CLINICAL STUDY

Association of bone microarchitecture with parathyroid hormone concentration and calcium intake in men: the STRAMBO study

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Abstract

Objective: In the elderly, vitamin D deficit, low calcium intake, and impaired bone microarchitecture are associated with higher risk of hip fracture. We assessed the association of bone microarchitecture with calcium intake and serum concentrations of 25-hydroxycholecalciferol (25OHD) and parathyroid hormone (PTH) in men.

Design: Cross-sectional analysis was performed in 1064 men aged 20–87 years not taking vitamin D or calcium supplements.

Methods: Daily calcium intake was assessed using a food frequency questionnaire. Bone microarchitecture was assessed at distal radius and tibia by high-resolution peripheral quantitative computed tomography. We measured serum and urinary levels of biochemical bone turnover markers (BTMs). Statistical models were adjusted for age, weight, height, and glomerular filtration rate.

Results: In 500 men aged < 65 years, lower 25OHD levels and low calcium intake were associated with lower trabecular volumetric bone mineral density (Dtrab) at the distal tibia, due to lower trabecular number (Tb.N). Low calcium intake was associated with lower cortical thickness (Ct.Th). Higher PTH level was associated with higher BTM levels. In 563 men aged ≥ 65 years, the highest PTH quartile was associated with lower Ct.Th (tibia), lower Dtrab (both sites), and lower Tb.N (radius) compared with the lowest quartile. Low calcium intake was associated with lower Tb.N and more heterogeneous trabecular distribution. BTM positively correlated with the PTH concentration.

Conclusion: In older men, elevated PTH concentration is associated with high bone turnover, poor trabecular microarchitecture (radius and tibia), and, at the distal tibia, lower Ct.Th. Low calcium intake is associated with lower Tb.N and more heterogeneous trabecular distribution.

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Introduction

Hip fractures in older men are a public health problem (1). The risk of hip fracture increases in subjects with impaired bone microarchitecture (cortical thinning, deterioration of trabecular connectivity) (2–5). It was also higher in older men with low serum 25-hydroxycholecalciferol (25OHD) concentration (6–8). The incidence of hip fracture was higher in the institutionalized elderly men who often suffer from vitamin D deficiency (8, 9). Finally, vitamin D and calcium supplementation reduced the fracture risk in vitamin D-deficient elderly individuals (10–12).

In older home-dwelling men, lower 25OHD level and higher parathyroid hormone (PTH) concentration were associated with lower areal bone mineral density (aBMD) measured by dual energy X-ray absorptiometry (DXA) and higher bone turnover (13). Accelerated bone turnover was associated with impaired bone microarchitecture in

men, mainly after 70 years (14). However, association of bone microarchitecture with concentrations of PTH and 25OHD is poorly studied (15).

Our aim was to analyze the relationship of the system PTH–vitamin D–calcium intake with bone microarchitecture at the distal radius and tibia in men aged 20 years and above. Our hypothesis was that bone microarchitecture is associated with the levels of PTH and 25OHD and dietary calcium intake in men regardless of age, weight and other confounders.

Materials and methods

Cohort

The STRAMBO study is a single-center prospective cohort study of skeletal fragility and its determinants in men (14). It is a collaboration between National

Institute of Health and Medical Research (INSERM) and Mutuelle de la Région Lyonnaise (MTRL). The study obtained authorization of the local ethics committee and was performed in agreement with the Helsinki Declaration of 1975 and 1983. Letters inviting participation were sent to randomly selected 4500 men aged 20–85 years living in greater Lyon. No specific exclusion criteria were used. The cohort of 1169 men was recruited in 2006–2008. All men provided informed consent, replied to an interviewer-administered epidemiological questionnaire, had bone microarchitecture evaluation, and blood and urine collection.

BMD and bone microarchitecture measurement

Volumetric BMD (vBMD) and bone microarchitecture were assessed at the nondominant distal radius and the right distal tibia using high-resolution peripheral quantitative computed tomography (HR-pQCT) (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland). If the participant reported a fracture at this site, the contralateral limb was measured. A stack of 110 parallel CT slices with an isotropic voxel size of 82 μm was obtained, thus delivering a 3D representation of a bone segment of ~ 9 mm of length in the axial direction. Quality control was monitored by daily scans of a phantom containing rods of hydroxyapatite (HA) embedded in a soft tissue equivalent resin (QRM, Moehrendorf, Germany). Details of measurements of microarchitecture parameters were previously described (16). Coefficient of variation (CV) for the phantom (100–800 mg HA/cm³) varied from 0.1 to 0.9%. CVs for the microarchitectural parameters assessed in 15 volunteers measured three times varied from 0.7 to 1.5% for cortical thickness (Ct.Th) and for vBMD: total density (Dtot), cortical density (Dcort), and trabecular density (Dtrab). CV varied from 2.5 to 4.4% for trabecular number (Tb.N), thickness (Tb.Th), spacing (Tb.Sp), and spacing standard deviation (Tb.Sp.SD). Scans of poor quality (movement, disrupted contour of cortical bone) were excluded (93 radii, i.e. 8%, 46 tibiae, i.e. 3.9%).

Bone turnover markers

Non-fasting serum and urine were collected at 1300 h and stored at -80°C (16). Serum PTH was measured using a human-specific two-site immunochimiluminescence assay (ELECSYS; Roche). The detection limit was 3 pg/ml. The intra- and inter-assay CVs were $< 5\%$. Serum 25OHD was measured with RIA (DiaSorin, Stillwater, MN, USA) after acetonitrile extraction. The detection limit was 3 ng/ml. Intraassay CV is 6.9, 5.9, and 4.9% for the concentrations of 10, 19, and 50 ng/ml respectively. Interassay CVs were 9–11%. Serum total osteocalcin (OC), N-terminal extension propeptide of type I collagen (PINP) and β -isomerized C-terminal telopeptide of collagen type I (β -CTX) were

measured with a human-specific two-site immunochimiluminescence assay (ELECSYS; Roche) (16). For OC, detection limit was 0.5 ng/ml. The interassay CV varied from 11.1 to 14.6% for concentrations from 21.4 to 220.5 ng/ml. For PINP, detection limit was 5 ng/ml. Interassay CV varied from 4.7 to 5.9% for concentrations from 41 to 766.7 ng/ml. For β -CTX, detection limit was 0.01 ng/ml. Interassay CV varied from 3.7 to 5% for concentrations from 0.3 to 2.8 ng/ml. Bone-specific alkaline phosphatase (BAP) was measured with an enzymatic immunoassay (Metra BAP; Quidel, San Diego, CA, USA). Detection limit was 0.7 $\mu\text{mol/l}$. Interassay CV varied from 2 to 5.9% for levels from 7.5 to 49.4 $\mu\text{mol/l}$. Urinary total deoxypyridinoline (DPD) was measured after acid hydrolysis by ELISA (Metra Total DPD; Quidel). Detection limit was 0.5 nmol/l. Interassay CV varied from 4.4 to 14.2% for concentrations between 4.3 and 9.7 nmol/l. Serum calcium, phosphorus, and creatinine were measured by standard laboratory methods (17). Glomerular filtration rate was estimated using the MDRD equation (18).

Covariates

Average time spent outdoors (h/wk) was estimated on the basis of the overall amount of time spent on walking, gardening, and participating in leisure sport activity including seasonal activities (19). Tobacco smoking was assessed as current smoker versus currently non-smoker. Calcium intake was estimated using a food-frequency questionnaire (FFQ) adapted to French alimentary habits (20).

Statistical analysis

Statistical analyses were made using the SAS 9.1 Software (Cary, NC, USA). Data are presented as mean $\pm$ s.d. or, for non-Gaussian distributed variables, as median and interquartile range. Age-related changes in PTH and 25OHD levels were modeled by the PROC LOESS using the option Automatic Smoothing Parameter Selection. This method provides robust fitting in the presence of outliers. In further analyses, variables with non-Gaussian distribution were log-transformed. Linear regression and simple correlation coefficient were calculated for various cutpoints in order to establish the threshold value of age associated with different age-related trends. Correlation was assessed by Pearson's partial correlation coefficient adjusted for age, weight, height, glomerular filtration rate, and season. Season was classified as four seasons (spring, summer, autumn, and winter) defined by the date of blood collection. The association of bone microarchitectural parameters with PTH and 25OHD quartiles was assessed by analysis of covariance. The preliminary models were adjusted for age, weight, height, glomerular filtration rate (all continuous), current

smoking status (yes versus no), time spent outdoors (log-transformed, quartiles), and season (four classes). As season and time spent outdoors were not significant ($P > 0.20$), they were not retained in the final models. Then, we calculated trend across the quartiles. The difference between the adjusted means in the fourth quartile and in the first quartile was expressed as percentage and number of s.d. and its statistical significance was calculated using the Dunnett–Hsu test. The analyses were also performed in groups classified using the generally accepted thresholds (21, 22): severe vitamin D deficiency (25OHD < 10 ng/ml), vitamin D deficiency (10–20 ng/ml), vitamin D insufficiency (20–30 ng/ml), and vitamin D sufficiency (> 30 ng/ml). The association between calcium intake and microarchitectural parameters was assessed, at first, across the quartiles of calcium intake. As these analyses showed similar average values of dependent variables in the three upper quartiles ($P > 0.7$), the analyses were repeated in two groups (lowest quartile versus three upper quartiles of calcium intake). The difference was expressed in percentage and number of s.d.

Results

Age-related changes in the levels of PTH and 25OHD

Men taking vitamin D or calcium supplementation ($n = 37$), 15 men who had increased serum levels of both calcium and PTH (possibility of primary hyperparathyroidism), Seven men who did not have biological measurements, one young man with extremely low 25OHD concentration as well as men who had scans of poor quality (93 radii, 46 tibiae) were excluded. Men who had scans of poor quality were older ($P < 0.01$), shorter ($P < 0.01$), and more obese ($P < 0.001$). Their PTH and 25OHD levels and their calcium intake did not differ from other men.

Serum 25OHD level was stable until 65 years ($r = 0.05$, $P = 0.38$) and then was correlated negatively with age ($r = -0.18$, $\beta = -0.29$ ng/ml per year,

$P < 0.001$; Table 1, Fig. 1). Serum PTH level correlated positively with age in men aged < 65 years ($r = 0.19$, $\beta = 0.23$ pg/ml per year, $P < 0.001$) and in men aged ≥ 65 years ($r = 0.27$, $\beta = 1.22$ pg/ml per year, $P < 0.001$). On the basis of the inspection of the LOESS curves, linear regression and correlation coefficients (Table 2), this threshold was most discriminative. Thus, the analyses were performed in the two age groups: < 65 years (480 radii, 500 tibiae) and ≥ 65 years (534 radii, 563 tibiae).

In older men, 25OHD and PTH concentrations varied according to the season ($P < 0.001$ and $P < 0.05$ respectively). In winter, 25OHD level was 34% lower (1.09 s.d., $P < 0.001$), whereas PTH concentration was 20% higher (0.53 s.d., $P < 0.05$) compared with summer. In younger men, 25OHD varied according to the season ($P < 0.001$), whereas PTH did not ($P = 0.18$). In winter, 25OHD level was 42% lower (1.21 s.d., $P < 0.001$) compared with summer. The age-adjusted partial correlation coefficient between 25OHD and log-transformed PTH varied from $r = 0.18$ ($n = 200$, $P < 0.01$) in summer to $r = 0.29$ ($n = 298$, $P < 0.001$) during the winter.

PTH and 25OHD versus bone microarchitecture and bone turnover marker before the age of 65 years

In men aged < 65 years, low calcium intake was associated with lower Ct.Th. Low calcium intake and low 25OHD were associated with poor trabecular microarchitecture. Higher PTH level was associated with higher bone turnover rate.

Cortical bone Total bone area (Tt.Ar) and medullary area (Ma.Ar) at the distal radius and tibia did not correlate with the PTH and 25OHD levels (Table 2). PTH level weakly correlated negatively with Ct.Th at the distal radius and tibia. In the multivariate models, PTH and 25OHD levels were not associated with Dtot, Dcort, and Ct.Th on both the skeletal sites ($P > 0.10$).

Table 1 Descriptive analysis of the 1064 men from the STRAMBO cohort included in the analysis presented in this study.

	Age (years)							
	20–30	31–40	41–50	51–60	61 to < 65	65–70	71–80	> 80
<i>n</i>	76	71	89	91	173	144	319	100
Weight (kg)	75 $\pm$ 10	78 $\pm$ 11	82 $\pm$ 14	80 $\pm$ 11	80 $\pm$ 10	80 $\pm$ 13	77 $\pm$ 12	77 $\pm$ 11
Height (cm)	178 $\pm$ 6	175 $\pm$ 7	176 $\pm$ 7	173 $\pm$ 6	171 $\pm$ 6	170 $\pm$ 7	167 $\pm$ 6	166 $\pm$ 5
Outdoor	7 $\pm$ 3	7 $\pm$ 4	7 $\pm$ 4	8 $\pm$ 5	9 $\pm$ 5	9 $\pm$ 5	8 $\pm$ 5	6 $\pm$ 4
Ca intake	886 $\pm$ 331	788 $\pm$ 291	790 $\pm$ 307	772 $\pm$ 279	777 $\pm$ 240	779 $\pm$ 239	749 $\pm$ 252	749 $\pm$ 256
Serum Ca	2.44 $\pm$ 0.13	2.43 $\pm$ 0.11	2.37 $\pm$ 0.13	2.38 $\pm$ 0.15	2.35 $\pm$ 0.17	2.37 $\pm$ 0.16	2.35 $\pm$ 0.19	2.38 $\pm$ 0.15
Phosphorus	1.17 $\pm$ 0.13	1.14 $\pm$ 0.15	1.11 $\pm$ 0.15	1.07 $\pm$ 0.16	1.07 $\pm$ 0.15	1.04 $\pm$ 0.16	1.03 $\pm$ 0.15	1.04 $\pm$ 0.17
GFR	102.5 $\pm$ 14.8	93.0 $\pm$ 14.3	88.8 $\pm$ 14.8	82.9 $\pm$ 14.2	81.7 $\pm$ 16.8	76.7 $\pm$ 16.3	73.1 $\pm$ 16.5	67.8 $\pm$ 17.3
PTH	34.5 $\pm$ 12.1	34.9 $\pm$ 9.9	40.5 $\pm$ 13.0	41.7 $\pm$ 15.2	41.5 $\pm$ 22.1	42.8 $\pm$ 15.2	50.1 $\pm$ 21.8	63.7 $\pm$ 37.6
25OHD	21.5 $\pm$ 10.4	24.9 $\pm$ 9.9	25.1 $\pm$ 10.1	23.4 $\pm$ 12.1	24.5 $\pm$ 11.1	22.8 $\pm$ 8.4	21.4 $\pm$ 9.5	17.6 $\pm$ 8.4

Outdoor, time spent outdoors (hours/week); Ca, calcium intake (mg/day); Serum Ca (mmol/l); phosphorus (mmol/l); GFR, glomerular filtration rate (ml/min); PTH, parathyroid hormone (pg/ml); 25OHD, 25 hydroxycholecalciferol (ng/ml).

Figure 1 Age-related changes of the concentrations of 25-hydroxycholecalciferol (25OHD) and parathyroid hormone (PTH) in 1063 men aged 20–87 years from the STRAMBO cohort.

Men in the lowest quartile of calcium intake had lower Ct.Th at the distal radius and distal tibia (5.2–6.0%, 0.29 s.d.) compared with men in the three higher quartiles (Table 3).

Trabecular bone Trabecular parameters of distal tibia correlated weakly but significantly with PTH and 25OHD concentrations (Table 2). After adjustment for confounders, trabecular parameters did not vary across PTH quartiles ($P > 0.15$).

At the distal tibia, Dtrab and Tb.N correlated weakly with 25OHD level. In multivariate models, both of them increased across the 25OHD quartiles ($P < 0.005$). Men in the lowest 25OHD quartile had lower Dtrab (6.7%, 0.33 s.d., $P < 0.05$) and Tb.N (5.0%, 0.32 s.d., $P < 0.01$) compared with the highest 25OHD quartile. Tb.Sp and Tb.Sp.SD decreased across the 25OHD quartiles ($P < 0.005$) and were 3.2% (0.35 s.d., $P < 0.05$) lower in the lowest quartile compared with the highest one. Men with 25OHD < 10 ng/ml ($n = 28$) had lower tibia Dtrab (10.7%, 0.52 s.d., $P < 0.05$) and lower Tb.N (7.0%, 0.43 s.d., $P < 0.05$) compared with men who had 25OHD > 30 ng/ml. They also had higher Tb.Sp (4.7%, 0.48 s.d., $P < 0.05$) and higher Tb.Sp.SD (4.4%, 0.44 s.d., $P < 0.05$). At the radius, trabecular parameters were not associated with 25OHD concentration.

On both the skeletal sites, men in the lowest quartile of calcium intake (< 600 mg/day) had lower Dtrab and Tb.N, but greater Tb.Sp and Tb.Sp.SD (Table 3).

Bone turnover markers Most of the bone turnover marker (BTM) correlated with the PTH level. After adjustment for confounders, serum OC, BAP, and β -CTX were 16–44% higher (0.35–0.70 s.d., $P < 0.005$) in the highest PTH quartile than in the lowest PTH quartile. In contrast, BTM levels did not vary across the 25OHD classes or between the calcium intake groups.

Associations between PTH, 25OHD, bone microarchitecture and BTM after the age of 65 years

Men aged ≥ 65 years with higher PTH level had lower Ct.Th and greater Ma.Ar at the distal tibia, poor trabecular microarchitecture on both skeletal sites, and higher bone turnover rate. Men with low calcium intake had poor trabecular microarchitecture on both skeletal sites.

Table 2 Correlation between PTH and 25OHD and bone microarchitectural parameters at the distal radius and distal tibia (adjusted for age, weight, height, glomerular filtration rate, and season) in two groups of men analyzed in this study.

	< 65 years ($n = 500$)		≥ 65 years ($n = 563$)	
	PTH	25OHD	PTH	25OHD
OC ^a	0.30 [†]	−0.03	0.32 [†]	−0.04
PINP ^a	0.19 [†]	−0.03	0.16 [†]	−0.06
BAP ^a	0.17 [†]	−0.09	0.20 [†]	−0.03
β -CTX ^a	0.32 [†]	−0.07	0.27 [†]	−0.06
DPD ^a	0.08	0.10	0.10	−0.01
Radius				
Tt.Ar	0.04	−0.01	−0.01	0.05
Dtot	−0.10	0.08	−0.07	0.03
DCort	−0.11	0.06	0.05	−0.01
Ct.Th	−0.13 [*]	0.08	0.01	0.01
Ma.Ar	−0.07	−0.05	−0.01	0.04
DTrab	−0.05	0.06	0.09	0.06
Tb.N	−0.01	0.02	−0.15 [*]	0.06
Tb.Th	−0.07	0.04	−0.11	0.04
Tb.Sp ^a	0.01	−0.02	0.16 [†]	−0.07
Tb.Sp.SD ^a	0.01	−0.03	0.16 [†]	−0.06
Tibia				
Tt.Ar	0.08	0.01	0.07	0.00
Dtot	−0.17 [†]	0.11	−0.14 [†]	0.05
DCort	−0.07	0.03	−0.07	0.04
Ct.Th	−0.17 [†]	0.10	−0.12 [†]	0.07
Ma.Ar	0.12	−0.04	0.09	−0.02
DTrab	−0.10	0.11	−0.14 [†]	0.03
Tb.N	−0.08	0.12 [*]	−0.06	0.03
Tb.Th	−0.10	0.03	−0.13 [†]	0.01
Tb.Sp ^a	0.09	−0.12 [†]	0.08	−0.03
Tb.Sp.SD ^a	0.08	−0.12 [†]	0.05	−0.02

^{*} $P < 0.005$; [†] $P < 0.001$; PTH, parathyroid hormone (pg/ml); 25OHD, 25 hydroxycholecalciferol (ng/ml); OC, osteocalcin (ng/ml); BAP, bone alkaline phosphatase ($\mu\text{mol/l}$); PINP, procollagen type 1 N-terminal propeptide (ng/ml); β -CTX, beta isomer of C-terminal telopeptide of type I collagen (ng/ml); DPD, total deoxypyridinoline (nmol/mg creatinine); Tt.Ar, total cross-sectional bone area (mm^2); Dtot, total density (mg HA/cm^3); DCort, cortical density (mg HA/cm^3); Ct.Th, cortical thickness (mm); Ma.Ar, medullary (marrow) area (mm^2); Dtrab, trabecular density (mg HA/cm^3); Tb.N, trabecular number (1/mm); Tb.Th, trabecular thickness (μm); Tb.Sp, trabecular separation (μm); Tb.Sp.SD, trabecular spacing standard deviation (μm).

^aNon-Gaussian distribution, median, and interquartile range.

Table 3 Bone microarchitectural parameters according to the daily calcium intake in men aged <65 years and in men aged ≥65 years (mean ± s.d.).

Parameter	Men aged <65 years			Men aged ≥65 years		
	QI <600 mg/d (n=122)	QII–IV ≥600 mg/d (n=378)	P ^a	QI <590 mg/d (n=140)	QII–IV ≥590 mg/d (n=423)	P ^a
Radius						
Tt.Ar	378 ± 66	375 ± 58	0.69	388 ± 56	398 ± 64	0.07
Dtot	315.4 ± 51.7	331.4 ± 58.3	<0.01	289.7 ± 64.5	291.5 ± 63.6	0.49
DCort	844 ± 52	849 ± 50	0.32	786 ± 72	789 ± 70	0.88
Ct.Th	0.78 ± 0.17	0.83 ± 0.20	<0.05	0.68 ± 0.21	0.69 ± 0.22	0.69
Ma.Ar	305 ± 67	297 ± 62	0.26	316 ± 60	322 ± 68	0.22
DTrab	180.8 ± 34.1	192.5 ± 34.8	<0.005	165.1 ± 42.1	173.5 ± 38.2	0.05
Tb.N	1.88 ± 0.21	1.93 ± 0.23	<0.05	1.80 ± 0.25	1.87 ± 0.25	<0.005
Tb.Th	80.1 ± 11.5	83.3 ± 11.8	<0.01	75.7 ± 13.4	77.1 ± 11.6	0.37
Tb.Sp ^b	454 (414; 500)	437 (396; 484)	<0.05	486 (423; 551)	456 (409; 506)	<0.01
Tb.Sp.SD ^b	188 (166; 218)	180 (160; 201)	<0.005	209 (178; 261)	196 (169; 226)	<0.01
Tibia						
Tt.Ar	828 ± 126	835 ± 122	0.50	842 ± 122	845 ± 129	0.59
Dtot	309.8 ± 52.0	323.2 ± 54.4	<0.05	279.5 ± 59.9	286.8 ± 56.6	0.17
DCort	877 ± 39	880 ± 41	0.55	828 ± 61	831 ± 59	0.61
Ct.Th	1.28 ± 0.25	1.35 ± 0.26	<0.05	1.17 ± 0.30	1.18 ± 0.29	0.73
Ma.Ar	681 ± 128	679 ± 127	0.88	706 ± 132	702 ± 134	0.73
DTrab	181.2 ± 38.2	191.2 ± 38.3	<0.01	166.9 ± 38.9	172.8 ± 37.4	0.08
Tb.N	1.78 ± 0.28	1.86 ± 0.29	<0.005	1.67 ± 0.28	1.76 ± 0.30	<0.005
Tb.Th	84.9 ± 12.4	85.8 ± 12.1	0.48	83.3 ± 13.9	82.7 ± 13.1	0.53
Tb.Sp ^b	477 (432; 548)	455 (403; 518)	<0.005	504 (448; 600)	495 (425; 555)	<0.005
Tb.Sp.SD ^b	224 (189; 259)	205 (176; 243)	<0.001	237 (205; 293)	228 (189; 270)	<0.005

OC, osteocalcin (ng/ml); BAP, bone-specific alkaline phosphatase (μmol/l); P1NP, N-terminal propeptide of type I procollagen (ng/ml); β-Ctx, beta-isomerized C-terminal telopeptide of type I collagen (ng/ml); DPD, total deoxypyridinoline (nmol/mg creatinine); Tt.Ar, total cross-sectional bone area (mm²); Dtot, total density (mg HA/cm³); DCort, cortical density (mg HA/cm³); Ct.Th, cortical thickness (mm); Ma.Ar, medullary (marrow) area (mm²); DTrab, trabecular density (mg HA/cm³); Tb.N, trabecular number (1/mm); Tb.Th, trabecular thickness (μm); Tb.Sp, trabecular separation (μm); Tb.Sp.SD, trabecular spacing standard deviation (μm).

^aAll the models are adjusted for age, weight, height, smoking, glomerular filtration rate, and 25OHD concentration.

^bVariables showing non-Gaussian distribution, presented as median and interquartile range, the analyses performed in the log-transformed variables.

Cortical bone Ct.Th at the distal tibia correlated negatively with PTH (Table 2). Otherwise, Tt.Ar, Ma.Ar, and cortical parameters at the distal radius and tibia did not correlate with the PTH and 25OHD levels.

In multivariate models, Ct.Th at the distal tibia decreased across the increasing PTH quartiles ($P < 0.05$; Fig. 2). It was 8.3% lower (0.39 s.d., $P < 0.005$) in the highest PTH quartile than in the lowest quartile. Also at the distal tibia, Ma.Ar increased across the PTH quartiles ($P < 0.05$). After adjustment for confounders, Ma.Ar was 4.3% higher (0.26 s.d., $P < 0.05$) in the highest PTH quartile compared with the lowest quartile. At the distal radius, cortical parameters did not vary across the PTH quartiles.

At both the skeletal sites, Dcort and Ct.Th did not correlate with the 25OHD level. They did not vary across the 25OHD quartiles either ($P > 0.25$). In multivariate models, cortical parameters did not differ between 45 men with severe vitamin D deficiency (<10 ng/ml) and 99 men with normal 25OHD (25OHD ≥ 30 ng/ml).

In similar models, Dcort and Ct.Th did not differ between men with the lower calcium intake (<590 mg/day, lowest quartile) and higher calcium intake except slightly lower Ct.Th at the tibia (Table 3).

Trabecular bone Dtrab correlated negatively with the PTH level and decreased across the PTH quartiles (Table 2, Fig. 2). At both the skeletal sites, men in the highest PTH quartile had 6.7–9.1% lower trabecular vBMD (0.31 and 0.44 s.d., $P < 0.001$) and 3.6–3.8% lower Tb.Th (0.24 s.d. and 0.25 s.d., $P < 0.05$) in comparison with the lowest PTH quartile. At the distal radius, men in the highest PTH quartile had also lower Tb.N and higher Tb.Sp.SD.

Trabecular parameters did not correlate with the 25OHD level. They did not vary according to the 25OHD quartiles nor according to the pre-defined groups.

At both the skeletal sites, men with low calcium intake (<590 mg/day) had lower Tb.N and slightly lower Dtrab, but higher Tb.Sp and Tb.Sp.SD compared with men who had higher calcium intake.

Bone turnover markers PTH was significantly correlated with the BTM levels (Table 2). In the highest PTH quartile, the BTM levels (except DPD) were 24–52% higher (0.42–1.45 s.d., $P < 0.001$) compared with the lowest PTH quartile (Fig. 3). In the lowest 25OHD quartile, BTM levels was slightly higher compared with the higher quartiles, but differences were not statistically significant. The BTM levels did not vary between the calcium intake groups.

Figure 2 Microarchitectural parameters at the distal radius (pointed bars) and distal tibia (hatched bars) according to quartiles of the PTH concentration in men aged ≥ 65 years: q1, <35 pg/ml, q2, 35–45.9 pg/ml, q3, 46–59 pg/ml, q4, >59 pg/ml. All the models are adjusted for age, weight, height, smoking, and glomerular filtration rate: (A) Dtot, total density ($\text{mg HA}/\text{cm}^3$): radius, $F=2.82$, $P<0.05$, P for trend = 0.06, tibia, $F=3.53$, $P<0.05$, P for trend <0.005 ; (B) Ct.Th, cortical thickness (mm): radius, $F=1.28$, $P=0.28$, P for trend = 0.41, tibia, $F=2.79$, $P<0.05$, P for trend <0.005 ; (C) Dtrab, trabecular density ($\text{mg HA}/\text{cm}^3$): radius, $F=4.32$, $P=0.005$, P for trend <0.005 , tibia, $F=3.35$, $P<0.05$, P for trend <0.01 ; (D) Tb.N, trabecular number (1/mm): radius, $F=3.73$, $P<0.05$, P for trend = 0.005, tibia, $F=1.47$, $P=0.22$, P for trend = 0.13; (E) Tb.Th, trabecular thickness (μm): radius, $F=2.74$, $P<0.05$, P for trend, $P<0.05$, tibia, $F=2.12$, $P=0.10$, P for trend <0.05 ; (F) Tb.Sp.SD, trabecular number distribution (μm): radius, $F=4.59$, $P<0.005$, P for trend <0.005 , tibia, $F=1.94$, $P=0.12$, P for trend = 0.06. Data are presented as adjusted mean and s.e.m. for (A) to (E) and as unadjusted median and interquartile range for (F). Symbols for the adjusted difference compared with the first quartile: * $P<0.01$, # $P<0.005$, § $P<0.001$.

Discussion

We found that early afternoon non-fasting PTH level correlated positively with age in men. Serum 25OHD level correlated negatively with age in men aged 65 years and above. Before the age of 65 years, men with low 25OHD level had lower Tb.N and higher Tb.Sp.SD at the distal tibia, whereas men with low calcium intake had lower Tb.N and higher Tb.Sp.SD at both the skeletal sites. After the age of 65 years, men with the increased PTH level had slightly lower Ct.Th at the distal tibia and lower Dtrab (due to lower Tb.N) at both the skeletal sites. Low calcium intake was associated with lower Tb.N and

higher Tb.Sp.SD. BTM levels correlated positively with the PTH level but not with 25OHD or calcium intake.

Age-related changes in the early afternoon PTH levels are similar to the PTH measurements in blood collected after overnight fasting, which is consistent with the importance of the endogenous regulation of the circadian PTH secretion (23, 24).

Before 65 years of age, 25OHD was positively associated with trabecular microarchitecture at the distal tibia. PTH and 25OHD were poorly associated with other bone microarchitectural parameters and these associations became non-significant in fully adjusted models. Vitamin D and calcium intake contribute to bone mineral accrual during growth (25, 26). By contrast, in adult men, current vitamin D status is weakly correlated with BMD (15, 27, 28). In young and middle-aged men, 25OHD was weakly correlated positively with BMD at the lumbar spine and the hip (13). The relationship between the PTH level and the BMD in young and middle-aged adults was weak or non-significant (15, 29, 30). Thus, current PTH secretion does not seem to be a significant determinant of bone mass and microarchitecture in young adult men, although these men may be in the phase of slow bone loss (31).

After the age of 65 years, high PTH level was associated with slightly lower bone mass mainly in the trabecular compartment of distal radius. The inverse associations of PTH level with Ct.Th at the distal tibia and with Tb.N at the distal radius suggest that higher bone resorption triggers this process. Positive

Figure 3 Biochemical bone turnover markers according to the quartiles of PTH concentration in men aged ≥ 65 years: q1, <35 pg/ml, q2, 35–45.9 pg/ml, q3, 46–59 pg/ml, q4, >59 pg/ml. All the models are adjusted for age, weight, height, smoking, and glomerular filtration rate: (A) osteocalcin, $F=21.1$, $P<0.001$, P for trend <0.001 ; (B) P1NP, $F=5.33$, $P<0.005$, P for trend <0.001 ; (C) β -CTX, $F=13.6$, $P<0.001$, P for trend <0.001 ; (D) total deoxypyridinoline, $F=1.18$, $P=0.32$, P for trend = 0.12. * $P<0.01$, # $P<0.005$, § $P<0.001$.

association between the levels of PTH and BTM in this study and poor bone microarchitecture in men with high bone turnover found previously show that secondary hyperparathyroidism may stimulate bone turnover and contribute to the deterioration of bone microarchitecture (14). At the distal tibia, Ct.Th decreased and Ma.Ar increased, whereas Tt.Ar did not vary across the PTH quartiles. It suggests that cortical thinning due to secondary hyperparathyroidism depends on endocortical resorption and trabecularization of the inner cortical bone (32). However, trabecularized cortical bone (now in the trabecular compartment) may have higher vBMD than the genuine trabecular bone. If so, trabecularization of the cortical bone would falsely increase D_{trab} in the highest PTH quartile and artifactually attenuate the inverse association of the PTH concentration with Tb.N or Tb.Th.

The relationship between bone microarchitecture and 25OHD level was weak as in the previous data (15). Data on the relationship between DXA-measured aBMD and 25OHD concentration in older people are discrepant (13, 33). Recently, the association of the 25OHD level with hip aBMD and with whole body bone mineral content in a large cohort of men and women was weakly positive, whereas it was not significant with quantitative ultrasound parameters (34).

In our study, lower calcium intake was associated with impaired trabecular microarchitecture irrespective of age. Data on the relationship between calcium intake and bone mass in adult men are discrepant (35, 36). Appropriate calcium intake seems to maintain bone mass in the elderly (37, 38). This relationship may depend not only on calcium itself but also on the better general nutritional status in people with higher calcium intake (39). The cross-sectional study does not allow to assess temporal relationship. The association of calcium intake with bone microarchitecture but not with the current bone turnover rate suggests that higher current calcium intake may reflect dietary patterns from the youth. In our study, calcium intake is probably underestimated and not accurate. However, our data raise a possibility that low calcium intake contributes to the deterioration of bone microarchitecture in older men.

Strengths of our study are the large cohort and assessment of bone microarchitecture at the weight-bearing tibia and non-weight-bearing radius. Our study has limitations. Home-dwelling volunteers may be sicker among young men and healthier among older men. Given the response rate of 26%, it can result in under-representation of older men with severe vitamin D deficit. The cross-sectional design limits inference on the cause and effect. Calcium intake assessment may be suboptimal due to simplified FFQ and approximate coefficients used to estimate calcium content of products. We collected biological samples in the standardised way in the early afternoon and after a non-standardised lunch that could contain dairy products. This limitation may influence mainly

concentrations of PTH and β -CTX (40). Although our data are in line with analyses based on the fasting blood samples, minor differences cannot be excluded. Low average 25OHD levels suggests that a substantial percentage of men are vitamin D deficient. However, considerable differences between laboratories exist and our laboratory was not cross-calibrated with other centers measuring 25OHD in the time of measurements (41). In the HR-pQCT, Tb.Th, Tb.Sp, and Ct.Th are calculated, not measured. In men with low Tb.Th, it may result in underestimation of Tb.N. Erroneous estimation of Ct.Th and D_{cort} may exist, mainly in old men with thin cortex. HR-pQCT underestimates Ct.Th compared with micro-CT; however, the correlation between the methods was very high (42). It indicates that the trends found in our study correspond most probably to the real biological trends; however, absolute values of the means are not accurate.

In conclusion, the association of bone microarchitecture with serum PTH and 25OHD levels and with calcium intake in men aged <65 years was relatively poor. Thus, in this age range, current calcium and vitamin D status may be weak determinants of bone microarchitecture. After 65 years of age, higher PTH level and lower calcium intake correlated weakly but significantly with poor bone microarchitecture, mainly in the trabecular compartment. Correlation of PTH level with BTM levels and with bone microarchitecture suggests the importance of current PTH secretion for bone status in this age group. In contrast, the association of calcium intake with trabecular microarchitecture, but not BTM levels, rather suggests the importance of long-term dietary habits. The poor association may partly depend on the cohort consisting of home-dwelling men who rarely have extreme vitamin D deficit. It also raises a possibility that a substantial part of the hip fracture risk related to vitamin D deficit depends on the lower muscle mass and higher risk of fall (43). However, despite the limitations of the cross-sectional study, our data support the view that, in the elderly, higher calcium intake and lower PTH concentrations can be beneficial for bone microarchitecture and bone strength.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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