 99 hemodialysis patients. FGF23 concentrations were increased in all hemodialysis patients, even in those without hyperphosphatemia, and positively correlated with serum phosphate but not with parathyroid hormone. Hemodialysis did not decrease the serum FGF23 concentration. We found no significant correlation between serum FGF23 levels and bone mineral density. Further analysis by gender or *T*-score did not modify these results. Serum markers of bone remodeling significantly correlated with parathyroid hormone but not with FGF23 levels. The increase in serum FGF23 concentration in hemodialysis patients cannot be solely ascribed to hyperphosphatemia. Our study suggests that the effects of FGF23 on bone mineralization are mainly due to hypophosphatemia and not a direct effect on bone.

Kidney International (2008) **73,** 102–107; doi:10.1038/sj.ki.5002622; published online 17 October 2007

KEYWORDS: bone; mineralization; fibroblast growth factor 23; phosphate; hemodialysis

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Received 3 May 2007; revised 9 July 2007; accepted 14 August 2007; published online 17 October 2007

Fibroblast growth factor 23 (FGF23) is a recently identified molecule involved in the control of phosphate homeostasis and calcitriol metabolism. This circulating 32 kDa peptide is secreted by bone cells in response to hyperphosphatemia and vitamin D therapy.[1–3] In healthy subjects, plasma FGF23 concentration begins to rise 6–8 h after a high-phosphate diet[4] and is found constantly elevated in chronic hyperphosphatemic states.[4,5] Administration of 1alpha,25-dihydroxyvitamin D3 ($1,25OH_2D_3$) in animals increases the expression of FGF23 mRNA in bone cells and subsequently its serum level.[6,7] This effect is mediated through the binding of $1,25OH_2D_3$ to the vitamin D-responsive elements present in the promoter region of the FGF23 gene.[8]

FGF23 reduces serum phosphate levels at least by two mechanisms: it decreases renal phosphate reabsorption by lowering renal sodium–phosphate transporter (NPT)2a and NPT2c expression and it diminishes plasma $1,25OH_2D_3$ levels by inhibiting 1α-hydroxylase and by stimulating 24–25 hydroxylase activities.[9–14] The reduction in $1,25OH_2D_3$ results in the diminution of intestinal sodium phosphate co-transporter NPT2b expression.[9,15]

Over-production of FGF23 in subjects with normal renal function induces hypophosphatemia, low plasma $1,25OH_2D_3$ levels, high serum parathyroid hormone (PTH) concentration, and severe bone demineralization.[16–18] In contrast, the decline of renal function in patients with chronic kidney disease (CKD) is associated with a progressive augmentation of circulating FGF23 concentration, which can be due to serum phosphate accumulation and/or the decrease of the renal clearance of FGF23.[19–21] Once on dialysis therapy, serum FGF23 levels are markedly increased and positively correlated with serum phosphate levels.[22] In addition, in dialysis patients, high serum FGF23 concentration seems to predict the occurrence of refractory secondary hyperparathyroidism as reported by several groups.[23,24]

FGF23 might directly interfere with bone mineralization independently of its hypophosphatemic effect and its negative regulation of plasma $1,25OH_2D_3$ levels. This is highly suggested by the presence of the fibroblast growth factor receptor type 1, which is one of the FGF23 putative co-receptors, in osteoblast and osteoclast cells,[25] and by the

rapidity and severity of bone demineralization occurring after administration of FGF23 in animal models, which cannot be exclusively explained by the renal effects.[26,27] If FGF23 modified bone mineralization by a direct action on bone cells, this effect should persist or even be magnified in hemodialysis patients with extremely high serum FGF23 levels and often markedly bone demineralization. Therefore, the purpose of this study was to determine whether serum FGF23 levels were associated with bone mineral content, as assessed by dual-emission X-ray absorptiometry and bone circulating biomarkers, in 99 patients on maintenance hemodialysis.

RESULTS

Circulating FGF23 levels and correlation with serum phosphate concentration

The main demographic and biological characteristics of the 99 hemodialysis patients participating in the study are presented in Table 1. The distribution of pre-dialysis serum phosphate concentration is shown in Figure 1a. Mean serum phosphate concentration was above the normal range; however, it was below the upper normal value (1.40 mM) in 24 patients and below normal values (<0.85 mM) in 4 of them (Figure 1a and b). Serum FGF23 concentrations were markedly increased in all of the patients (mean $\pm$ s.d.: $15\,692 \pm 17\,496$ RU ml^{-1}; range 1030–69 050 RU ml^{-1}). They positively correlated with serum phosphate (Figure 1b) and calcium concentrations and with serum calcium phosphate product (Figure 1c) but not with serum PTH concentration (Figure 1d).

Table 1 | Patient characteristics

Age (years)	58.6 $\pm$ 14, range 21–87, median 60
Gender (M/F) (number)	69/30
Duration of dialysis (years)	6.8 $\pm$ 6.9, range 1–29, median 4.0
Pre-dialysis serum total calcium (mmol l^{-1})	2.34 $\pm$ 0.19, range 1.77–2.90
Pre-dialysis serum phosphate (mmol l^{-1})	1.94 $\pm$ 0.63, range 0.52–3.48
Post-dialysis serum phosphate (mmol l^{-1})	0.94 $\pm$ 0.22, range 0.42–1.50
Intact PTH (pg ml^{-1})	304 $\pm$ 290, range 3–1155
Plasma 25OHD$_3$ (ng ml^{-1})	13 $\pm$ 8, range 4–47
Plasma calcitriol (pg ml^{-1})	13 $\pm$ 10, range 4–88
Bone-specific alkaline phosphatase (ng ml^{-1})	15 $\pm$ 12, range 2–81
Cross-laps (nmol l^{-1})	18 $\pm$ 14, range 2–95

F, female; M, male; 25OHD$_3$, hydroxyl vitamin D3; PTH, parathyroid hormone. All results are presented as mean $\pm$ s.d.

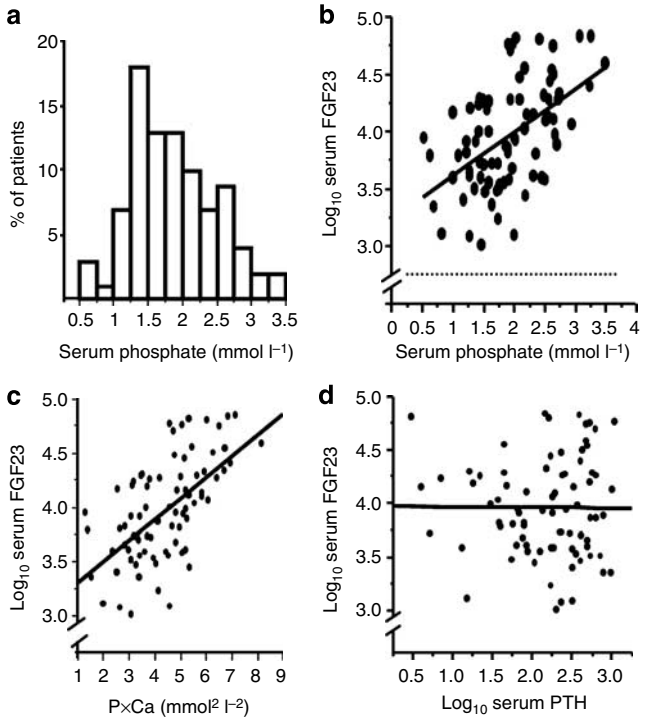

Figure 1 | Serum phosphate and FGF23 concentrations in hemodialysis patients. (**a**) Distribution of pre-dialysis serum phosphate concentration in 99 patients on maintenance hemodialysis. (**b**) Correlation between pre-dialysis serum phosphate and FGF23 concentration expressed as $\log_{10}$ values ($n = 99$, $P < 0.0001$, $r^2 = 0.259$). (**c**) Correlation between serum calcium phosphate ion product (Ca $\times$ P) and FGF23 concentration expressed as $\log_{10}$ values ($n = 99$, $P < 0.0001$, $r^2 = 0.344$). (**d**) Correlation between pre-dialysis serum PTH and FGF23 concentration, both expressed as $\log_{10}$ values ($n = 99$, $P = 0.881$, $r^2 = 0.0003$).

Effect of hemodialysis procedure on serum FGF23 concentration

To determine if the hemodialysis procedure decreased FGF23 levels, we measured serum FGF23 concentration before starting and at the end of the hemodialysis session in 23 patients. As shown in Figure 2, serum FGF23 concentration was significantly higher after the hemodialysis procedure (pre-dialysis $16\,241 \pm 14\,432$; post-dialysis $20\,791 \pm 19\,366$). This statistically significant difference still persisted after correction for the hemoconcentration following the dialysis ultrafiltration.[28] FGF23 was undetectable in the dialysis fluid exiting the dialyzer filter. Furthermore, pre-dialysis serum FGF23 levels, in the whole population, did not correlate with the dialysis efficacy as assessed by the percentage of reduction in plasma urea after the dialysis and the K_t/V.[29]

Correlation between serum FGF23 concentration and bone mineralization

We report here the measurement of bone mineral density (BMD) at four sites: lumbar spine, femoral neck, mid-radius, and total body. The distributions of BMD expressed as *Z*-scores at various sites in the present population of hemodialysis patients were close to the normal distribution (Figure 3). *Z*-score was lower than -2.5 in 5, 10, and 45% of patients at femoral neck, lumbar and radius sites, respectively (Figure 3a–c). We found no significant correlation between serum FGF23 concentrations and BMD, either using absolute values or *T*-scores or *Z*-scores at any site (Figure 4). The analysis of the correlations by gender or by *T*-score did not modify the results. We also considered the relation between serum FGF23 and PTH concentrations and serum bone-

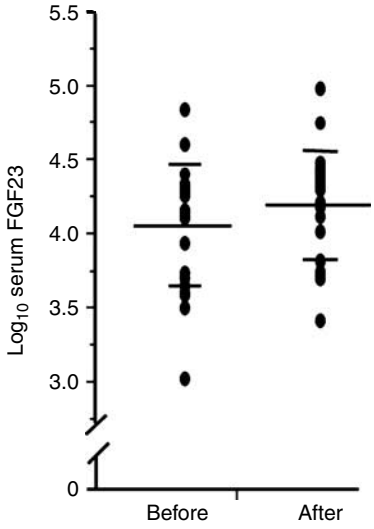

Figure 2 | Concentration of serum FGF23 concentration before starting and at the end of the 4-h hemodialysis procedure in 23 patients, expressed as $\log_{10}$ values ($n = 23$, $P < 0.0001$, Student's paired *t*-test).

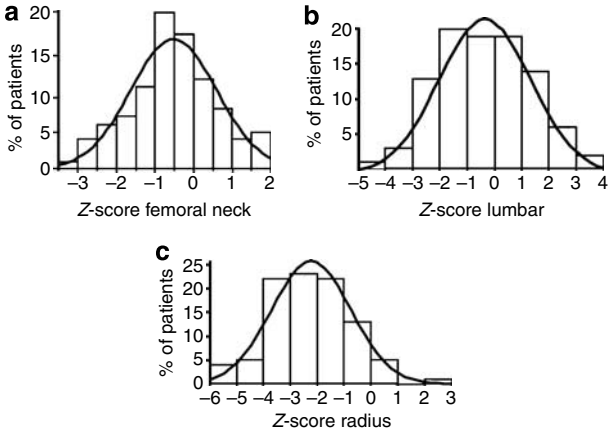

Figure 3 | Distribution of BMD expressed as *Z*-scores at three sites. BMD was measured by dual-emission X-ray absorptiometry at the (**a**) femoral neck, (**b**) lumbar spine (vertebrae L2–L4), and (**c**) mid-radius.

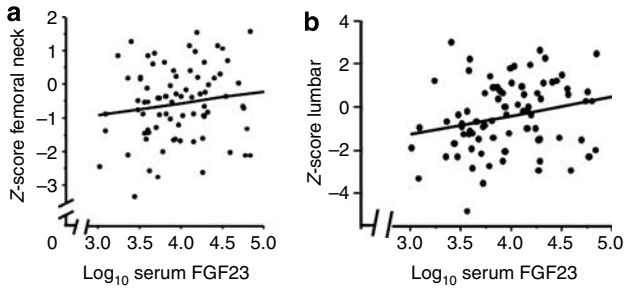

Figure 4 | Correlation between serum FGF23 concentration, expressed as $\log_{10}$ values, and BMD expressed as *Z*-scores at the (a) femoral neck ($n = 99$, $P = 0.239$, $r^2 = 0.01$) and (b) lumbar spine (vertebrae L2–L4) ($n = 99$, $P = 0.063$, $r^2 = 0.045$).

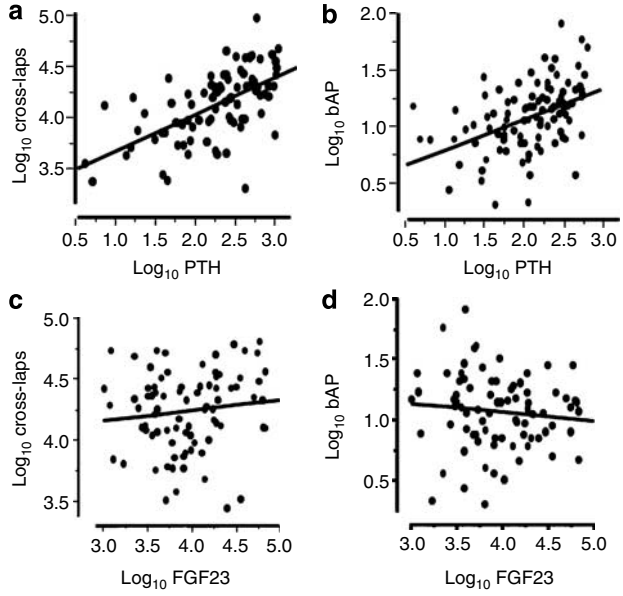

Figure 5 | Correlation of circulating markers of bone remodeling with serum PTH and FGF23 concentration. (**a**) Correlation between serum cross-laps and serum PTH levels ($n = 99$, $P < 0.0001$, $r^2 = 0.349$). (**b**) Correlation between serum bone-specific alkaline phosphatase (bAP) and serum PTH ($n = 99$, $P < 0.0001$, $r^2 = 0.190$). (**c**) Correlation between serum cross-laps and serum FGF23 levels ($n = 99$, $P = 0.317$, $r^2 = 0.014$). (**d**) Correlation between bAP and FGF23 concentration ($n = 99$, $P = 0.392$, $r^2 = 0.01$). All values are expressed as $\log_{10}$ because of their known uneven distribution.

specific alkaline phosphatase and cross-laps levels, two circulating markers of bone remodeling. Both markers significantly correlated with serum PTH concentration (Figure 5a and b) but not with serum FGF23 levels (Figure 5c and d).

Correlation between serum C-terminal and intact FGF23 levels

To check that elevated FGF23 concentrations were not due to the accumulation of C-terminal FGF23 by-products, we measured serum intact FGF23 levels with a specific sandwich ELISA (Kainos Laboratories Inc., Tokyo, Japan) in a subgroup of 14 hemodialysis patients. The mean value was $8223 \pm 2084\ \mathrm{pg\,ml^{-1}}$ (normal values $< 40\ \mathrm{pg\,ml^{-1}}$). There was an excellent positive correlation between serum C-terminal and intact fibroblast growth factor (FGF23) ($n = 14$, $P < 0.0001$, $r^2 = 0.804$) (Figure 6).

DISCUSSION

In this study, we extend previous data demonstrating that serum FGF23 levels are extremely elevated in all CKD hemodialysis patients, including subjects with low and normal pre-dialysis serum phosphate concentration, and that those levels positively correlated with serum FGF23 concentrations. For the first time, we demonstrate that FGF23 is not cleared by the dialysis membrane, and that

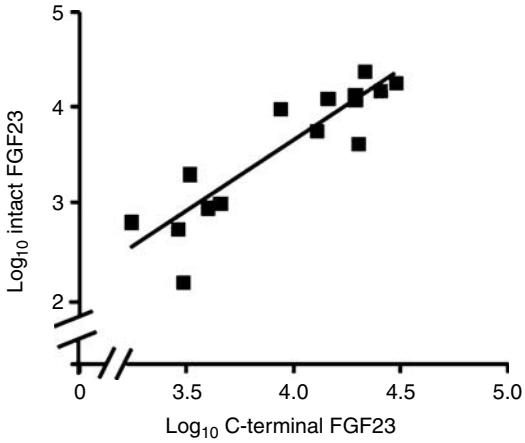

Figure 6 | Correlation between the logarithm of serum C-terminal and intact FGF23 concentrations in a subgroup of 14 hemodialysis patients ($n = 14$, P<0.0001, $r^2 = 0.804$). Serum FGF23 concentrations were determined by two assays: the Immutopics assay recognizes an epitope located in the C-terminal part of FGF23 peptide and measures intact and C-terminal FGF23 peptides; the assay from Kainos measured only the intact (full-length) FGF23 peptide.

serum FGF23 levels are not reduced by the dialysis procedure in spite of the significant reduction of serum phosphate concentration. Moreover, no correlation exists between serum FGF23 levels and BMD at any of the four skeletal sites explored, nor with several circulating biomarkers of bone remodeling.

Serum FGF23 levels increase at the early stage of renal diseases to compensate for the reduction of phosphate excretion accompanying the decrease in glomerular filtration rate. When these patients arrive at the end stage of renal disease and are treated by hemodialysis, the increase in serum FGF23 levels is more pronounced than what it is expected for the degree of hyperphosphatemia. This is also illustrated by our results showing that hyperphosphatemia cannot be the explanation for the elevation of serum FGF23 levels in hemodialysis patients with normal or low serum phosphate concentrations. In these patients, serum FGF23 levels were more than 10 times above the higher values observed in healthy subjects and they only weakly correlated with serum phosphate levels. Thus, a significant fraction of the elevated serum FGF23 comes probably from its accumulation in the organism because of the lack of its renal catabolism or its renal clearance in these hemodialysis patients with practically no residual renal function. In addition, despite its relative low molecular weight of 32 kDa, FGF23 could not be removed by the dialysis procedure. There was even a statistically significant increase in serum FGF23 levels after the dialysis procedure, which will need further investigation to understand the mechanisms. The possibility that the kidney could participate in FGF23 elimination is also suggested by the fact that FGF23 was detected in the urine of one healthy volunteer.[19]

In the hemodialysis population, the increase in serum FGF23 concentration could also reflect an impaired regulation of the synthesis and/or secretion of FGF23, similar to the over-production of PTH observed in the case of autonomous hyperparathyroidism. This is also suggested by the state of tertiary hyperphosphatoninism recently described in hypophosphatemic kidney transplanted patients.[30] Although some authors suggested that PTH stimulates the synthesis of FGF23, our findings, showing no correlation between PTH and serum FGF23 levels, argue against any important role of PTH in the augmentation of serum FGF23 levels.

Vitamin D therapy could also be involved in the augmentation of serum FGF23 concentrations; indeed, administration of calcitriol in hemodialysis patients significantly increases serum FGF23 concentration.[2] However, we did not observe any correlation between serum levels of FGF23 and plasma levels of vitamin D and calcitriol or the treatment by vitamin D analogs.

Bone demineralization and fractures are frequent in hemodialysis patients. The mechanism of bone demineralization in CKD patients is complex and not completely understood.[31–33] High PTH and low plasma $1,25(OH)_2D_3$ levels certainly contribute to the pathogenesis of this disorder but cannot completely explain it.[34] High levels of serum FGF23, which can predict the severity and resistance of secondary hyperparathyroidism in dialysis patients, have now been added to the list of these factors.[23,24]

Although high serum FGF23 concentrations cannot induce hypophosphatemia in dialysis patients, it is unknown whether the massive accumulation of FGF23 could directly affect bone mineralization. We did not find any correlation between serum FGF23 levels and BMD nor with bone remodeling biomarkers in this study population. Our results suggest that FGF23 may not have any direct effect on bone mineralization in CKD dialysis patients. The decrease in bone mineral content observed in subjects with normal renal function is highly probable secondary to the renal phosphate loss and hypophosphatemia.

In conclusion, in CKD patients on maintenance hemodialysis, FGF23 accumulates in the circulation and correlates with serum phosphate levels but is not associated with decreased BMD.

MATERIALS AND METHODS

A total of 99 adult, Caucasian patients on maintenance hemodialysis (30 females and 69 males) were included in this cross-sectional study in accordance with the national guidelines for research on human subjects. Mean duration of hemodialysis treatment was 6.8 ± 6.9 years. All the patients were treated by conventional 4–5 h hemodialysis, thrice a week, using hollow-fiber dialyzers, against a dialysis bath containing 32–36 mmol l^{-1} of bicarbonate, 0.85 mmol l^{-1} of magnesium, 1.50–1.75 mmol l^{-1} of calcium, and 2–3 mmol l^{-1} of potassium. The underlying kidney diseases were chronic glomerulonephritis (24), diabetes (15), chronic interstitial nephritis (12), congenital nephropathy (7), polycystic kidney disease (13), hypertensive nephrosclerosis (7), and other and unknown diseases (21). Patient characteristics are presented in Table 1.

At the time of evaluation, none of the patients were receiving or had received within the 3 months before the study estrogen, calcitonin, bisphosphonate, or recombinant PTH. Two patients were receiving less than 5 mg day^{-1} of corticoids for β-2-microglobulin amyloid arthropathy, 13 patients were receiving vitamin D derivatives either orally or intravenously, and 46 patients were receiving 0.5–3 g day^{-1} of calcium carbonate. Seven patients have had previous kidney transplantation. There was no clinical or biological evidence for other bone diseases such as osteomalacia, aluminum-related bone disease, and Paget's disease. The main clinical and biochemical features of patients are presented in Table 1.

BIOCHEMICAL ANALYSIS

Unless otherwise stated, all measurements were performed on blood samples obtained just before starting hemodialysis session and after a 12-h fast. Plasma calcium was determined using atomic absorption spectrometry and plasma phosphorus using a Technicon Auto Analyzer. Plasma PTH concentration was measured using a commercial radioimmunometric assay for intact human PTH 1–84 (Elecsys, from Roche Diagnostic, Meylan, France). The range of normal values was between 15 and 65 pg ml^{-1}. Serum bone-specific alkaline phosphatase was measured using a radioimmunometric assay (Tandem-R, Ostase) provided by Hybritech Europe SA, Belgium. The mean ± s.d. value obtained in normal adult individuals was 11.8 ± 4.3 ng ml^{-1} (normal range 4.0–25.0 ng ml^{-1}). Plasma vitamin D and $1,25(OH)_2D_3$ were measured by radiocompetition assays. Normal values for plasma vitamin D were 10–40 ng ml^{-1} and for $1,25(OH)_2D_3$, 20–60 pg ml^{-1} for $1,25(OH)_2D_3$. Serum cross-laps levels were measured using an ELISA from Osteometer, Biotech, Denmark. Normal values were 1.74 ± 0.74 nmol l^{-1} in premenopausal women and 3.01 ± 1.55 nmol l^{-1} in postmenopausal women.

Serum FGF23 levels were measured using an ELISA kit from Immutopics (cterm FGF23, San Clemente, CA, USA). Normal values ranged between 0 and 130 RU ml^{-1}. We also measured serum intact FGF23 by a sandwich ELISA purchased from Kainos Laboratories Inc. (Tokyo, Japan) as described previously.[35] This FGF23 assay utilizes specific antibodies directed against the N- and C-terminal regions of human FGF23 that allow the detection of only full-length FGF23. Normal values for this assay ranged between 0 and 40 pg ml^{-1}.

BMD MEASUREMENTS

BMD was measured with a dual-energy X-ray absorptiometry (Lunar DPX densitometer) from Lunar Corporation (Madison, WI, USA). All the BMD measurements were performed by the same experienced operator. The densitometer was calibrated everyday with a standard phantom specimen. The following sites were examined: the total body, lumbar spine (vertebrae L2–L4), the femoral neck, trochanter, and mid-radius. Scans were performed at the two forearms but only the results at the non-dominant forearm were considered, with the exception in the case of arteriovenous fistula influence (three patients). BMD results are presented as *T*-score. *T*-score is the number of standard deviations from the mean of a healthy young adult population (20–40 years old); it is used for the definition of osteopenia (*T*-score between −1.0 and −2.5) and osteoporosis (*T*-score < -2.5). The reference values were obtained from a French normal population aged between 20 and 89 years, issued from several centers and provided by Lunar France.

CONFLICT OF INTEREST

Dr Ureña Torres reports receiving consulting and lecture fees from Abbott, Amgen, Astra Zeneca, and Shire.

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