Bone turnover predicts change in volumetric bone density and bone geometry at the radius in men

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**Abstract**

**Purpose**

To determine prospective change in bone density and geometry at the radius in men, and examine the influence of bone turnover markers and sex hormones on that change.

**Methods**

Men aged 40-79 years were recruited from population registers in Manchester (UK) and Leuven (Belgium). At baseline, markers of bone formation (P1NP and Osteocalcin) and resorption (β-cTX and ICTP ) were assessed. Total and bioavailable testosterone, and oestradiol were also measured. Peripheral quantitative computed tomography (pQCT) was used to scan the radius at distal and midshaft sites at baseline and a median of 4.3 years later.

**Results**

514 men, mean (SD) age 59.6 (10.5) years, contributed data. At the midshaft site there was a significant decrease in mean cortical volumetric BMD (vBMD) (-0.04%/year), bone mineral content (BMC) (-0.1%/year) and cortical thickness (-0.4%/year), whilst total and medullary area increased (+0.5%/year, +2.4%/year, respectively). At the distal radius, total vBMD declined (-0.5%/year) and radial area increased (+0.6%/year). Greater plasma concentrations of bone resorption and formation markers were associated with greater decline in BMC and cortical area at the midshaft and total vBMD at the distal site. Increased bone resorption was linked with an increase in total and medullary area and decrease in cortical thickness at the midshaft. Sex hormone levels were unrelated to change in pQCT parameters.

**Conclusions**

Age-related changes in vBMD and bone geometry are greater in men with higher biochemical markers of bone turnover at baseline. Sex hormones have little influence on change in pQCT parameters.

**Mini abstract**

Peripheral quantitative computed tomography scans of the distal and mid-shaft radius were performed in 514 European men aged 40-79 years at baseline and a median of 4.3 years later. Age-related changes in vBMD and bone geometry were greater in men with higher biochemical markers of bone turnover at baseline.

**Introduction**

Osteoporosis in men is a considerable public health problem with the lifetime risk of fracture in men after age 50 years estimated at ~20% [[1](#_ENREF_1)]. Most studies examining changes in bone health with age have focused on ‘areal’ bone mineral density (g/cm2; aBMD) [[2](#_ENREF_2)] as measured by dual-energy X-ray absorptiometry (DXA) [[3-8](#_ENREF_3)]. However bone strength is influenced not only by bone mineral content but also bone shape and mineral distribution and the loading conditions to which the bone is subjected. In addition, DXA tends to overestimate aBMD in larger, and underestimate in smaller, bones [[9](#_ENREF_9)]. Peripheral quantitative computed tomography (pQCT) allows assessment of both bone geometry and volumetric bone mineral density (vBMD). Data from cross-sectional studies, including data from the European Male Aging Study (EMAS), suggest variously a lower distal radius vBMD and BMC, thinner cortices and greater cross-sectional bone area with increasing age [[10-17](#_ENREF_10)]. However, there are limitations to estimating true longitudinal change in bone parameters from cross-sectional data [[18](#_ENREF_18), [19](#_ENREF_19)]. In contrast to our understanding about prospective change in DXA aBMD, there are relatively few data concerning prospective change in pQCT parameters in middle-aged and elderly men, [[18](#_ENREF_18), [20](#_ENREF_20), [21](#_ENREF_21)] with few data on change in bone geometry at the midshaft and distal radius.

In older men, cross-sectional studies suggest increased bone turnover markers are associated with lower aBMD[[22](#_ENREF_22), [23](#_ENREF_23)] and, more recently, microarchitectural parameters[[24](#_ENREF_24)]. In line with these findings, prospective data suggest that higher levels of bone remodeling may be associated with increased rates of bone loss [[25](#_ENREF_25), [26](#_ENREF_26)] however, there are no data linking bone turnover markers to changes in bone geometry in older men.

Levels of sex steroids are known to be associated with aBMD in men, as assessed using DXA, and also rate of bone loss [[7](#_ENREF_7), [22](#_ENREF_22), [27-33](#_ENREF_27)]. The contribution of oestradiol (E2) to BMD has been reasonably well established but the effect of testosterone (T) is less clear, as are the effects of sex hormones on bone structural parameters [[34](#_ENREF_34)]. Khosla (2005) [[16](#_ENREF_16)], showed that E2 was the most constant predictor of BMD and some geometrical variables, assessed by QCT, and similarly in the MINOS cohort, E2 was related to aBMDand cortical thickness [[27](#_ENREF_27)]. Using data from the baseline EMAS survey we showed a weak association between vBMD and E2, while the association of T with bone geometry was inconsistent [[17](#_ENREF_17)].

The aims of this prospective study were: firstly to characterise longitudinal changes in bone density and structure at the radius in middle-aged and elderly European men; secondly to determine the relationship between bone turnover markers and subsequent change in BMD and bone structure; and thirdly to determine the association between sex hormones and change in BMD and structure.

**Materials and methods**

# Subjects

The subjects included in this analysis were recruited for participation in the EMAS, a prospective study of ageing in European community-dwelling men. Detailed methods have been described previously [[35](#_ENREF_35)]. Briefly, men were recruited from population-based sampling frames in 8 centres between 2003 and 2005. Stratified random sampling was used with the aim of recruiting equal numbers of men in each of four 10-year age bands: 40-49 years, 50-59 years, 60-69 years, and 70-79 years. Letters of invitation were sent to subjects asking them to attend for health assessments by a range of health questionnaires, physical performance tests, anthropometry and a fasting blood sample. In two centres, Manchester (UK) and Leuven (Belgium), subjects had pQCT measurements performed at the radius. The men were invited to participate in a follow-up assessment a median of 4.3 years later. Ethics approval for the study was obtained in accordance with local institutional requirements in each centre, and each participant gave written informed consent.

*Peripheral Quantitative Computed Tomography (pQCT)*

Peripheral QCT measurements of the non-dominant radius were made in men recruited to the Manchester and Leuven centres at both baseline and follow-up using XCT-2000 scanners (Stratec, Pforzheim, Germany). At the distal (4%) site, total and trabecular vBMD (mg/cm3) and bone cross sectional area (mm2) were measured (voxel size 0.4mm); the slice location at the 4% and 50% site was more distal in Leuven compared to Manchester; the reference line was placed at the distal border of the radial endplate in Leuven, in Manchester the line is placed to bisect the lateral border of the endplate. These differences result in a scan site difference approximately 1-2mm between centers. At the diaphysis (50% site, voxel size 0.6mm), cortical BMD (mg/cm3), bone mineral content (BMC mg/mm), total, cortical and medullary areas (mm2), cortical thickness (mm) and stress strain index (SSI, mm3) were measured. SSI provides a measure of a bone’s torsional strength [[36](#_ENREF_36), [37](#_ENREF_37)]. A detailed methodology for these measurements has been described previously [[38](#_ENREF_38)].

For cross-calibration between Leuven and Manchester the European Forearm Phantom (EFP) was measured [[39](#_ENREF_39)]. There were no differences greater than precision error for trabecular, total and cortical BMD, BMC or cortical area, therefore no cross calibration was performed between the two centres [[17](#_ENREF_17)]. The short term precision of 2 repeat radius measurements with repositioning in adults were: Manchester (n = 22) *Leuven (n = 40)* trabecular BMD 1.27%, *1.42%*; total BMD 2.1%, *1.3%*; cortical BMD 0.77%, *0.71%;* cortical area 2.4%, *1.3%.* Manufacturer’s standard quality assurance procedures were followed in both centres.

*Bone marker measurement*

Bone turnover markers were measured at baseline on the Elecsys 2010 automated analyser (Roche Diagnostics GmbH, Manheim, Germany). To assess bone resorption, serum beta C-telopeptide of type I collagen (β-cTX) was measured at baseline using the ß-Crosslaps/serum reagents [[40](#_ENREF_40)]. This assay is specific for cross-linked ß-isomerized type I collagen C-telopeptide fragments and uses two monoclonal antibodies, each recognizing the Glu-Lys-Ala-His-ßAsp-Gly-Gly-Arg peptide (Crosslaps antigen). The intra-assay coefficient of variation (CV) evaluated by repeated measurements of several serum samples was <5.0%. The detection limit was 10 pg/mL. Carboxyterminal telopeptide region of type I collagen (ICTP) was measured using the competitive radioimmunoassay technique. A known amount of labelled ICTP and an unknown amount of unlabelled ICTP in the sample compete for the limited number of high affinity binding sites of the antibody. After separating the free antigen, the amount of labelled ICTP in the sample tube is inversely proportional to the amount of ICTP in the sample. The concentrations in unknown samples are obtained from a calibration curve. The intra-assay CV was <9% and the lower detection limit <0.4 μg/l. To evaluate bone formation, measurements were performed on the Elecsys 2010 with a 2-site assay using monoclonal antibodies raised against intact human P1NP purified from human amniotic fluid. This assay detects both intact mono- and trimeric forms (total P1NP), as previously described [[41](#_ENREF_41)]. The intra-assay CV was <3.0% and the lower detection limit <5 ng/mL. The Elecsys N\_MID Osteocalcin assay uses two monoclonal antibodies specifically directed against epitopes on the N-MID fragment as well as the intact osteocalcin. The test is non-dependent on the unstable C-terminal-fragment of the osteocalcin molecule and thus ensures constant measurement results under routine conditions in the laboratory. The intra-assay CV was <4% and the lower detection limit <0.5 ng/mL.

# Sex hormone measurement

A single fasting morning (before 10.00 h) venous blood sample was obtained from all subjects at the baseline assessment. Serum was separated immediately after phlebotomy and stored at -80°C until assay at the end of the baseline study. Measurement of T and E2 were carried out by gas chromatography mass spectrometry (GC-MS) as described in Labrie et al [[42](#_ENREF_42), [43](#_ENREF_43)]. The lower limit of T quantitation was 0.17 nmol/L and E2 was 7.34 pmol/L. The coefficients of variation of T measurements were 2.9% within runs and 3.4% between runs, and for E2, were 3.5% within runs and 3.7% between runs. SHBG was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) as previously described[[44](#_ENREF_44)]. Free and bio T and E2 levels were derived from total T, total E2, SHBG and albumin concentrations using mass action equations and associations constants of Vermeulen et al. and Van Pottelbergh et al. [[30](#_ENREF_30), [45](#_ENREF_45)].

# Statistical analysis

Descriptive statistics were used to summarise subject characteristics at baseline. Change in pQCT parameters was calculated as percentage change per year ([follow-up value – baseline value]/baseline value x 100/time between scans). Differences between baseline and follow-up pQCT parameters were assessed using paired t-tests. Linear regression analysis was used to investigate the association of change in pQCT parameters with markers of bone turnover (Osteocalcin, P1NP and ICTP and B-cTX), and sex hormones including total and bioavailable E2 and T. In the linear regression analyses, bone turnover markers and sex hormones were standardised (z-score), so results represent the change in pQCT parameters per standard deviation increase in the independent variable. Adjustments were made in these analyses for age, height, weight and centre and the results expressed as standardised (z-score) β coefficients and 95% confidence intervals (CI). Statistical analysis was performed using STATA version 13 (StataCorp, College Station, TX).

**Results**

# Subject characteristics

Five hundred forty men had baseline and follow up assessments. Of these 26 were excluded because of therapy which may have impacted on bone including sex hormones, anti-osteoporotic therapies and glucocorticoids. Of the 514 included in the analysis the mean (standard deviation) age was 59.6 (10.5) years and the mean (standard deviation) BMI was 27.3 (3.8) kg/m2 see Table 1.

*Change in bone mass and geometry*

There was significant change in most pQCT parameters over the course of the study, see Table 2. At the midshaft radius, mean cortical BMC and vBMD decreased by -0.1% (*P*=0.03) and -0.04% (*P*=0.007) per year respectively, while the medullary and total area increased by 2.4% (*P*=0.0001) per year and 0.5% (*P*=0.0001) per year, respectively. Cortical thickness declined by 0.4% (*P*<0.001) per year, with no significant change in cortical area or SSI. At the distal radial site there was a significant reduction in total vBMD (0.5% per year, *P*<0.0001) while radial area increased (0.6% per year, *P*<0.0001). In this sample of men age 40-79 years, there was no association between age and the rate of change of the pQCT parameters (data not shown).

*Influence of bone turnover on change in pQCT parameters*

1. Mid shaft

After adjustment for age, height, weight and centre, an increase in bone resorption markers (ICTP and β-cTX) as well as bone formation markers (PINP and Osteocalcin) were associated with a significant reduction in cortical BMC, see Table 3. An increase in ICTP was associated with a significant increase in both total area (β per SD change = 0.25% per year) and medullary area (β per SD change = 0.93 % per year). Markers of bone resorption (β-cTX and ICTP) were associated with a greater decline in cortical thickness in the adjusted model. P1NP, osteocalcin and β-cTX were associated with a greater decline in cortical area, see Table 3.

1. Distal radius

After adjustment for age, height, weight and centre, an increase in β-cTX and P1NP was associated with a reduction in total vBMD (β per SD change =-0.14% and -0.16% per year, respectively). β-cTX was also associated with a reduction in trabecular vBMD (β per SD change = -0.13 % per year).

*Influence of sex hormones on change pQCT parameters*

The association between free and bioavailable fractions of T and E2 with pQCT parameters were broadly similar so here we present data for the total and bioavailable hormone relationships (bioE2, bioT), see Table 4. There was no association between bio T or E2, nor SHBG (data not shown) on change in any of the pQCT parameters in the adjusted models.

**Discussion** Our data show evidence in middle-aged and elderly men of a longitudinal change in bone mass and geometry at the radial midshaft with a decline in cortical BMD, BMC and cortical thickness and an increase in medullary and total area. At the distal radius site there was a decline in the total volumetric BMD and an increase in radial area. A higher rate of bone turnover at baseline (formation and resorption) was associated with a greater reduction in cortical BMC and cortical area at the midshaft and total vBMD at the distal radius. Increased resorption markers were associated with an increase in total and medullary area, and decrease in cortical thickness at the midshaft, and a greater rate of decline in trabecular vBMD at the distal radius. In contrast, sex hormones, within the normal range in our community-dwelling sample of men, appeared to have little influence on the change in vBMD and geometry as measured by pQCT.

A number of cross-sectional studies have looked at the influence of age on pQCT parameters in men [[10-17](#_ENREF_10)]. In a cross-sectional study of 202 men aged 20-99 years, and using high resolution pQCT, trabecular area/height at the radius increased with age by 28%, while other parameters decreased with increasing age, including trabecular BMD (-32%), trabecular thickness (-16%), cortical area/height (-5%), cortical BMD ( -15%) and cortical thickness (-21%) [[11](#_ENREF_11)]. In another cross-sectional study using HR-pQCT of men aged 20-80 years, compared to younger men (≤35 yrs), older men (mean age 80 yrs) had larger total area, thinner trabeculae and lower total and trabecular BMD at the radius [[10](#_ENREF_10)]. There are, however, limitations in interpreting these data given their cross-sectional design [[18](#_ENREF_18)]. There are few prospective studies which have looked at change in bone mass and geometry. Data from the Gothenburg Osteoporosis and Obesity Study [[46](#_ENREF_46)] showed change in radial pQCT parameters in younger men, around the time of accrual of peak bone mass, however, there are limited data in older men (over 60 years of age). In a 7.5 year prospective study Specker (2015) described rates of change at the 4% and 20% distal radial sites in three distinct populations of 20-66 year-old men [[21](#_ENREF_21)]. There were increases in bone cross sectional area, cortical thinning and decreasing bone strength (at older ages) during follow up. In the InChianti study [[18](#_ENREF_18)], Lauretani (2008), using tibial pQCT data in 345 men (age 21yrs – 101 yrs) reported a decline in BMD and an increase in medullary and total bone area. In a study using HR-pQCT, Shanbhogue (2016) reported an increase in trabecular vBMD at the distal radius in men aged 50 years and older over a median follow up of 3 years, with no significant change in total vBMD or cortical area though the number of men who were studied was relatively small (88) [[47](#_ENREF_47)].

Given the paucity of prospective data concerning change in pQCT it is not surprising that there are few data which have looked at the link between bone turnover markers and bone structural change at the distal radius. Using data from the GOOD study, Darelid et al. (2015) reported that OC was a positive predictor of an increase in aBMD and BMC at the radius between the ages of 19 and 24 years; also men in the highest quartile of OC at baseline were more likely to gain in radial cross-sectional area and trabecular vBMD than men in the lowest quartile[[48](#_ENREF_48)]. These findings, particularly in relation to BMD differ from our findings; however, this almost certainly reflects the fact the GOOD study focused on a much younger cohort of men. Our results suggest that increased turnover, and particularly bone resorption, is linked with structural decay and vBMD loss in older men. Whilst there are some similarities to bone loss in women, it is important to recognise there is a sexual dimorphism in patterns of bone ageing. It seems plausible that the reduction in cortical thickness from endosteal bone resorption would impair bone strength if increased strains did not lead to compensatory periosteal expansion to redistribute the bone over a larger cross-sectional area as a mechanism to maintain bone strength. We observed no overall change in stress strain index suggesting that biomechanical stability persisted despite the loss in cortical thickness. Redistribution of bone is due to periosteal apposition (indicated by an increase in bone area), and our data are in line with previous studies suggesting that periosteal bone formation in old age may largely be driven in response to endosteal resorption [[34](#_ENREF_34)]. In any case, the maintenance of bone strength via this mechanism may be one reason why the incidence of wrist fracture in men, in contrast to women, remains low until later life, though further studies are needed [[1](#_ENREF_1), [15](#_ENREF_15)]. There is some evidence, at least in mice and rats, that T may increase periosteal apposition (and thereby increase total area), and certainly in adolescents T increases periosteal growth [[34](#_ENREF_34)]. Szulc (2004) using DXA data suggested an increase in periosteal apposition with age, though not via an action of T [[27](#_ENREF_27)]. In contrast, Khosla (2005) found an inverse association in men with higher levels of T linked with reduced bone area [[16](#_ENREF_16)]. Our results, however, showed no significant association between either testosterone or estrogen and change in bone geometry, suggesting that these are not the primary driver of structural bone decay in community-dwelling men. Evidence from observational and clinical studies support the view that oestrogen is the most important sex steroid in determining bone mass in men [[7](#_ENREF_7), [22](#_ENREF_22), [28](#_ENREF_28), [30](#_ENREF_30), [33](#_ENREF_33)], with some evidence of a threshold effect, though studies so far are inconclusive [[16](#_ENREF_16), [49](#_ENREF_49)]. All but eight men in our cohort had total E2>37 pmol/L. Given the low prevalence of clinically significant hypogonadism in EMAS, however, the study may have been underpowered to examine associations between sex steroids and longitudinal pQCT changes.

The strengths of our study were the population sample and the prospective design. There are however some limitations which need to be considered when interpreting the results. The response rate for participation in the baseline survey in Manchester and Leuven was 38.8% and 38.6%, respectively [[35](#_ENREF_35)]**.** It is possible that those who did not take part may have differed with respect to their pQCT measurements and also bone turnover markers and also sex steroid levels resulting in an over or under estimation with respect to the true population value and so caution is required in interpreting the absolute levels of these measurements. However, the main findings, in relation to the relationship between bone turnover markers and sex steroid levels and change in pQCT parameters were based on internal comparisons among responders and so selection factors are unlikely to have influenced the strength of the observed biological relationships. One of the key factors in designing the study was to ensure standardisation of the study instruments used in the different participating centres. Hormone and bone turnover marker measurements were performed in a central reference laboratory to minimise assay variability, and gold standard mass spectrometry methods were applied. The same pQCT scanner type and model was used in each centre and after testing scanner differences with the EFP, no cross calibration was necessary. The data however were derived from a European Caucasian population and so the results may not necessarily be extrapolated beyond this setting**.**

In conclusion, our study provides the first longitudinal characterization of the gradual BMD and bone geometry changes with age at the radius in middle-aged and elderly European men. Increased bone turnover in such men is predictive of bone loss as measured by pQCT. Sex hormones in the normal range however appeared to have no influence on the change in pQCT parameters.

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Table 1. Subject characteristics.

|  |  |
| --- | --- |
|  | Mean (SD) |
| Age at interview (years) | 59.6 (10.5) |
| Height (cm) | 174.9 (7.1) |
| Weight (kg) | 83.5 (13.2) |
| Body mass index (kg/m2) | 27.3 (3.8) |
| Testosterone (nmol/L) | 18.2 (6.0) |
| Free testosterone (pmol/L) | 320.0 (87.1) |
| Bioavailable testosterone (nmol/L) | 8.0 (2.3) |
| Oestradiol (pmol/L) | 77.8 (24.9) |
| Free oestradiol (pmol/L) | 1.3 (0.4) |
| Bioavailable oestradiol (pmol/L) | 54.6 (17.5) |
| SHBG (nmol/L) | 42.8 (18.4) |
| P1NP (ng/mL) | 42.7 (20.3) |
| Osteocalcin (ng/mL) | 22.3 (7.9) |
| β-cTX (pg/mL) | 327.5 (155.6) |
| ICTP (ng/ml) | 3.1 (0.9) |

SD: standard deviation, SHBG: Sex hormone-binding globulin, P1NP: serum N-terminal propeptide of type 1 procollagen, β-cTX: β-C-terminal cross-linked telopeptide, ICTP: carboxyterminal telopeptide of type I collagen

Table 2. Radial pQCT parameters: baseline and follow up

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Mean (SD) value at baseline | Mean (SD) value at follow up | Mean (SD) % change per year | *P\** | |
| **Midshaft radius** |  |  |  | |  |
| Cortical BMD (mg/cm3) | 1214.6 (29.9) | 1212.7 (30.1) | -0.04 (0.3) | | 0.007 |
| Cortical BMC (mg/mm) | 124.0 (17.3) | 123.5 (17.6) | -0.1 (1.3) | | 0.03 |
| Total area (mm2) | 150.3 (21.6) | 152.5 (21.1) | 0.5 (2.1) | | 0.0001 |
| Cortical area (mm2) | 107.5 (13.9) | 107.3 (14.1) | -0.06 (1.2) | | 0.18 |
| Cortical thickness (mm) | 3.3 (0.4) | 3.2 (0.4) | -0.4 (2.3) | | <0.0001 |
| Medullary area (mm2) | 42.7 (17.0) | 45.2 (17.2) | 2.4 (6.7) | | 0.0001 |
| Stress strain index (mm3) | 342.9 (66.5) | 341.5 (64.1) | -0.006 (1.8) | | 0.3 |
| **Distal radius** |  |  |  | |  |
| Total vBMD (mg/cm3) | 398.7 (73.2) | 391.6 (73.6) | -0.5 (1.4) | | <0.0001 |
| Radial area (mm2) | 381.1 (68.0) | 387.8 (69.9) | 0.6 (2.5) | | <0.0001 |
| Trabecular vBMD (mg/cm3) | 207.1 (42.3) | 206.7 (41.8) | -0.02 (1.4) | | 0.3 |

SD: standard deviation, BMD: bone mineral density (mg/cm3), BMC: bone mineral content (mg/mm), vBMD: volumetric bone mineral density

\*P-value for difference between baseline and follow-up value using a paired t-test

Table 3. Influence of bone turnover markers on change in pQCT parameters (% change / yr) at the radius

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | P1NP (per (SD) | | Osteocalcin (per SD) | | β-cTX (per SD) | | ICTP (per SD) | |
| pQCT parameters (% change / yr) | β coefficient (95%CI) | | | | | | | |
| Unadjusted | Adjusteda | Unadjusted | Adjusteda | Unadjusted | Adjusteda | Unadjusted | Adjusteda |
| **Midshaft radius** |  |  |  |  |  |  |  |  |
| Cortical BMD (mg/cm3) | -0.01 (-0.04, 0.01) | -0.01 (-0.04, 0.02) | -0.02 (-0.05, 0.01) | -0.02 (-0.05, 0.01) | -0.02 (-0.04, 0.01) | -0.01 (-0.04, 0.01) | 0.00 (-0.03, 0.03) | -0.01 (-0.04, 0.02) |
| Cortical BMC (mg/mm) | -0.14 (-0.25, -0.03)\* | -0.14 (-0.24, -0.03)\* | -0.15 (-0.26, -0.04)\* | -0.14 (-0.25, -0.03)\* | -0.18 (-0.29, -0.07)\* | -0.17 (-0.28, -0.06)\* | -0.14 (-0.25, -0.03)\* | -0.14 (-0.25, -0.02)\* |
| Total area (mm2) | 0.02 (-0.17, 0.22) | 0.04 (-0.16, 0.23) | 0.03 (-0.16, 0.22) | 0.10 (-0.10, 0.29) | 0.12 (-0.07, 0.32) | 0.16 (-0.03, 0.36) | 0.33 (0.14, 0.52)\* | 0.25 (0.05, 0.45)\* |
| Cortical area (mm2) | -0.13 (-0.23, -0.02)\* | -0.12 (-0.22, -0.01)\* | -0.13 (-0.24, -0.03)\* | -0.11 (-0.22, 0.00)\* | -0.16 (-0.26, -0.05)\* | -0.14 (-0.24, -0.03)\* | -0.09 (-0.19, 0.02) | -0.09 (-0.20, 0.02) |
| Cortical thickness (mm) | -0.19 (-0.39, 0.01) | -0.19 (-0.38, 0.01) | -0.16 (-0.36, 0.04) | -0.19 (-0.39, 0.01) | -0.27 (-0.47, -0.07)\* | -0.27 (-0.47, -0.08)\* | -0.38 (-0.58, -0.18)\* | -0.33 (-0.53, -0.13)\* |
| Medullary area (mm2) | 0.16 (-0.44, 0.77) | 0.16 (-0.45, 0.78) | 0.07 (-0.54, 0.68) | 0.18 (-0.46, 0.81) | 0.35 (-0.26, 0.96) | 0.42 (-0.21, 1.04) | 1.00 (0.40, 1.61)\* | 0.93 (0.29, 1.58)\* |
| Stress strain index (mm3) | -0.08 (-0.23, 0.07) | -0.08 (-0.23, 0.07) | -0.10 (-0.25, 0.05) | -0.10 (-0.26, 0.06) | -0.10 (-0.25, 0.06) | -0.09 (-0.25, 0.06) | -0.03 (-0.18, 0.12) | -0.03 (-0.20, 0.13) |
| **Distal radius** |  |  |  |  |  |  |  |  |
| Total vBMD (mg/cm3) | -0.16 (-0.29, -0.04)\* | -0.16 (-0.29, -0.04)\* | -0.07 (-0.20, 0.06) | -0.08 (-0.21, 0.05) | -0.13 (-0.25, 0.00) | -0.14 (-0.27, -0.01)\* | -0.06 (-0.19, 0.06) | -0.05 (-0.18, 0.09) |
| Radial area (mm2) | -0.03 (-0.25, 0.19) | -0.03 (-0.26, 0.19) | -0.08 (-0.31, 0.14) | -0.09 (-0.32, 0.15) | -0.09 (-0.31, 0.13) | -0.09 (-0.32, 0.14) | 0.00 (-0.22, 0.22) | -0.01 (-0.24, 0.23) |
| Trabecular vBMD (mg/cm3) | -0.08 (-0.20, 0.03) | -0.08 (-0.20, 0.03) | -0.10 (-0.22, 0.01) | -0.09 (-0.21, 0.03) | -0.13 (-0.25, -0.02)\* | -0.13 (-0.24, -0.01)\* | 0.06 (-0.05, 0.17) | 0.02 (-0.10, 0.14) |

aChange in pQCT parameters per standard deviation increase in bone turnover parameter marker. pQCT parameters are % change per year

bAdjusted for age, centre, height, weight

SD: standard deviation, P1NP: serum N-terminal propeptide of type 1 procollagen, β-cTX: β-C-terminal cross-linked telopeptide, ICTP: carboxyterminal telopeptide of type I collagen, vBMD: volumetric bone mineral density, BMC: bone mineral content, CSMA: cross-sectional muscle area

\**P*<0.05

**Table 4**. Influence of sex hormones on change in pQCT parameters (% change / yr) at the radius

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dependent variable  pQCT parameters (% change / yr) | Total testosterone (per SD) | | Bioavailable testosterone (per SD) | | Total oestradiol (per SD) | | Bioavailable oestradiol (per SD) | |
| Standardised (z-score) β-coefficient (95% CI) | | | | | | | |
| Unadjusted | Adjusteda | Unadjusted | Adjusteda | Unadjusted | Adjusteda | Unadjusted | Adjusteda |
| **Midshaft radius** |  |  |  |  |  |  |  |  |
| Cortical BMD (mg/cm3) | 0.00 (-0.03, 0.02) | 0.00 (-0.03, 0.03) | -0.01 (-0.04, 0.02) | 0.01 (-0.03, 0.04) | 0.03 (0.00, 0.05) | 0.02 (-0.01, 0.05) | 0.02 (-0.01, 0.05) | 0.02 (-0.01, 0.05) |
| Cortical BMC (mg/mm) | -0.03 (-0.15, 0.08) | -0.01 (-0.13, 0.12) | 0.06 (-0.06, 0.17) | 0.04 (-0.10, 0.17) | -0.03 (-0.15, 0.08) | -0.01 (-0.12, 0.11) | 0.02 (-0.09, 0.14) | 0.03 (-0.09, 0.14) |
| Total area (mm2) | -0.09 (-0.28, 0.10) | -0.12 (-0.33, 0.08) | -0.19 (-0.38, 0.00) | -0.14 (-0.37, 0.08) | -0.09 (-0.28, 0.10) | -0.09 (-0.28, 0.09) | -0.10 (-0.29, 0.08) | -0.07 (-0.27, 0.12) |
| Cortical area (mm2) | -0.06 (-0.17, 0.05) | -0.04 (-0.15, 0.08) | 0.03 (-0.08, 0.14) | 0.02 (-0.11, 0.15) | -0.06 (-0.16, 0.05) | -0.03 (-0.14, 0.08) | 0.01 (-0.10, 0.11) | 0.01 (-0.10, 0.12) |
| Cortical thickness (mm) | 0.00 (-0.20, 0.21) | 0.08 (-0.14, 0.29) | 0.16 (-0.05, 0.36) | 0.14 (-0.10, 0.38) | -0.02 (-0.23, 0.18) | 0.01 (-0.19, 0.20) | 0.05 (-0.15, 0.25) | 0.03 (-0.17, 0.24) |
| Medullary area (mm2) | -0.38 (-0.97, 0.22) | -0.49 (-1.15, 0.16) | -0.42 (-1.01, 0.18) | -0.45 (-1.16, 0.27) | -0.05 (-0.64, 0.54) | -0.06 (-0.66, 0.54) | 0.01 (-0.58, 0.60) | 0.04 (-0.58, 0.65) |
| Stress strain index (mm3) | -0.03 (-0.19, 0.12) | 0.00 (-0.17, 0.18) | -0.02 (-0.18, 0.14) | -0.01 (-0.20, 0.18) | -0.03 (-0.19, 0.12) | -0.04 (-0.20, 0.12) | -0.01 (-0.17, 0.14) | -0.04 (-0.20, 0.13) |
| **Distal radius** |  |  |  |  |  |  |  |  |
| Total vBMD (mg/cm3) | -0.02 (-0.14, 0.11) | -0.01 (-0.15, 0.13) | 0.04 (-0.09, 0.17) | 0.04 (-0.12, 0.19) | -0.05 (-0.18, 0.08) | -0.06 (-0.19, 0.07) | -0.03 (-0.16, 0.10) | -0.05 (-0.18, 0.09) |
| Radial area (mm2) | 0.02 (-0.19, 0.24) | 0.07 (-0.17, 0.31) | 0.08 (-0.14, 0.30) | 0.09 (-0.18, 0.35) | 0.09 (-0.13, 0.31) | 0.09 (-0.13, 0.31) | 0.12 (-0.10, 0.34) | 0.09 (-0.14, 0.32) |
| Trabecular vBMD (mg/cm3) | 0.04 (-0.08, 0.17) | 0.03 (-0.11, 0.17) | -0.02 (-0.15, 0.11) | 0.04 (-0.12, 0.19) | 0.07 (-0.05, 0.20) | 0.09 (-0.04, 0.21) | 0.04 (-0.08, 0.17) | 0.09 (-0.04, 0.22) |

aChange in pQCT parameters per standard deviation increase in bone turnover parameter marker. pQCT parameters are % change per year

bAdjusted for age, centre, height, weight

SD: standard deviation , BMD: bone mineral density, BMC: bone mineral content, vBMD: volumetric bone mineral density

References

1. van Staa TP, Dennison EM, Leufkens HG, Cooper C (2001) Epidemiology of fractures in England and Wales. Bone 29:517-522

2. Engelke K, Gluer CC (2006) Quality and performance measures in bone densitometry: part 1: errors and diagnosis. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 17:1283-1292

3. Burger H, de Laet CE, van Daele PL, Weel AE, Witteman JC, Hofman A, Pols HA (1998) Risk factors for increased bone loss in an elderly population: the Rotterdam Study. American journal of epidemiology 147:871-879

4. Jones G, Nguyen T, Sambrook P, Kelly PJ, Eisman JA (1994) Progressive loss of bone in the femoral neck in elderly people: longitudinal findings from the Dubbo osteoporosis epidemiology study. Bmj 309:691-695

5. Szulc P, Delmas PD (2007) Bone loss in elderly men: increased endosteal bone loss and stable periosteal apposition. The prospective MINOS study. Osteoporosis Int 18:495-503

6. Hannan MT, Felson DT, Anderson JJ (1992) Bone mineral density in elderly men and women: results from the Framingham osteoporosis study. J Bone Miner Res 7:547-553

7. Khosla S, Melton LJ, Atkinson EJ, O'Fallon WM (2001) Relationship of serum sex steroid levels to longitudinal changes in bone density in young versus elderly men. J Clin Endocr Metab 86:3555-3561

8. Kaptoge S, Reid DM, Scheidt-Nave C, et al. (2007) Geographic and other determinants of BMD change in European men and women at the hip and spine. A population-based study from the Network in Europe for Male Osteoporosis (NEMO). Bone 40:662-673

9. Carter DR, Bouxsein ML, Marcus R (1992) New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137-145

10. Hansen S, Shanbhogue V, Folkestad L, Nielsen MMF, Brixen K (2014) 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. Calcified Tissue Int 94:269-281

11. Macdonald HM, Nishiyama KK, Kang JA, Hanley DA, Boyd SK (2011) Age-Related Patterns of Trabecular and Cortical Bone Loss Differ Between Sexes and Skeletal Sites: A Population-Based HR-pQCT Study. J Bone Miner Res 26:50-62

12. Dalzell N, Kaptoge S, Morris N, Berthier A, Koller B, Braak L, van Rietbergen B, Reeve J (2009) Bone micro-architecture and determinants of strength in the radius and tibia: age-related changes in a population-based study of normal adults measured with high-resolution pQCT. Osteoporosis Int 20:1683-1694

13. Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, Peterson JM, Rouleau PA, McCollough CH, Bouxsein ML, Khosla S (2004) Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 19:1945-1954

14. Khosla S, Riggs BL, Atkinson EJ, Oberg AL, McDaniel LJ, Holets M, Peterson JM, Melton LJ (2006) Effects of sex and age on bone microstructure at the ultradistal radius: A population-based noninvasive in vivo assessment. J Bone Miner Res 21:124-131

15. Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, Oberg AL, Rouleau PA, McCollough CH, Khosla S, Bouxsein ML (2006) Population-based analysis of the relationship of whole bone strength indices and fall-related loads to age- and sex-specific patterns of hip and wrist fractures. J Bone Miner Res 21:315-323

16. Khosla S, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, Oberg AL, Rouleau PA, Riggs BL (2005) Relationship of volumetric BMD and structural parameters at different skeletal sites to sex steroid levels in men. J Bone Miner Res 20:730-740

17. Ward KA, Pye SR, Adams JE, et al. (2011) Influence of age and sex steroids on bone density and geometry in middle-aged and elderly European men. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 22:1513-1523

18. Lauretani F, Bandinelli S, Griswold ME, Maggio M, Semba R, Guralnik JM, Ferrucci L (2008) Longitudinal changes in BMD and bone geometry in a population-based study. J Bone Miner Res 23:400-408

19. Wey HE, Binkley TL, Beare TM, Wey CL, Specker BL (2011) Cross-Sectional versus Longitudinal Associations of Lean and Fat Mass with pQCT Bone Outcomes in Children. J Clin Endocr Metab 96:106-114

20. Riggs BL, Melton LJ, Robb RA, Camp JJ, Atkinson EJ, McDaniel L, Amin S, Rouleau PA, Khosla S (2008) A population-based assessment of rates of bone loss at multiple skeletal sites: Evidence for substantial trabecular bone loss in young adult women and men. J Bone Miner Res 23:205-214

21. Specker BL, Wey HE, Binkley TL, Beare TM, Minett M, Weidauer L (2015) Rural vs. non-rural differences and longitudinal bone changes by DXA and pQCT in men aged 20-66 years: A population-based study. Bone 79:79-87

22. Khosla S, Melton LJ, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL (1998) Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: A key role for bioavailable estrogen. J Clin Endocr Metab 83:2266-2274

23. Boonen S, Pye SR, O'Neill TW, et al. (2011) Influence of bone remodelling rate on quantitative ultrasound parameters at the calcaneus and DXA BMDa of the hip and spine in middle-aged and elderly European men: the European Male Ageing Study (EMAS). Eur J Endocrinol 165:977-986

24. Chaitou A, Boutroy S, Vilayphiou N, Munoz F, Delmas PD, Chapurlat R, Szulc P (2010) Association between bone turnover rate and bone microarchitecture in men: the STRAMBO study. J Bone Miner Res 25:2313-2323

25. Gielen E, O'Neill T, Pye S, et al. (2015) Bone turnover markers predict hip bone loss in elderly European men: results of the European Male Ageing Study (EMAS). Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 26:617-627

26. Szulc P, Montella A, Delmas PD (2008) High bone turnover is associated with accelerated bone loss but not with increased fracture risk in men aged 50 and over: the prospective MINOS study. Annals of the rheumatic diseases 67:1249-1255

27. Szulc P, Uusi-Rasi K, Claustrat B, Marchand F, Beck TJ, Delmas PD (2004) Role of sex steroids in the regulation of bone morphology in men. The MINOS study. Osteoporosis Int 15:909-917

28. Center JR, Nguyen TV, Sambrook PN, Eisman JA (1999) Hormonal and biochemical parameters in the determination of osteoporosis in elderly men. The Journal of clinical endocrinology and metabolism 84:3626-3635

29. Szulc P, Munoz F, Claustrat B, Garnero P, Marchand F, Duboeuf F, Delmas PD (2001) Bioavailable estradiol may be an important determinant of osteoporosis in men: The MINOS study. J Clin Endocr Metab 86:192-199

30. Van Pottelbergh I, Goemaere S, Kaufman JM (2003) Bioavailable estradiol and an aromatase gene polymorphism are determinants of bone mineral density changes in men over 70 years of age. J Clin Endocr Metab 88:3075-3081

31. Fink HA, Ewing SK, Ensrud KE, Barrett-Connor E, Taylor BC, Cauley JA, Orwoll ES, G OFMS (2006) Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. J Clin Endocr Metab 91:3908-3915

32. Hsu BJM, Cumming RG, Seibel MJ, Naganathan V, Blyth FM, Bleicher K, Dave A, Le Couteur DG, Waite LM, Handelsman DJ (2015) Reproductive Hormones and Longitudinal Change in Bone Mineral Density and Incident Fracture Risk in Older Men: The Concord Health and Aging in Men Project. J Bone Miner Res 30:1701-1708

33. Gennari L, Merlotti D, Martini G, et al. (2003) Longitudinal association between sex hormone levels, bone loss, and bone turnover in elderly men. J Clin Endocr Metab 88:5327-5333

34. Vanderschueren D, Laurent MR, Claessens F, Gielen E, Lagerquist MK, Vandenput L, Borjesson AE, Ohlsson C (2014) Sex steroid actions in male bone. Endocrine reviews 35:906-960

35. Lee DM, O'Neill TW, Pye SR, et al. (2009) The European Male Ageing Study (EMAS): design, methods and recruitment. Int J Androl 32:11-24

36. Augat P, Reeb H, Claes LE (1996) Prediction of fracture load at different skeletal sites by geometric properties of the cortical shell. J Bone Miner Res 11:1356-1363

37. Schiessl H, Ferretti JL, TysarczykNiemeyer G, Willnecker J (1996) Noninvasive bone strength index as analyzed by peripheral quantitative computed tomography (pQCT). Int Congr Ser 1105:141-146

38. Ward KA, Roberts SA, Adams JE, Mughal MZ (2005) Bone geometry and density in the skeleton of pre-pubertal gymnasts and school children. Bone 36:1012-1018

39. Ruegsegger P, Kalender WA (1993) A Phantom for Standardization and Quality-Control in Peripheral Bone Measurements by Pqct and Dxa. Phys Med Biol 38:1963-1970

40. Garnero P, Borel O, Delmas PD (2001) Evaluation of a fully automated serum assay for C-terminal cross-linking telopeptide of type I collagen in osteoporosis. Clin Chem 47:694-702

41. Garnero P, Vergnaud P, Hoyle N (2008) Evaluation of a fully automated serum assay for total N-terminal propeptide of type I collagen in postmenopausal osteoporosis. Clin Chem 54:188-196

42. Labrie F, Belanger A, Belanger P, et al. (2006) Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. J Steroid Biochem 99:182-188

43. Labrie F, Belanger A, Belanger P, et al. (2007) Metabolism of DHEA in postmenopausal women following percutaneous administration. J Steroid Biochem 103:178-188

44. Wu FCW, Tajar A, Pye SR, et al. (2008) Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: The European Male Aging Study. J Clin Endocr Metab 93:2737-2745

45. Vermeulen A, Verdonck L, Kaufman JM (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocr Metab 84:3666-3672

46. Ohlsson C, Darelid A, Nilsson M, Melin J, Mellstrom D, Lorentzon M (2011) Cortical Consolidation due to Increased Mineralization and Endosteal Contraction in Young Adult Men: A Five-Year Longitudinal Study. J Clin Endocr Metab 96:2262-2269

47. Shanbhogue VV, Brixen K, Hansen S (2016) Age- and Sex-Related Changes in Bone Microarchitecture and Estimated Strength. A Three-Year Prospective Study Using HR-pQCT. J Bone Miner Res

48. Darelid A, Nilsson M, Kindblom JM, Mellstrom D, Ohlsson C, Lorentzon M (2015) Bone turnover markers predict bone mass development in young adult men: a five-year longitudinal study. The Journal of clinical endocrinology and metabolism 100:1460-1468

49. Finkelstein JS, Lee H, Leder BZ, et al. (2016) Gonadal steroid-dependent effects on bone turnover and bone mineral density in men. The Journal of clinical investigation 126:1114-1125