Published in final edited form as:
Bone. 2006 October ; 39(4): 915–921. doi:10.1016/j.bone.2006.03.014.

Correlates of bone quality in older persons

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Abstract

Purpose of the study—In a population-based sample of older persons, we studied the relationship between tibial bone density and geometry and factors potentially affecting osteoporosis.

Methods—Of the 1260 participants aged 65 years or older eligible for the InCHIANTI study, 1155 received an interview and 915 (79.2%) had complete data on tibial QCTscans and other variables used in the analysis presented here. The final study population included 807 persons (372 men and 435 women, age range 65–96 years) after exclusion of participants affected by bone diseases or treated with drugs that interfere with bone metabolism.

Results—In both sexes, calf cross-sectional muscle area (CSMA) was significantly and independently associated with total bone cross-sectional area (tCSA) and cortical bone cross-sectional area (cCSA) but not with trabecular or cortical volumetric bone mineral density (vBMD). Bioavailable testosterone (Bio-T) was independently associated with both trabecular and cortical vBMD in both sexes. In women, independently of confounders, 25(OH)-vitamin D was positively associated with tCSA and cortical vBMD, while PTH was negatively associated with cortical vBMD. IL-1 beta was negatively correlated with cortical vBMD in women, while TNF-alpha was associated with enhanced bone geometrical adaptation in men.

Conclusions—Physiological parameters that are generically considered risk factors for osteoporosis were associated with specific bone parameters assessed by tibial QCT. Factors known to be associated with increased bone reabsorption, such as 25(OH)-vitamin D, PTH and Bio-T, affected mainly volumetric BMD, while factors associated with bone mechanical stimulation, such as CSMA, affected primarily bone geometry. Our results also suggested that pro-inflammatory cytokines might be considered as markers of bone resorption.

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Keywords

Bone geometry and density; pQCT; Age-related bone loss; Osteoporosis

Introduction

Most epidemiological evidence on factors affecting bone tissue mineralization in older persons is based on DXA-derived estimates of bone tissue mineral content relative to volume [1]. These studies neglect to consider that trabecular and cortical bones have different physiological characteristics and, therefore, are likely to be affected by different factors [2-5]. Age-related bone loss is accompanied by an increase in bone diameter, a putative adaptive mechanism that tends to maintain bone strength [6-8]. While it is well established that geometrical characteristics contribute to bone strength, whether they are affected by specific behavioral, hormonal and metabolic factors is unknown.

There is robust evidence that estrogens are important for the maintenance of bone mineralization over the life span. However, because of their adverse effects on cardiovascular outcomes, estrogens are no longer considered the first choice intervention for the prevention and treatment of osteoporosis in menopausal women [9,10]. Therefore, it is important to study physiological factors other than estrogens that may affect bone characteristics in older persons and which may be suitable targets for alternative interventions.

There is strong evidence that exercise is beneficial to the skeleton, probably because it is associated with a substantial increment of the mechanical stimulation of bone both directly and indirectly through the enhancement of muscle strength [11]. Accordingly, it has been demonstrated that strength training reduces the risk of fractures and slows down the age-associated decline of mineral density [12]. However, whether the beneficial effects of exercise and muscle strength affect trabecular or cortical bone and/or bone geometry is still unclear. Likewise, vitamin D deficiency is frequent in older persons [13], especially in older women and, together with a compensatory elevation of PTH serum levels, potentially contributes to accelerate bone loss in older individuals [14]. In a recent study, vitamin D but not estradiol or free testosterone predicted fractures in older men [15]. Other anabolic hormones, such as dehydroepiandrosterone sulfate (DHEAS), and GH/IGF-1 may have beneficial effects on bone [16-18]. Again, how these hormones selectively affect trabecular, cortical bone and/ or bone geometry is unknown. Finally, there is now a large body of evidence suggesting that pro-inflammatory cytokines may enhance bone reabsorption and contribute to the disruption of the bone micro-architecture that is often observed in older persons [19]. Accordingly, experimental and clinical studies indicate the existence of an important link between pro-inflammatory cytokines and postmenopausal bone loss [20]. Whether pro-inflammatory cytokines affect mainly trabecular or cortical bone is unclear.

Using data on trabecular and cortical bone density and bone geometry collected by peripheral quantitative computerized tomography, we studied the relationship between muscle strength, muscle mass, physical activity, parathyroid hormone (PTH), bioavailable testosterone (Bio-T), dehydroepiandrosterone sulfate (DHEAS), total insulin-like growth factors-1 (IGF-1) and pro-inflammatory cytokines and bone strength in a representative sample of older persons. In particular, we aimed at differentiating correlates of trabecular and cortical bone characteristics and bone geometrical structure.

Methods

Study population

InCHIANTI is an epidemiological study of risk factors for mobility disability in old age performed on a representative sample of the population living in Greve in Chianti and Bagno a Ripoli, two small towns located in the Chianti countryside of Tuscany, Italy. The study design and data collection have been previously described elsewhere [21]. Of the 1260 participants 65 years old or older, 1155 received a home interview. Of these, 915 (79.2%) participants had complete data on pQCT scans and other variables used in the analysis presented here. We excluded 46 participants affected by bone diseases such as osteopetrosis or Paget's disease, 58 participants treated with drugs that interfere with bone metabolism such as calcium, vitamin D, bisphosphonates, calcitonin, corticosteroids, anticonvulsive therapy and lithium and 4 participants with primary hyperparathyroidism.

The final study population included 807 persons (372 men and 435 women, age range 65–96 years). The study protocol was examined and approved by our institutional review board. All participants received a detailed description of the purpose and design of the study, and all signed an informed participation consent.

Measures

After the home interview, participants received a functional evaluation and an examination in a clinical center.

Tibial pQCT

The peripheral quantitative computed tomography (pQCT) was performed by the XCT 2000 device (Stratec Medizintechnik, Pforzheim, Germany). A detailed description of the tibial QCT examination has been published elsewhere [3]. The precision error of the XCT2000 is below 1% for volumetric trabecular and cortical density and between 1 and 3% for composite geometry parameters [22].

The cross-sectional images obtained from the pQCT were analyzed using the *BonAlyse* software (BonAlyse Oy, Jyvaskyla, Finland), a software program for processing pQCT scans that automatically identifies bone tissue (cortical and trabecular) and assesses its density and geometry. The following bone parameters were derived from the pQCT images:

- Trabecular volumetric BMD (vBMDt) (mg/cm^3): assessed as the average density of the trabecular bone area detected at the 4% site. Cortical bone was excluded from the measurement.
- Cortical volumetric BMD (vBMDc) (mg/cm^3): a selective measure of the apparent volumetric density of cortical bone measured at the 38% site, which is a good marker of bone material property.
- Total bone cross-sectional area (tCSA) (mm^2): assessed as the area within the circumference that delimited all cortical bone tissues with a density higher than 180 mg/cm^3 measured at the 38% site, which is a measure of bone size.
- Cortical bone cross-sectional area (cCSA) (mm^2): assessed as the cross-sectional area of the voxels with a density higher than 710 mg/cm^3, measured at the 38% site. The cortical bone area is a good measure of total cortical bone mass and a valid marker of bone resistance against compression and tensile loads [22].

Calf muscle cross-sectional area (CSMA) was evaluated from a transverse scan performed at 66% of the tibia length from the distal tip of the tibia, which is the level of largest outer calf diameters, with consistency across individuals [22].

Laboratory measures

Blood samples were drawn in the morning after a 12-h overnight fast and after the participants had been sitting for at least 15 min, carefully avoiding red cell hemolysis. All the routine hematological tests were performed on fresh blood.

The assays of 25(OH)-vitamin D, PTH, total IGF-1, total testosterone, interleukin-6 (IL-6) and tumor necrosis factors alpha (TNF-alpha) were performed on specimens previously stored at −80°C. 25(OH)-vitamin D was measured by RIA (DiaSorin Inc., Stillwater, MN, USA), after extraction of samples with acetonitrile. Intra- and interassay CVs were 8.1 and 10.2%, respectively. Serum intact parathyroid hormone (PTH) was measured using a two-site immunoradiometric assay kit (N-tact PTHSP, DiaSorin Inc., Stillwater, MN, USA). The assay uses two affinity-purified polyclonal antibodies, one specific for the amino-terminal 1–34 portion of PTH molecule and the second specific for the 39–84 sequence of the hormone. The assay sensitivity was 1.2 ng/l. Intra- and interassay CVs were <3.0 and 5.5%, respectively. Serum levels of total IGF-1 were measured in duplicate by immunoradiometric assay, using commercial reagents (DSL, Webster, TX). Interassay and intraassay coefficients of variation (CV) for 3 concentrations (low, medium, high) were all less than 10%. Total testosterone was assayed using commercial radioimmunologic kits (Diagnostic Systems Laboratories, Webster, TX). For total testosterone, the minimum detection limit was 0.03 nmol/l; intraassay and interassay coefficients of variation for 3 different concentrations were 9.6%, 8.1% and 7.8%, and 8.6%, 9.1% and 8.4%, respectively. Sex hormone-binding globulin (SHBG) was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) which has a sensitivity of 0.04 nmol/l and interassay and intraassay coefficients of variation for 3 concentrations (10.8 nmol/l, 64 nmol/l, 116 nmol/l) 3.1%, 5.3% and 6.9%, and 2.8%, 3.0% and 3.6%, respectively. Concentrations of bioavailable testosterone (Bio-T) were calculated using the Vermeulen formula [24]. Dehydroepiandrosterone sulfate (DHEAS) was assayed using commercial radioimmunologic kits (Diagnostic Systems Laboratories, Webster, TX). For DHEAS, the minimum detection limit was 1.7 μg/dl; intraassay and interassay coefficients of variation for 3 different concentrations were 4.1%, 5.3% and 4.7%, and 4.8%, 7.0% and 4.6%, respectively. Serum levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta) were measured in duplicate by high sensitivity enzyme-linked immunoabsorbent assays (ELISA) using commercial kits (BIOSOURCE International, Camarillo, CA). The lower detectable limit was 0.1 pg/ml for IL-6, 0.09 pg/ml for TNF-alpha and 0.01 pg/ml for IL-1 beta. The interassay coefficient of variation was less than 7% for all cytokines.

Other measures

Weight was measured to the nearest 0.1 kg using a high precision mechanical scale, with the participant wearing light clothes and without shoes. Standing height without shoes was measured to the nearest 0.1 cm.

Body mass index (BMI) was calculated as [weight (kg)] / [height $(m)^2$]. The circumferences were taken using a flexible, fiberglass tape, while the participant was standing. Waist circumference was measured at the narrowest area of the waist, usually 1 cm above the iliac crest, while hip circumference was measured at the maximum posterior extension of the buttocks while the participant was standing. Waist to hip ratio (WHR) was calculated as: waist circumference (cm) divided by the hip circumference (cm).

Ankle extension strength was measured with a hand-held dynamometer (Nicholas Muscle Tester, Sammon Preston Inc., Chicago). Participants, lying in lateral decubitus (opposite to the examined limb) with the hip and the knee extended and the ankle in neutral position, were asked to perform the task twice with the right foot. The average of the results obtained was used for the present analyses. This measure has been extensively used as a muscular strength parameter of lower limb [25]. In previous studies, the intraclass correlation coefficients for duplicate measures of ankle extension strength were 0.93 for inter-rater reliability and 0.93 for test–retest reliability [26]. The level of physical activity in the year prior to the interview was classified on an ordinal scale based on responses to a modified standard questionnaire [27] into: (1) hardly any physical activity; (2) mostly sitting (occasionally walks, easy gardening); (3) light exercise (no sweat) 2–4 h/week; (4) moderate exercise (sweat) 1–2 h/week (level 4); (5) moderate exercise >3 h/week; (6) intense exercise (at the limits) >3 times/week. For analytical purposes, we grouped the participants as: 1–3 inactive or having light physical activity; 4–5 having moderate physical activity; 6 having intense activity.

Data on dietary intake were collected by administering the food frequency questionnaire created for the European Prospective Investigation into Cancer and nutrition (EPIC) study [28]. Although the EPIC questionnaire was originally developed for and validated in middle-aged persons, our previous study suggested that this tool also provides good estimates of dietary intake when administered to the older population [29]. Participants were asked to specify how frequently (weekly, monthly, yearly) each specific food and beverage was consumed in the last year. Participants were asked to report the quantity of food consumed, using for reference colored photographs with different sizes of portions for the main dishes. Specific software created for EPIC transformed data on food consumption into daily intake of energy, macro and micronutrients.

Statistical analyses

All analyses were performed separately in men and women. To account for secular trend of increasing height in later born generations and sex differences in height, tCSA, cCSA and calf cross-sectional muscle area (CSMA) were adjusted by tibial length [7]. These three variables were divided by tibial length squared in each subject and multiplied by the sample mean tibial length squared. Ankle extension strength was adjusted by BMI [30]. Variables distributed normally were reported as mean values ± standard deviations and categorical values as percentages. The 25(OH)-vitamin D, PTH, Bio-T, DHEAS, total IGF-1, IL-6, IL-1 beta and TNF-alpha were described by reporting median values and interquartile ranges due to skewness. To approximate normal distributions, log-transformed values for 25(OH)-vitamin D, PTH, Bio-T, DHEAS, IGF-1, IL-6, IL-1 beta and TNF-alpha were used in the analysis and back-transformed for data presentation. Differences across sexes were analyzed using age-adjusted linear or multinomial logistic regression models, as appropriate.

Factors statistically correlated with bone parameters were identified using age-adjusted partial correlation coefficient and Spearman partial rank–order correlation coefficients, as appropriate. Parsimonious models obtained by backward selection from initial fully adjusted models were used to identify independent factors of tCSA, cCSA, vBMDt and vBMDc.

SAS 8.2 statistical package was used for all analyses (SAS Institute, Inc., Cary, NC, USA).

Results

The characteristics of the study population according to sex are reported in Table 1. Education, waist circumference, all selected bone parameters (tCSA, cCSA, vBMDt and vBMDc), calf CSMA, ankle extension strength, current physical activity, energy intake, protein intake, calcium intake, creatinine clearance, serum 25(OH)-vitamin D, Bio-T, total IGF-1 and IL-6

were significantly higher in men than in women. Conversely, BMI and serum PTH were higher in women than in men. Ionized calcium, DHEAS, IL-1 beta and TNF-alpha were not significantly different between sexes.

After adjusting for age, BMI positively correlated with tCSA, cCSA and vBMDt in both sexes, WHR correlated with the same parameters of bone in women, while in men only with vBMDt (Table 2). Noteworthy, calf CSMA was positively correlated with all bone parameters examined in both sexes. Ankle extension strength was associated with parameters expressing bone geometry (tCSA and cCSA) in women and with vBMDt in men. Current physical activity was associated with vBMDc in women and with cCSA and vBMDt in men. Creatinine clearance was associated with tCSA in men. Only in women serum PTH was negatively correlated with vBMDc, while 25(OH)-vitamin D was positively correlated with tCSA and vBMDc. Unexpectedly, Bio-T was positively correlated with all bone parameters in women and with vBMDc in men. Furthermore, serum DHEAS was positively associated with tCSA and vBMDc in women. Surprisingly, TNF-alpha was positively correlated with tCSA and cCSA in both sexes, while as expected, IL-6 and IL-1 beta were negatively correlated, respectively, with cCSA and vBMDc. Finally, total IGF-1 was not correlated with any bone parameters examined, while DHEAS appeared to be positively correlated with both cortical bone characteristics cCSA and vBMDc in women.

To further explore factors independently affecting the different bone parameters, we fitted parsimonious models, including only independent variables that were statistically associated with the dependent variable, namely tCSA and cCSA (Table 3) and vBMDt and vBMDc (Table 4), in the age- and sex-adjusted analyses reported above.

Calf CSMA appeared to be an independent factor for both the geometrical bone parameters examined, namely tCSA and cCSA, in both sexes. Both BMI and 25(OH)-vitamin D were independently associated with tCSA and cCSA in women, while TNF-alpha appeared to be an independent factor of tCSA and cCSA in men (Table 3).

Bio-T was confirmed to be an independent correlate of both vBMDt and vBMDc in both sexes (Table 4). CSMA was also an independent correlate of the vBMDc in women, while ankle extension strength and current physical activity are factors associated with vBMDt in men. In women but not in men, 25(OH)-vitamin D was positively associated with vBMDc and serum PTH was negatively associated with vBMDc.

Clearance creatinine remained an independent factor of volumetric bone mineral density, in particular of the vBMDt in women and of the vBMDc in men. Furthermore, BMI was a significant independent correlate of vBMDt and vBMDc in men and of vBMDc in women. Finally, IL-1 beta was negatively correlated with vBMDc in women.

Discussion

Using data collected in a representative sample of the older population, we studied the relationship between variables that are widely and generically considered as risk factors for osteoporosis and structural and geometrical characteristics of cortical and trabecular bone. In both men and women, calf CSMA was a strong independent correlate of tCSA and cCSA (indexes of bone geometry) while Bio-T was more evidently associated with vBMDt and vBMDc. Calciotrophic hormones were strong independent correlates of cortical bone characteristics in women, being 25 (OH)-vitamin D positively associated with cCSA and vBMDc and serum PTH inversely associated with vBMDc. Both trabecular and cortical bone density were lower in old age in both sexes; while changes in bone geometry were substantially different in two sexes. In men, cortical density but not cortical area was lower in old age, with

a significant increase in bone size. In women, both cortical bone area and density were lower in old age, but the increase in total bone area was less pronounced than in men.

Our findings are in accordance with observational studies and intervention trials showing that muscle mass primarily affects bone size and not volumetric density in the loaded skeletal sites [11,12]. Furthermore, our findings are concordant with studies showing that testosterone positively affects bone mineral density [16]. In fact, androgen deprivation therapy in adult men with advanced prostate cancer is followed by rapid bone loss, similar to the loss of skeletal integrity in women after surgical ovariectomy (OVX) or during early menopause. Significant bone loss, albeit to a lesser extent, has also been observed at appendicular skeletal sites, including the hip and the radius, after cessation of androgen therapy [31]. The removal of the androgen stimulation causes primarily a deterioration of the trabecular bone architecture of the distal tibia, as shown by high-resolution magnetic resonance micro-imaging performed in untreated hypogonadal men [31]. An important role of testosterone in bone physiology is also suggested by a recent study demonstrating that free testosterone level is positively associated with the cortical bone area in young men, while estrogen appears to be negatively correlated [32]. Moreover, both in older men and women, Bio-E_2 and Bio-T were significantly associated with cortical bone at different sites [33,34].

The relationship of PTH on cortical vBMD found in our study is somewhat complex and sex-specific. While in men PTH was not associated with vBMDc, in women, serum PTH and vBMDc were strongly and inversely correlated. Since estrogens enhance the osteoclast-promoting activity of PTH and all the women in our study population were postmenopausal, we speculated that the inverse association between PTH and vBMDc might be also mediated by estrogen deficiency. This is also in accordance with findings reported by Riggs et al., indicating that estrogen deficiency is indirectly responsible for the secondary hyperparathyroidism observed in late postmenopausal women [35]. Our data are also in agreement with Neer et al. who suggested that PTH prevent a proximal femur and total body bone loss in young women with induced estrogen deficiency [36]. Given that teriparatide, a PTH analogue, is currently tested in several clinical trials, understanding the bone correlates of PTH is very important [37-39]. In fact, the mechanism of action of teriparatide on the bone surfaces is still controversial [40]. Our results suggest that changes in the sex hormone milieu in older women may modify the effects of bone trophic factors such as PTH, facilitating the sex-specific change in both cortical bone mineral density.

We also found an association between pro-inflammatory cytokines and cortical bone loss. In particular, IL-1 beta appeared to be related to the loss in cortical bone density in women. Our findings are in accordance with the suggestion that decline in ovarian function with menopause is associated with increases in pro-inflammatory cytokines [19]. A puzzling result of our study was that TNF-alpha was positively associated with bone geometry parameters in men. Although these findings contrast with those reported by other studies, it is possible that increase in bone area is an attempt to compensate for the reduction in apparent vBMD. In fact, in our data, age-related bone density loss was accompanied by an increase in bone size [8]. Hence, our results further strength the notion of pro-inflammatory cytokines as a marker of bone resoption in older persons.

The main limitation of this study is its cross-sectional design, which precludes the determination of causality between observed phenomena. Although we considered several factors known to influence bone characteristics, we cannot exclude that other factors not considered in this analysis, such as estradiol, may have confounded our findings. Finally, although the findings were obtained at the tibial shaft, recent studies confirm that fractures at almost every site are associated with low BMD. Our study also has strengths. Tibial QCT is a precise and inexpensive technique for studying bone quality which has been seldom applied

in epidemiological studies. The availability of many physiological factors potentially affecting bone physiology in a large representative sample of the older population in addition to the pQCT data is somewhat unique of the InCHIANTI study.

In conclusion, many factors known to predict the development of osteoporosis were associated with specific bone parameters assessed by tibial QCT. Factors known to be associated with increased bone resorption (Bio-T, 25(OH)-vitamin D and PTH) affected mainly volumetric BMD, while others associated with delivering mechanical forces on bone such as CSMA affected primarily bone geometry. Since these physiological factors are potentially affected by interventions used in the prevention or treatment of osteoporosis, the present findings may help to predict which bone characteristics can be affected by a particular intervention. For instance, based on our data, we may hypothesize that an increase in muscle mass obtained by means of progressive resistance training will have a greater effect on bone geometry parameters measured by QCT rather than on volumetric BMD. Similarly, the effects of a treatment with vitamin D compounds or teriparatide will be effective in modulating vBMD rather than geometric parameters.

Acknowledgements

The InCHIANTI study was supported as a “targeted project” (ICS 110.1\RS97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (contracts N01-AG-916413 and N01-AG-821336 and contracts 263 MD 9164 13 and 263 MD 821336) and in part by the Intramural Research Program, NIA-NIH, USA.

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Table 1

pQCT-derived bone parameters and bone strength potential correlates in men and women InCHIANTI participants

	Men (*n* = 372)	Women (*n* = 435)	*P*[a]
Age (years)	74.0 ± 6.5	75.4 ± 7.0	<0.0001
Education (years)	6.3 ± 3.7	4.7 ± 2.8	<0.0001
BMI (kg/m^2)	27.1 ± 3.3	27.8 ± 4.7	0.0062
WHR	95.2 ± 8.9	90.5 ± 11.0	<0.0001
Total bone area (tCSA) (mm^2)	413 ± 60	364 ± 55	<0.0001
Cortical bone area (cCSA) (mm^2)	334 ± 52	270 ± 58	<0.0001
vBMDt (mg/cm^3)	281 ± 51	236 ± 48	<0.0001
vBMDc (mg/cm^3)	1018 ± 65	985 ± 73	<0.0001
CSMA (cm^2)	64 ± 13	61 ± 13	<0.0001
Ankle extension strength (kg)	35.7 ± 10.2	25.5 ± 4.7	<0.0001
Physical activity			<0.0001
Sedentary (%)	7.8	21.4	
Light (%)	82.8	76.1	
Moderate/High (%)	9.4	2.5	
Energy intake (kcal/day)	2205 ± 553	1731 ± 473	<0.0001
Protein intake (g/day)	84 ± 20	70 ± 19	<0.0001
Vitamin D intake (μg/day)	2.0 ± 0.80	1.8 ± 0.9	<0.0001
Calcium intake (mg/day)	866 ± 284	801 ± 313	0.0026
Creatinine clearance (ml/min)	70 ± 18	60 ± 19	<0.0001
Serum PTH (pmol/l) (median and IQ range)	20.7 [15.2–27.4]	23.8 [16.2–33.1]	0.0004
Serum 25(OH)-vitamin D (nmol/l) (median and IQ range)	55.2 [35.9–75.9]	34.7 [24.5–21.0]	<0.0001
Ca++ (mg/dl)	4.1 [4.0–4.2]	4.1 [4.0–4.3]	0.051
Bio-T (ng/dl) (median and IQ range)	90.7 [68.7–116.4]	9.6 [6.1–15.0]	<0.0001
Total IGF-1 (ng/dl) (median and IQ range)	122.1 [89.3–152.8]	100.9 [70.0–137.5]	<0.0001
DHEAS (μg/dl) (median and IQ range)	70.8 [43.6–11.4]	64.5 [35.7–114.8]	0.34
IL-6 (pg/ml) (median and IQ range)	1.5 [0.9–2.4]	1.3 [0.8–2.0]	<0.0001
IL-1 beta (pg/ml) (median and IQ range)	0.0 [0.0–0.40]	0.0 [0.0–0.85]	0.23
TNF-alpha (pg/ml) (median and IQ range)	0.0 [1.46–3.44]	1.93 [1.51–3.07]	0.31

Data are reported as mean value ± standard deviations if not otherwise specified.

[a] From age-adjusted linear or multinomial logistic regression models as appropriate. Variables with a skewed distribution are presented as value and interquartile range and were rank-transformed for the analysis.

Table 2

Age-adjusted Spearman partial rank–order correlation coefficients of potential factors of bone strength with total bone area, cortical bone area, trabecular volumetric BMD and cortical volumetric BMD, according to sex

	tCSA		cCSA		vBMDt		vBMDc	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
Education (years)	−0.1	−0.18 **	−016 **	−0.14 **	0	0.03	0.04	0.01
BMI (kg/m^2)	0.17 **	0.36 **	0.19 **	0.35 **	0.19 **	0.18 **	−0.05	0.09
WHR (cm)	0.04	0.24 **	0.04	0.24 **	0.14 *	0.16 **	−0.1	0.08
CSMA (cm^2)	0.31 **	0.58 **	0.42 **	0.55 **	0.11 *	0.14 *	0.14 *	0.12 *
Ankle extension strength (kg)	−0.04	0.15 **	−0.03	0.12 *	0.12 *	−0.09	−0.01	−0.04
Physical activity (3 levels)	0.08	0.07	0.12 *	0.1	0.13 3	0.02	0.1	0.13 *
Energy intake (kcal/day)	0.01	−0.02	0.04	−0.06	−0.04	−0.03	0.03	−0.03
Protein intake (g/day)	0.02	−0.03	−0.01	−0.08	−0.06	−0.05	0.04	−0.01
Vitamin D intake (μg/day)	0.06	−0.01	0.06	−0.02	0.02	0.02	0.07	0.02
Calcium intake (mg/day)	0.05	−0.1	0.02	−0.6	−0.07	−0.07	0.01	−0.06
Creatinine clearance (ml/min)	−0.03	0.18 **	0.02	0.14	0.05	0.02	0.07	0.10 *
Serum PTH (pmol/l)	−0.02	0.06	−0.08	−0.04	0.01	0.04	0.05	−0.17 **
Serum 25(OH)-vitamin D (nmol/l)	0.02	0.13 *	0.05	−0.05	0	0.04	0.06	0.18 **
Ca++ (mg/dl)	0.03	−0.02	−0.05	−0.05	0.1	0.01	0	−0.07
Bio-T (ng/dl)	−0.01	0.20 **	0.07	0.27 **	0.06	0.17 **	0.24 **	0.16 **
Total IGF-1 (ng/dl)	0.04	−0.03	0.09	−0.03	0.06	0.01	0.05	0.09
DHEAS (μg/dl)	0.01	0.06	0.09	0.13 *	0.01	0.11	0.07	0.12 *
IL-6 (pg/ml)	0.02	0.09	−0.04	0.11 *	−0.08	−0.02	−0.07	0.05
IL-1 beta (pg/ml)	−0.02	0.02	−0.04	0.05	0.01	0.02	0.04	0.14 **
TNF-alpha (pg/ml)	0.14 *	0.12 *	0.15 *	0.10 *	0.01	−0.05	0.08	0.03

* $P < 0.05$.

** $P < 0.01$.

Table 3

Independent factors of total and cortical bone area in older men and women

	Dependent variable tCSA				Dependent variable cCSA			
	Men		Women		Men		Women	
	b + SE	*P*	*b* + SE	*P*	*b* + SE	*P*	*b* + SE	*P*
Age (years)	1.76 ± 0.54	0.0011	−0.17 ± 0.36	0.63	1.41 ± 0.45	0.0020	−1.57 ± 0.34	<0.0001
Education (years)			−1.98 ± 0.83	0.0197	−1.84 ± 0.78			
BMI (kg/m^2)			1.41 ± 0.56	0.0120			1.27 ± 0.56	0.0253
CSMA (cm^2)	1.6 ± 0.3	<0.0001	2.0 ± 0.1	<0.001	1.8 ± 0.2	<.0001	2.1 ± 0.2	<0.0001
Serum 25(OH)-vitamin D (nmol/l)			11.7 ± 4.3	0.0067			7.8 ± 2.4	0.0009
TNF-alpha (pg/ml)	10.5 ± 4.1	0.0118			8.20 ± 3.40	0.0165		

Parsimonious models obtained by backward selection method from initial models including all covariates reported in the table and WHR, ankle extension strength, current physical activity, calcium intake, creatinine clearance, DHEAS, PTH, Bio-T, IL-6.

Bone. Author manuscript; available in PMC 2009 February 22.

Table 4

Independent factors of trabecular and cortical bone mineral density in older men and women

	Dependent variable vBMDt				Dependent variable vBMDc			
	Men		Women		Men		Women	
	b **+ SE**	***P***	***b*** **+ SE**	***P***	***b*** **+ SE**	***P***	***b*** **+ SE**	***P***
Age (years)	−0.08 ± 0.44	0.84	−1.95 ± 0.45	<0.0001	−1.20 ± 0.70	0.09	−1.28 ± 0.53	0.0156
BMI (kg/m^2)	3.42 ± 0.88	<0.0001	2.39 ± 0.65	<0.0001	3.9 ± 1.3	0.0029		
CSMA (cm^2)					1.0 ± 0.3	0.0010		
Ankle extension strength (kg * 100)	0.04 ± 0.01	0.0012	0.02 ± 0.01	0.094				
Physical activity								
Sedentary	−32.7 ± 13.8	0.0182						
Light	20.0 ± 9.4	0.0348						
Moderate/High	Reference							
Creatinine clearance (ml/min)			0.48 ± 0.20	0.0297	0.55 ± 0.27	0.042		
Serum 25 (OH)-Vitamin D (nmol/l)							20.1 ± 6.97	0.0042
Serum PTH (pmol/l)							−22.5 ± 8.7	0.0098
Bio-T (ng/dl)	13.1 ± 2.4	<0.0001	5.9 ± 2.4	0.013	26.1 ± 6.2	<0.0001	9.74 ± 3.6	0.0074
IL-1 beta (pg/ml)							−2.9 ± 0.92	0.0015

Parsimonious model obtained by backward selection method from initial models including all covariates reported in the table plus education, WHR, calcium intake, DHEAS, TNF-alpha.