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Increase in lumbar spine but not distal radius bone mineral density in adults after pancreas kidney transplantation

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ABSTRACT

Osteoporosis occurs in every third individual after simultaneous pancreas kidney transplantation (SPKT). Currently used bone measures insufficiently predict their fracture risk. Lumbar spine Trabecular bone score (TBS) and distal radius areal and volumetric bone mineral density (BMD) were monitored for the first time in patients with type 1 diabetes and chronic renal failure after SPKT with steroid-sparing protocol. In 33 subjects (mean age 43.4 ± 9.8 years), dual-energy X-ray absorptiometry and peripheral quantitative computed tomography were performed just after SPKT (baseline) and one and three years later. While TBS *Z*-scores increased (−1.1 ± 1.2 and −0.3 ± 1.0; p<0.001, at baseline and year three, respectively), trabecular volumetric BMD *Z*-scores at distal radius metaphysis did not change during the study (−1.3 ± 1.3 and −1.3 ± 1.0; $p = 0.38$). Similarly, areal BMD *Z*-scores increased at lumbar spine, total hip and femoral neck (all $p < 0.01$), but not at the distal radius. SPKT induced bone measures' improvement at lumbar spine and hip but not at distal radius. Before suggesting changes in current clinical care, predictive value of individual bone measures or its combination for fracture risk assessment remains to be elucidated.

1. Introduction

Simultaneous pancreas and kidney transplantation (SPKT) is the treatment of choice for most patients with type 1 diabetes (T1D) and renal failure due to diabetic nephropathy (Kukla et al., 2021). SPKT successfully restores normoglycaemia and kidney function. However, other metabolic as well as skeletal complications become prominent with improved patient and graft survival (Lauria and Ribeiro-Oliveira Jr., 2016). Post-transplant osteoporosis with increase fracture risk is an acknowledged long-term complication after solid organ transplantation (Anastasilakis et al., 2019). In SPKT, post-transplant bone impairment occurs due to a combination of pre-transplant conditions (diabetic osteopathy and chronic kidney disease-mineral and bone disorder (Hygum et al., 2019; Ebeling, 2009)) and post-transplant risk factors (suboptimal graft function, the use of corticosteroids and calcineurin inhibitors or vitamin D deficiency (Lan et al., 2015)). In addition, hyperparathyroidism may persist in post-transplant period despite normalization of metabolic abnormalities (Malluche et al., 2010; Vangala et al., 2018). Active diagnostic approach is recommended since symptoms like bone pain or accentuated height loss are not generally present and fracture might be the first clinical sign of advanced osteoporosis.

A clear association has been found between areal bone mineral density (aBMD) assessed by Dual energy X-ray absorptiometry (DXA) and fracture risk in females and males (Arlot et al., 1997; Kanis et al., 2001). Based on the scientific evidence, the International Society for Clinical Densitometry (ISCD) produced Official Positions, where anterio-posterior scan of lumbar spine, total hip, femoral neck and, in specific cases (such as hyperparathyroidism), 33 % radius, were established as appropriate skeletal sites to diagnose osteoporosis (Binkley et al., 2006). However, it is an acknowledged fact that DXA-assessed aBMD represents only a minor contributor of fracture risk in an individual and that other (more predictive) risk factors such as age, previous fracture, glucocorticoid treatment, etc., needs to be accounted for (Kanis, 2002). Therefore, other bone quality and strength surrogates have recently been introduced aiming to improve fracture prediction in health and disease. Among them, DXA-derived trabecular bone score (TBS) assessed from the lumbar spine scan and bone-size independent trabecular volumetric BMD (vBMD) assessed by peripheral quantitative computerized

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Abbreviations

BMD	bone mineral density
aBMD	areal bone mineral density
vBMD	volumetric bone mineral density
BMI	body mass index
CKD	chronic kidney disease
DCCT	Diabetes Control and Complications Trial
DR	distal radius
DXA	dual energy x-ray absorptiometry
FN	femoral neck
GFR	glomerular filtration rate
HbA1c	glycated haemoglobin
HPLC	high performance liquid chromatography
IFCC	International Federation of Clinical Chemistry
LS	lumbar spine
MA	muscle area
MDRD	modification of diet in renal disease
PTH	parathormone
pQCT	peripheral quantitative computed tomography
SPKT	simultaneous pancreas and kidney transplantation
SSI	polar strength-strain index
TBS	trabecular bone score
TH	total hip
T1D	diabetes mellitus type 1

tomography (pQCT) at the radius or tibia seems to be promising (McCloskey et al., 2016; Krohn et al., 2019; Samelson et al., 2019).

Data pertaining to BMD after SPKT are scarce and contradictory. While Smets et al. demonstrated BMD decline at both lumbar spine and femoral neck during the first 6 months after SPKT (Smets et al., 2004), more recent studies report no changes (Torregrosa et al., 2015) or even improvements in BMD T-scores at the same sites (Rocha et al., 2016; Pereira et al., 2010). So far, there have been no reports on development of novel bone strength measures like TBS or development of BMD at the peripheral skeleton (i.e., radius), in subjects after SPKT.

The aim of our three-year-observational study was to explore the effect of SPKT on bone health in subjects with diabetic nephropathy by prospectively capturing recently established measures of bone strength (in particular: DXA-assessed TBS at lumbar spine and pQCT-assessed trabecular vBMD at distal radius). Secondary outcomes were to complement the findings with observations of the aBMD assessed by DXA at the lumbar spine (LS), total hip (TH), femoral neck (FN) and 33 % radius (DR), cortical vBMD, polar Strength-strain index and muscle area, as assessed by pQCT, biochemical parameters of bone mineral metabolism and organ transplant function, and incident fractures.

2. Materials and methods

2.1. Design

Three-year prospective observational single-centre study.

2.2. Subjects

Thirty-six individuals with T1D and advanced diabetic nephropathy (CKD G 4–5) who underwent their first SPKT and agreed with the protocol entered the study. All SPKTs were performed between the end of November 2011 and November 2014 at the only national transplantation diabetes centre in the Czech Republic. Three participants refused to continue after the baseline assessment. Thirty-three remaining participants completed the study protocol and were analysed. Of them, three and one patients missed pQCT densitometry at year 1 and 3, respectively. The study was conducted according to the principles of the Declaration of Helsinki and its later amendments and approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (approval number: G 11-06-06, July 1, 2011, Prague, Czech Republic). Informed consent was obtained from all individual participants included in the study.

2.3. Transplantation procedure

The SPKT procedure was performed using grafts from deceased donors with portal venous drainage and enteric drainage of the pancreatic duct. A standard immunosuppressive protocol was followed (Girman et al., 2019). Induction immunosuppressive therapy included 2 doses of methylprednisolone (500 and 250 mg) and 3 or 4 doses of anti-T-lymphocyte globulin. Maintenance immunosuppression consisted of tacrolimus in combination with either mycophenolate mofetil or sirolimus. Oral prednisone was gradually tapered from an initial dose of 20 mg per day and completely withdrawn within 4 weeks of transplantation, unless reintroduced in selected cases due to graft rejection or, temporarily, due to intolerance to maintenance immunosuppressive agents. Episodes of acute rejection were treated with methylprednisolone pulses or with polyclonal anti-T-cell antibodies. All patients obtained the obligatory cumulative glucocorticoid dose of 750 mg of methylprednisolone plus 330 mg of prednisone within the first 4 weeks after SPKT. In 21 subjects, median dose 1250 mg (interquartile range 435–1733 mg) of methylprednisolone/prednisone was additionally administered during the first post-transplant year, while in 10 subjects, a median dose of 856 mg (interquartile range 491–2006 mg) was administered in the second and third years of the study.

2.4. Study protocol

Routine clinical follow-up consisted of physical examination, body mass index (BMI) calculation, laboratory assessment of graft function (serum creatinine level, estimated glomerular filtration rate, fasting glycaemia and glycated haemoglobin) and bone metabolism (serum calcium, phosphate, intact parathyroid hormone and 25-OH vitamin D). DXA and pQCT were performed within 2 month and then 1 and 3 years after SPKT. The occurrence of symptomatic radiologically confirmed fractures was documented.

2.5. Bone metabolism modulating therapy

In addition to routine laboratory follow-up, calcium, phosphorus, PTH and vitamin D level is evaluated initially in all subjects after SPKT and in those intended to treat with bone metabolism modification therapy every 6–12 months later on. Markers of bone remodeling (P1NP and beta cross-laps) are monitored every 6 months in subjects treated with antiresorptive therapy. The aim of the follow-up is to maintain calcium and phosphorus levels in normal range, PTH level adequate to renal function and to reach vitamin D level at least 75 nmol/l. When GFR declines below 0.6 ml/s, we switch from bisphosphonates to denosumab (not a case of our study).

Oral calcium substitution (500–600 mg/day) was prescribed to correct hypocalcaemia in altogether 21 subjects (64 %) during the study period. Cholecalciferol was administered orally in subjects with a 25-OH vitamin D (25-OHD) serum concentration < 75 nmol/l (< 30 ng/ml) unless contraindicated by conditions such as hypercalcaemia or hyperphosphataemia. In total, 31 subjects (94 %) received cholecalciferol substitution (5000–20,000 IU/week) during the study period. Calcitriol (25 μg/day) was used to treat secondary hyperparathyroidism in cases

where the effect of cholecalciferol administration was insufficient (7 subjects, 21 %). Cinacalcet (30–60 mg/day) was prescribed in three subjects (9 %) with tertiary hyperparathyroidism and hypercalcaemia; two of these patients later underwent parathyroidectomy. In subjects with aBMD in the osteoporotic range (T-score at either site ≤ -2.5 according to the World Health Organization criteria (Assessment of fracture risk and its application to screening for postmenopausal osteoporosis, 1994)), bisphosphonates were prescribed after kidney function stabilisation (GFR > 0.6 ml/s). This treatment was introduced in 5 subjects during the first post-transplant year and in another 3 subjects during the second post-transplant year.

2.6. Laboratory measurements

Total serum calcium, phosphate and creatinine values were analysed spectrophotometrically using automated analysers. Ionised calcium was measured by direct potentiometry, intact PTH by using electrochemiluminescence immunoassay (Elecsys PTH, Roche diagnostics GmbH Sandhoferstrasse 116, D-68305 Mannheim). Concentrations of 25-OHD were measured by RIA (using kits from DIAsource Immunoassays S.A., Louvain-la-Neuve, Belgium) and glycated haemoglobin (HbA1c) by HPLC, as calibrated to the International Federation of Clinical Chemistry (IFCC) reference procedure (International Federation of Clinical C et al., 2007). Both IFCC and calculated Diabetes Control and Complications Trial (DCCT) values are presented. The estimated glomerular filtration rate (GFR) was calculated using the MDRD formula (Levey et al., 2007).

2.7. Bone densitometry – dual energy X-ray absorptiometry (DXA)

All subjects underwent bone densitometry (DXA, Lunar Prodigy Primo, GE Healthcare) of the L1-L4 lumbar spine (LS), total hip (TH), femoral neck (FN) and 33 % radius (distal radius, DR). Values were evaluated using enCORE software, version 13.60.033 (GE Healthcare) including the USA combined NHANES/Lunar reference population. Results were expressed in absolute values (g/cm^2) and as *Z*-scores (standard deviation score from the age- and sex-specific mean of a healthy population), as the majority of study subjects was younger than 50 years. Instrument quality control on the DXA scanner was performed daily using a standard spine phantom. Trabecular bone score (TBS) from the LS scan was assessed with TBS iNsight software version 3.0.3.0 (GE Healthcare). Due to the lack of both the device-incorporated and local population-specific reference values, TBS *Z*-scores were calculated from the published Australian reference values (Anderson et al., 2019).

2.8. Bone densitometry – peripheral quantitative computed tomography (pQCT)

An XCT 2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) was used to obtain measurements at the non-dominant radius. Trabecular vBMD was assessed at the distal radius (4 % site) while cortical vBMD, polar Strength Strain Index were assessed at the proximal radius (65 % site). Technical details and scanner setting were published previously from our group (Soucek et al., 2018). The muscle area (MA) parameter, representing the cross-sectional area of the forearm muscles at the 65 % site, was computed by subtracting areas of the radius, ulna and subcutaneous fat tissue (Rauch and Schoenau, 2008). Precision errors for pQCT measurements were very low at the radius (Soucek et al., 2011). Raw values were transformed into age- and sex-specific *Z*-scores based on published references (Rauch and Schoenau, 2008; Rauch and Schoenau, 2005).

2.9. Statistical analysis

Statistical analysis was performed in statistical computing environment R (R Core Team, 2018). Two-sample comparisons were based on the two-sample *t*-test with Welch correction for degrees of freedom. Post-transplant development of biochemical and bone parameters was modelled using a mixed linear regression model, including fixed effects for age, sex, and time (as factor) and assuming a normally distributed subject-specific random intercept. This longitudinal model is used to evaluate the effects of further predictors by testing the statistical significance of additional explanatory variables (interaction between age and time, BMI, bisphosphonate treatment history, mycophenolate vs. sirolimus, cumulative glucocorticoid dosage). Maximum likelihood estimates were calculated in R library nlme (Pinheiro et al., 2018), while *p*-values were obtained from corresponding likelihood ratio tests. Changes in bone parameter *Z*-scores from baseline to year 3 were evaluated using the paired *t*-test. The dependency of improvements in bone parameters (from baseline to year 3) on enhanced biochemistry markers (from baseline to year 3) was evaluated by testing the effect of the marker changes using a linear regression model adjusted for sex and age.

3. Results

3.1. Baseline clinical characteristics

Baseline anthropometric and selected laboratory characteristics are shown in Table 1. Men prevailed in our study and were significantly older than women. T1D duration and control were comparable between the sexes. Twenty-five subjects received dialysis treatment before transplantation with a mean dialysis time of 20.2 (±14.4) months. The remaining 8 subjects underwent pre-emptive transplantation.

3.2. Novel bone strengths surrogates

Mean TBS *Z*-score was decreased at the beginning of the study but increased significantly and normalized during the first year of follow up (Table 2, Fig. 1). No subsequent changes were observed between year 1 and year 3. Mean trabecular vBMD *Z*-score was low at study baseline and remained unchanged during the follow-up period (Table 2, Fig. 2).

Table 1
Patient's anthropometric and biochemistry characteristics at the time of transplantation.

Participants	*N* = 33		
	Males *N* = 23 (69.7 %)	Females *N* = 10 (30.3 %)	*P*-value[a]
Age (years)	46.3 ± 9.8 (23.8; 62.5)	36.8 ± 6.1 (27.8; 48.2)	0.002
Height (cm)	179.0 ± 7.5 (168; 195)	165.9 ± 6.6 (156; 178)	<0.001
Weight (kg)	78.9 ± 10.8 (61;98)	60.0 ± 10.5 (43; 74)	<0.001
BMI (kg/m^2)	24.6 ± 3.0 (18.6; 29.8)	21.8 ± 3.5 (15.2; 26.1)	0.042
Diabetes duration (years)	26.5 ± 10.3 (14.7; 50.4)	21.8 ± 4.7 (15.8; 30.1)	0.084
IFCC HbA1c (mmol/mol) (reference range: 20–42)	65.7 ± 13.2 (43; 95)	72.3 ± 15.3 (54; 99)	0.256
DCCT HbA1c (%) (reference range: 4–6)	8.2 ± 1.2 (6.1; 10.8)	8.8 ± 1.4 (7.1; 11.2)	
Creatinine (μmol/l) (reference range: men 64–104; women 49–90)	525.5 ± 243.8 (214.7; 1187.0)	533.4 ± 153.5 (334.8; 807.3)	0.911
Glomerular filtration rate (ml/s/1.73 m^2)	0.21 ± 0.10 (0.21; 0.01)	0.15 ± 0.05 (0.08; 0.24)	0.011

Mean ± SD and (min; max) values are shown. BMI, body mass index; IFCC, International Federation of Clinical Chemistry; DCCT, Diabetes Control and Complications Trial.

[a] *t*-Test with Welch correction was used to analyse differences between sexes.

Table 2
Development of bone density and strength parameters during the post-transplant period.

		Baseline	Year 1		Year 3		Overall trend *P*-value [a]
				p-Value (vs. baseline)		p-Value (vs. year 1)	
Lumbar spine aBMD	g/cm^2	1.063 ± 0.154	1.122 ± 0.140	<0.001	1.183 ± 0.156	<0.001	<0.001
	Z-score	−1.1 ± 1.3**	−0.6 ± 1.2**	<0.001	−0.1 ± 1.2	<0.001	<0.001
Total hip aBMD	g/cm^2	0.830 ± 0.119	0.835 ± 0.114	0.597	0.859 ± 0.129	0.017	0.007
	Z-score	−1.5 ± 0.9***	−1.5 ± 0.8***	0.446	−1.3 ± 0.9***	0.002	<0.001
Femoral neck aBMD	g/cm^2	0.806 ± 0.112	0.813 ± 0.118	0.462	0.832 ± 0.129	0.061	0.025
	Z-score	−1.4 ± 0.9***	−1.4 ± 0.8***	0.689	−1.2 ± 0.8***	0.010	0.005
Distal radius aBMD	g/cm^2	0.891 ± 0.087	0.882 ± 0.086	0.198	0.873 ± 0.100	0.199	0.034
	Z-score	−0.7 ± 0.8***	−0.8 ± 0.8***	0.262	−0.9 ± 0.8***	0.262	0.075
Trabecular bone score (LS scan)	No unit	1.201 ± 0.118	1.292 ± 0.108	<0.001	1.275 ± 0.115	0.441	<0.001
	Z-score	−1.1 ± 1.2***	−0.2 ± 1.0	<0.001	−0.3 ± 1.0	0.830	<0.001
Trabecular vBMD	mg/cm^3	161.4 ± 48.3	159.0 ± 42.9	0.262	158.3 ± 38.5	0.412	0.494
(distal radius, pQCT)	Z-score	−1.3 ± 1.3***	−1.3 ± 1.2***	0.513	−1.3 ± 1.0***	0.177	0.379
Cortical vBMD	mg/cm^3	1119.0 ± 45.1	1107.0 ± 46.0	0.021	1107.0 ± 49.8	0.626	0.008
(prox. Radius, pQCT)	Z-score	−0.2 ± 1.4	−0.6 ± 1.4*	0.022	−0.5 ± 1.5	0.671	0.010
Strength-strain index	mm^3	362.6 ± 104.3	368.8 ± 122.0	0.555	370.3 ± 102.7	0.979	0.782
(prox. radius, pQCT)	Z-score	−0.2 ± 1.2	−0.2 ± 1.3	0.807	−0.1 ± 1.0	0.685	0.785
Muscle area	mm^2	3192.0 ± 844.0	3458.0 ± 820.1	<0.001	3499.0 ± 936.6	0.595	<0.001
(prox. radius, pQCT)	Z-score	−2.2 ± 1.5***	−1.5 ± 1.2***	<0.001	−1.6 ± 1.4***	0.637	<0.001

Mean ± SD values are shown. The one-sample *t*-test was used to test differences in mean Z-scores from zero: * p<0.05, **p<0.01, ***p<0.001.
aBMD areal bone mineral density; vBMD volumetric bone mineral density.
All measures were assessed in all time points except for pQCT parameters in three subjects at year 1 and one subject at year 3.
[a] A mixed linear regression model adjusted for age and sex was used to test the effect of time.

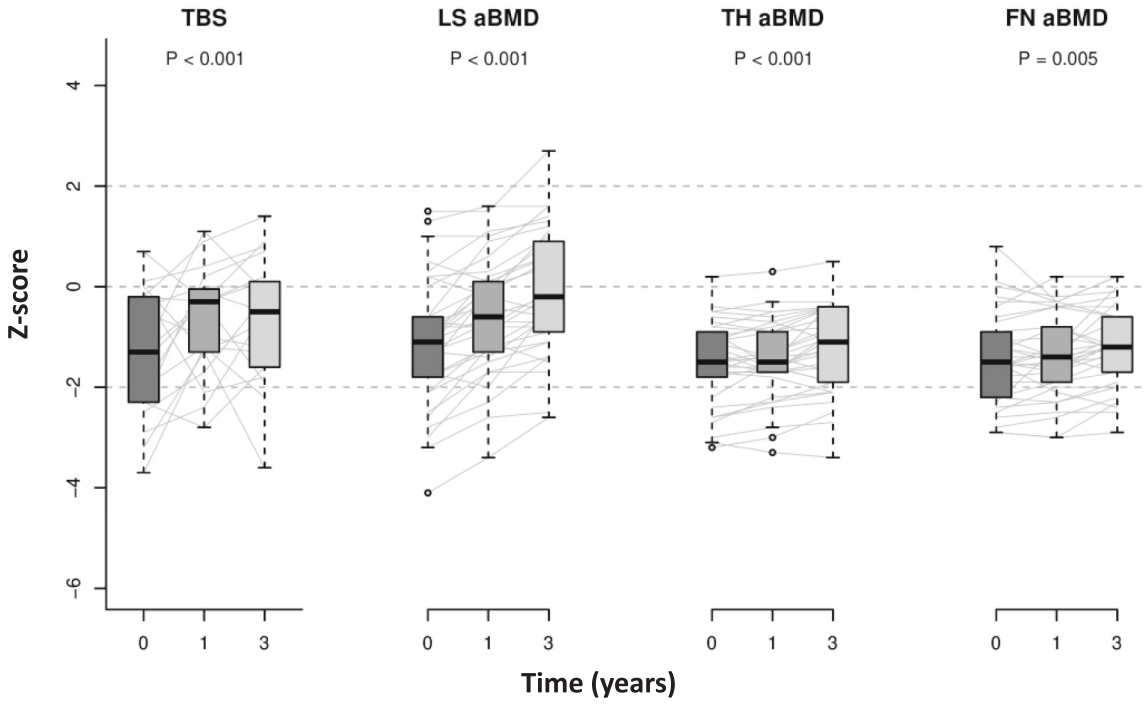

Fig. 1. Post-transplant development of DXA bone measures at the weight-bearing skeleton sites.
Spaghetti plot showing development in individual patients. Boxplots summarizing the median (thick middle line), first and third quartile (lower and upper box margin), minimum and maximum (whiskers) and outliers (>Q3 + 1.5 × IQR or <Q1 − 1.5 × IQR).
Mixed linear regression model adjusted for age and sex was used to test the effect of time.
aBMD, areal bone mineral density; Q1, first quartile; Q3, third quartile; IQR, interquartile range
TBS, trabecular bone score; LS, lumbar spine; TH, total hip; FN, femoral neck.

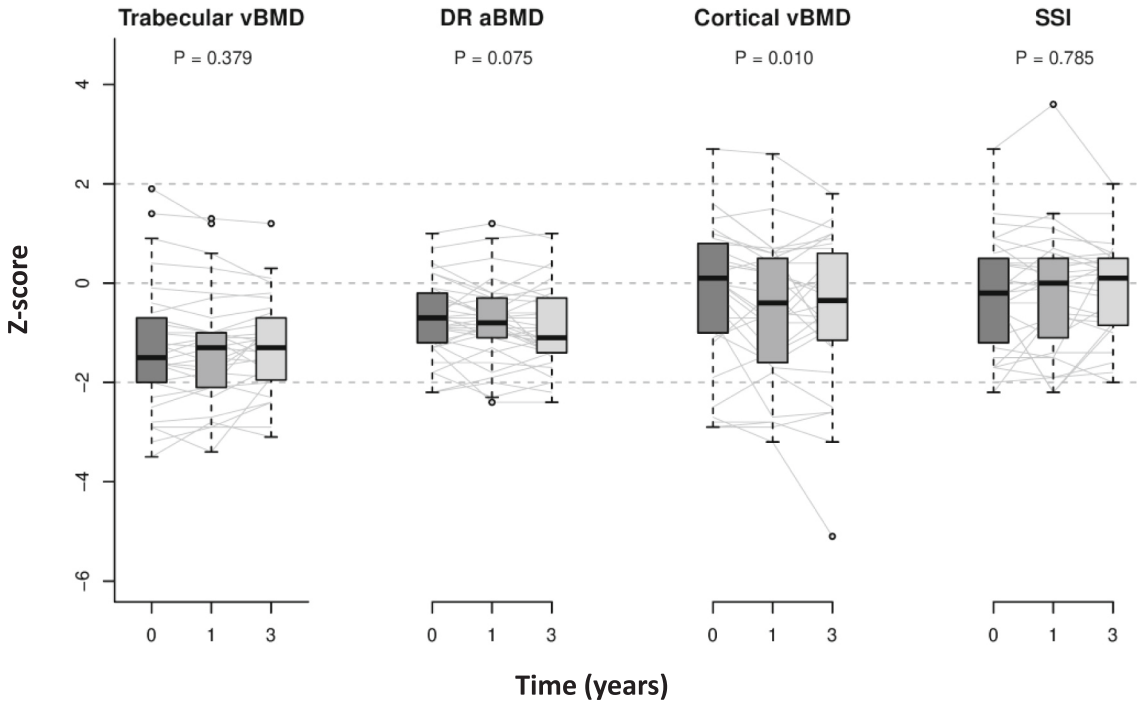

Fig. 2. Post-transplant development of DXA and pQCT bone measures at non-weight-bearing radius.
Spaghetti plot showing development in individual patients. Boxplots summarizing the median (thick middle line), first and third quartile (lower and upper box margin), minimum and maximum (whiskers) and outliers (>Q3 + 1.5 × IQR or <Q1 − 1.5 × IQR).
Mixed linear regression model adjusted for age and sex was used to test the effect of time.
aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; Q1, first quartile; Q3, third quartile; IQR, interquartile range
SSI, polar strength-strain index.

3.3. Traditional and complementary bone strength measures

Even though all DXA-assessed aBMD *Z*-scores were low at baseline and all except the DR significantly increased during follow-up, only mean LS aBMD *Z*-score had normalized by the end of the study (Table 2, Fig. 1). Mean LS aBMD *Z*-score increased continuously during the follow-up period with maximal increment during the first post-transplant year, whereas TH and FN aBMD *Z*-scores increased significantly only between years 1 and 3 (Table 2, Fig. 1).

Mean cortical vBMD Z-score assessed at radial diaphysis by pQCT was normal at baseline and remained normal throughout the follow up, despite statistically significant decrease during the first post-transplant year (Table 2, Fig. 2). Mean polar SSI Z-score remained normal for the whole study period (Table 2, Fig. 2). The muscle area of the forearm exhibited a significant increase over the follow-up period, despite the score remaining low (p<0.001) at all time points (Table 2).

3.4. Calcium-phosphate metabolism development

While mean calcium and phosphate serum concentrations remained normal during the follow-up period, the mean PTH serum concentration, which was elevated at baseline, declined significantly during the first post-transplant year (Table 3). However, PTH serum concentration was above the upper reference limit for the assay (i.e., > 6.9 pmol/l) in 25 subjects (75.8 %) at year 1 and in 27 (81.8 %) subjects at year 3. At the beginning of the study, 25-OHD serum concentration was within the deficiency range (< 30 nmol/l) in 19 subjects (61.3 %) and within the insufficiency range (30–50 nmol/l) in 11 subjects (35.5 %) according to current guidelines (Giustina et al., 2019) (two patients had missing values). The concentrations increased significantly (Table 3) so that only 3 (11.5 %) were deficient and 6 (23.1 %) were insufficient at study end (seven patients with missing values).

3.5. Graft function

Kidney graft function was established in all subjects within the first post-transplant month (Table 3). We recorded only one case of kidney graft failure (due to chronic active antibody mediated rejection), which occurred 30 months after transplantation. Mean glycated haemoglobin improved significantly after SPKT (Table 3). In one recipient, the pancreas graft was explanted one week after SPKT due to graft thrombosis. This patient underwent pancreas re-transplantation 9 months after the first transplant. Apart from this case, full function of the pancreas graft (not requiring antidiabetic therapy, HbA1c < 47 mmol/l) was documented in 27 subjects across the entire study period. In 5 subjects, while still having the functioning pancreas graft with significantly positive C peptide level, anti-diabetic therapy was required (gliptins in 2 patients and small doses of insulin + gliptins in 3 patients).

3.6. Predictors of bone measures' development

Age was a significant predictor of BMD. The younger the patient, the larger the increase in LS, TH and FN aBMD *Z*-scores (by 0.03 ± 0.01, $p < 0.001$; 0.01 ± 0.01, $p = 0.01$; and 0.02 ± 0.01, $p = 0.035$; respectively, in the first year of follow-up). Contrarily, changes in bone parameters were not associated with changes in creatinine, MDRD, HbA1c, PTH or BMI.

As expected, at the start of the study subjects treated with bisphosphonates had lower LS, FN and TH aBMD *Z*-scores as compared to untreated subjects (by −2.3 ± 0.4, −1.5 ± 0.3 and −1.6 ± 0.2, respectively, all $p < 0.001$). However, mean Z-scores increased similarly between the two groups (*p*-values 0.112, 0.537 and 0.391, respectively). The only parameter to be considerably affected by bisphosphonate treatment was TBS (Z-scores as well as crude values), which improved more in the treated group than in the untreated group (the difference in Z-scores was −0.9 ± 0.4, $p = 0.035$ at study start and 0.2 ± 0.4, $p = 0.6$

Table 3
Development of selected biochemistry parameters during the post-transplant period.

	Time from transplantation				
	Week 1	Month 1	Year 1	Year 3	P-value[a]
Calcium (mmol/l) (reference range: 2.15–2.55)	2.09 ± 0.17 (1.79; 2.57)	2.42 ± 0.14 (2.16; 2.78)	2.49 ± 0.10 (2.22; 2.72)	2.42 ± 0.12 (2.11; 2.62)	<0.001
Ionised calcium (mmol/l) (reference range: 1.15–1.29)	1.21 ± 0.08 (1.07; 1.40)	1.28 ± 0.06 (1.16; 1.50)	1.26 ± 0.05 (1.19; 1.35)	1.25 ± 0.05 (1.09; 1.35)	<0.001
Phosphate (mmol/l) (reference range: 0.71–1.23)	1.10 ± 0.62 (0.47; 2.81)	0.92 ± 0.21 (0.40; 1.27)	1.03 ± 0.19 (0.74; 1.51)	1.03 ± 0.18 (0.50; 1.45)	0.185
Parathormone (pmol/l) (reference range: 1.6–6.9)	19.5 ± 15.4 (7.2; 73.5)	11.4 ± 7.6 (3.1; 44.5)	9.7 ± 4.7 (3.7; 21.6)	11.7 ± 8.1 (3.6; 42.6)	<0.001
25-Hydroxyvitamin D (ng/ml) (reference range: 9.2–45.2)	11.4 ± 4.8 (5.2; 21.6)	N/A N/A	29.2 ± 11.4 (10.7; 57.7)	26.6 ± 11.3 (5.1; 49.0)	<0.001
25-Hydroxyvitamin D (nmol/l) (reference range: 23–113 nmol/l)	28.5 ± 12.0 (13.0; 54.0)		73.0 ± 28.5) (26.8; 144.3)	66.5 ± 28.3 (12.8; 122.5)	
Creatinine (μmol/l) (reference range: men 64–104; women 49–90)	263.7 ± 240.2 (51.6; 852.0)	137.6 ± 39.2 (61.7; 238.5)	121.3 ± 32.7 (75.4; 239.0)	136.7 ± 68.9 (81.0; 477.6)	<0.001
Glomerular filtration rate (ml/s/1.73 m^2)	0.69 ± 0.48 (0.09; 1.92)	0.83 ± 0.29 (0.32; 1.56)	0.92 ± 0.22 (0.41; 1.3)	0.86 ± 0.28 (0.14; 1.32)	<0.001
HbA1c (IFCC) (mmol/mol) (reference range: 20–42)	N/A N/A	45.2 ± 8.0 (3.0; 67.0)	38.9 ± 5.0 (26.0; 53.0)	38.8 ± 6.9 (28.0; 69.0)	<0.001
HbA1c (DCCT) (%) (reference range: 4–6)		6.2 ± 0.7 (5.4; 8.3)	5.7 ± 0.5 (4.5; 7.0)	5.7 ± 0.6 (4.7; 8.5)	
Fasting glycaemia (mmol/l) (reference range: 3.6–5.59)	N/A[a]	5.8 ± 1.1 (4.4; 9.6)	5.5 ± 1.2 (3.7; 10.2)	5.5 ± 1.1 (4.4; 9.2)	0.322

Mean ± SD and (min; max) values are shown; (a) fasting glycaemia was not evaluated at week 1 because of parenteral nutrition with concomitant insulin administration in several subjects.
IFCC, International Federation of Clinical Chemistry; DCCT, Diabetes Control and Complications Trial.
All measures were assessed in all time points except for 25-hydroxyvitamin D level in 2 subjects at year 1 and 7 subjects at year 3.
[a] A mixed linear regression model adjusted for age and sex was used to test the effect of time.

at study end, after adjusting for age and sex).

The (logarithmically transformed) cumulative glucocorticoid dose was negatively associated with changes in cortical vBMD and MA Z-scores (-0.4 ± 0.2, $p = 0.044$ and -0.4 ± 0.2, $p = 0.032$, respectively). Immunosuppression type (mycophenolate vs. sirolimus) did not have a significant effect on bone measures' development after the transplantation.

3.7. Fractures

Twelve subjects had a history of previous fracture, no other clinical sign of any bone impairment was present. Across the entire study period, there were three incident symptomatic fractures in two subjects. One patient suffered a distal radius fracture and another patient sustained two unrelated fractures: of the distal radius and of the metatarsal in the left foot due to osteomyelitis. The fracture rate was too low to permit any other analysis.

4. Discussion

This is the first study to describe the development of TBS of the lumbar spine and areal as well as volumetric BMD of the distal radius in patients with T1D and diabetic nephropathy during the first three years after SPKT. We found that: a) TBS as well as aBMD at lumbar spine, total hip and femoral neck were low at the time of SPKT and increased during the follow-up, b) improvements occurred earlier in the trabecular bone (in the first post-transplant year, TBS and LS aBMD) than in the cortical bone (between years 1 and 3, TH and FN aBMD), c) both areal and trabecular volumetric BMD assessed at distal radius did not improve, d) bone strength surrogates at the diaphysis of the radius (cortical vBMD and SSI polar) were normal, and e) bone measures' improvements at lumbar spine, total hip and femoral neck were negatively related to age of the patients.

4.1. TBS and aBMD at established osteoporotic skeletal sites

Despite that TBS was proved to improve fracture prediction compared to aBMD alone (Silva et al., 2015), guidelines adept at distinguishing physiological and pathological TBS values have not been established yet. By considering *Z*-scores >-2.0 and <2.0 to be "within range" (i.e., similar approach as to BMD values), 21 % of our study participants would have been below the expected range at baseline, whereas all of them would have had normal TBS Z-scores by the end of the study. Similarly to TBS, aBMD at LS, TH and FN was low at baseline and improved significantly during the follow up. Low aBMD at the time of transplantation is in accordance with previously published cross-sectional and longitudinal studies in subjects before (Kratochvilova et al., 2019) and after SPKT (Smets et al., 2004; Torregrosa et al., 2015; Rocha et al., 2016; Pereira et al., 2010; Smets et al., 1998). However, the favourable aBMD development observed in our study contrasts the findings of several previously published papers. In individuals who underwent SPKT between the years 1995 and 1997, rapid LS aBMD reduction within the first six months after transplantation (−6.0 %) and a failure to reach baseline values up to 2.5–4.0 years after SPKT were documented (Smets et al., 2004). Contrarily, subjects transplanted between the years 1998 and 1999 presented with stable LS aBMD one and 10 years after SPKT (Torregrosa et al., 2015) and patients transplanted between the years 2000 and 2009 showed increases in LS aBMD T-scores by 0.8 three years after SPKT (Rocha et al., 2016). As currently used immunosuppressive protocols consist of much lower doses of glucocorticoids compared to protocols used in the '90s, and glucocorticoids are a known deleterious bone agent, this protocol change could at least partially explain the contradictory findings. Moreover, some reports suggest that now widely used maintenance immunosuppressant tacrolimus may have a less deteriorating effect on bone remodeling compared to formerly administered cyclosporine (Anastasilakis et al., 2019; Lan et al., 2015). In addition, we may speculate that enteric drainage of the pancreatic duct (used in latter studies as well as in our study) may be superior to bladder drainage (used in the mid-90s study (Smets et al., 2004)), where exocrine pancreatic secretion into the urinary bladder may lead to bicarbonate loss and metabolic acidosis, thus exacerbating metabolic bone disease. Significant improvements in TBS and LS, TH and FN aBMD, observed in our patients, may thus reflect bone-sparing SPKT protocol changes that were introduced over the past decades.

4.2. The impact of cortical bone content

Whereas TBS and LS aBMD, both largely reflecting trabecular bone, increased significantly already in the first post-transplant year, TH and

FN aBMD, which both predominantly include cortical bone, did not improve until the last two years of follow-up. Similarly to LS aBMD, previous findings on FN aBMD in subjects after SPKT are inconsistent and probably influenced by the different treatment protocols used (Smets et al., 2004; Rocha et al., 2016; Pereira et al., 2010). Cortical bone has substantially slower rate of metabolism compared to trabecular bone (Hart et al., 2020), which may explain why TH and FN aBMD improved later on during the follow up. In addition, as there were 76 % of subjects at year 1 and 82 % of subjects at year 3 who had their PTH values above the upper reference limit, hyperparathyroidism, which is quite common in patients with a history of renal failure (Wolf et al., 2016) and which predominantly affects cortical bone (Dempster et al., 2007; Iyer et al., 2014; Nickolas et al., 2013), probably mitigated the positive effect of SPKT on cortical bone sites. It is not known yet how changes in cortical bone quality affect the fracture risk and how to effectively manage persistent hyperparathyroidism after SPKT to reduce fracture risk in these patients.

4.3. Bone strength at non-weight bearing peripheral skeleton

Forearm is among the most frequently fractured sites both in general population (Collaborators, 2021) and in individuals after kidney transplantation (Iseri et al., 2020). Still, this is the first study to explore bone measures at the radius in patients after SPKT. In our patients, both distal radius aBMD and trabecular vBMD at the distal metaphysis of the radius were low at baseline and did not increase over the follow up. Osteoporosis is a systemic disease that undoubtedly affects the whole skeleton. However, the discrepancy between distal radius and LS (and TH and FN) aBMD development suggest that distinct skeletal sites may react differentially to altered metabolic conditions after SPKT, probably at least partially due to hyperparathyroidism. Also kidney transplant recipients, who were treated with similar immunosuppressive protocol with early corticosteroid withdrawal, showed no change of LS and TH aBMD but decrease of distal radius aBMD within the first post-transplant year (Iyer et al., 2014). Moreover, cortical vBMD and polar SSI (resistance to torsion and bending) at the diaphysis of the radius were normal at study baseline and remained normal during the follow up in our patients after SPKT. This was accompanied by a significant increase of muscle area at the forearm, which presumably reflects enhanced physical activity and wellbeing of patients. It remains to be elucidated whether the consequent increased mechanical loading contributed to the finding that weight-bearing sites exhibited more profound positive effects of SPKT than non-weight-bearing sites.

4.4. Predictors of development of bone strength surrogates

Identifying predictors of skeletal outcomes aims to improve or individualize patient care. Our study showed that younger patients gained more LS, TH and FN aBMD after SPKT than older subjects. This might be due to physiological age-related bone turnover rate suppression (Jorgensen et al., 2017) or other yet unknown factor. Nevertheless, the clinical implication is that the sooner the transplantation the better the bone outcome to be expected. Bisphosphonate treatment lead to SPKT-independent TBS improvement, but the relevance for diminishing the fracture risk is not clear yet. Further studies are needed to establish the indication criteria and effectiveness of bisphosphonate treatment in patients after SPKT.

4.5. Fractures

Fracture rate was low during the study and both subjects had a history of fracture before SPKT. Distal radius fractures occurred after the fall from standing position during routine daily activities suggesting that patients in risk should be advised to adopt intensive fall prevention measures in addition to general lifestyle modification to reduce bone loss.

4.6. Study strengths and limitations

The strengths of our study were the prospective longitudinal design, complex bone densitometry assessment including parallel evaluation of multiple skeletal sites, utilizing volumetric BMD of the radius and exploring recently established bone strength surrogates. The limitation was a lack of control group. As young patients with T1D and CKD are always considered for SPKT in our centre unless they have a living kidney donor (thus first undergo kidney transplantation and then get pancreas transplant during a second surgery) (Girman and Saudek, 2011), there were no subjects available that would stand as controls to SPKT. Kidney transplant recipients with T2D are not appropriate due to older age and different pathophysiology of bone impairment.

4.7. Conclusions

While patients with T1D and renal failure due to diabetic nephropathy presented with low BMD at multiple skeletal sites, SPKT with corticoid sparing protocol lead to rapid improvement in metabolic parameters and increase in TBS and aBMD at major osteoporotic sites (i.e. LS, FN and TH). Trabecular bone measures (TBS and LS aBMD) showed normalization already within the first post-transplant year, but predominantly cortical sites (FN and TH aBMD) increased to lesser extent and significantly not until the second and third years of follow up, which might be due to persistent hyperparathyroidism in majority of the patients. We are the first to demonstrate that systemic changes induced by both the disease and treatment may be less pronounced at non-weight-bearing skeletal sites like radius. By including both bone assessment at the radius and rigorous record of incident fractures as a standard of care in patients after SPKT we anticipate better understanding of the development of skeletal health, which may direct our future diagnostic and treatment approaches to osteoporosis in these patients. Studies involving a larger population of subjects after SPKT are needed in order to clarify which bone measures (or combination of measures) are predictive of subsequent fractures and warrant anti-osteoporotic treatment.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The study was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (approval number: G 11-06-06, July 1, 2011, Prague, Czech Republic). Informed consent was obtained from all individual participants included in the study.

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CRediT authorship contribution statement

Simona Kratochvílová: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Klara Maratova:** Writing – review & editing, Investigation, Data curation. **Zdenek Sumnik:** Writing – review & editing, Supervision, Formal analysis. **Jana Brunová:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Zdeněk Hlávka:** Writing – review & editing, Visualization, Formal analysis. **Peter Girman:** Writing – review & editing, Supervision, Formal analysis. **František Saudek:** Writing –

review & editing, Supervision, Formal analysis. **Ondrej Soucek:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

References

Anastasilakis, A.D., Tsourdi, E., Makras, P., Polyzos, S.A., Meier, C., McCloskey, E.V., Pepe, J., Zillikens, M.C., 2019. Bone disease following solid organ transplantation: a narrative review and recommendations for management from the European calcified tissue society. Bone 127, 401–418.

Anderson, K.B., Holloway-Kew, K.L., Hans, D., Kotowicz, M.A., Hyde, N.K., Pasco, J.A., 2019. Reference ranges for trabecular bone score in Australian men and women: a cross-sectional study. JBMR Plus 3, e10133.

Arlot, M.E., Sornay-Rendu, E., Garnero, P., Vey-Marty, B., Delmas, P.D., 1997. Apparent pre- and postmenopausal bone loss evaluated by DXA at different skeletal sites in women: the OFELY cohort. J. Bone Miner. Res. 12, 683–690.

(1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser 843: 1–129.

Binkley, N., Bilezikian, J.P., Kendler, D.L., Leib, E.S., Lewiecki, E.M., Petak, S.M., International Society for Clinical D, 2006. Official positions of the International Society for Clinical Densitometry and Executive Summary of the 2005 position development conference. J. Clin. Densitom. 9, 4–14.

Collaborators, G.B.D.F., 2021. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990-2019: a systematic analysis from the global burden of disease study 2019. Lancet Healthy Longev 2, e580–e592.

Dempster, D.W., Muller, R., Zhou, H., Kohler, T., Shane, E., Parisien, M., Silverberg, S.J., Bilezikian, J.P., 2007. Preserved three-dimensional cancellous bone structure in mild primary hyperparathyroidism. Bone 41, 19–24.

Ebeling, P.R., 2009. Approach to the patient with transplantation-related bone loss. J. Clin. Endocrinol. Metab. 94, 1483–1490.

Girman, P., Saudek, F., 2011. The IKEM pancreas and islet transplant program as part of healthcare for type 1 diabetes patients: retrospective analysis of outcome from 1983 to 2010. Rev. Diabet. Stud. 8, 35–43.

Girman, P., Lipar, K., Kocik, M., Voska, L., Koznarova, R., Marada, T., Lanska, V., Saudek, F., 2019. Sirolimus vs mycophenolate mofetil (MMF) in primary combined pancreas and kidney transplantation. Results of a long-term prospective randomized study, Am J Transplant.

Giustina, A., Adler, R.A., Binkley, N., Bouillon, R., Ebeling, P.R., Lazaretti-Castro, M., Marcocci, C., Rizzoli, R., Sempos, C.T., Bilezikian, J.P., 2019. Controversies in vitamin D: summary Statement from an international conference. J. Clin. Endocrinol. Metab. 104, 234–240.

Hart, N.H., Newton, R.U., Tan, J., Rantalainen, T., Chivers, P., Siafarikas, A., Nimphius, S., 2020. Biological basis of bone strength: anatomy, physiology and measurement. J. Musculoskelet. Neuronal Interact. 20, 347–371.

Hygum, K., Starup-Linde, J., Langdahl, B.L., 2019. Diabetes and bone. Osteoporos Sarcopenia 5, 29–37.

International Federation of Clinical C, Laboratory Medicine ISD, Mosca, A., et al., 2007. Global standardization of glycated hemoglobin measurement: the position of the IFCC working group. Clin. Chem. Lab. Med. 45, 1077–1080.

Iseri, K., Carrero, J.J., Evans, M., Fellander-Tsai, L., Berg, H.E., Runesson, B., Stenvinkel, P., Lindholm, B., Qureshi, A.R., 2020. Fractures after kidney transplantation: incidence, predictors, and association with mortality. Bone 140, 115554.

Iyer, S.P., Nikkel, L.E., Nishiyama, K.K., et al., 2014. Kidney transplantation with early corticosteroid withdrawal: paradoxical effects at the central and peripheral skeleton. J. Am. Soc. Nephrol. 25, 1331–1341.

Jorgensen, N.R., Mollehave, L.T., Hansen, Y.B.L., Quardon, N., Lylloff, L., Linneberg, A., 2017. Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population. Osteoporos. Int. 28, 2103–2113.

Kanis, J.A., 2002. Diagnosis of osteoporosis and assessment of fracture risk. Lancet 359, 1929–1936.

Kanis, J.A., Johnell, O., Oden, A., Dawson, A., De Laet, C., Jonsson, B., 2001. Ten year probabilities of osteoporotic fractures according to BMD and diagnostic thresholds. Osteoporos. Int. 12, 989–995.

Kratochvilova, S., Brunova, J., Wohl, P., Lanska, V., Saudek, F., 2019. Retrospective analysis of bone metabolism in patients on waiting list for simultaneous pancreas-kidney transplantation. J. Diabetes Res. 2019, 5143021.

Krohn, K., Schwartz, E.N., Chung, Y.S., Lewiecki, E.M., 2019. Dual-energy X-ray absorptiometry monitoring with trabecular bone score: 2019 ISCD official position. J. Clin. Densitom. 22, 501–505.

Kukla, A., Ventura-Aguiar, P., Cooper, M., et al., 2021. Transplant options for patients with diabetes and advanced kidney disease: a review. Am. J. Kidney Dis. 78, 418–428.

Lan, G.B., Xie, X.B., Peng, L.K., Liu, L., Song, L., Dai, H.L., 2015. Current status of research on osteoporosis after solid organ transplantation: pathogenesis and management. Biomed. Res. Int. 2015, 413169.

Lauria, M.W., Ribeiro-Oliveira Jr., A., 2016. Diabetes and other endocrine-metabolic abnormalities in the long-term follow-up of pancreas transplantation. Clin Diabetes Endocrinol 2, 14.

Levey, A.S., Coresh, J., Greene, T., Marsh, J., Stevens, L.A., Kusek, J.W., Van Lente, F., Chronic Kidney Disease Epidemiology C, 2007. Expressing the modification of diet in renal disease study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin. Chem. 53, 766–772.

Malluche, H.H., Monier-Faugere, M.C., Herberth, J., 2010. Bone disease after renal transplantation. Nat. Rev. Nephrol. 6, 32–40.

McCloskey, E.V., Oden, A., Harvey, N.C., et al., 2016. A Meta-analysis of trabecular bone score in fracture risk prediction and its relationship to FRAX. J. Bone Miner. Res. 31, 940–948.

Nickolas, T.L., Stein, E.M., Dworakowski, E., et al., 2013. Rapid cortical bone loss in patients with chronic kidney disease. J. Bone Miner. Res. 28, 1811–1820.

Pereira, S., Pedroso, S., Martins, L., et al., 2010. Bone mineral density after simultaneous kidney-pancreas transplantation: four years follow-up of 57 recipients. Transplant. Proc. 42, 555–557.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2018. nlme: linear and nonlinear mixed effects Models_. R package version 3.1-137. https://CRAN.R-project.org/package=nlme.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rauch, F., Schoenau, E., 2005. Peripheral quantitative computed tomography of the distal radius in young subjects - new reference data and interpretation of results. J. Musculoskelet. Neuronal Interact. 5, 119–126.

Rauch F, Schoenau E (2008) Peripheral quantitative computed tomography of the proximal radius in young subjects–new reference data and interpretation of results. J. Musculoskelet. Neuronal Interact. 8:217–226.

Rocha, A., Martins, L.S., Malheiro, J., Dores, J., Santos, C., Henriques, C., 2016. Changes in bone mineral density following long-term simultaneous pancreas-kidney transplantation. J. Bone Miner. Metab. 34, 209–215.

Samelson, E.J., Broe, K.E., Xu, H., et al., 2019. Cortical and trabecular bone microarchitecture as an independent predictor of incident fracture risk in older women and men in the bone microarchitecture international consortium (BoMIC): a prospective study. Lancet Diabetes Endocrinol. 7, 34–43.

Silva, B.C., Broy, S.B., Boutroy, S., Schousboe, J.T., Shepherd, J.A., Leslie, W.D., 2015. Fracture risk prediction by non-BMD DXA measures: the 2015 ISCD official positions part 2: trabecular bone score. J. Clin. Densitom. 18, 309–330.

Smets, Y.F., van der Pijl, J.W., de Fijter, J.W., Ringers, J., Lemkes, H.H., Hamdy, N.A., 1998. Low bone mass and high incidence of fractures after successful simultaneous pancreas-kidney transplantation. Nephrol. Dial. Transplant. 13, 1250–1255.

Smets, Y.F., de Fijter, J.W., Ringers, J., Lemkes, H.H., Hamdy, N.A., 2004. Long-term follow-up study on bone mineral density and fractures after simultaneous pancreas-kidney transplantation. Kidney Int. 66, 2070–2076.

Soucek, O., Lebl, J., Snajderova, M., Kolouskova, S., Rocek, M., Hlavka, Z., Cinek, O., Rittweger, J., Sumnik, Z., 2011. Bone geometry and volumetric bone mineral density in girls with turner syndrome of different pubertal stages. Clin. Endocrinol. (Oxf) 74, 445–452.

Soucek, O., Schonau, E., Lebl, J., Willnecker, J., Hlavka, Z., Sumnik, Z., 2018. A 6-year follow-up of fracture incidence and volumetric bone mineral density development in girls with turner syndrome. J. Clin. Endocrinol. Metab. 103, 1188–1197.

Torregrosa, J.V., Sanchez-Escuredo, A., Fuster, D., et al., 2015. DXA variations and fractures after simultaneous pancreas-renal transplantation: results of a long-term follow-up. Clin. Nucl. Med. 40, e232–e235.

Vangala, C., Pan, J., Cotton, R.T., Ramanathan, V., 2018. Mineral and bone disorders after kidney transplantation. Front Med (Lausanne) 5, 211.

Wolf, M., Weir, M.R., Kopyt, N., Mannon, R.B., Von Visger, J., Deng, H., Yue, S., Vincenti, F., 2016. A prospective cohort study of mineral metabolism after kidney transplantation. Transplantation 100, 184–193.