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

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