Bioavailable testosterone is positively associated with bone mineral density in male kidney transplantation candidates

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Keywords

Bone density; Chronic kidney disease; Hypogonadism; Osteoporosis; Gonadal sex steroids

Abstract

Background

Low levels of sex hormones are common in patients with chronic kidney disease (CKD) and may be a contributing factor to bone fragility. We investigated associations between levels of sex hormones and bone mineral density (BMD) in adult kidney transplantation candidates.

Methods

Volumetric BMD of spine and hip were measured by computed tomography. Parathyroid hormone (PTH), Testosterone (T), Estradiol (E) and sexual hormone binding protein (SHBG) were measured from fasting morning blood samples. Bioavailable (Bio) T and E were calculated based on constants for protein binding.

Results

A total of 146 patients (102 men, 44 women) were included in analyses. Median age was 54 years (range 32 to 72), 32% were diabetics and 36% received maintenance dialysis therapy. In men, Bio T was positively associated with BMD at the lumbar spine (β=5.02, p=0.002), total hip (β=6.35, p=0.001) and femoral neck (β=13.9, p=0.002) independently of age, body mass index, dialysis, diabetes type 1 and 2, parathyroid hormone, and steroid exposure. Bio E was positively associated with BMD at lumbar spine (β=0.23, p=0.03) and femoral neck (β=0.61, p=0.04) using the same fully adjusted model. In post-menopausal women, Bio T was positively correlated to lumbar spine BMD (r=0.46, p=0.02).

Conclusions

High endogenous levels of sex hormones were associated with greater bone density in male kidney transplantation candidates. Disturbances in the gonadal axis may contribute to skeletal fragility in men with late stage CKD.

Introduction

Gonadal dysfunction is common in chronic kidney disease (CKD), particularly in late stages[1]. Testosterone (T) and estradiol (E) decrease bone resorption in adults[2,3], and low levels are known to cause osteopenia and osteoporosis in both men and women in the general population[4]. Patients with CKD suffer renal bone disease with changes in bone quantity and quality[5], resulting in an increased risk of fractures[6]. Disturbances in the gonadal axis could be a contributing factor to bone fragility in these patients.

The effects of sex hormone levels on bone health in CKD has not been well described. Low levels of E are associated with reduced BMD in both men[7] and women[8,9] on hemodialysis, and BMD has been shown to improve with hormone replacement therapy in women[10]. In male patients, it has been suggested that the action of sex hormones on BMD could be mediated through the receptor activator of nuclear factor κB (RANK) and RANK ligand (RANKL)-system[11,12], though others found no correlation between levels of T and BMD[7].

Only small percentages of T and E circulate unbound. The greater fractions are either tightly bound to sex hormone binding globulin (SHBG), or more loosely bound to albumin[13]. A bioavailable fraction, the sum of free and albumin bound hormone concentrations, can be calculated based on constants for protein binding[14–16]. It is still unclear whether the biological actions of T and E are best represented by the total, bioavailable, or unbound forms[17]. This issue may be particularly relevant in CKD, where changes in the gonadal axis occur at several levels[18] and disturbances in albumin levels are frequent[19].

As there is a lack of knowledge of the relationship between sex hormones and bone density in CKD, we investigated the associations between the total and bioavailable fractions of T and E and

volumetric bone mineral density (BMD) in a cohort of kidney transplantation candidates. We hypothesized that there would be a positive relationship between endogenous levels of sex hormones and BMD in men and women with late stage CKD.

Methods

Study participants

Adult kidney transplantation candidates were consecutively enrolled from four centers in Denmark from February 2011 through January 2014. The inclusion criteria were indication for cardiovascular screening before kidney transplantation by one or more of the following characteristics: age >40 years, diabetes mellitus (DM), dialysis treatment >5 years, kidney transplant waiting list >3 years, or symptoms of cardiovascular disease. Patients with unstable angina pectoris were excluded.

Written informed consent was obtained from all patients prior to study participation. The study followed the principles in the declaration of Helsinki and was registered at ClinicalTrials.gov (NCT01344434). The study protocol was approved by the Central Denmark Region Committee on Health Research Ethics and The Danish Data Protection Agency.

Bone density measurements

An angiographic CT-scan of chest, abdomen, and pelvis was performed on a dual-source CT-scanner (SOMATOM Definition Flash; Siemens, Erlangen, Germany). Details of the CT protocol have been published previously[20]. Briefly, tube energy was set at 100 or 120 kVp depending on patient size, tube current was dose-modulated and set at 250 mAs, slice thickness was 3.0 mm, and images were reconstructed with a standard soft tissue kernel. A set dose of 95 mL of the x-ray contrast media Ioversol was administered intravenously (Optiray, Mallinckrodt, Germany). BMD was analyzed from the contrast-enhanced images.

The commercially available software *QCT Pro* version 5.1 (Mindways Software Inc, TX, US) was used to determine BMD of lumbar spine and proximal femur. A calibration phantom (Mindways Solid; Mindways Software Inc, TX, US) was scanned separately at regular intervals to provide calibration data for asynchronous BMD-analysis[21].

At the lumbar spine, an oval region of interest was placed in the anterior part of three consecutive vertebrae between L1 and L4, keeping clear of the posterior venous plexus. Fractured and visibly deformed vertebrae were excluded from analyses. L1-L3 was preferred, though in 17 patients L2-L4 was analyzed. At the proximal femur, the semi-automatic function provided by the software was used for analyses of left hip BMD, with visual control and manual adjustment if needed. In 13 patients the right hip was analyzed due to previous fracture, metallic prosthesis or incomplete image of the left hip. *T*- and *Z*-scores were calculated from the two-dimensional hip-projection (CTXA) compared to reference data supplied by the software manufacturer. In recent guidelines, CTXA was recommended for use in diagnosing osteoporosis[22]. Coefficients of variations (CV) were 0.68% at the lumbar spine, 1.85% at the total hip and 2.30% at the femoral neck.

Fractures

Fracture status was determined by previous clinical fractures and prevalent vertebral fractures. All low-trauma fractures occurring in adult life were included. High-trauma fractures, as well as fractures of fingers, toes, face, and skull were excluded. Data on clinical fractures were extracted from patient interview and chart review, and all fractures were confirmed by x-ray images or radiology reports. Prevalent VFs were diagnosed from two-dimensional reconstructions of CT images of the thoracolumbar spine by an experienced radiologist, according to Genant's method[23].

Biochemical measurements

Fasting morning blood samples were collected. Plasma intact parathyroid hormone (PTH), phosphate, ionized calcium, and albumin were analyzed throughout the study period. Blood samples

for analyses of sex hormones and bone turnover markers were stored at -80°C and analyzed in a single batch at study completion. We analyzed two markers of bone formation: Bone specific alkaline phosphatase (BSAP; enzyme-linked immunosorbent assay, CV 10%) and procollagen type 1 N-terminal propeptide trimer (P1NP; radioimmunoassay IDS-iSYS, CV 10%) and one marker of bone resorption: Tartrate resistant alkaline phosphatase (TRAP5b; enzyme-linked immunosorbent assay, CV 3%). Total T and Total E were measured by high performance liquid chromatography, with a CV of 10%. Follicle stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone binding globulin (SHBG) were measured by sandwich immunometric assay. The limit of detection was 0.12 nmol/L for total T (*n* = 7 below), 0.018 nmol/L for total E2 (*n* = 14 below), and 0.30 IU/L for both LH (*n* = 1 below), and FSH (none below). Below detection-values were estimated by dividing the limit of detection by the square root of 2. Upper limit of detection was 200 IU/L for both LH (*n* = 1 above) and FSH (*n* = 4 above), and values were estimated by adding 10%. Bio T and Free T were calculated based on Vermeulens algorithm[14], using the actual concentrations of plasma albumin rather than the suggested set value of 4.3 g/L. Bio E and Free E were similarly calculated based on constants for protein binding[15]. As correlation coefficients were close to 1 between the bioavailable and free fractions for both men (Bio T and Free T, $r = 0.95$; Bio E and Free E, $r = 0.93$) and women (Bio T and Free T, $r = 0.99$; Bio E and Free E, $r = 0.995$), only the bioavailable fractions are reported. Further details on the analytic methods and assays used are given in Supplementary Table 1.

Statistical analyses

Statistical analyses were performed with standard software package STATA/IC 13.1 for Windows (StataCorp LP, TX, US). Continuous variables were visually evaluated for normality by QQ-plots. Skewed variables were transformed to their natural logarithm (BSAP, P1NP, TRAP5b, Total T, Total E2, SHBG, PTH) to enable parametric statistical testing. Severely skewed variables (LH,

FSH, Prolactin) were not transformed, and non-parametric testing was used. Normally distributed variables are presented as mean±SD, and skewed variables as median with interquartile range. Associations between continuous variables were evaluated by pairwise univariate correlation or Spearman's rank correlation. Multiple linear regression analysis was then used to adjust for age, BMI, dialysis therapy, type 1 DM, type 2 DM, and steroid exposure. Steroid exposure was defined as previous treatment with either >10 mg prednisolone for 3 months, or > 5 mg prednisolone for 12 months, or current prednisolone use. All analyses involving sex hormones were stratified by gender and menopausal status. A two-sided *p*-value < 0.05 was considered statistically significant.

Results

Demographic data

Of 167 included adult kidney transplantation candidates, 10 were excluded due to: Withdrawn consent (*n* = 5), major cardiovascular event (*n* = 4), or kidney transplantation (*n* = 1) prior to first study visit. Further 11 were excluded due to inability to draw blood for biochemical analyses (*n* = 8), or current hormone replacement therapy (2 women and 1 man); leaving a total of 146 patients.

Baseline data including levels of sex hormones are given in Table 1. Underlying causes of CKD were: DM type 1 or 2 (26%), hypertension or glomerulosclerosis (25%), glomerulonephritis or vasculitis (23%), adult polycystic kidney disease (14%), and other/unknown (11%). Fifty-three patients were on maintenance dialysis therapy (> 3 months); 37 received hemodialysis and 16 peritoneal dialysis therapy. Median time on dialysis was 24 months (IQR 6 to 66, range 3 to 240). Pre-dialysis patients (*n* = 93) were all considered close to initiating dialysis by their primary clinician. They had a median estimated glomerular filtration rate (eGFR) of 11 ml/min/m^3 (IQR 9 to 14, range 4 to 31). Only four patients had an eGFR above 20 ml/min/m^3 (at 21, 23, 31, and 31); of these, one patient had a high variability in serum creatinine, with the lowest eGFR at 14 ml/min/m^3, one had a 24 hour urine creatinine clearance of 15 ml/min, and one was amputated, with likely an

overestimated eGFR. The remaining patient had DM type 1 with multiple diabetic complications, and was being referred for a simultaneous pancreas-kidney transplantation.

One male patient had recently initiated bisphosphonate-therapy (1 month prior to study inclusion) and two men currently received calcimimetics. Forty patients had been exposed to steroids, by previous treatment (n = 22), or by currently taking prednisolone (n = 18). The majority of patients on active steroid treatment were on a low daily dose of either 2.5 or 5 mg/day (n = 16), most commonly due to previous kidney transplantation (n = 13). Other indications included connective tissue disease (n = 2), glomerulonephritis (n = 2), and lung transplant (n = 1). Percentages exposed to steroids were: 24% of men vs. 36% of women (p = 0.16), and 24% of pre-dialysis patients vs. 34% of dialysis patients (p = 0.18). Steroid exposure was associated with significantly reduced Z-scores at all areas: Lumbar spine (-0.99±1.38 vs. -0.28±1.40, p = 0.004), total hip (-1.56±0.86 vs. -1.13±1.12, p = 0.03), and femoral neck (-1.41±0.72 vs. -0.94±0.99, p = 0.008).

Results for male patients

The prevalence of hypogonadism defined as a Total T below 10 nmol/L was 22% (n = 22), with levels below 8 nmol/L in 13% (n = 13). Men with low levels of Total T had BMD comparable to those with normal T levels (Figure 1). However, a trend towards reduced BMD was seen in men with Bio T levels beneath the 25th (3.9 nmol/L) or 10th (3.25 nmol/L) percentiles (Figure 1).

Patients on dialysis had significantly higher levels of T compared to pre-dialysis patients (Figure 2). Patients with DM type 1 and 2 had similar levels of T as non-diabetics. Bio T was 6.3±2.4 nmol/L in type 1 DM, 4.9±2.1 nmol/L in type 2 DM, and 5.7±2.0 nmol/L in non-diabetics (ANOVA p = 0.18). Levels of E were higher in DM; Bio E was 89±41 ρmol/L in type 1 DM, 84±38 ρmol/L in type 2 DM, and 67±26 ρmol/L in non-diabetics (ANOVA p = 0.01). The difference between type 1 DM and non-diabetics was significant (p = 0.005), and remained so after adjustment for age, BMI,

and dialysis therapy ($p = 0.001$). The difference between type 2 DM and non-diabetics was not significant; $p = 0.11$ and $p = 0.10$ in the unadjusted and adjusted model, respectively.

Scatterplots between sex hormones and BMD are shown in Figure 3, and univariate correlations between sex hormones, BMD, and bone turnover markers are shown in Table 2. Both Bio T and Bio E were positively associated with lumbar spine BMD, but not with BMD at the proximal femur. To explore these relationships further, we performed multiple linear regression analyses with BMD as the outcome variable, and the bioavailable fractions of sex hormones as explanatory variables (Table 3). In the adjusted models, Bio T was positively associated with BMD at both spine and hip, while Bio E was positively associated with lumbar spine BMD. Bio T remained significantly associated with BMD at both spine and hip after adjusting for levels of Bio E (Table 4).

Bone turnover markers were not correlated to levels of Bio T or E (Table 3). Total T was positively associated with markers of bone formation; BSAP and P1NP. However, these associations were no longer significant after adjusting for age, BMI, and dialysis therapy (BSAP: $p = 0.07$, and P1NP: $p = 0.18$). Similarly, Total E was positively correlated with bone resorption marker TRAP5b, but not significantly so in the adjusted model ($p = 0.17$).

Men with a previous fragility fracture ($n = 17$) had Total T 16 [IQR 9, 19] nmol/L, Bio T of 5.3±2.3 nmol/L, and Bio E of 82±44 ρmol/L. This was not significantly different from patients with no history of fracture: Total T 13 [10, 16] nmol/L (difference 2%, $p = 0.87$), Bio T 5.8±2.1 nmol/L (difference: 0.5 nmol/L, $p = 0.36$), and Bio E 73±30 ρmol/L (difference: 9 ρmol/L, $p = 0.30$). Levels of sex hormones were also not associated with fracture status in a multivariate logistic regression model adjusted for age, BMI, and dialysis therapy (results not shown).

Results for female patients

By patient interview, 12 women were pre-menopausal, 22 were post-menopausal, and 10 had unknown status. The ten women with unknown status were categorized as post-menopausal if FSH-levels were >100 IU/L (n = 5) or age were >60 years with undetectable Total E (n = 1). The remaining four women were 42, 45, 45, and 49 years old, with Total E values well above the post-menopausal range (>200 nmol/L), and they were categorized as premenopausal.

BMD and *Z*-scores for pre- and post-menopausal women are shown in Table 1. Lumbar spine BMD was significantly reduced in post-menopausal compared to pre-menopausal women, with a difference of -52 mg/cc (CI -76 to -28, $p < 0.001$). In contrast, BMD was comparable at the proximal femur, with a difference of -25 mg/cc at the total hip (CI -57 to 6, $p = 0.11$), and -28 mg/cc at the femoral neck (CI -65 to 9, $p = 0.14$). TRAP5b was 31% (CI 3 to 51%, $p = 0.03$) higher in postmenopausal women, while there were no differences in BSAP (-7%, CI -35 to 25, $p = 0.57$) or P1NP (10%, CI -33 to 40, p=0.58) based on menopausal status.

Univariate correlations between levels of sex hormones and BMD of spine and hip in women are shown in Table 5. In post-menopausal women, levels of T were positively correlated with BMD of the spine. In pre-menopausal women LH was the only hormone significantly correlated to BMD.

There were no significant correlations between biochemical markers of bone turnover and levels of sex hormones for either pre- or post-menopausal women (data not shown).

Pre-menopausal women with a previous fragility fracture (n = 4) had lower levels of Bio T than women without fracture (median 57 [52, 74] vs. 144 [113, 208] ρmol/L, $p = 0.04$), while levels of Bio E were comparable (median 270 [140, 330] vs. 145 [96, 215] ρmol/L, $p = 0.67$). Post-menopausal women with fracture (n = 4) had similar levels of Bio T (median 23 [18, 246] vs. 137

[48, 246] ρmol/L, $p = 0.12$) and Bio E (median 7 [6, 24] vs. 8 [IQR 6, 20] ρmol/L, $p = 0.68$) as women without fracture.

Discussion

In this study we demonstrate a positive association between levels of sex hormones and BMD in male kidney transplantation candidates. Specifically, these associations were seen with the biologically active fractions of the hormones. In female patients testosterone levels were positively correlated to bone density of the spine in post-menopausal, but not pre-menopausal women.

Male patients

Surprisingly, patients on maintenance dialysis therapy had elevated levels of T compared to pre-dialysis patients. This is in contrast to previous studies, where prevalence of hypogonadism increased with decreasing kidney function[24,25]. Possible explanations include a higher number of Type 1 DM among pre-dialysis compared to dialysis patients (30 vs. 11%), as these patients had a trend towards higher levels of T. However, including DM 1 and 2 as explanatory variables in the multivariate models did not fully explain the differences in T-levels between dialysis and pre-dialysis patients. Albumin levels were lower in pre-dialysis patients; however this would only be expected to affect Bio T and not Total T-levels. Our maintenance dialysis patients were a highly selected group, as they were all considered kidney transplantation candidates. This was reflected by high proportions of patients having been less than 12 months on dialysis (32%) and retaining some residual kidney function (67%). We speculate that an improvement in gonadal axis function may occur with the initiation of dialysis therapy, but this issue will need further investigations.

This is the first study to demonstrate an association between T levels and bone density in male patients with severe CKD. Such a positive relationship is well known in otherwise healthy elderly men[26,27], where low levels of T increase the risk of osteoporosis and are associated with loss of BMD over time[27]. In CKD, only one study previously reported on this issue. Mirfakhraee *et al.*

found no significant correlations between Total T or Bio T and *Z*-scores in male hemodialysis patients[7]. The study did find that Free E was positively associated with hip and forearm BMD, with correlations of the same magnitude as seen in our cohort; however, these associations were no longer significant after adjustment for body size. In contrast, the relationship between BMD and both Bio T and Bio E in our cohort were robust through adjustments for several possible confounders, including age, body size, diabetes, maintenance dialysis therapy, hyperparathyroidism, and steroid exposure.

We did not find any differences in sex hormone status between men based on fracture status. However, the cross-sectional nature of our analyses, and the low number of events, may have prevented us from detecting a true association.

The mechanism of T on bone is believed to be mediated through stimulation of the androgen receptor on both osteoblast and osteoclast lineage cells[3]. We found positive correlations between levels of sex hormones and bone formation markers, while there were no significant associations with bone resorption markers. In addition, a previous study including male hemodialysis patients found a negative association between Total T and RANKL concentrations [11,12]. RANKL is secreted by osteoblasts and binds to its receptor RANK on osteoclasts, stimulating osteoclast proliferation and activation; higher levels are therefore associated with increased bone resorption. Thus, a direct stimulation of osteoblast function, coupled with an indirect effect on osteoclast-function through the RANK-RANKL system may be the possible mechanism of the effect of T on the skeleton in CKD. Unfortunately, we did not have the data to further investigate this hypothesis.

Bio E was positively associated with bone density of the lumbar spine. This is in line with several recent studies in otherwise healthy elderly men, demonstrating strong, positive, associations between endogenous levels of E and BMD, leading authors to speculate that E could be as powerful

a regulator of bone metabolism in men, as it is in women [28–31]. This hypothesis is supported by results from interventional studies selectively blocking the effects of T and E and measuring the skeletal response by QCT or bone turnover markers[32,33].

Female patients

In women we found the expected differences of reduced BMD and increased bone resorption after menopause. High levels of T were associated with greater BMD at the lumbar spine in post-menopausal women, which is consistent with findings in post-menopausal women with normal kidney function[34]. Pre-menopausal women with previous fragility fractures had lower levels of T, and the same trend was seen in post-menopausal women. Thus, similar to otherwise healthy women, the reduction of endogenous sex hormone levels after menopause could cause a loss of BMD and consequently a reduction in bone strength in CKD. Unfortunately, the low number of women in each group prevented any meaningful multivariate analyses.

Strengths and limitations

We consider our choice of method for measuring BMD a strength as CT yields high-resolution, three-dimensional images, enabling precise measurement of volumetric BMD and avoiding artefacts from surrounding tissues. Major limitations include the cross-sectional design, and a relatively small number of patients, particularly women. We used single-measurement of sex hormones in both men and women, and further, we did not time our analyses according to the menstrual cycle in pre-menopausal women. The equations used to calculate free and bioavailable fractions have not been validated for patients with CKD. There was great heterogeneity in the cohort with regards to the cause, stage, and treatment of CKD; we attempted to adjust for this by including DM Type 1 and 2, as well as dialysis therapy in our multivariate models. On the other hand, the cohort was a selected population of end stage kidney disease patients considered for

kidney transplantation, which may limit the generalizability of our results. As more than 95% of our cohort was Caucasian, results may not be transferrable to other ethnic groups.

Conclusion

A positive relationship between sex hormones and BMD was found in male kidney transplantation candidates. Disturbances in the gonadal axis may contribute to skeletal fragility in men with late stage CKD.

Disclosure

Conflicts of interest: Hanne S Jørgensen: None, Simon Winther: None, Lars Rejnmark: None, Morten Bøttcher: None, Ellen M Hauge: None, My Svensson: None, Per Ivarsen: None

Acknowledgements

The results published here have previously been presented in abstract form at ASN Kidney Week 2016 (Chicago, IL).

The authors thank all study participants and the following co-workers: Birgitte Kildevæld Sahl (Renal Research Lab, Aarhus University Hospital) for biochemical analyses and clinical examinations; Birgitte Bang (Department of Nephrology, Aalborg University Hospital) for her long-standing assistance; and staff at Departments of Nephrology, Viborg Hospital and Holstebro Hospital for including patients.

This study received financial support from the following sources: Aarhus University, Danish Society of Nephrology, Danish Kidney Association, Central Denmark Health Region, Karen Elise Jensen Foundation, Helen and Ejnar Bjørnow Research Fund, Søster and Verner Lippert Research Fund, Cand. polyt. Frode V Nyegaard and Wife Research Fund and MD Søren Segel and Wife Johanne Wiibroe Segel Research Fund

Supplementary material

Supplementary Table 1
Details of analytic methods and assays used

Supplementary Table 2
Levels of sex hormones with normal ranges by gender

Supplementary information is available at KI Report's website.

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Tables

Table 1 Baseline data of participating kidney transplant candidates by gender

	Men (*n* = 102)	Pre-menopausal women (*n* = 16)	Post-menopausal women (*n* = 28)
Age, years	54 (11)	42 (6)	60 (7)
Body mass index, kg/m²	25.7 (3.7)	25.4 (7.7)	25.9 (3.7)
Active smoker	34 (33%)	5 (31%)	6 (21%)
Diabetes mellitus type 1	24 (24%)	7 (44%)	2 (7%)
Diabetes mellitus type 2	11 (11%)	1 (6%)	1 (4%)
Maintenance dialysis therapy	36 (35%)	8 (50%)	9 (32%)
Previous kidney transplantation	14 (14%)	5 (31%)	5 (18%)
Medical treatment			
Previous steroid exposure	16 (16%)	1 (6%)	5 (18%)
Current steroid treatment	8 (8%)	4 (25%)	6 (21%)
Phosphate-binder	74 (73%)	13 (81%)	16 (57%)
Active vitamin D receptor activator	75 (74%)	10 (63%)	20 (77%)
Calcium metabolism			
Parathyroid hormone, ρmol/L	21.5 [14.5, 29.3]	16.9 [11.9, 27.0]	19.8 [11.4, 31.4]
Phosphate, mmol/L	1.59 (0.37)	1.59 (0.44)	1.48 (0.36)
Ionized calcium, mmol/L	1.22 (0.08)	1.24 (0.09)	1.23 (0.06)
Bone specific alkaline phosphatase, u/L	25 [20, 32]	30 [25, 41]	24 [22, 37]
Pro-collagen type I N-terminal propeptide, μg/L	61.0 [40.5, 85.3]	78.1 [37.4, 107]	77.8 [44.9, 113]
Tartrate resistant alkaline phosphatase type 5b, U/L	4.1 [2.7, 5.8]	3.5 [2.1, 5.2]	5.0 [3.2, 6.3]
Sex hormones			
Total Testosterone, nmol/L	13 [10, 17]	0.5 [0.3, 0.7]	0.58 [0.16, 0.95]
Bioavailable Testosterone, nmol/L	5.71 (2.14)	0.130 [0.074, 0.176]	0.124 [0.030, 0.246]
Total Estradiol, nmol/L	114 (42)	300 [174, 692]	16 [13, 47]
Bioavailable Estradiol, ρmol/L	74 (33)	167 [96, 270]	8.3 [5.8, 19.8]
Sex hormone binding globulin, nmol/L	35 [26, 48]	61 [48, 81]	59 [40, 84]
Bone density			
Lumbar spine vBMD, mg/cm³	116 (35)	167 (30)	116 (42)
Lumbar spine *Z*-score	-0.81 (1.20)	0.42 (1.20)	0.24 (1.56)
Total hip vBMD, mg/cm³	227 (41)	250 (66)	225 (37)
Total hip *Z*-scores	-1.11 (1.08)	-1.81 (1.34)	-1.42 (0.66)
Femoral neck vBMD, mg/cm³	228 (46)	261 (73)	233 (48)
Femoral neck *Z*-score	-0.96 (0.92)	-1.45 (1.17)	-1.26 (0.80)
Femoral neck aBMD, mg/cm²	0.593 (0.093)	0.593 (0.143)	0.541 (0.100)

Data are mean(SD), median[IQR] or n(%). Abbr.: aBMD=areal bone mineral density, vBMD=volumetric bone mineral density

Table 2 Univariate correlations between levels of sex hormones, bone density, and bone turnover markers in male kidney transplantation candidates

	Lumbar spine vBMD		Total hip vBMD		Femoral neck aBMD		BSAP		TRAP5b		P1NP	
	rho		*rho*		*rho*		*rho*		*rho*		*rho*	
Total Testosterone, nmol/L	0.02		-0.10		-0.12		0.26	†	0.18	(†)	0.24	†
Bioavailable Testosterone, nmol/L	0.25	†	0.14		0.10		0.18	(†)	-0.02		0.16	
Total Estradiol, nmol/L	0.21	†	-0.06		-0.02		0.14		0.22	†	0.19	(†)
Bioavailable Estradiol, ρmol/L	0.31	†	0.07		0.09		-0.02		0.03		0.05	
Sex hormone binding globulin, nmol/L	-0.23	†	-0.22	†	-0.22	†	0.28	†	0.34	†	0.20	†
Follicle stimulating hormone, U/L	-0.22	†	-0.07		-0.09		0.16		0.05		-0.02	
Luteinizing hormone, U/L	-0.09		-0.08		-0.11		0.03		-0.01		-0.09	

Data are Spearman's correlation coefficients, *rho*, (†) = $p < 0.10$, † = $p < 0.05$

Abbr.: aBMD=areal bone mineral density, vBMD=volumetric bone mineral density, BSAP=Bone specific alkaline phosphatase (U/L), TRAP5b=Tartrate resistant alkaline phosphatase (U/L), P1NP=Pro-collagen type 1 N-terminal propeptide (ug/L)

Table 3 Association between bioavailable sex hormones and bone mineral density (BMD) of spine and hip in male kidney transplant candidates

	Lumbar spine vBMD			*Total hip vBMD*			*Femoral neck aBMD*		
	β	*95% CI*	*p*	*β*	*95% CI*	*p*	*β*	*95% CI*	*p*
Bioavailable Testosterone, nmol/L									
Minimal model	4.79	1.59 to 8.00	0.004	4.94	0.82 to 9.06	0.02	10.3	0.72 to 19.9	0.04
Complete model	5.02	1.95 to 8.10	0.002	6.35	2.56 to 10.1	0.001	13.9	5.18 to 22.5	0.002
Bioavailable Estradiol, nmol/L									
Minimal model	0.23	0.03 to 0.43	0.02	0.09	-0.17 to 0.34	0.51	0.30	-0.29 to 0.89	0.32
Complete model	0.23	0.02 to 0.44	0.03	0.20	-0.06 to 0.47	0.12	0.61	0.03 to 1.20	0.04

Data are multiple linear regression coefficients, β, with corresponding 95% confidence intervals and -values. Minimal model = age, body mass index, and dialysis therapy. Complete model = age, body mass index, dialysis therapy, type 1 diabetes mellitus, type 2 diabetes mellitus, parathyroid hormone, and steroid exposure
Abbr.: aBMD=areal bone mineral density, vBMD=volumetric bone mineral density

Table 4 Multiple linear regression analysis of the association between sex hormones and bone density in male kidney transplant candidates

	Lumbar spine vBMD (Adj. $R^2 = 0.27$, $p < 0.001$)			Total hip vBMD (Adj. $R^2 = 0.22$, $p < 0.001$)			Femoral neck aBMD (Adj. $R^2 = 0.24$, $p < 0.001$)		
	β	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
Age, years	-1.28	-1.93 to -0.63	<0.001	-0.61	-1.41 to 0.19	0.14	-2.05	-3.88 to -0.23	0.03
Body mass index, kg/m²	1.46	-0.26 to 3.19	0.10	1.75	-0.40 to 3.90	0.11	2.15	-2.77 to 7.07	0.39
Dialysis therapy, y/n	-12.8	-26.8 to 1.25	0.07	-14.4	-31.8 to 3.01	0.10	-54.3	-94.0 to -14.5	0.008
Diabetes mellitus type 1, y/n	9.04	-26.4 to 8.36	0.31	-34.7	-56.2 to -13.2	0.002	-111.8	-161 to -62.6	<0.001
Diabetes mellitus type 2, y/n	4.77	-15.4 to 24.9	0.64	18.3	-6.62 to 43.2	0.15	13.0	-43.2 to 69.1	0.65
Steroid exposure, y/n	-19.3	-33.7 to -4.80	0.01	-17.3	-35.2 to 0.59	0.06	-52.6	-92.8 to -12.3	0.01
Parathyroid hormone, ρmol/L	-8.86	-16.0 to -1.69	0.02	-13.3	-22.6 to -3.96	0.006	-19.8	-40.8 to 1.20	0.06
Bioavailable Testosterone, nmol/L	4.32	0.87 to 7.76	0.02	6.21	1.95 to 10.5	0.005	12.1	2.39 to 21.8	0.02
Bioavailable Estradiol, ρmol/L	0.10	-0.12 to 0.33	0.37	0.22	-0.26 to 0.30	0.88	0.27	-0.37 to 0.90	0.40

Data are multiple linear regression *β*-coefficients with 95% confidence intervals (CI) and *p*-values

Table 5 Univariate correlations between levels of sex hormones and bone mineral density (BMD) of spine and hip in female kidney transplant candidates

	Pre-menopausal women (n = 16)			Postmenopausal women (n = 28)		
	Lumbar spine vBMD	Total hip vBMD	Femoral neck aBMD	Lumbar spine vBMD	Total hip vBMD	Femoral neck aBMD
	Rho	*Rho*	*Rho*	*Rho*	*Rho*	*Rho*
Total Testosterone, nmol/L	0.18	-0.01	-0.01	0.46 †	0.17	0.16
Bioavailable Testosterone, nmol/L	0.12	0.09	0.09	0.46 †	0.23	0.23
Total Estradiol, nmol/L	0.09	-0.04	-0.05	0.18	-0.02	-0.11
Bioavailable Estradiol, nmol/L	0.22	0.14	0.12	0.17	-0.00	-0.02
Sex hormone binding globulin, nmol/L	0.02	-0.15	-0.14	0.06	-0.05	-0.04
Follicle stimulating hormone, U/L	0.24	-0.05	0.04	0.01	-0.02	-0.21
Luteinizing hormone, U/L	0.53 †	0.41	0.52 †	-0.12	-0.01	-0.18

Data are Spearman's correlation coefficients, *rho*, † = $p < 0.05$

Abbr.: vBMD=volumetric bone mineral density, aBMD=areal bone mineral density

Legends to figures

Figure 1 Bone mineral density (BMD) in male kidney transplantation candidates with low levels of total and bioavailable testosterone. Mean values with standard errors, *p* = Student's *t* test

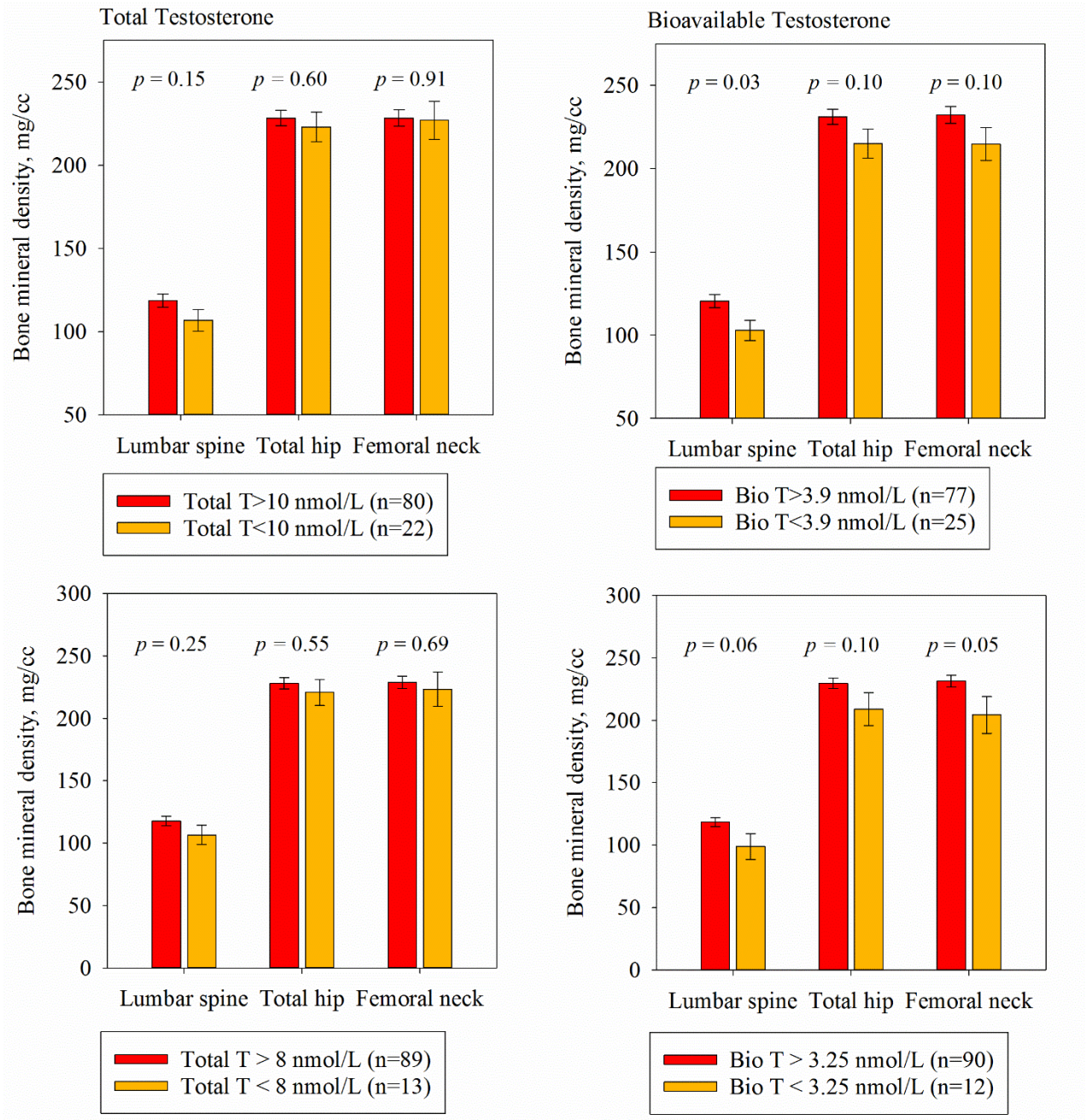

Figure 2 Sex hormone levels in male kidney transplantation candidates, by dialysis status. Box plots with median, interquartile range, and whiskers at 5 and 9%, *p* = Student's *t* test

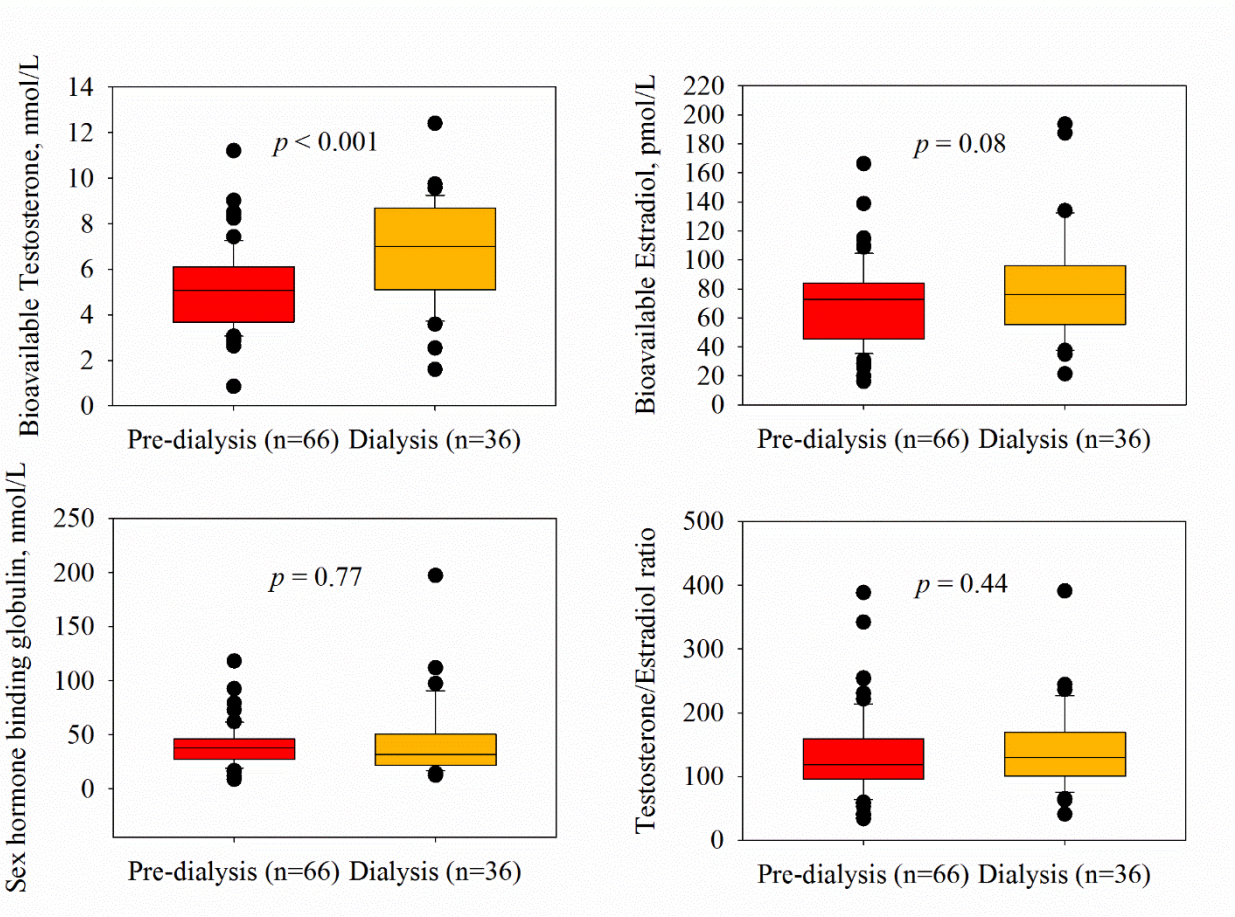

Figure 3 Scatterplots on the association between bioavailable fractions of testosterone and estradiol and bone mineral density (BMD) in male kidney transplantation candidates. Full line = dialysis patients, dotted line = pre-dialysis patients