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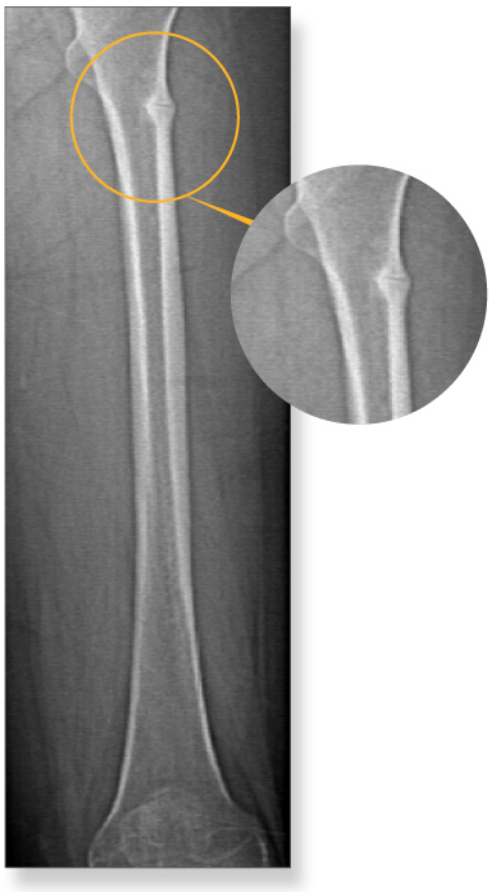

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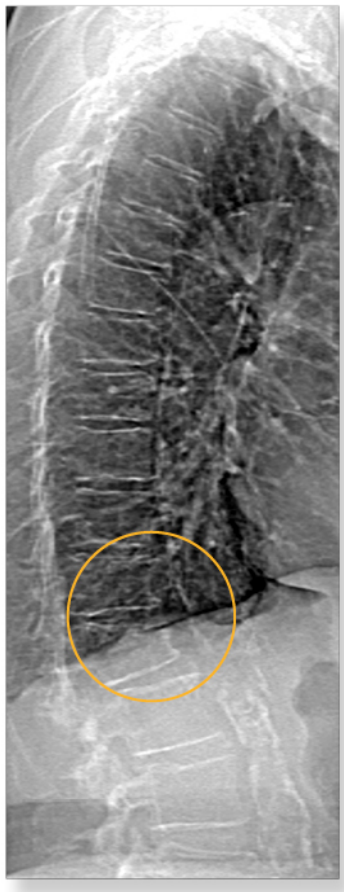

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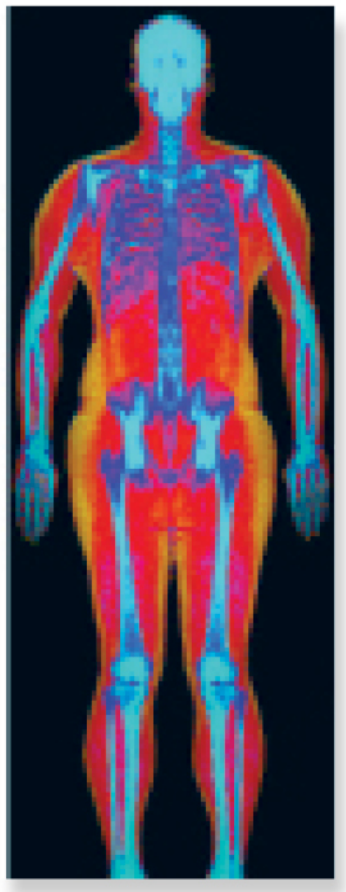

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ORIGINAL ARTICLE

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Bone Geometry, Volumetric Density, Microarchitecture, and Estimated Bone Strength Assessed by HR-pQCT in Klinefelter Syndrome

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ABSTRACT

Although the expected skeletal manifestations of testosterone deficiency in Klinefelter's syndrome (KS) are osteopenia and osteoporosis, the structural basis for this is unclear. The aim of this study was to assess bone geometry, volumetric bone mineral density (vBMD), microarchitecture, and estimated bone strength using high-resolution peripheral quantitative computed tomography (HR-pQCT) in patients with KS. Thirty-one patients with KS confirmed by lymphocyte chromosome karyotyping aged 35.8 ± 8.2 years were recruited consecutively from a KS outpatient clinic and matched with respect to age and height with 31 healthy subjects aged 35.9 ± 8.2 years. Dual-energy X-ray absorptiometry (DXA) and HR-pQCT were performed in all participants, and blood samples were analyzed for hormonal status and bone biomarkers in KS patients. Twenty-one KS patients were on long-term testosterone-replacement therapy. In weight-adjusted models, HR-pQCT revealed a significantly lower cortical area ($p < 0.01$), total and trabecular vBMD ($p = 0.02$ and $p = 0.04$), trabecular bone volume fraction ($p = 0.04$), trabecular number ($p = 0.05$), and estimates of bone strength, whereas trabecular spacing was higher ($p = 0.03$) at the tibia in KS patients. In addition, cortical thickness was significantly reduced, both at the radius and tibia (both $p < 0.01$). There were no significant differences in indices of bone structure, estimated bone strength, or bone biomarkers in KS patients with and without testosterone therapy. This study showed that KS patients had lower total vBMD and a compromised trabecular compartment with a reduced trabecular density and bone volume fraction at the tibia. The compromised trabecular network integrity attributable to a lower trabecular number with relative preservation of trabecular thickness is similar to the picture found in women with aging. KS patients also displayed a reduced cortical area and thickness at the tibia, which in combination with the trabecular deficits, compromised estimated bone strength at this site.

KEY WORDS: KLINEFELTER SYNDROME; vBMD; HR-pQCT; FINITE ELEMENT ANALYSIS; TRABECULAR NUMBER

Introduction

Klinefelter syndrome (KS), occurring in approximately 1 in 660 live male births,[1] is the most common congenital abnormality leading to male primary hypogonadism and is owing to the presence of an extra X chromosome, resulting in a 47,XXY genotype. Although the classical phenotypical presentations are infertility, eunochoid body habitus with small testis, gynaecomastia, learning disabilities, and androgen deficiency,[2] subjects with KS can present with fewer stigmata than the full syndrome.[3]

The expected skeletal manifestation of testosterone deficiency is low bone mass,[4,5] as reflected in the areal bone mineral density (aBMD). Studies have shown a positive correlation between serum testosterone levels and BMD in KS,[6–8] and accordingly, most[9–12] but not all studies[13,14] have revealed a higher prevalence of osteopenia and osteoporosis in these patients when compared with age-matched healthy control subjects. Histomorphometric analysis of iliac crest biopsies in KS patients showed a markedly reduced trabecular bone volume and a profound depression in osteoblastic activity.[15] This study, however, was limited by the small number of KS subjects studied ($n = 5$) and the lack of control subjects. There are currently limited data on bone microarchitecture and bone compartment-specific BMD in patients with KS.

The refinement of bone imaging methods such as high-resolution peripheral quantitative computed tomography (HR-pQCT) has made it possible to achieve visualization and structural characterization of the distal radius and tibia with an isotropic image voxel size of 82 μm. This allows evaluation of

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cortical and trabecular microarchitecture in very high detail and simultaneously determine the three-dimensional volumetric BMD (vBMD) and bone geometry. Also, finite element analysis (FEA), a biomechanical computation model, can be applied to HR-pQCT images to obtain estimates of bone strength. Thus, it is now possible to gain insights into elements of bone microarchitecture and aspects of skeletal pathophysiology without the need for an invasive bone biopsy.

In this cross-sectional study, we aimed to assess bone geometry, cortical and trabecular compartmental vBMD and microarchitecture, as well as to evaluate bone strength in patients with KS when compared with age- and height-matched healthy men. We hypothesized that patients with KS would have a compromised bone structure with smaller bones and reduced cortical and trabecular thickness when compared with their healthy counterparts, leading to a reduction in bone strength estimates.

Materials and Methods

Participants

A sample of 31 subjects with lymphocyte chromosome karyotyping–confirmed KS were recruited consecutively between July 2011 and July 2012 from the KS outpatient clinic at the Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark. Twenty-nine subjects had a karyotype of 47,XXY, whereas 2 subjects were mosaics with a karyotype of 46,XY/47,XXY and 46,XY/47,XXY/48,XXXY. KS males were diagnosed at the age of 28.0 ± 9.6 (0 to 45) years. All patients presented with hypergonadotropic hypogonadism, although to a varying degree. Twenty-one KS patients were on long-term testosterone substitution therapy at the time of the study with a median duration of treatment of 6.7 (2.4 to 25.9) years. Four patients were on treatment for less than 2 years. Of the remaining 6 patients, 4 patients had never received testosterone treatment, whereas 2 patients had been previously treated for less than 2 years but were currently not on therapy. The majority ($n = 19$) received intramuscular testosterone injections (1000 mg given on average every 12th week), 5 KS patients received transdermal testosterone gel given daily, and 1 patient was on daily oral testosterone tablets. Five patients had been operated for cryptorchidism. Subjects with known metabolic bone disease (other than osteoporosis), overt endocrine disease (including type 1 or type 2 diabetes), kidney or liver disease, or those on medication known to affect bone metabolism (prior or present anti-osteoporotic medications or systemic glucocorticoids for more than 3 months) were excluded. The protocol was approved by the Regional Scientific Ethical Committees for Southern Denmark (ID S-2010-0115).

A total of 31 control subjects were included from a cohort of 236 healthy men participating in a separate study aimed at establishing HR-pQCT reference data in the adult Danish population.[16] The protocol was approved by the The Regional Scientific Ethical Committees for Southern Denmark (ID S-20090069). Each KS subject was matched with a control subject from this cohort based on the closest age (± 3 years) and height (± 5 cm).

All participants provided informed consent, and the study was performed according to the guidelines from the Declaration of Helsinki. All examinations and scans were performed at the Department of Endocrinology at Odense University Hospital.

Medical and fracture history, pharmacological therapy with testosterone, and calcium intake based on the amount of milk and dairy products consumed daily were detailed through interview and self-administered questionnaires.

Body weight was measured in all participants dressed in casual indoor clothing and barefoot to the nearest 0.1 kg on a Seca model 708 scale (Seca, Hamburg, Germany), whereas body height was measured to the nearest 0.1 cm on a wall-mounted Harpenden stadiometer (Holtain Ltd., Crymich, UK).

DXA

aBMD was measured in all subjects at the lumbar spine and hip using dual-energy X-ray absorptiometry (DXA) (Hologic Discovery, Waltham, MA, USA). The coefficient of variation (CV) is 1.5% at both the spine and hip in our unit.

HR-pQCT

Bone geometry, vBMD, and microarchitecture were assessed at the nondominant distal radius and distal tibia (the opposite limb in the presence of a previous fracture) using a HR-pQCT system (Xtreme CT, Scanco Medical, AG, Brüttisellen, Switzerland). The image acquisition, analysis, and validation of the method have been described in detail previously.[17–19] Briefly, the manufacturer's default protocol was applied for in vivo scanning, the first CT slice starting at 9.5 mm and 22.5 mm from the endplate of the distal radius and tibia, respectively. Each measurement included 110 parallel slices in the axial direction corresponding to 3D representation of 9.02-mm-thick cross sections with an isotopic image voxel size of 82 μm. First, the manufacturer's standard evaluation protocol was used where trabecular (Tb) and cortical (Ct) compartments were distinguished by means of filtering and thresholding as described by Laib and colleagues.[19] Tb vBMD was calculated as the average mineral density in the Tb volume of interest, and Tb bone volume fraction (BV/TV) was derived from it, assuming fully mineralized bone to have a mineral density of 1200 mg HA/cm^3. Trabecular number (Tb.N) was directly measured using a 3D distance transformation method.[18] Trabecular thickness (Tb.Th) and trabecular spacing (Tb.Sp) were then derived from BV/TV and Tb.N. The standard deviation of 1/Tb.N was also measured, serving as an index of trabecular network inhomogeneity. Second, an extended evaluation of the cortical compartment was performed where the periosteal and endosteal surfaces of the cortex were extracted using a fully automated three-step algorithm, allowing 3D measurement of cortical thickness (Ct.Th) and cortical porosity (Ct.Po) as described by others.[20,21] Third, the mechanical properties of the radius and tibia were estimated by using a micro-finite element (FE) analysis solver provided by the manufacturer (Finite Element Analysis Software v1.15, Scanco Medical). All bone elements were assigned isotopic material properties, a Young's Modulus of 10 GPa, and a Poisson's ratio of 0.3. An estimate of bone failure load was calculated as described by Pistoia and colleagues[22] on the assumption that bone failure occurs if >2% of the elements are strained beyond 0.7% strain. Total bone stiffness was also assessed as an index for bone strength as described previously.[23]

A total of three scans per anatomic site were allowed to obtain images of optimum quality. Before analysis, the image quality was graded by one of the authors (SH) based on a five-step scale, 1 being the best and 5 being the worst,[24] and images more than grade 3 were disregarded in the analysis.

The CVs for geometry, density, and microarchitecture parameters ranged from 0.4% to 7.2% in our unit.[25]

Biochemical analyses

Blood samples from KS patients were drawn between 8 a.m. and 10 a.m. in the fasting state and stored at −80°C until analysis. Bone turnover markers were measured by a fully automated immunoassay system (iSYS, Immunodiagnostic Systems Ltd., Boldon, UK); C-telopeptide of type 1 collagen (CTX-I), procollagen type 1 amino-terminal propeptide (PINP), and osteocalcin by a chemiluminescence method and bone-specific alkaline phosphatase (bone ALP) by photometric method. The samples were analyzed in a single run with the same batch of the reagents/assay. The limit of quantitation for CTX-I was 0.033 μg/L and the measurement range was 0.033-6 μg/L. Intra- and interassay CV were <5% and 7% to 10%, respectively. Intact PINP assay had a quantitation limit of <1.0 μg/L and the measurement range of 2 to 230 μg/L. Intra- and interassay CV were 3% and 5% to 8%, respectively. The limit of quantitation for osteocalcin was 1.57 μg/L and the measurement range 2 to 200 μg/L. Intra- and interassay CV were 3% and 6% to 9%, respectively. Bone ALP had a limit of quantitation of 1.0 μg/L and the measurement range of 1 to 75 μg/L. Intra- and interassay CV were <2% and 5% to 10%, respectively. The reference ranges given for the bone turnover markers are all based on the same assays as used for the analysis of the patient samples. They were obtained from data from 907 healthy Danish men aged 25 to 79 years without any known bone metabolic diseases.

Serum levels of 17β-estradiol (detection limit: 18.4 pmol/L; intra- and interassay CV: 6.1% and 7.0%), testosterone (detection limit: 87 pmol/L; intra- and interassay CV: 2.8 and 3.2%), follicular stimulating hormone (FSH) (detection limit: 0.1 mU/L; intra- and interassay CV: 2.8% and 3.7%), lutinising hormone (LH) (detection limit: 0.1 mU/L; intra- and interassay CV: 0.9% and 3.2%), and sex hormone binding globulin (SHBG) (detection limit: 0.35 nmol/L; intra- and interassay CV: 1.7% and 4.0%) were measured on a Cobas e601 using electrochemiluminescence immunoassays according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Statistical analysis was performed using SPSS statistical package version 19 (IBM SPSS Statistics, Chicago, IL, USA) and STATA version 12 StataCorp., College Station, TX, USA). All data are expressed as mean ± standard deviation or median (interquartile range) as appropriate. Comparisons between KS and control subjects were assessed using chi-square test for categorical variables and independent unpaired Student's *t* test or Mann-Whitney U test for normally or non-normally distributed continuous variables, respectively. Multiple linear regression analysis with group (KS or control) as independent variable was used to assess differences between KS and control subjects adjusted for body weight. Also, in those with KS, age- and weight-adjusted multiple regression analyses were performed to assess the association of DXA and HR-pQCT parameters (dependent variables) and testosterone, estrogen, FSH, LH, duration of testosterone therapy, and testosterone treatment status (independent variables). Testosterone and estrogen values were converted into *Z*-scores to obtain comparable numbers. In the latter analysis, treatment status was defined as current testosterone therapy for more than 2 years. Histograms and normal probability plots of residuals were used to check model assumptions. Transformation of the dependent variable according to a Box-Cox analysis was performed where necessary. Throughout the study, a p value less than 0.05 was considered statistically significant.

Results

General presentation

General characteristics of KS subjects and control subjects are presented in Table 1. Mean age, body height, body weight, and body mass index (BMI) did not differ significantly between the groups. Of the 31 KS subjects, 10 had never received testosterone therapy or had received therapy for less than 2 years, whereas 10 patients commenced replacement therapy before age 20 years and 11 patients commenced therapy after the age of 20 years. Because there were no significant differences in the bone parameters between the groups of KS patients in whom treatment was initiated before and after the age of 20 years, their data were pooled. Ten patients with KS and 6 control subjects had a history of prior fractures, but neither this, nor smoking, alcohol consumption, and daily calcium intake differed significantly between the groups.

DXA measurements of spine and hip

Total spine aBMD (L_1 to L_4) and total hip aBMD were significantly lower in KS patients compared with controls (Table 1). These differences remained statistically significant in weight-adjusted regression models ($p < 0.01$ at the spine and hip).

HR-pQCT measurements of the distal radius and distal tibia

Radius and tibia images with adequate quality were acquired in all KS patients (grade 1, $n = 40$; grade 2, $n = 14$; grade 3, $n = 8$) as well as controls (grade 1, $n = 41$; grade 2, $n = 19$; grade 3, $n = 2$).

Bone geometry

At the radius, cortical area was 10% (interquartile range 4% to 19%) lower in KS subjects compared with controls, but this did not reach statistical significance ($p = 0.07$, after adjusting for body weight) (Table 2). Other geometrical parameters were comparable between the two groups. At the tibia, however, cortical area was 19% (interquartile range 8% to 25%) lower in KS compared with controls, which was statistically significant in weight-adjusted models ($p < 0.01$). Total and trabecular bone areas as well as endosteal and periosteal perimeters were not significantly different between the groups.

Volumetric BMD

None of the density parameters were significantly different between patients with KS and controls at the radius in weight-adjusted models (Table 2). At the tibia, total vBMD and Tb vBMD were 9% ± 3% and 7% ± 25% lower in KS subjects after adjusting for body weight, ($p = 0.02$ and $p = 0.04$, respectively). There was a trend toward lower Ct vBMD in patients with KS ($p = 0.11$).

Microarchitecture

In weight-adjusted models at the radius, Ct.Th was lower by 18% ± 28% ($p < 0.01$) in KS patients, whereas Ct.Po was similar between groups. No differences were observed in the trabecular microarchitectural parameters between the groups. Similar to

Table 1. General Characteristics and aBMD of the Whole Cohort

	KS	Control	*p* Value
Number	31	31	–
Age (years)	35.8 ± 8.2	35.9 ± 8.2	0.98
Weight (kg)	95.6 ± 16.8	89.1 ± 13.4	0.10
Height (cm)	185.9 ± 8.3	185.1 ± 7.1	0.69
BMI (kg/m^2)	27.6	26.0	0.13
Karyotype 47XXY/mosaics	29/2	–	–
Testosterone treatment (*n*), never/after age 20 yr/before age 20 yr	10/11/10	–	–
Any previous fracture (*n*), yes/no	10/21	6/21	0.25
Current smoking (*n*), yes/no	15/16	13/18	0.61
Alcohol consumption[a] (*n*), yes/no	4/27	1/28	0.15
Daily calcium intake (mg)	800 (600–1050)	600 (400–1100)	0.55
DXA			
Spine aBMD (g/cm^2)	0.97 ± 0.11	1.04 ± 0.12	**0.02***
Spine *T*-score	−1.17 ± 0.81	−0.6 ± 0.86	**0.03***
Hip aBMD (g/cm^2)	0.97 ± 0.13	1.05 ± 0.15	**0.02***
Hip *T*-score	−0.74 ± 1.03	−0.09 ± 1.10	**0.01***

KS = Klinefelter syndrome.

Data are expressed as mean ± SD or in numbers. Significant *p* values are shown in bold.

Between-group differences were assessed using unpaired Student's *t* test and chi-square tests as appropriate.

[a]No = < 14 units of alcohol per week; yes = > 14 units of alcohol per week.

*Remained significant after adjusting for weight.

the radius, Ct.Po at the tibia was comparable between groups, whereas Ct.Th was 24% ± 9% lower in KS subjects ($p < 0.01$). In contrast to the radius, at the tibia, evidence of compromised trabecular integrity was found in KS subjects with Tb BV/TV and Tb.N being 7% ± 21% and 3% ± 10% lower ($p = 0.04$ and $p = 0.05$), respectively, whereas Tb.Sp was 5% ± 15% higher ($p = 0.03$) compared with controls. The thickness of individual trabeculae was similar between the groups, but KS subjects had a 7% increase in trabecular network inhomogeneity ($p = 0.04$) (Fig. 1).

Estimated bone strength

Although total bone stiffness and estimated failure load were not significantly different between groups at the radius, both these parameters were reduced to a similar extent (both $p < 0.01$) at the tibia in KS subjects in weight-adjusted models (Fig. 1).

Biochemistry

Although biochemical markers of bone formation and resorption were numerically higher in KS patients on testosterone replacement compared with those not on therapy, these differences were not statistically significant (Table 3). KS patients on treatment had higher serum testosterone levels and lower SHBG levels than those not on treatment, but this did not reach statistical significance ($p = 0.09$ and $p = 0.08$, respectively). Serum levels of LH and FSH were significantly lower in KS patients on treatment ($p < 0.01$ and $p = 0.02$, respectively), whereas the level of serum estrogen was similar between the groups.

Relationship between hormonal levels, treatment status, and bone parameters in KS

In age- and weight-adjusted multiple regression analysis, there were significant positive associations between testosterone level and estimated failure load in both the radius and tibia (β1 coefficients of 0.35 kN/1 SD, $p = 0.01$, and 1.02 kN/1 SD, $p < 0.01$, respectively). Similarly, significant associations of estrogen and Ct.Th (β1 coefficient 56.5 μm/1 SD, $p = 0.03$) at the radius and Ct vBMD (β1 coefficient 17.1 mg.cm^{-3}/1 SD, $p = 0.03$) and Ct.Th (β1 coefficient 119.2 μm/1 SD, $p = 0.02$) at the tibia were observed. There was no significant association between estrogen and the trabecular parameters and no associations between testosterone and any of the cortical or trabecular parameters (Table 4). None of the parameters were significantly different at the radius and tibia between the treated and untreated KS groups (Fig. 2).

Discussion

This study assessed bone structural parameters and estimated bone biomechanics of the appendicular skeleton utilizing HR-pQCT in adult patients with KS in comparison to healthy controls. KS subjects had a lower total BMD and a compromised trabecular compartment with a reduced trabecular density and bone volume fraction, lower trabecular number leading to more widely spaced trabeculae, and higher network inhomogeneity at the tibia. KS subjects also had a reduced cortical area and thickness at the tibia, which in combination with the trabecular deficits compromised estimated bone strength at this site. Although cortical thickness was reduced at the radius, no other differences were observed in the structural, density, or estimated strength parameters at this site.

Our findings of a marked reduction in trabecular bone volume in tibia in KS are consistent with the histomorphometric study by Delmas and colleagues,[15] reporting a picture similar to postmenopausal osteoporosis with a marked reduction in trabecular bone volume. These results are comparable with those of Jackson and colleagues,[26] who assessed bone histology in 6 male patients with hypogonadism not suffering

Table 2. Bone Geometry, vBMD, Microarchitectural Characteristics, and Estimated Bone Strength by HR-pQCT at the Radius and Tibia

	Radius			Tibia		
	KS ($n = 31$)	Control ($n = 31$)	p Value	KS ($n = 31$)	Control ($n = 31$)	p Value
Geometry						
Total bone area (mm^2)	386.2 ± 68.2	387.2 ± 70.7	0.96	931.0 ± 157.4	959.0 ± 165.8	0.50
Cortical area (mm^2)	66.9 (60.1–77.0)	74.3 (61.4–82.5)	0.28	127.0 (117.7–157.1)	157.3 (139.1–171)	<0.01[a]
Trabecular area (mm^2)	313.2 ± 68.1	309.2 ± 72.1	0.82	793.5 ± 166.2	797.9 ± 172.8	0.92
Periosteal perimeter (mm)	86.0 ± 8.4	87.2 ± 9.8	0.62	123.6 ± 12.8	126.7 ± 16.2	0.42
Endosteal perimeter (mm)	80.3 ± 8.7	80.6 ± 9.7	0.89	112.2 ± 11	113.8 ± 11.7	0.57
Volumetric BMD						
Total bone density (mg/cm^3)	325.8 ± 58.5	338.0 ± 61.5	0.43	297.4 ± 55.9	324.2 ± 54.2	0.06[b]
Cortical density (mg/cm^3)	854.8 ± 46.8	862.4 ± 46.9	0.52	847.1 ± 42	864.3 ± 41.6	0.11
Trabecular density (mg/cm^3)	197.3 ± 40.1	199.6 ± 38.6	0.82	197.2 ± 40.7	210.2 ± 32.6	0.17[c]
Microarchitecture						
Trabecular parameters						
Trabecular bone volume/total volume	0.165 ± 0.033	0.166 ± 0.032	0.83	0.164 ± 0.034	0.175 ± 0.027	0.17[d]
Trabecular number (1/mm)	2.16 ± 0.30	2.11 ± 0.25	0.45	2.19 ± 0.31	2.26 ± 0.28	0.35[e]
Trabecular thickness (mm)	0.076 ± 0.012	0.079 ± 0.013	0.38	0.075 ± 0.012	0.078 ± 0.008	0.33
Trabecular spacing (mm)	0.397 ± 0.072	0.402 ± 0.056	0.74	0.392 ± 0.068	0.372 ± 0.058	0.23[f]
SD.1/Tb.N (mm)	0.15 (0.13–0.18)	0.17 (0.14–0.19)	0.28	0.15 (0.14–0.19)	0.14 (0.13–0.18)	0.35[g]
Cortical parameters						
Cortical thickness (mm)	0.80 ± 0.14	0.94 ± 0.18	<0.01[a]	1.05 ± 0.22	1.30 ± 0.24	<0.01[a]
Cortical porosity (%)	1.76 (1.44–2.19)	1.78 (1.23–2.6)	0.91	5.9 ± 1.6	5.8 ± 2.4	0.86
Estimated bone strength						
Total bone stiffness (kN/mm)	112.7 ± 18.5	115.9 ± 22.0	0.54	256.6 (242–292)	296.5 (275.1–320.2)	<0.01[a]
Estimated failure load (kN)	5.7 ± 0.9	5.9 ± 1.1	0.65	13.0 (12.2–14.7)	15.0 (13.9–15.8)	<0.01[a]

KS = Klinefelter syndrome; SD.1/Tb.N (mm) = standard deviation of 1/trabecular number.

Data are expressed as mean ± SD, median (interquartile range).

Between-group differences were assessed using independent Student t test and Mann Whitney U test where appropriate. Data are adjusted for body weight after log-transformation where required.

The p values of the differences after adjusting for weight are as follows: [a]$p < 0.01$, [b]$p = 0.02$, [c]$p = 0.04$, [d]$p = 0.04$, [e]$p = 0.05$, [f]$p = 0.03$, [g]$p = 0.04$.

from KS and reported changes similar to those seen in postmenopausal women. We also observed that the deterioration in trabecular bone volume and trabecular network integrity was attributable to a reduction in trabecular number with relative preservation of trabecular thickness. Our study does not allow us to discriminate if these changes were owing to lack of bone accretion or increased bone loss; however, our findings are to some extent consistent with age-related bone loss observed in postmenopausal women.[27,28]

The different pattern of trabecular involvement at the radius (similar Tb.N and Tb.Th between controls and KS) when compared with the pattern at the tibia (lower Tb.N) may have important implications on bone strength. Biomechanical studies suggest that removal of entire structural elements of bone, as observed with an absolute loss of trabeculae, has a two- to fivefold larger detrimental impact on bone strength when compared with thinning of the trabeculae for the same volume of bone lost.[29] We found that total bone stiffness, an excellent predictor of bone strength,[23] was significantly lower at the tibia in KS compared with controls in the finite element model. The estimated failure load was also lower in KS, suggesting lower bone strength relative to applied mechanical loads in weight-adjusted models. Although aBMD is used as a surrogate marker of bone strength in clinical practice, it is not a comprehensive measure of this. In a study using cadaveric radius, finite element estimates of bone strength improved estimation of bone strength in comparison to measurement of bone mass by DXA.[22]

Testosterone regulates male bone metabolism during the critical stage of bone acquisition during puberty and maintains it during adult life. Testosterone acts directly by its action on osteoblasts through the androgen receptor and, indirectly, by serving as a substrate for aromatization to estrogen.[5] We found a reduced cortical bone area, cortical thickness, and a trend toward a lower total bone area with similar trabecular areas at the tibia in KS subjects in comparison to their normal counterparts. This could, in part, reflect a decreased periosteal apposition and increased endocortical resorption secondary to testosterone deficiency.[30] Even though we did find an association between serum testosterone levels and estimated failure load, there were no differences in the individual parameters or estimated bone strength when comparing the treated and untreated KS patients. The lack of a uniform time interval between the dose of testosterone and serum testosterone sampling, the small numbers in the subgroups, and the fact that those treated may have had a more severe disease phenotype make it difficult to draw strong inferences in this regard. Further, the dose of testosterone may have been too low or not initiated sufficiently early in life to restore and/or maintain bone geometry and cortical and trabecular topography. Although duration of testosterone treatment showed no correlation with indices of bone geometry, microarchitecture, or bone strength, our study was not designed to elucidate the effect of therapy, and therefore, the possibility of a window of opportunity for commencement of testosterone treatment remains.[31]

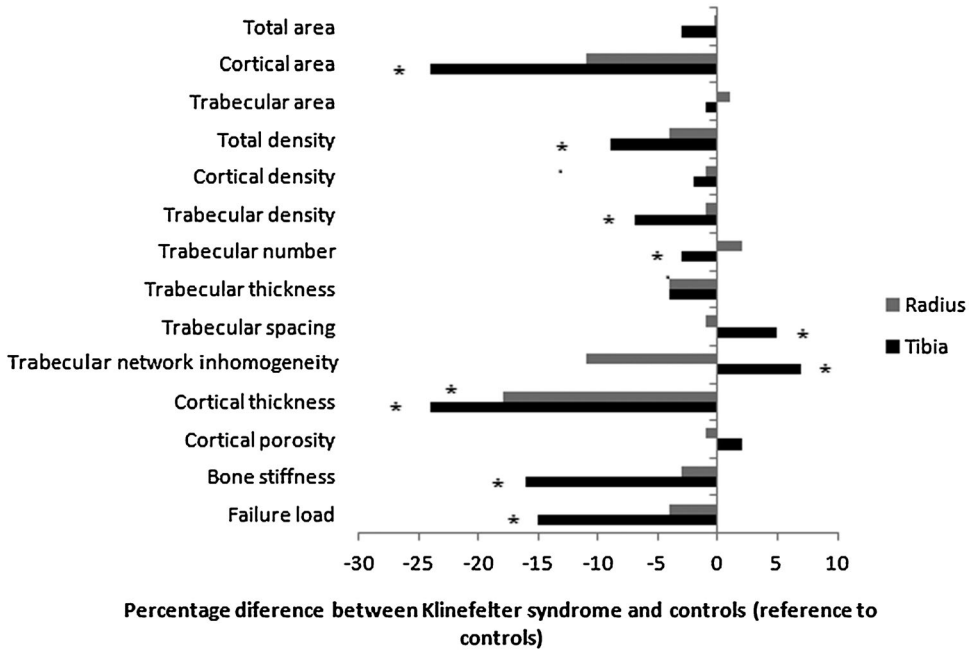

Fig. 1. Differences in mean/median of bone parameters between Klinefelter syndrome patients and control subjects (using controls as the reference group) expressed as percentage. Black bars represent tibia, and gray bars represent radius. *Denotes a significance difference with $p < 0.05$.

We found no differences in geometry, density, microarchitectural, or estimated strength parameters at the radius in KS and controls except for a reduced cortical thickness. This disparate involvement of weight-bearing and non-weight-bearing skeletal sites in KS may be related to a threshold effect of bioavailable estradiol on the male skeleton, as described by Khosla and colleagues.[32] Non-weight-bearing bones may have a lower sensitivity to estrogen and testosterone (and thus a high threshold to withstand sex hormone deficiency) for maintaining trabecular integrity. The traditional notion that testosterone is most closely associated with bone loss in men has been refuted by evidence indicating that estrogen has a more dominant role on the mature male skeleton.[32] The circulating levels of estrogen in KS may be sufficiently high to maintain trabecular integrity at the radius but not at the tibia.

We found that biochemical markers of bone turnover were similar in KS patients with and without testosterone therapy. Although serum testosterone levels were at the lower end of normal in both groups (17.59 versus 12.61 [normal range 9 to 34] nmol/L in the treated and nontreated groups, respectively), the slightly higher levels in the treated group were sufficient to suppress the LH and FSH to normal, consistent with previous studies.[33]

Our finding that bone microarchitecture and estimated strength did not differ significantly between KS subjects receiving testosterone replacement and those who were not,

Table 3. Biochemistry in Testosterone-Treated and -Untreated KS patients.

	Testosterone untreated ($n = 10$)	Testosterone treated ($n = 21$)	p Value
PINP[a] (19.4–89.2 μg/L)	74.4 ± 36.5	75.4 ± 33.3	0.9
Osteocalcin[a] (7.5–33.3 μg/L)	23.4 ± 10.3	25.1 ± 11.7	0.2
CTXI[a] (0.16–1.31 μg/L)	0.50 ± 0.28	0.69 ± 0.44	0.7
Bone ALP[a] (7.5–27.4 μg/L)	12.0 ± 4.9	13.8 ± 7.3	0.5
Testosterone[b] (9–34 nmol/L)	12.6 (7.8–16.8)	17.6 (12.8–20.9)	0.09
SHBG[b] (13–76 nmol/L)	41.5 (25.5–48.3)	27.81 (23.0–39.7)	0.08
LH[b] (1.7–8.6 IU/L)	22.8 (4.5–25.9)	1.4 (0.0–10.2)	**<0.01**
FSH[b] (1.2–15.8 IU/L)	23.1 (11.1–38.6)	2.8 (0.6–21.1)	**0.02**
17β-estradiol[b] (E2) (28–156 pmol/L)	77 (39–101)	83 (60–100)	0.44

KS = Klinefelter's syndrome; LH = leutinising hormone; FSH = follicle-stimulating hormone; SHBG = sex hormone binding globulin; PINP = amino-terminal propeptide of type I procollagen; CTXI = cross-linked C-telopeptide 1.

Data expressed as mean ± SD, median (interquartile range). Significant p values are shown in bold.

Between-group differences were tested using independent Student t test and Mann Whitney U test where appropriate.

[a]The reference range indicated is for normal 25- to 79-year-old males.

[b]The reference range indicated is for normal 20- to 50-year-old males.

Table 4. β_1 Coefficients and *p* Values for Testosterone, 17β-estradiol, and Duration of Testosterone Therapy Derived From Age- and Weight-Adjusted Multiple Regression Models of Selected DXA and HR-pQCT Parameters in KS Subjects[a]

	Testosterone	17β-estradiol	Duration of testosterone therapy	Testosterone treatment yes/no[b]
No. of subjects in analysis	31	31	25	21/10
DXA				
Spine BMD (g/cm^2)	**0.05; $p<0.01$**	0.04; $p=0.06$	0.001; $p=0.85$	0.07; $p=0.10$
Total hip BMD (g/cm^2)	0.04; $p=0.08$	0.03; $p=0.20$	−0.002; $p=0.54$	0.05; $p=0.27$
HR-pQCT radius				
Total density (mg/cm^3)	1.94; $p=0.83$	7.95; $p=0.38$	2.54; $p=0.120$	5.66; $p=0.78$
Cortical density (mg/cm^3)	3.52; $p=0.69$	15.9; $p=0.07$	1.41; $p=0.40$	0.65; $p=0.97$
Ct.Th (μm)	16.8; $p=0.53$	**56.5; $p=0.03$**	5.72; $p=0.27$	25.7; $p=0.67$
Ct. Po (%)	0.013; $p=0.93$	0.052; $p=0.72$	0.018; $p=0.49$	0.12; $p=0.71$
BV/TV (%)	0.38; $p=0.47$	−0.033; $p=0.95$	0.083; $p=0.39$	0.27; $p=0.82$
Tb.N (1/mm)	0.055; $p=0.32$	0.011; $p=0.85$	0.0088; $p=0.39$	0.047; $p=0.71$
Tb.Th (μm)	−0.50; $p=0.78$	−0.82; $p=0.65$	0.055; $p=0.86$	−0.78; $p=0.85$
Failure load (kN)	**0.35; $p=0.01$**	0.20; $p=0.18$	−0.018; $p=0.52$	0.23; $p=0.49$
HR-pQCT tibia				
Total density (mg/cm^3)	4.51; $p=0.62$	15.3; $p=0.09$	1.00; $p=0.55$	4.57; $p=0.83$
Cortical density (mg/cm^3)	2.09; $p=0.79$	**17.1; $p=0.03$**	1.73; $p=0.21$	−7.37; $p=0.68$
Ct.Th (μm)	30.9; $p=0.55$	**119.2; $p=0.02$**	3.72; $p=0.70$	−9.08; $p=0.94$
Ct. Po (%)	−0.37; $p=0.25$	−0.56; $p=0.08$	−0.017; $p=0.77$	−0.91; $p=0.21$
BV/TV (%)	0.52; $p=0.32$	0.30; 0.57	0.014; $p=0.88$	0.91; $p=0.44$
Tb.N (1/mm)	0.065; $p=0.20$	0.032; $p=0.54$	−0.010; $p=0.25$	0.12; $p=0.29$
Tb.Th (μm)	−0.17; $p=0.92$	0.21; $p=0.91$	0.44; $p=0.15$	0.51; $p=0.90$
Failure load (kN)	**1.02; $p<0.01$**	0.52; $p=0.54$	−0.036; $p=0.65$	0.79; $p=0.45$

[a]The β_1 coefficient represents the change in parameter in question with an increase of 1 standard deviation in testosterone or estrogen. For duration of testosterone therapy, the β_1 coefficient represents the change in parameter per additional year of therapy. The right column shows the difference in parameter in question between those treated versus those not treated with testosterone (reference to those not treated). Significant *p* values are shown in bold.

[b]Defined as treated if currently on testosterone treatment for >2 years.

Note that cortical thickness and trabecular thickness are expressed in μm in this table.

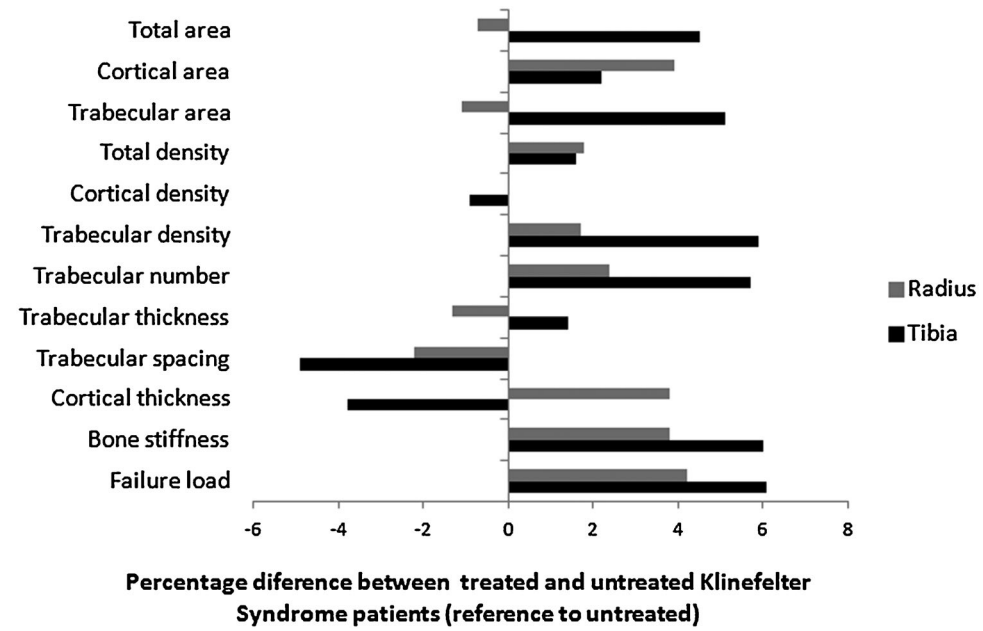

Fig. 2. Differences in the estimated marginal means (after adjusting for age and weight in ANCOVA) between treated and untreated Klinefelter syndrome patients (using untreated KS patients as the reference group) expressed as percentage. Black bars represent tibia, and gray bars represent radius. None of the parameters were significantly different between the groups.

coupled with the presence of tall stature from early childhood,[34] could also indicate that factors other than hypogonadism may play a role. The trabecular involvement may be related to an overdosage of the short-stature homeobox-containing gene (SHOX) located on each of the sex chromosomes. SHOX is a transcription factor only expressed in the hypertrophied chondrocytes of the pubertal growth plate orchestrating the right balance between proliferation and apoptosis during bone development.[35] Overexpression of SHOX, as seen in KS, may lead to imbalance in the coordinated process of chondrocyte differentiation and apoptosis in normal bone development. These chondrocytes lie on the inside of the bone and are eventually replaced by trabecular bone and bone marrow,[36] leading to the involvement of trabecular architecture in KS.

A potential limitation of our study was the application of the HR-pQCT standard patient protocol for image acquisition, which entails a fixed offset from the extremity endplate. The measurement site would thus vary with differing lengths of the radius and tibia, being more distally located in taller subjects, leading to a relative over- and underrepresentation of trabecular and cortical bone, respectively. The body height of control and KS subjects in our study was comparable, although KS subjects may potentially have disproportionately longer lower limbs in relation to height.[37] Although the trends in density and Tb bone volume fraction at the radius and tibia were similar, it is uncertain if the lack of data on limb lengths in KS patients and controls has a bearing on structural comparisons. Another limitation is the lack of data on body composition in KS subjects. The magnitude of the influence of total body fat and muscle mass on bone structure in KS is still unknown. Moreover, we cannot draw reliable conclusions regarding the impact of testosterone treatment on bone structure because of the cross-sectional design of the study and the small number of participants in the testosterone treatment groups.

Nevertheless, this is the first study looking at bone compartment–specific structural parameters and their corroboration with noninvasive estimation of bone strength in KS. Patients with KS were recruited consecutively from a routine outpatient clinic, thus reducing selection bias. We also have a large HR-pQCT database of healthy control subjects, facilitating selection of control subjects matched for age and height to KS subjects.

In summary, our study showed that individuals with KS have a compromised bone structure in the form of reduced total and trabecular vBMD at the tibia primarily because of a thinner cortex and a deficit in trabecular number, respectively. Also, cortical bone area was lower in patients with KS at the tibia. Although the cortical thickness was reduced, there were no other significant differences in structural parameters at the radius. Estimates of bone strength in tibia were lower in KS subjects after adjusting for weight, indicating lower bone strength relative to applied mechanical loads. These findings could partly explain the observed increased fracture risk in these subjects.[38] Further studies are necessary to elucidate the consequences of appropriate testosterone treatment and to examine the possibility of a critical window of opportunity to initiate treatment.

Disclosures

All authors state that they have no conflicts of interest with respect to the submitted manuscript. Financial support not pertaining to the submitted work: SH has received speaker fee payment from Eli Lilly. KB has received grants from MSD, AMGEN, Novartis, and NPS; speaker fee payments from SERVIER, AMGEN, GSK, and Novartis; and travel and accommodation expenses and consultancy fee from MSD. CHG has received grants from Novo Nordisk Foundation and speaker fee payments from Bayer Pharmaceuticals and Novo Nordisk. There are no restrictions on access to raw data and statistical analysis.

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Authors' roles: Study design: SH, KB, and CG. Study conduct: SH and VS. Data collection: SH and VS. Data analysis: VS, NJ, and SH. Data interpretation: VS, SH, KB, and CG. Drafting manuscript: VS. Revising manuscript content: VS, SH, NJ, KB, and CG. Approving final version of manuscript: VS, SH, NJ, KB, and CG. VS and SH take responsibility for the integrity of the data analysis.

References

1. Bojesen A. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab. 2003; 88(2):622–6.
2. Smyth CM, Bremner WJ. Klinefelter syndrome. Arch Int Med. 1998 Jun 22;158(12):1309–14.
3. Simpson JL, de la Cruz F, Swerdloff RS, et al. Klinefelter syndrome: expanding the phenotype and identifying new research directions. Genet Med. 2003 Nov–Dec;5(6):460–8.
4. Liu H, Paige NM, Goldzweig CL, et al. Screening for osteoporosis in men: a systematic review for an American College of Physicians guideline. Ann Int Med. 2008 May 6;148(9):685–701.
5. Ferlin A, Schipilliti M, Di Mambro A, Vinanzi C, Foresta C. Osteoporosis in Klinefelter's syndrome. Mol Hum Reprod. 2010 Jun;16(6):402–10.
6. Eulry F, Bauduceau B, Lechevalier D, Magnin J, Flageat J, Gautier D. [Early spinal bone loss in Klinefelter syndrome. X-ray computed tomographic evaluation in 16 cases]. Revue du rhumatisme. 1993 Apr;60(4):287–91.
7. Choi HR, Lim SK, Lee MS. Site-specific effect of testosterone on bone mineral density in male hypogonadism. J Korean Med Sci. 1995 Dec; 10(6):431–5.
8. Foresta C, Ruzza G, Mioni R, Meneghello A, Baccichetti C. Testosterone and bone loss in Klinefelter syndrome. Hormone Metab Res. 1983 Jan;15(1):56–7.
9. van den Bergh JP, Hermus AR, Spruyt AI, Sweep CG, Corstens FH, Smals AG. Bone mineral density and quantitative ultrasound parameters in patients with Klinefelter's syndrome after long-term testosterone substitution. Osteoporos Int. 2001;12(1):55–62.
10. Wong FH, Pun KK, Wang C. Loss of bone mass in patients with Klinefelter's syndrome despite sufficient testosterone replacement. Osteoporos Int. 1993 Jan;3(1):3–7.
11. Kubler A, Schulz G, Cordes U, Beyer J, Krause U. The influence of testosterone substitution on bone mineral density in patients with Klinefelter's syndrome. Exp Clin Endocrinol. 1992;100(3):129–32.
12. Horowitz M, Wishart JM, O'Loughlin PD, Morris HA, Need AG, Nordin BE. Osteoporosis and Klinefelter's syndrome. Clin Endocrinol. 1992 Jan;36(1):113–8.
13. Luisetto G, Mastrogiacomo I, Bonanni G, et al. Bone mass and mineral metabolism in Klinefelter's syndrome. Osteoporos Int. 1995;5(6): 455–61.
14. Hieronimus S, Lussiez V, Le Duff F, Ferrari P, Bstandig B, Fenichel P. Klinefelter's syndrome and bone mineral density: is osteoporosis a constant feature? Annales d'endocrinologie. 2011 Feb;72(1):14–8.

15. Delmas P, Meunier PJ. [Osteoporosis in Klinefelter's syndrome: Quantitative bone histological data in 5 cases and relationship with hormonal deficiency (author's transl)]. La Nouvelle presse medicale. 1981 Feb 28;10(9):687–90.
16. Hansen S, Shanbhogue V, Folkestad L, Nielsen MM, Brixen K. Bone microarchitecture and estimated strength in 499 adult Danish women and men: a cross-sectional, population-based high-resolution peripheral quantitative computed tomographic study on peak bone structure. Calcif Tissue Int. 2014 Mar;94(3):269–81.
17. Laib A, Ruegsegger P. Calibration of trabecular bone structure measurements of in vivo three-dimensional peripheral quantitative computed tomography with 28-microm-resolution microcomputed tomography. Bone. 1999 Jan;24(1):35–9.
18. Laib A, Hildebrand T, Hauselmann HJ, Ruegsegger P. Ridge number density: a new parameter for in vivo bone structure analysis. Bone. 1997 Dec;21(6):541–6.
19. Laib A, Hauselmann HJ, Ruegsegger P. In vivo high resolution 3D-QCT of the human forearm. Technol Health Care. 1998 Dec; 6(5–6):329–37.
20. Burghardt AJ, Kazakia GJ, Ramachandran S, Link TM, Majumdar S. Age- and gender-related differences in the geometric properties and biomechanical significance of intracortical porosity in the distal radius and tibia. J Bone Miner Res. 2010 May;25(5):983–93.
21. Nishiyama KK, Macdonald HM, Buie HR, Hanley DA, Boyd SK. Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. J Bone Miner Res. 2010 Apr;25(4):882–90.
22. Pistoia W, van Rietbergen B, Lochmuller EM, Lill CA, Eckstein F, Ruegsegger P. Image-based micro-finite-element modeling for improved distal radius strength diagnosis: moving from bench to bedside. J Clin Densitom. 2004; Summer;7(2):153–60.
23. Macneil JA, Boyd SK. Bone strength at the distal radius can be estimated from high-resolution peripheral quantitative computed tomography and the finite element method. Bone. 2008 Jun;42(6): 1203–13.
24. Pialat JB, Burghardt AJ, Sode M, Link TM, Majumdar S. Visual grading of motion induced image degradation in high resolution peripheral computed tomography: impact of image quality on measures of bone density and micro-architecture. Bone. 2012 Jan;50(1):111–8.
25. Hansen S, Hauge EM, Beck Jensen JE, Brixen K. Differing effects of PTH 1-34, PTH 1-84, and zoledronic acid on bone microarchitecture and estimated strength in postmenopausal women with osteoporosis: an 18-month open-labeled observational study using HR-pQCT. J Bone Miner Res. 2013 Apr;28(4):736–45.
26. Jackson JA, Kleerekoper M, Parfitt AM, Rao DS, Villanueva AR, Frame B. Bone histomorphometry in hypogonadal and eugonadal men with spinal osteoporosis. J Clin Endocrinol Metab. 1987 Jul;65(1):53–8.
27. Aaron JE, Makins NB, Sagreiya K. The microanatomy of trabecular bone loss in normal aging men and women. Clin Orthop Relat Res. 1987 Feb(215);260–71.
28. Parfitt AM, Mathews CH, Villanueva AR, Kleerekoper M, Frame B, Rao DS. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest. 1983 Oct;72(4):1396–409.
29. Silva MJ, Gibson LJ. Modeling the mechanical behavior of vertebral trabecular bone: effects of age-related changes in microstructure. Bone. 1997 Aug;21(2):191–9.
30. Leder BZ, LeBlanc KM, Schoenfeld DA, Eastell R, Finkelstein JS. Differential effects of androgens and estrogens on bone turnover in normal men. J Clin Endocrinol Metab. 2003 Jan;88(1):204–10.
31. Groth KA, Skakkebaek A, Host C, Gravholt CH, Bojesen A. Clinical review: Klinefelter syndrome—a clinical update. J Clin Endocrinol Metab. 2013 Jan;98(1):20–30.
32. Khosla S, Melton LJ 3rd, Robb RA, et al. Relationship of volumetric BMD and structural parameters at different skeletal sites to sex steroid levels in men. J Bone Miner Res. 2005 May;20(5): 730–40.
33. Bojesen A, Birkebaek N, Kristensen K, et al. Bone mineral density in Klinefelter syndrome is reduced and primarily determined by muscle strength and resorptive markers, but not directly by testosterone. Osteoporos Int. 2011 May;22(5):1441–50.
34. Aksglaede L, Skakkebaek NE, Juul A. Abnormal sex chromosome constitution and longitudinal growth: serum levels of insulin-like growth factor (IGF)-I, IGF binding protein-3, luteinizing hormone, and testosterone in 109 males with 47,XXY, 47,XYY, or sex-determining region of the Y chromosome (SRY)-positive 46,XX karyotypes. J Clin Endocrinol Metab. 2008 Jan;93(1):169–76.
35. Marchini A, Marttila T, Winter A, et al. The short stature homeodomain protein SHOX induces cellular growth arrest and apoptosis and is expressed in human growth plate chondrocytes. J Biol Chem. 2004 Aug 27;279(35):37103–14.
36. Olsen BR, Reginato AM, Wang W. Bone development. Ann Rev Cell Dev Biol. 2000;16:191–220.
37. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet. 2004 Jul 17–23;364(9430):273–83.
38. Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. J Clin Endocrinol Metab. 2006 Apr;91(4):1254–60.