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ORIGINAL ARTICLE

Body composition, adipokines, FGF23-Klotho and bone in kidney transplantation: Is there a link?

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Abstract

Background Kidney transplantation—associated mineral and bone disorder (KT-MBD) still represents a black box on the long-term due to scarce available data. We aimed to investigate the impact of non-classical bone regulating factors (body composition, adipokines, inflammatory markers, fibroblast growth factor 23—FGF23 and α-Klotho) in long-standing kidney transplant (KT) recipients compared to the general population.

Methods Our cross-sectional study, enrolling 59 KT patients and age, sex and body mass index—matched healthy general population volunteers, assessed the predictive role of the body composition, serum adipokines (leptin, adiponectin, resistin), inflammatory markers (erythrocyte sedimentation rate, C-reactive protein) and parathyroid hormone (PTH)—FGF23/α-Klotho axis upon bone mineral density (BMD) and osteocalcin, using correlation and linear multiple regression.

Results The 59 KT recipients (mean transplantation span of 57.7 ± 7.2 months) had similar body composition but significantly lower BMD ($p < 0.01$) compared to the general population group. Total lean mass was independently associated with BMD in both groups. In KT patients, age, time spent on dialysis and PTH were the main negative independent predictors of BMD, after adjusting for possible confounders. Resistin and α-Klotho also negatively predicted lumbar bone density ($p < 0.001$), while adiponectin and α-Klotho positively predicted osteocalcin levels ($p < 0.001$) in KT recipients, independently of inflammatory markers. No significant associations were found between FGF23 and bone parameters in any of the groups.

Conclusions Age, PTH, time on dialysis and lean mass are among the main bone density predictors in long-standing KT patients. The bone impact of adipokine dysregulation and of α-Klotho merits further investigations in KT-MBD. Preserving lean mass for improved bone outcomes should be part of KT-MBD management on the long-term.

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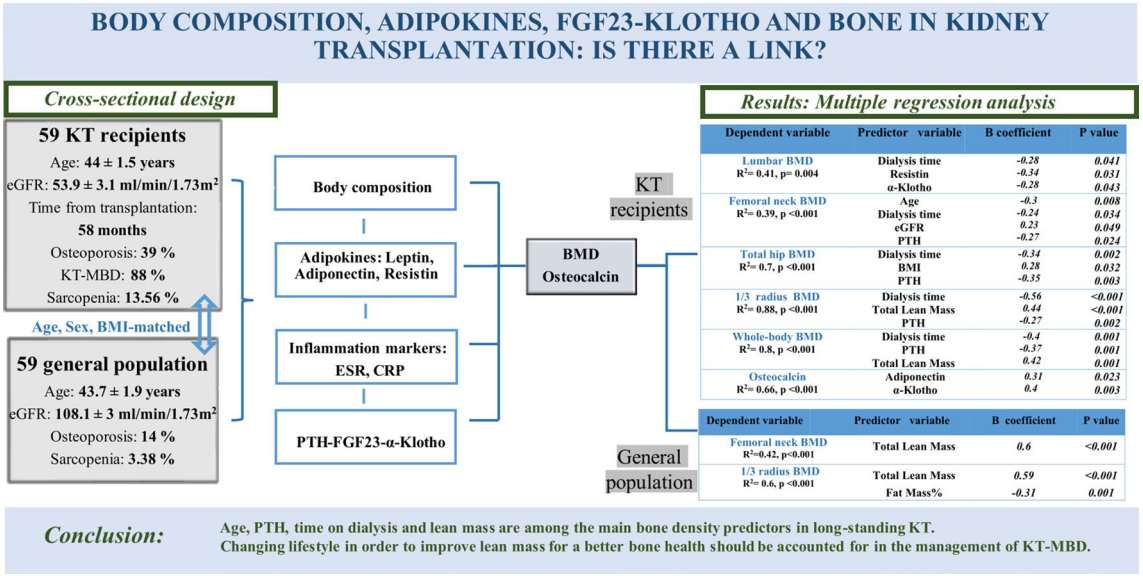
Graphic abstract



Keywords Kidney transplantation · Adipokines · FGF23 · α-Klotho · Bone mineral density

Introduction

Chronic kidney disease—mineral and bone disorder (CKD-MBD) is a major cause of morbidity that proceeds after renal transplantation. Laboratory abnormalities, such as persistent secondary hyperparathyroidism, are common even beyond 12 months post-transplantation [1]. Bone metabolism in kidney transplant (KT) recipients is challenged by both the legacy of the pre-transplantation CKD-MBD (changes in bone turnover, mineralization and volume due to low serum levels of 1,25-dihydroxy vitamin D and the consequent hypocalcemia and hyperparathyroidism) and by the new risk factors that also join in: the immunosuppressive therapy, proton pump inhibitor (PPI) treatment to prevent corticosteroid-induced peptic ulcer, electrolyte disturbances (such as hypophosphatemia or drug-induced hypomagnesemia and hypercalciuria), reduced physical activity and sarcopenia further impair bone mineralization and promote uncoupling of bone resorption and formation (high bone resorption coexisting with a low formation rate) [2–5].

Age, body weight and parathyroid hormone (PTH) are classical bone mass regulators in the general population and also in KT patients [6]. Non-classical bone regulators such as body composition, adipokines, fibroblast growth factor 23 (FGF23) and α-Klotho may also have a significant impact upon the KT—associated mineral and bone disorder (KT-MBD) development.

Sarcopenia is one of the risk factors for osteoporosis in the elderly general population [7]. Sarcopenia is common among CKD patients, being inversely related to the estimated glomerular filtration rate (eGFR) and reaching up to 50% prevalence in hemodialysis patients [8]. A significant more-than-expected fat mass relative to body mass, accompanied by an inappropriately low muscle mass—similar to sarcopenic obesity—is also reported in KT [9]. The true impact of lean and fat tissue, respectively, upon the bone in KT lacks full understanding, as changes in body composition also correlate with the bone active adipokines [10]. Besides adipokines malfunction, the systemic chronic inflammation—common in KT [11]—might be involved in changes in the bone-fat-muscle crosstalk, as recently reviewed by Kirk et al. [12].

Finally, higher FGF23 concentrations at the time of kidney transplantation are associated with bone loss in the first year post-transplantation [13]. FGF23 decreases faster than PTH after kidney transplantation but remains inappropriately elevated when related to phosphate concentration [2]. This "tertiary hyperphosphatoninism", as described by Kanaan et al. [13], may have a detrimental impact upon bone mineralization in KT. Moreover, certain α-Klotho polymorphisms are associated with an increased co-receptor function for FGF23 in KT, possibly contributing to bone loss via enhanced inhibition of 1α-hydroxylase [14]. Therefore, the pathophysiology of the FGF23/α-Klotho—phosphate imbalance and its bone consequences in KT patients is yet to be clarified.

We aimed at evaluating the predictive role of non-classical bone regulators, such as body composition, inflammatory and adipose—related markers, FGF23 and α-Klotho, upon bone mineral density (BMD) in long standing KT subjects. A secondary objective of our study was to compare these parameters between KT patients and the general population.

Materials and methods

Study design

We performed a cross-sectional study in which KT patients aged between 20 and 60-years-old with a successful kidney transplant were included, irrespective of the donor type, history, type of dialysis before transplantation or immunosuppressive therapy, respectively. Exclusion criteria were represented by pregnancy, dialysis, acute graft rejection, urinary tract infections, bone active therapy other than vitamin D preparations, diabetes mellitus, menopause (defined as more than 12 months since natural cessation of menstrual cycles), secondary causes of osteoporosis other than CKD-MBD (e.g., primary hyperparathyroidism, thyrotoxicosis, hypercortisolism, severe bone trauma, inflammatory bowel disease, congenital bone disease, anorexia nervosa, malignancy).

Three hundred forty-seven KT patients who attended the Nephrology Department for regular follow-up between April and November 2019 were invited to participate in the study. After the exclusion of those that did not fulfill the study criteria or refused to take part, 59 KT subjects were enrolled.

To perform a comparative analysis regarding serum and bone parameters, 59 general population volunteers referred by the general practitioner to our outpatient department for health check-up were included in an age, sex and body mass index (BMI) one-to-one matched control group. All general population volunteers had a normal eGFR (≥ 90 ml/min/1.73 m^2), followed the same exclusion criteria and were selected by individual matching in the same period as the KT subjects.

All subjects underwent a comprehensive medical evaluation with a complete history (cross-checked with the medical record) and physical examination. During a one-time visit, a fasting morning blood draw of 12 ml for serum determinations (serum aliquots were stored at – 80 °C until analysis) and Dual X-ray Absorptiometry (DXA) for bone and body composition assessment were performed.

Measurements

BMI was calculated as weight (kg)/[height (m)]^2. Serum levels of leptin, adiponectin, resistin, FGF23, α-Klotho and osteocalcin were quantified using commercially available ELISA research kits (Elabscience Biotechnology, USA). Serum PTH was quantified by electrochemiluminescence, using commercial kits (Advia Centaur Intact PTH Assay, Siemens Healthcare Diagnostics Inc., USA). Serum concentrations of calcium, phosphate and magnesium were determined by colorimetry with a Cobbas 6000 (Roche) automated analyzer. Serum creatinine was measured by a kinetic colorimetric assay and eGFR was calculated using the validated CKD-EPI equation [15]. Erythrocyte sedimentation rate (ESR) was assessed by capillary photometry (ALIFAX ROLLER 20 LC) and C-reactive protein (CRP) was determined via the latex immunoturbidimetric method (ALINITY I). Alfacalcidol does not apparently modify serum 25(OH)D, despite increasing 1,25-dihydroxyvitamin D [16]. Since all KT were under alfacalcidol supplementation, we did not evaluate, however, endogenous levels of serum 25(OH)D as the results would not have been relevant for interpretation.

BMD at the lumbar spine, femoral neck, total hip, 1/3 radius and whole-body levels were measured via DXA (Hologic Delphi A; Hologic Inc., USA). Osteoporosis was defined as Z-score ≤ -2 for premenopausal women and men under 50 years of age and T-score ≤ -2.5 for men above 50 years of age [17].

Body composition parameters (total fat mass, trunk and lower limbs fat mass, total and appendicular lean mass) were measured along with the BMD during the whole-body DXA scan. Appendicular lean mass index was calculated as appendicular lean mass (kg)/[height (m)]^2. Sarcopenia was defined as appendicular lean mass index < 7.23 kg/m^2 in men and < 5.67 kg/m^2 in women, respectively [18]. Trunk-to-leg fat ratio was calculated as trunk fat mass (g)/lower limbs fat mass (g). Measurements were made by two internationally certified technicians according to standard protocol.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS version 18.0 for Windows, IBM SPSS Inc.). Data are expressed as mean ± standard error of the mean. Normal distribution was assessed using the Shapiro–Wilk test. Student's paired t test (for normally distributed data) and the non-parametric Mann–Whitney U test (for skewed data) were employed to evaluate between-group differences. Linear relationships were assessed using Pearson correlations for normally distributed data and Spearman correlations for skewed data, respectively. Significant correlations were introduced in multiple regression analysis in order to evaluate independent predictors of bone parameters after adjusting for potential confounders. The level of significance was established for a p-value < 0.05.

Results

Descriptive results

The 59 KT patients with a mean age of 44 years and a mean eGFR of 53.9 ± 3.1 ml/min/1.73m^2 (corresponding to CKD stage G3aT) had a mean time elapsed from transplantation of 58 months, during which a mean cumulative corticosteroids dose of approximately 11 g prednisone equivalent was administered (Table 1). At the time of the study, the 59 patients were under immunosuppression therapy with calcineurin inhibitors—cyclosporine ($n = 22$) or tacrolimus ($n = 37$), and almost all of them ($n = 55$) also associated mycophenolate mofetil (3 of the remaining patients associated either mTOR inhibitor Sirolimus ($n = 2$) or azathioprine ($n = 1$), while one patient was under tacrolimus therapy alone). 54 of the 59 KT recipients were also under a low daily maintenance dose of 5 mg prednisone (for which 29 of them also associated PPI drugs). All KT subjects were under alfacalcidol supplementation, and 4 of them had undergone parathyroidectomy before transplantation (Fig. 1); none received calcium supplements, antiresorptive drugs (bisphosphonates and denosumab) or cinacalcet therapy. Only 5 of the 59 KT recipients were taking diuretics which may interfere with calcium metabolism, such as furosemide ($n = 2$) and indapamide ($n = 3$), respectively. Mean serum

Table 1 The descriptive characteristics of the study participants

Parameter	KT ($n = 59$)	GP ($n = 59$)	p value
Age (y)	44 ± 1.5	43.7 ± 1.9	0.11
Men:Women	30:29	30:29	–
Time from CKD diagnosis to transplantation (mo)	86.5 ± 12.6	–	–
Dialysis time (mo)	36.8 ± 6.5	–	–
Time elapsed from transplantation (mo)	57.7 ± 7.2	–	–
Cumulative corticosteroid dose (prednisone equivalent mg)	10,834.51 ± 7799.40	–	–
BMI (kg/m^2)	25.4 ± 0.5	25.4 ± 0.6	0.96
Total fat mass (kg)	22.96 ± 1.04	21 ± 1.2	0.23
Fat mass (%)	30.8 ± 1.02	28.3 ± 1.22	0.11
Total lean mass (kg)	48.8 ± 1.37	50.3 ± 1.5	0.45
Lean mass (%)	66.3 ± 0.98	68.7 ± 1.17	0.11
Trunk-to-leg fat ratio	1.2 ± 0.1	1.1 ± 0.1	0.32
Lumbar BMD (g/cm^2)	0.92 ± 0.2	0.96 ± 0.02	0.11
Femoral neck BMD (g/cm^2)	0.75 ± 0.02	0.84 ± 0.02	0.005
Total hip BMD (g/cm^2)	0.88 ± 0.02	0.97 ± 0.02	0.002
1/3 radius BMD (g/cm^2)	0.67 ± 0.01	0.74 ± 0.01	0.002
Whole-body BMD (g/cm^2)	1.03 ± 0.02	1.09 ± 0.01	0.007
Creatinine (mg/dl)	1.67 ± 0.1	0.85 ± 0.03	< 0.001
eGFR (ml/min/1.73m^2)	53.9 ± 3.1	108.1 ± 3	0.002
Ca (mg/dl)	9.2 ± 0.1	–	–
P (mg/dl)	3 ± 0.2	–	–
Mg (mmol/l)	0.74 ± 0.03	–	–
Osteocalcin (ng/ml)	63.5 ± 6.4	–	–
Leptin (ng/ml)	14.1 ± 2.5	50 ± 0.8	< 0.001
Adiponectin (ug/ml)	43.3 ± 0.9	9 ± 0.6	< 0.001
Resistin (ng/ml)	41.7 ± 3.6	5.7 ± 0.35	< 0.001
PTH (pg/ml)	115.4 ± 31.8	37.47 ± 15.46	0.018
FGF23 (pg/ml)	78.7 ± 8.1	73 ± 1.8	0.5
α-Klotho (ng/ml)	1.37 ± 0.13	–	–
ESR (mm/h)	20.41 ± 1.98	–	–
CRP (mg/dl)	0.47 ± 0.08	–	–

Data are expressed as mean ± standard error of the mean

BMD bone mineral density, *BMI* body mass index, *Ca* serum calcium, *CKD* chronic kidney disease, *CRP* C-reactive protein, *eGFR* estimated glomerular filtration rate, *ESR* erythrocyte sedimentation rate, *FGF23* fibroblast growth factor 23, *GP* general population group, *KT* kidney transplant group, *mo* months, *Mg* serum magnesium, *P* serum phosphate, *PTH* parathyroid hormone, *y* years

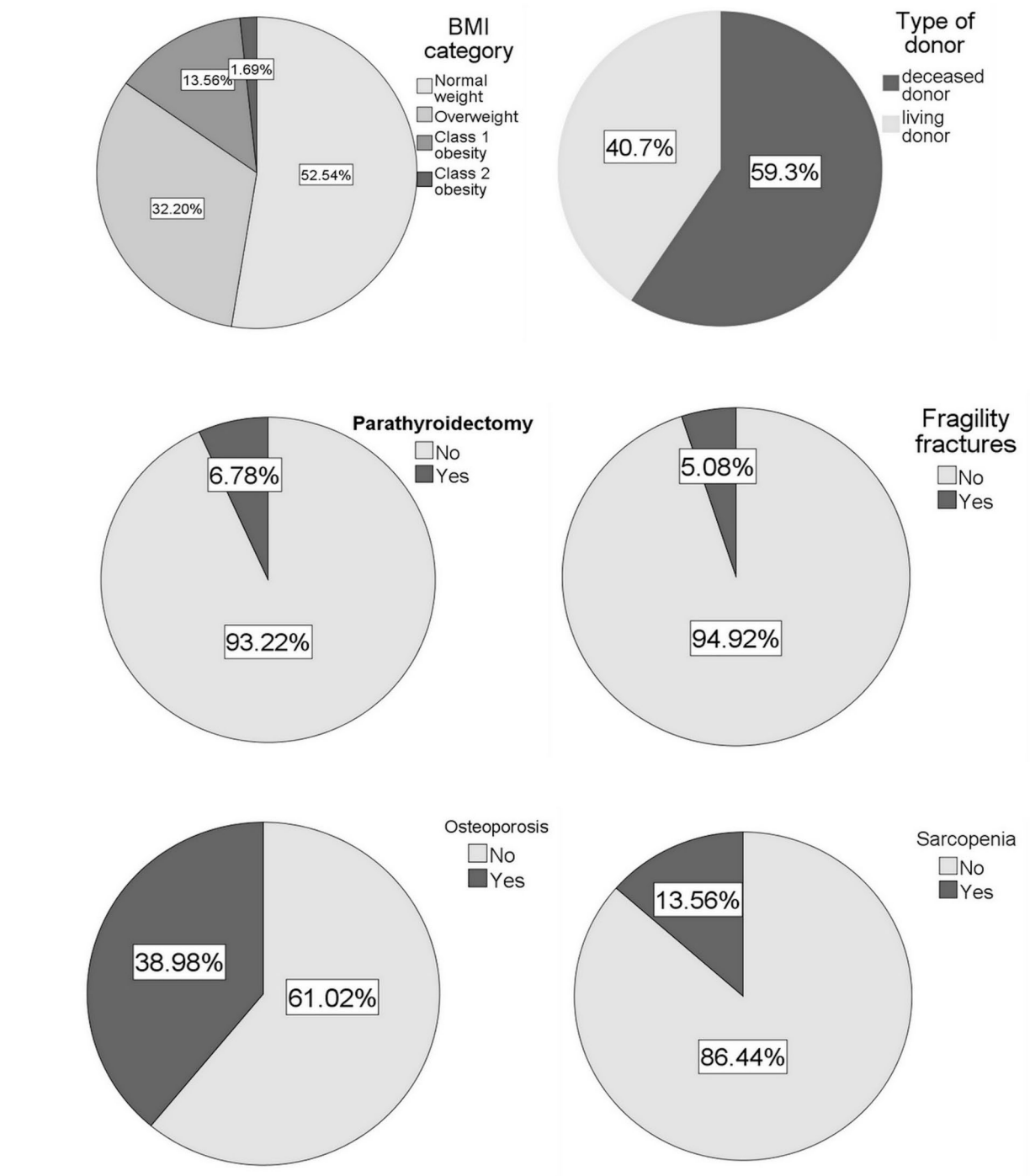


Fig. 1 The descriptive characteristics of the study participants

CRP levels were within the normal range in the KT patients, while ESR was slightly elevated (Table 1).

The KT recipients had a significantly lower BMD at the femoral neck, total hip, forearm, and whole-body levels compared to the general population ($p < 0.01$ for all, Table 1). Eighty-eight percent ($n = 52$) of the KT subjects had evidence of KT-MBD. In the KT group, 38.98% had osteoporosis and 5.08% had a history of fragility fractures (Fig. 1), compared to 13.56% cases of osteoporosis and no fragility fractures in the general population group. In KT subgroup analysis, BMD, serum calcium and PTH did not differ significantly between KT patients under PPI treatment and KT patients not taking PPI, respectively (data not shown).

Although comparative analysis confirmed similar total fat mass and lean mass in the KT and general population groups, sarcopenia was more prevalent in the KT group (13.56% vs. 3.38%, respectively—Table 1; Fig. 1). All sarcopenia cases in both groups associated low BMD.

Regardless of the presence of normocalcemic and normophosphatemic secondary hyperparathyroidism in the KT compared to the general population subjects, serum FGF23 did not differ significantly between the two groups (Table 1). However, the serum levels of adiponectin, leptin and resistin were considerably different in the KT when compared to the general population group, despite similar BMI and fat mass (Table 1).

Correlation analysis

Significant correlations in the KT group are shown in Table 2. Age, time spent on dialysis, fat mass percentage, adipokine (leptin, adiponectin and resistin) concentrations, PTH and α-Klotho negatively correlated with BMD at various sites. In contrast, eGFR, BMI and lean mass were all positively related to BMD. No significant relationship was found between FGF23 and BMD at any site (Table 2). Also, serum magnesium concentrations were not related neither to BMD and osteocalcin, nor to PTH (data not shown). Leptin, adiponectin, and α-Klotho were positively correlated with osteocalcin (Table 2). Moreover, the serum levels of PTH ($r = -0.38$, $p < 0.01$), leptin ($r = -0.47$, $p = 0.001$) and adiponectin ($r = -0.4$, $p < 0.01$) were inversely related to the eGFR.

Regarding inflammation markers in the KT patients, ESR negatively correlated with lumbar BMD, while no significant correlations were found between CRP and bone parameters. ESR—but not CRP—levels were negatively correlated with BMI ($r = -0.27$, $p = 0.05$) and lean mass ($r = -0.39$, $p = 0.004$), respectively (Table 2). No significant correlations were found between inflammation markers and adipokines (Table 2).

In the general population group, fat mass percentage was negatively related to bone density, while lean mass

Table 2 Results of correlation analysis in KT patients (only significant correlations are shown)

Variable	Lumbar BMD	Femoral neck BMD	Total hip BMD	1/3 radius BMD	Whole-body BMD	Osteocalcin	ESR	CRP
Age	–	− 0.41**	− 0.27*	–	–	–	–	–
Dialysis time	− 0.28*	− 0.3*	− 0.43**	− 0.54***	− 0.47***	–	–	–
Time from CKD diagnosis to transplantation	–	–	–	− 0.38**	− 0.34**	–	–	–
Time elapsed from transplantation	–	–	–	–	–	–	–	–
Cumulative corticosteroid dose	–	–	–	–	–	–	–	–
eGFR	–	0.39**	0.36**	0.32*	0.36**	–	–	–
BMI	–	–	0.29*	–	–	–	− 0.27*	–
Total lean mass	0.28*	–	0.33*	0.55***	0.53***	–	− 0.39*	–
Lean mass (%)				0.35**	0.27*	–	–	–
Total fat mass	–	–	–	–	–	–	–	–
Fat mass (%)	–	–	–	− 0.39**	− 0.31*	–	–	–
Trunk-to-leg fat ratio	–	–	–	–	–	–	–	–
Leptin	–	–	–	− 0.4 **	− 0.42**	0.28*	–	–
Adiponectin	− 0.35*	–	–	–	–	0.54***	–	–
Resistin	− 0.39**	–	–	− 0.28*	–	–	–	–
PTH	− 0.32**	− 0.55***	− 0.48***	− 0.39**	− 0.45***	–	–	–
FGF23	–	–	–	–	–	–	–	–
α-Klotho	− 0.31*	–	–	–	–	0.57***	–	–
ESR	− 0.35**	–	–	–	–	–		–
CRP	–	–	–	–	–	–	–	

BMD bone mineral density, *BMI* body mass index, *CKD* chronic kidney disease, *CRP* C-reactive protein, *eGFR* estimated glomerular filtration rate, *ESR* erythrocyte sedimentation rate, *FGF23* fibroblast growth factor 23, *KT* kidney transplant, *PTH* parathyroid hormone

*$p < 0.05$

**$p < 0.01$

***$p < 0.001$

positively correlated with BMD at all sites ($p < 0.05$ for all, data not shown). No significant correlations were observed between adipokines or FGF23, respectively, and BMD (data not shown).

Multiple regression analysis

Significant independent BMD and osteocalcin predictors in KT after adjusting for possible confounders such as sex, cumulative dose of corticosteroids, time from CKD diagnosis to transplantation, time elapsed from transplantation, inflammatory indices and smoking are depicted in Table 3. The regression curves of the main independent associations found in KT are presented in Fig. 2.

Age, dialysis time and PTH were the main negative independent predictors of BMD in the KT group. Resistin and α-Klotho also proved significant negative independent predictors of lumbar BMD. eGFR, BMI and total lean mass were positive independent predictors of femoral neck BMD, total hip BMD and whole-body BMD, respectively. Adiponectin and α-Klotho positively predicted osteocalcin levels in the KT group (Table 3; Fig. 2).

Table 3 Results of multiple regression analysis in KT patients after adjusting for sex, cumulative dose of corticosteroids, time from CKD diagnosis to transplantation, time elapsed from transplantation, ESR, CRP and smoking (only significant associations are shown)

Dependent variable	Predictor variable	B coefficient	*p* value
Lumbar BMD $R^2 = 0.41$, $p = 0.004$	Dialysis time	− 0.28	0.041
	Resistin	− 0.34	0.031
	α-Klotho	− 0.28	0.043
Femoral neck BMD $R^2 = 0.39$, $p < 0.001$	Age	− 0.3	0.008
	Dialysis time	− 0.24	0.034
	eGFR	0.23	0.049
	PTH	− 0.27	0.024
Total hip BMD $R^2 = 0.7$, $p < 0.001$	Dialysis time	− 0.34	0.002
	BMI	0.28	0.032
	PTH	− 0.35	0.003
1/3 radius BMD $R^2 = 0.88$, $p < 0.001$	Dialysis time	− 0.56	< 0.001
	Total lean mass	0.44	< 0.001
	PTH	− 0.27	0.002
Whole-body BMD $R^2 = 0.8$, $p < 0.001$	Dialysis time	− 0.4	0.001
	PTH	− 0.37	0.001
	Total lean mass	0.42	0.001
Osteocalcin $R^2 = 0.66$, $p < 0.001$	Adiponectin	0.31	0.023
	α-Klotho	0.4	0.003

BMD bone mineral density, *BMI* body mass index, *CKD* chronic kidney disease, *CRP* C-reactive protein, *eGFR* estimated glomerular filtration rate, *ESR* erythrocyte sedimentation rate, *KT* kidney transplant, *PTH* parathyroid hormone

In the general population group, total lean mass (positive) and fat mass percentage (negative) were independent BMD predictors (Table 4).

Discussion

Our study confirms the significant role of BMI and lean mass—besides that of the graft functionality—on maintaining a healthy bone mass in KT recipients. Mild systemic inflammation may negatively impact lean mass and thus contribute to sarcopenia, as shown by our study. Age, time spent on dialysis and PTH seem to have more deleterious effects upon the bone mass on the long-term in comparison with the duration of the CKD, the cumulative dose of corticosteroids or inflammation. Moreover, we found adipokine dysregulation to share bone-active properties, independently of inflammatory markers. Interestingly, α-Klotho—but not FGF23—was observed to have a significant impact upon bone mass and metabolism.

Osteosarcopenia was encountered in KT recipients in a few recent studies, being associated with time spent on dialysis, but also with the lack of physical exercise after transplantation [19]. The beneficial influence of lean mass upon bone is unanimously reported [20] and was observed both in the KT patients and general population in our study. Muscle contraction activates the mechanosensors in the osteocytes, thus triggering bone cortex osteoformation [21]. Regardless of similar BMI, sarcopenia was more frequently encountered in the KT than in the general population group, associating low bone mass in all cases. One of the causes of sarcopenia may be the adipocytic chronic inflammation related to the oxidative stress in the CKD setting which generates dysfunctional adipokine secretion and conversion of white adipose tissue to brown fat tissue, thus promoting energy loss [22]. Moreover, low-grade systemic inflammation was negatively related to lean mass in the KT patients from our study, similar to the geriatric population [23]. The absence of a direct correlation between inflammatory indices and the serum concentration of adipokines in our study does not necessarily exclude adipocytic inflammatory injury.

Low bone mass is frequent among KT patients [6]. Age significantly predicts bone mass in all populations [24], including in KT patients, as confirmed by our study. As previously reported [19], we showed that time spent on dialysis also has a major impact upon bone density in KT subjects, mirroring the long-lasting maladaptation to the uremic milieu [2].

Although still debated, the immunosuppressive therapy in our KT group might influence bone mass or turnover. Calcineurin inhibitors seem to increase both osteoclast and osteoblast activity, while promoting hypercalciuria, and

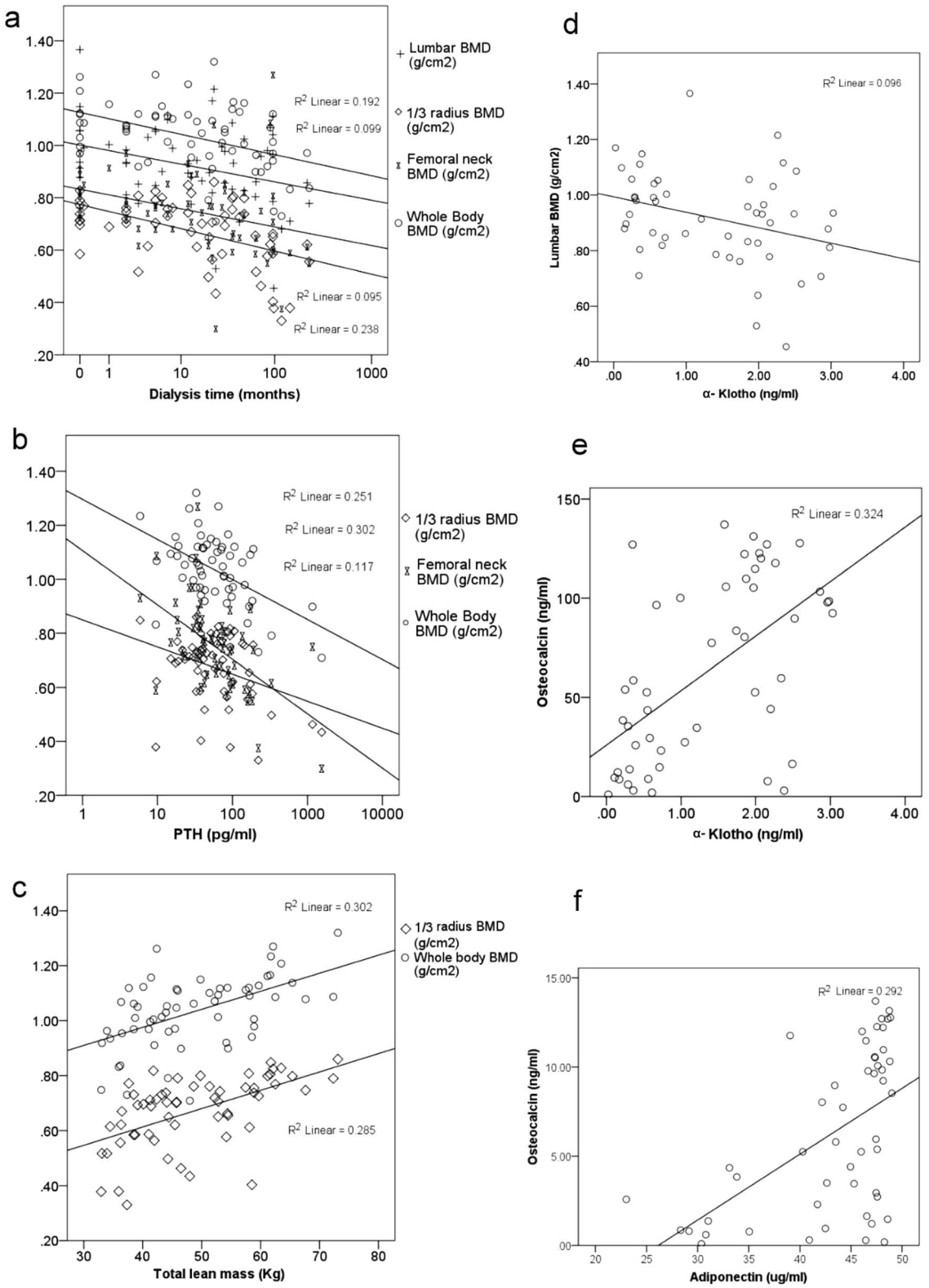


Fig. 2 Regression curves of the main independent associations found. **a**–**b**: Data displayed on a logarithmic scale; **c**–**f**: Data displayed on a linear scale. *PTH* parathyroid hormone

Table 4 Results of multiple regression analysis in the general population group after adjusting for age, sex and smoking (only significant associations are shown)

Dependent variable	Predictor variable	B coefficient	*p* value
Femoral neck BMD $R^2=0.42$, $p<0.001$	Total lean mass	0.6	< 0.001
1/3 radius BMD $R^2=0.6$, $p<0.001$	Total lean mass	0.59	< 0.001
	Fat mass%	− 0.31	0.001

BMD bone mineral density

mTor inhibitors increase osteoclast apoptosis. Mycophenolate mofetil and azathioprine have no influence upon bone [2].

Corticosteroid—based treatment regimen is described as one of the main detrimental factors upon bone metabolism in KT-MBD, due to their marked inhibitory effects upon the osteoblasts; in addition, corticosteroids mediate osteoclastic resorption and promote calcium loss [25]. The lack of association between the cumulative corticosteroid dose and bone parameters in our study is not surprising, considering the minimization of corticosteroid dosage in long-lasting KT. The elevated remodeling rate related to post-transplantation hyperparathyroidism also promotes bone loss, triggering further cortical and trabecular decline [26].

The excessive PTH secretion and low eGFR were indeed among the main independent predictors of BMD decline in KT recipients in our study. The mild normocalcemic, normophosphatemic hyperparathyroidism identified in our long-standing KT group may reflect the long-lasting pre-transplantation parathyroid diffuse hyperplasia, leading to downregulation of the calcium-sensing and vitamin D receptors (VDR), respectively [1]; however, one cannot exclude the effect of post-transplantation de novo CKD, a per se cause of secondary hyperparathyroidism.

PPI therapy frequently associated in KT for preventing corticosteroid-induced peptic ulcer, was reported to interfere with the calcium-PTH-bone axis. Electrolyte disturbances secondary to PPI include hypomagnesemia and hypocalcemia, with consequent secondary hyperparathyroidism or even gastrin-induced parathyroid hyperplasia [4]. Data regarding potential bone detrimental effects of PPIs are very heterogeneous: a recent meta-analysis in 2019 failed to find a significant correlation between PPI and bone loss, but identified an associated increased risk of fracture in the general population [27]. In our study we found no significant impact of PPIs upon KT-MBD. The electrolyte and bone disorders specific to CKD and KT, respectively, probably outweigh the potential detrimental effects of PPI.

Both PPIs and calcineurin inhibitors associate hypomagnesemia as a common side-effect, a potential contributor to post-transplantation osteoporosis [5]. Although magnesium levels are thought to interfere with bone mineralization and the PTH-calcitriol axis, we did not find any significant relationship between serum magnesium and bone parameters or PTH, respectively, in KT patients (with normal magnesemia).

Albeit controversial, emerging data suggest FGF23 and α-Klotho may directly influence bone mass. Altered FGF23 homeostasis is linked to bone abnormalities, even slight elevations being detrimental for the osteoblasts [28]. The FGF23/α-Klotho complex blocks the Wnt bone formation signaling pathway, while FGF23 has an additive effect to that of PTH upon bone resorption [29]. Moreover, osteocyte-specific deletion of *Klotho* in vivo results in a marked increase in bone formation and volume, without any significant change in circulating FGF23 concentrations and *FGF23* transcripts [30], suggesting independent bone regulating actions of α-Klotho; however, this effect was not observed in rodent models of renal osteodystrophy [30].

Our study is among the forefront to investigate the relationship between FGF23 and α-Klotho and bone parameters, respectively, in long-standing KT recipients. In the study of Kanaan et al. [13], high levels of FGF23 were associated with a low bone density in the early post-transplantation period. FGF23 serum concentrations were similar in long-standing KT individuals versus the general population in our study and were not significantly correlated with bone density in any of the groups. We found, however, that α-Klotho is related to increased osteocalcin and low lumbar BMD in KT recipients and may therefore negatively impact bone mass by increasing bone turnover. Data interpretation needs caution because of poor standardization and agreement among available α-Klotho assays [31]. Nevertheless, the role of FGF23/α-Klotho axis in bone mineralization and particularly in KT-MBD needs further investigation.

The available data regarding the dynamics of adipokines secretion and their presumed direct bone-active properties in KT recipients are scarce and contradictory [32]. Although adiponectin is rather reported to have a negative impact upon bone mineralization both in the general population and in CKD patients [33, 34], we and other authors with a similar approach [35] failed to find any significant associations between adiponectin and bone density in both KT and general population individuals. The hyperadiponectinemia in our KT group, also reported by others [22], might be due to impaired clearance, but one cannot preclude a certain degree of adiponectin resistance due to a post-receptor signaling downstream effect [32]. Nonetheless, we describe an independent osteocalcin-adiponectin association in KT which is similar to the general [33] and CKD [34, 35] populations, respectively. Higher osteocalcin levels are known to increase adiponectin production, whereas adiponectin facilitates at its turn the osteogenic differentiation of bone mesenchymal stem cells [36].

The effects of leptin on bone are bidirectional in the general population [37], in hemodialysis patients [38] and also in KT, where data are rather scarce [32]. In our study, the relationship between leptin and bone parameters lost statistical significance in multiple regression analysis when analyzed together with PTH, time spent on dialysis and body composition. Moreover, leptin was not related to bone density in the general population either. Likewise, in the similarly designed study of Marchelek-Mysliwiec et al. [35], leptin failed to correlate with femoral neck and lumbar spine *T*-scores or osteocalcin, respectively, in KT recipients.

KT patients were reported to have high serum levels of the proinflammatory adipokine resistin, possibly linked to reduced clearance and inflammation [39]. Literature data regarding a possible impact of resistin upon bone are rather scarce [40]. The potential harmful bone effects of high resistinemia identified in this study need further investigations, as it may explain bone loss in KT recipients via inflammation, compared to the general population [39].

In our low-grade inflammation KT patients, no independent associations between inflammatory indices (ESR and CRP) and bone mass were found. The detrimental bone effect of systemic inflammation may become apparent in the context of important inflammatory states, such as ankylosing spondylitis [41] or Crohn's disease [42]. Also, more specific parameters such as IL-6 and tumor necrosis factor α (TNF-α) involved in osteoporosis [43] may be more relevant, as they are associated with resistin values in the general population [44].

Our study is limited by the relatively small sample size, the cross-sectional nature of the research, as well as by the lack of α-Klotho, osteocalcin, and inflammatory markers assessment in the general population, respectively. Evaluation of urinary electrolytes to better describe KT-MBD was equally not performed. In addition, the interpretation of our data is limited by the co-existence of post-transplantation specific factors (immunosuppressive therapy here included) together with the de novo development of CKD, both having mineral and bone deleterious effects. While alfacalcidol administration may be thought to limit the interpretation of the results, vitamin D deficit and the consequent supplementation is a common finding in KT, thus reflecting a real-life situation. Also, DXA bone density evaluation does not provide compartmental (cortical versus trabecular) or bone quality assessment. However, growing evidence shows that DXA BMD results are associated with fracture risk in KT recipients [45].

Nonetheless, the current research is among the forefront to globally evaluate the bone impact of body composition, serum adipokines, inflammation and FGF23/α-Klotho axis in long-standing KT recipients, a less-studied population exhibiting rare, specific and critical bone disorders [35]. The presence of a comparison group from the general population is one of the strengths of the study. Our work is among the first to outline the major role of lean mass preservation also in KT-MBD. Ample KT clinical studies are needed to clarify the inconsistencies regarding the effect of the adipokines and FGF23/α-Klotho upon bone mass and metabolism.

Conclusions

Age, PTH, eGFR, time spent on dialysis and lean mass are among the main BMD predictors in long-standing KT recipients. Adipokine dysregulation and α-Klotho may also independently impact bone mass and metabolism, but this needs to be confirmed by further investigations. Low-grade inflammation, although present, does not seem to have independent bone deleterious effects in KT patients. Finally, changing lifestyle in order to improve lean mass and to minimize the deleterious effects of malfunctioning adipose tissue for a better bone health should be accounted for in the management of KT-MBD.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval The study adhered to the Declaration of Helsinki and the Declaration of Istanbul. The institutional ethics committee approved the protocol (20.03.2019).

Consent to participate All individual participants gave written informed consent before entering the study.

Consent for publication All individual participants gave written informed consent regarding publishing their data before entering the study.

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