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ORIGINAL ARTICLE

Bone density and hemoglobin levels in older persons: results from the InCHIANTI study

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Abstract Hypoxemia has been recognized as a risk factor for bone loss. The aim of the present study is to investigate the relationship of bone mass and density measures with anemia and hemoglobin levels in a large sample of older community-dwelling persons. The study is based on data from 950 participants enrolled in the "Invecchiare in Chianti" (Aging in the Chianti area, InCHIANTI) study. All the analyses were performed considering continuous hemoglobin levels as well as the dichotomous anemia variable (defined according to WHO criteria as hemoglobin <12 g/dl in women and <13 g/dl in men). A peripheral quantitative computerized tomography (pQCT) scan of the right calf was performed in all participants to evaluate total bone density, trabecular bone density, cortical bone density, and the ratio between cortical and total bone area. Linear regression analyses were used to assess the multivariate relationship of pQCT bone measures with anemia and hemoglobin levels after adjustment for demographics, chronic conditions, muscle strength and biological variables. Participants were 75.0 (SD 6.9) years old. In our sample, 101 participants (10.6%) were anemic. In women, coefficients from adjusted linear regression analyses evaluating the association between pQCT bone measures (per SD increase) and hemoglobin levels/anemia showed significant associations of anemia with total bone density ($\beta=-0.335$, SE $=0.163$; $P=0.04$) and cortical bone density ($\beta=-0.428$, SE $=0.160$; $P=0.008$). Relationships with borderline significance were found for the associations of anemia with trabecular bone density and the ratio between cortical and total bone area. Significant associations were found between hemoglobin levels and trabecular bone density ($\beta=0.112$, SE $=0.049$; $P=0.02$), total bone density ($\beta=0.101$, SE $=0.046$; $P=0.03$), cortical bone density ($\beta=0.100$, SE $=0.046$; $P=0.03$) and the ratio between cortical bone and total area ($\beta=0.092$, SE $=0.045$; $P=0.04$). In men, significant associations were found for hemoglobin levels with total bone density ($\beta=0.076$, SE $=0.036$; $P=0.03$) and cortical bone density ($\beta=0.095$, SE $=0.41$; $P=0.02$). A borderline significance was reported for the association between anemia and cortical bone density. We concluded that anemia and low hemoglobin levels are negatively and independently associated with bone

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mass and density. The bone loss associated with hemoglobin levels mainly occurs in the cortical bone. Women with lower hemoglobin levels demonstrate a higher bone loss than male counterparts.

Keywords Anemia · Bone density · Cortical bone density · Hemoglobin

Introduction

Anemia, a common condition that affects approximately 13% of persons aged 70 years or older [1], has been defined by the World Health Organization (WHO) [2] as a hemoglobin level lower than 12 g/dl for women and 13 g/dl for men. The reported decrease in hemoglobin concentrations that occur during the aging process might be due to a lower erythropoietin secretion [3] or a reduced hematopoietic reserve [4]. However, a strong association between anemia and comorbidity has also been shown, which has led to the recommendation that hemoglobin levels below normal ranges should always be investigated in clinical practice [5]. Late-life anemia has been associated with clinical outcomes such as mortality [5], disability [6, 7, 8], loss of physical performance [9] and poor quality of life [10]. Moreover, anemia is so frequently found in frail older persons that it has been defined as a biological marker of the frailty syndrome [11].

Lower levels of bone mass with age have been widely documented [12]. This decline, more evident in women than in men [13], is responsible for a weaker resistance of the bone to external forces [12, 14]. Osteoporosis, characterized by bone mass reduction and by a higher risk of consequent fractures, is a common condition in older persons, and its prevalence increases with age [12, 15]. The consequent increased risk of fractures is an important issue in geriatrics, given the associated clinical and socio-economic burden [8, 12, 16, 17].

Several reports in specific populations (such as in thalassemia and hemodialysis patients) are available in the literature and suggest a direct relationship of anemia and hemoglobin levels with bone density [16, 18, 19, 20, 21]. Moreover, hypoxemia has been recognized as a risk factor for bone loss [20]. Nevertheless, this topic has not yet been deeply studied in the general older population. Furthermore, to our knowledge, studies that have analyzed the association between hemoglobin levels and bone density have not distinguished between the trabecular and the cortical bone, despite reports of different changes that occur with age in these two components [13]. The aim of the present study is to investigate the relationship of bone mass and density measures, assessed by peripheral quantitative computerized tomography (pQCT), with anemia and hemoglobin levels in a large sample of older community-dwelling persons.

Methods

The study is based on data from the "Invecchiare in Chianti" (Aging in the Chianti area, InCHIANTI) study. The InCHIANTI study is a prospective population-based study of older people, designed by the Laboratory of Clinical Epidemiology of the Italian National Research Council of Aging (INRCA, Florence, Italy).

The InCHIANTI study population includes 1,156 participants aged 65 to 102 years, randomly selected from residents in two towns of the Chianti geographic area (Greve in Chianti and Bagno a Ripoli, Tuscany, Italy). The data collection started in September 1998 and was completed in March 2000. A detailed description of the sampling procedure and data collection method has been previously published [22]. The Italian National Research Council on Aging Ethical Committee ratified the entire study protocol.

The present analyses were performed on 950 participants, after exclusion of those subjects in whom hemoglobin levels and pQCT measures were not assessed ($n = 206$). The only sociodemographic characteristic in which excluded participants differed from those considered for the present analyses was older age (80.3 vs 75.0 years, $P < 0.001$).

Anemia

Blood samples were obtained from participants after a 12-h fast. Participants had also been sedentary in the sitting or supine position for at least 15 min before the blood draw. Hemoglobin levels were analyzed with the hematology automated Autoanalyzer DASIT SE 9000 (Sysmex Corporation, Kobe, Japan). For the study we used: (1) a dichotomous anemia indicator, defined by the WHO criteria [2] as hemoglobin levels lower than 12 g/dl for women and 13 g/dl for men; (2) a continuous hemoglobin level variable; (3) a categorical variable that distinguished hemoglobin levels into: (a) anemia as defined by the WHO criteria [2]; (b) Hemoglobin levels between 0 and 0.9 g/dl above the anemia cut-off point; (c) Hemoglobin levels between 1 and 1.9 g/dl above the anemia cut-off point; (d) Hemoglobin levels ≥2 g/dl above the anemia cut-off point.

pQCT measures

We performed a right-leg pQCT scan on all participants with a recent generation device (XCT-2000, Stratec, Pforzheim, Germany) to evaluate several bone mass and density parameters. Data presented here are derived from standard 2.1 mm-thick transverse scans obtained at 4% (where trabecular bone is most abundant) and 38% (where the cortex is thick enough to allow the accurate measurement of cortical bone density) of the tibial length, starting from the tibio-tarsal joint.

The bone density (in milligrams per centimeter cubed) and the bone area (in millimeters squared) were calculated with BonAlyse software, version 1.3 (BonAlyse, Jyväskylä, Finland). Different tissues in the analysis were separated according to different density thresholds. Areas with density values above 710 mg/cm^3 were considered as "cortical bone", while areas with density values between 180 and 710 mg/cm^3 were considered as "trabecular bone" [13, 23]. The precision error of the XCT-2000 is below 1% for volumetric trabecular and cortical density and for cortical bone area [13, 23, 24]. The following pQCT bone measures were considered for the present analyses: total bone density (4%); trabecular bone density (4%); total bone density (38%); cortical bone density (38%); ratio between cortical and total bone area (38%).

Covariates

Covariates included sociodemographic variables [age, gender, study site, smoking habit, Mini Mental State Examination (MMSE) score, education]; height; weight; comorbidity (adjudicated diagnoses of hypertension, angina, myocardial infarction, stroke, cancer, diabetes, congestive heart failure, chronic obstructive pulmonary disease); physical activity (defined as moderate-to-high-intensity exercise performed for at least 1–2 h per week or light-intensity exercise performed for more than 4 h per week), as well as biological parameters [serum albumin, creatinine, calcium, 25(OH)-vitamin D, and parathyroid hormone (PTH)]. Adjudicated disease diagnoses were based on self-reported history, clinical assessment by the study geriatrician, and medication use. Serum albumin (as a percentage) was detected by electrophoresis (Hydragel 7 Protein, Sebia, France; mean inter-assay coefficient 0.8%), and its concentration was calculated from serum total proteins (Roche Diagnostics, inter-assay coefficient of variation was lower than 1%). Serum creatinine was detected by a standard creatinine Jaffe method (Roche Diagnostics, Mannheim, Germany); inter-assay coefficient of variation was less than 2.5%. The serum calcium level was determined by a colorimetric assay.

The inter-assay coefficient of variation and the analytical sensitivity were 1.5% and 0.2 mg/dl, respectively. A proportion (two 0.5 ml aliquots) of serum sample was frozen and stored at –80°C for 25(OH)-vitamin D and PTH determination. Serum levels of 25(OH)-vitamin D were measured by RIA (DiaSorin, Stillwater, Minn., USA), after extraction of the samples with acetonitrile. Intra-assay and inter-assay coefficients of variation were 8.1% and 10.2%, respectively. Serum intact PTH levels were measured with a two-site immunoradiometric assay (N-tact PTHSP, DiaSorin) kit. The assay uses two affinity-purified polyclonal antibodies, one specific for the amino-terminal 1–34 portion of the PTH molecule, and the second specific for the 39–84 sequence of the hormone. The assay sensitivity was 1.2 ng/l. Intra-assay and inter-assay coefficients of variation were $<3.0\%$ and 5.5%, respectively.

Ankle extension strength: The ankle extension strength, measured with a hand-held dynamometer (Nicholas Muscle Tester, Sammon Preston, Chicago, USA), was used in the study as a potential confounder of the association between hemoglobin levels and the pQCT bone measures. Participants, lying in lateral decubitus (opposite to the examined limb) with the hip and the knee extended and the ankle in a neutral position, were asked to perform the task twice with the right foot. The average of the results obtained was used for the analyses. This measurement has been used as a muscular strength parameter of the lower limb [25]. In our sample, this measure is highly correlated with other performance measures, such as hand grip strength or knee extension strength (Spearman correlation $r=0.647$ and $r=0.790$, respectively; both $P<0.001$), which have been shown to predict mortality and disability in the elderly [26, 27].

Interleukin-6 (IL-6): IL-6 was separately considered as an additional potential explanatory covariate in the adjusted models to determine whether inflammation was able to explain the association between hemoglobin levels and pQCT bone measures. In fact, it has been suggested that IL-6, a multifunctional cytokine and a marker of the geriatric syndrome of frailty [11], might be involved in bone turnover [11, 28, 29]. IL-6 was quantified with immunoassay kits (BioSource Cytoscreen human IL-6 UltraSensitive kit). The minimum detectable concentration was 0.10 pg/ml. The inter-assay coefficient of variation was 7%. All assays were done in duplicate and repeated if the second measurement was more than 10% greater or less than the first. The average of the two measurements was used in the analyses. Because of the non-normal distribution of serum IL-6, its log values were used in the analyses.

Statistical analyses

Differences in proportions and means of covariates according to anemia status were assessed with chi-square and ANOVA statistics, respectively. Median values with the 25th-75th percentile ranges and P values based on Mann–Whitney U statistics were reported for non-normally distributed variables. All variables found to be different, with a P value <0.10 in univariate analyses, were used as adjustment covariates in subsequent multivariate analyses. Gender interactions were assessed by the testing of the interaction term added to the adjusted models as a covariate. We used linear regression analyses to identify regression coefficients (with standard error) in calf pQCT measures for continuous hemoglobin levels as well as for the dichotomous anemia variable. We performed analyses of covariance to assess differences in adjusted means of bone measurements according to hemoglobin levels.

A regression coefficient (β) from a linear regression model is a constant that represents the slope of the

regression line between two variables (i.e., y = bone density measure, x = hemoglobin). The regression line is provided by the equation: $y = \beta x + a$, where a represent the intercept of the regression line on the y axis. A positive regression coefficient indicates a direct relationship, a negative regression coefficient indicates an inverse relationship. The regression coefficients reported in the present paper provide the estimates of expected changes in bone density measures (per SD increase) for 1 g/dl increments for hemoglobin levels and for the difference between non-anemic vs anemic participants.

Results

The mean age of the sample population ($n = 950$) was 75.0 (SD 6.9) years and the prevalence of women was 55.8%. A total of 101 (10.6%) subjects had anemia according to the WHO criteria. The prevalence of anemia was similar in women (10.6%) and men (10.7%).

Anemic men and women were older, had lower levels of albumin, were more likely to have a history of stroke and reported worse results of muscular strength than did their non-anemic counterparts (Table 1). Anemic women also had higher levels of PTH than non-anemic women, while anemic men were less educated, more likely to have gastric ulcers and higher levels of creatinine and IL-6 than men with normal levels of hemoglobin. No significant differences were found for physical activity levels according to the presence of anemia in both men and women. The use of some medications able to affect bone mass parameters (such as hormone replacement therapy, bisphosphonates, or calcium supplements) was not included in the list of potential confounders, given the extremely low prevalence found in our sample (hormone replacement therapy: no participants; bisphosphonates: three participants, 0.3%; calcium supplements: six participants, 0.6%; vitamin D supplements: 12 participants, 1.3%).

Because of the significant interactions between hemoglobin levels and gender with total bone density ($P = 0.04$) and the ratio between cortical bone area and total bone area ($P = 0.001$), all the analyses were stratified for gender. Similar significant interactions were also found when we tested the interaction between the anemia dichotomous variable and gender with pQCT bone measures.

Table 1 Characteristics [mean ± SD, %, or median (interquartile range)] of the population, stratified for gender

Characteristic	Women ($n = 530$)			Men ($n = 420$)		
	No anemia ($n = 474$)	Anemia ($n = 56$)	P	No anemia ($n = 375$)	Anemia ($n = 45$)	P
Sociodemographic						
Age (years)	74.9 ± 6.7	81.7 ± 7.6	< 0.001	73.8 ± 6.2	78.9 ± 8.1	< 0.001
Site (Bagno a Ripoli)	54.6	42.9	0.10	51.7	51.1	0.94
Height (cm)	153.1 ± 6.8	150.0 ± 7.9	0.003	165.8 ± 7.2	161.8 ± 8.7	0.001
Weight (kg)	65.5 ± 11.4	58.1 ± 10.5	< 0.001	75.0 ± 11.4	67.6 ± 12.2	< 0.001
Smoking			0.19			0.70
Never	81.2	91.1		29.6	26.7	
Former	10.8	5.4		53.6	60.0	
Current	8.0	3.6		16.8	13.3	
Education (years)	4.8 ± 2.8	4.3 ± 3.4	0.26	6.4 ± 3.7	4.8 ± 2.9	0.006
Physical activity[a]	26.9	19.6	0.24	53.6	44.4	0.24
Comorbidity						
Coronary heart disease	7.7	13.2	0.17	10.5	15.9	0.28
Congestive heart failure	6.8	7.1	0.91	4.8	11.1	0.08
Diabetes	9.5	3.6	0.14	12.0	11.1	0.86
Stroke	4.6	12.5	0.01	5.9	24.4	< 0.001
Pulmonary disease	3.4	-	0.16	10.1	15.6	0.27
Hypertension	57.0	62.3	0.46	46.1	43.2	0.71
Gastric ulcer	2.7	5.4	0.28	5.1	31.1	< 0.001
Cancer	7.7	7.5	0.97	4.0	9.1	0.13
Peripheral artery disease	8.2	8.9	0.86	8.0	11.1	0.48
Serum biological markers						
Albumin (g/dl)	4.2 ± 0.3	4.0 ± 0.3	< 0.001	4.3 ± 0.3	4.1 ± 0.4	< 0.001
Creatinine (mg/dl)	0.9 ± 0.2	0.9 ± 0.5	0.01	1.0 ± 0.2	1.1 ± 0.4	< 0.001
Calcium (mg/dl)	9.5 ± 0.5	9.3 ± 0.5	0.001	9.4 ± 0.4	9.3 ± 0.4	0.006
Parathyroid hormone (pmol/l)[b]	23.5 (16.0–33.1)	28.4 (17.8–39.7)	0.02	20.8 (15.5–27.7)	22.6 (13.5–30.5)	0.97
25(OH)-vitamin D (nmol/l)[b]	35.2 (24.0–51.0)	31.0 (21.7–40.9)	0.06	54.2 (35.9–74.6)	43.4 (29.7–76.4)	0.25
Interleukin-6 (pg/ml)[b]	1.3 (0.8–2.0)	1.5 (0.9–2.8)	0.20	1.5 (0.9–2.4)	2.1 (1.1–5.5)	0.005
Muscle strength						
Ankle extension strength (kg)	24.4 ± 10.1	15.8 ± 11.8	< 0.001	33.3 ± 12.4	25.5 ± 15.6	< 0.001

[a]Percentage of participants that reported moderate-to-high intensity exercise performed for at least 1–2 h per week or light intensity exercise performed for more than 4 h per week
[b]Median (interquartile range)

In univariate unadjusted analyses, a significant difference, according to anemia status, was reported for cortical bone density for both genders (Table 2). Anemic women were also more likely to present lower levels of total bone density, trabecular bone density and a reduced ratio between cortical bone area and total bone area.

Table 3 shows coefficients (adjusted for age, site, height, weight, education, congestive heart failure, stroke, gastric ulcer, serum albumin, creatinine, calcium, 25(OH)-vitamin D, PTH, and ankle extension strength) from linear regression analyses evaluating the association between pQCT bone measures (per SD increase) and hemoglobin levels/anemia. In women, significant associations were found between anemia, and total bone density and cortical bone density. Borderline significances were found for the associations between anemia, and trabecular bone density and the ratio between cortical and total bone area. Significant associations were found between hemoglobin levels, and trabecular bone density, total bone density, cortical bone density, and the ratio between cortical bone and total area. In men, a significant association was found for the associations between hemoglobin levels, and total bone density and cortical bone density. A borderline significance was reported for the association between anemia and cortical bone density.

In order to test whether IL-6, an inflammatory cytokine, was able to explain part of the link between

Table 2 pQCT bone measurements, according to anemia status and gender, in crude unadjusted analyses

Parameter	No anemia Mean ± SD	Anemia Mean ± SD	*P*
Women ($n = 530$)			
Scan performed at 4% of the tibial length			
Total bone density (mg/cm^3)	238.4 ± 47.9	224.3 ± 47.8	0.04
Trabecular bone density (mg/cm^3)	199.3 ± 59.4	178.9 ± 76.3	0.02
Scan performed at 38% of the tibial length			
Total bone density (mg/cm^3)	856.6 ± 98.7	780.8 ± 108.7	< 0.001
Cortical bone density (mg/cm^3)	987.6 ± 70.6	934.5 ± 95.5	< 0.001
Cortical bone area/total bone area	0.74 ± 0.10	0.66 ± 0.12	< 0.001
Men ($n = 420$)			
Scan performed at 4% of the tibial length			
Total bone density (mg/cm^3)	282.0 ± 51.0	267.7 ± 49.9	0.08
Trabecular bone density (mg/cm^3)	220.3 ± 53.3	211.0 ± 59.1	0.29
Scan performed at 38% of the tibial length			
Total bone density (mg/cm^3)	914.7 ± 73.2	893.6 ± 87.7	0.07
Cortical bone density (mg/cm^3)	1,018.9 ± 61.8	992.5 ± 86.9	0.01
Cortical bone area/total bone area	0.81 ± 0.06	0.81 ± 0.06	0.99

Table 3 Adjusted[a] regression coefficients (with SE) for the association of anemia and hemoglobin levels with pQCT bone measures[b]

Parameter	Anemia		Hemoglobin levels	
	Regression coefficient (SE)	*P*	Regression coefficient (SE)	*P*
Women ($n = 530$)				
Scan performed at 4% of the tibial length				
Total bone density (mg/cm^3)	−0.109 (0.147)	0.45	0.074 (0.041)	0.07
Trabecular bone density (mg/cm^3)	−0.304 (0.173)	0.08	0.112 (0.049)	0.02
Scan performed at 38% of the tibial length				
Total bone density (mg/cm^3)	−0.335 (0.163)	0.04	0.101 (0.046)	0.03
Cortical bone density (mg/cm^3)	−0.428 (0.160)	0.008	0.100 (0.046)	0.03
Cortical bone area / Total bone area	−0.271 (0.160)	0.09	0.092 (0.045)	0.04
Men ($n = 420$)				
Scan performed at 4% of the tibial length				
Total bone density (mg/cm^3)	−0.112 (0.176)	0.53	0.043 (0.042)	0.30
Trabecular bone density (mg/cm^3)	−0.116 (0.158)	0.94	0.010 (0.037)	0.79
Scan performed at 38% of the tibial length				
Total bone density (mg/cm^3)	−0.152 (0.149)	0.31	0.076 (0.036)	0.03
Cortical bone density (mg/cm^3)	−0.319 (0.170)	0.06	0.095 (0.041)	0.02
Cortical bone area / Total bone area	0.074 (0.128)	0.56	0.028 (0.031)	0.36

[a]Adjusted for age, site, height, weight, education, congestive heart failure, stroke, gastric ulcer, serum albumin, creatinine, calcium, parathyroid hormone (logarithmic value), 25(OH)-vitamin D (logarithmic value), ankle extension strength

[b]Per SD increase. SDs: total bone density (4%) 53.854 mg/cm^3; trabecular bone density (4%) 59.353 mg/cm^3; total bone density (38%) 96.561 mg/cm^3; cortical bone density (38%) 72.982 mg/cm^3; cortical bone area/total bone area (38%) 0.095

hemoglobin and bone density, we entered IL-6 in the adjusted models. No significant discrepancy from previous findings was reported. In fact, when we added the inflammatory marker to the models, it did not modify the associations of anemia with trabecular bone density ($\beta = -0.304$, SE = 0.174; $P = 0.08$), total bone density (scan performed at the 38% tibial length; $\beta = -0.336$, SE = 0.164; $P = 0.04$), cortical bone density ($\beta = -0.428$, SE = 0.162; $P = 0.008$) and the ratio between cortical and total bone area ($\beta = -0.268$, SE = 0.161; $P = 0.09$) and of hemoglobin levels with trabecular bone density ($\beta = 0.112$, SE = 0.049; $P = 0.02$), total bone density (scan performed at the 4% tibial length; $\beta = 0.076$, SE = 0.042; $P = 0.07$; scan performed at the 38% tibial length; $\beta = 0.101$, SE = 0.049; $P = 0.03$), cortical bone density ($\beta = 0.099$, SE = 0.046; $P = 0.03$) and the ratio between cortical and total bone area ($\beta = 0.092$, SE = 0.046; $P = 0.04$) previously found in women.

Similarly, in men, the reported significant association between anemia and cortical bone density was not influenced by IL-6 levels ($\beta = -0.317$, SE = 0.170; $P = 0.06$), or the associations of hemoglobin levels with total bone density (scan performed at the 38% tibial length: $\beta = 0.074$, SE = 0.036; $P = 0.04$) and cortical bone density ($\beta = 0.095$, SE = 0.041; p = 0.02).

Finally, we performed adjusted analyses of covariance to evaluate the relationships of hemoglobin levels above and below the anemia cut-off points in men and women with pQCT bone measures (Fig. 1). A significant difference in cortical bone density was found between men with hemoglobin levels ≥2 g/dl above the anemia cut-off point and anemic men (1,026.3 mg/cm^3 vs 992.3 mg/cm^3; $P = 0.01$). In women, significant differences according to hemoglobin levels were found for trabecular bone density (anemia: 180.8 mg/cm^3; hemoglobin ≥2 g/dl above anemia cut-off point: 207.7 mg/cm^3; $P = 0.02$), total bone density (anemia: 820.6 mg/cm^3; hemoglobin 1–1.9 g/dl above anemia cut-off value: 859.7 mg/cm^3, $P = 0.02$; hemoglobin ≥2 g/dl above anemia cut-off point: 860.0 mg/cm^3; $P = 0.03$), cortical bone density (anemia: 955.4 mg/cm^3; hemoglobin < 1 g/dl above anemia cut-off value: 985.0 mg/cm^3, $P = 0.02$; hemoglobin 1–1.9 g/dl above anemia cut-off: 987.0 mg/cm^3, $P = 0.01$; hemoglobin ≥2 g/dl above anemia cut-off: 988.9 mg/cm^3; $P = 0.01$), and cortical/total bone area ratio (anemia: 0.713; hemoglobin 1–1.9 g/dl above anemia cut-off point: 0.745, $P = 0.05$; hemoglobin ≥2 g/dl above anemia cut-off: 0.746; $P = 0.05$).

Discussion

To our knowledge ours is the first study exploring the relationship between hemoglobin levels and anemia and pQCT bone measures in a representative sample of the older population. We measured separately the cortical and trabecular components of the bone. Participants with lower levels of hemoglobin or with anemia had lower levels of bone density, especially in the cortical bone. Such associations were more evident in women than in men.

Several mechanisms that lead to bone loss during the aging-process have been suggested. Hormone-related mechanisms have been documented to be responsible for the decline of bone density in postmenopausal women [30, 31, 32] as well as in older men [19, 30, 33]. Nutritional factors may also contribute to the bone loss [12]. Moreover, several studies have directly or indirectly suggested an association of hemoglobin levels with bone density [16, 18, 19, 21]. However, to our knowledge, this issue has never been specifically evaluated in the older general population. In fact, current evidence is extremely limited and derives mainly from special populations, such as thalassemic patients [18, 21, 34].

An explanation for the relationship of anemia and hemoglobin levels with bone density might be provided by a study conducted by Fujimoto et al. [20], in which, by combining results from animal and human models, they suggested that hypoxemia can affect mineral density and might be a risk factor for bone loss. In fact, the authors showed that patients with low PaO_2 presented a decreased bone mineral density. Furthermore, to exclude the hypothesis that limited exercise was influencing their results, they conducted an animal experiment and showed that a significantly lower bone density was found in hypoxemic rats than in normoxemic rats.

During the aging process a reduction in bone mass has been well documented [12]. The age-related rate of bone loss is higher in women than in men [35] and specifically affects cortical and total bone density. The age-related reduction of trabecular bone density seems to be similar in men and women [13]. Even if limited by the cross-sectional design of the study, our results are substantially in agreement with those previous findings. We have reported a stronger association of hemoglobin levels with the cortical bone. In fact, we have found significant differences in cortical bone density (and cortical bone area only in women) according to hemoglobin levels. It has been demonstrated that age-related bone remodeling and bone loss occur mainly in the cortical bone that becomes "trabecularized" due to an increased intracortical porosity, preferentially occurring in cortical regions adjacent to the marrow cavity [30, 36, 37]. This might provide an explanation for the particularly strong association between hemoglobin levels and bone density in that site. In our analyses we also reported a small but significant difference in trabecular bone density between women with anemia and those with high hemoglobin levels.

Several diseases characterized by low hemoglobin levels or anemia have been associated with an increased risk of bone loss or osteoporosis. In fact, it has been demonstrated that pernicious anemia is associated with a twofold to threefold increased risk of osteoporotic fractures [38, 39]. A reduced bone density associated with low hemoglobin levels has also been demonstrated in hemodialysis patients [18]. Hens et al. [40] have shown that, as chronic obstructive pulmonary disease becomes

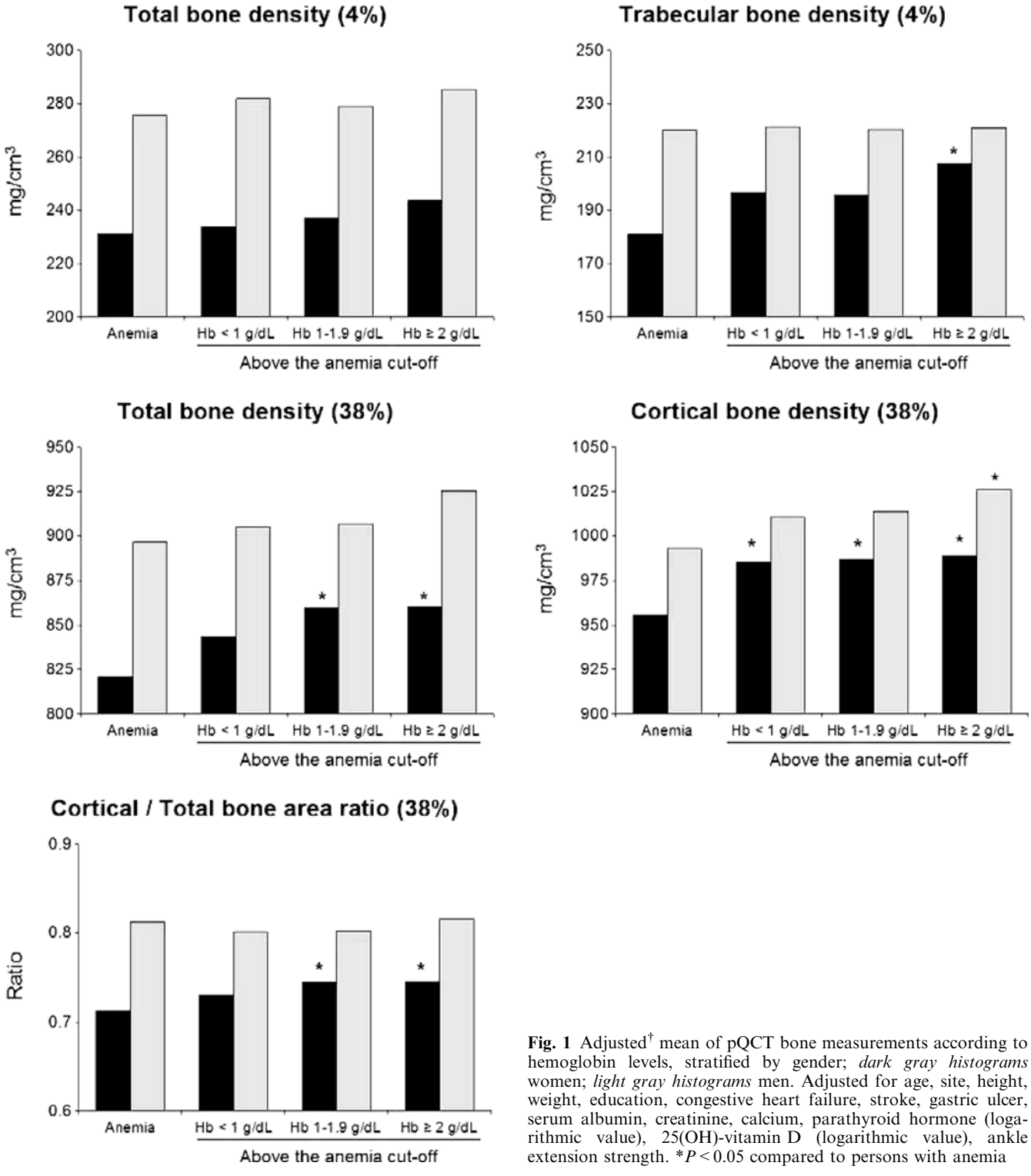

Fig. 1 Adjusted† mean of pQCT bone measurements according to hemoglobin levels, stratified by gender; *dark gray histograms* women; *light gray histograms* men. Adjusted for age, site, height, weight, education, congestive heart failure, stroke, gastric ulcer, serum albumin, creatinine, calcium, parathyroid hormone (logarithmic value), 25(OH)-vitamin D (logarithmic value), ankle extension strength. *$P < 0.05$ compared to persons with anemia

more severe, the prevalence of osteoporotic patients increases. A direct correlation between bone density and the severity of airway obstruction has also been found [41]. Patients with thalassemia, a disease characterized by anemia due to defective hemoglobin synthesis, often present skeletal morbidity [42]. Even if a multifactorial etiology has been shown to be responsible for these pathological bone changes in thalassemia, low hemoglobin levels and anemia have been recognized among the risk factors [16, 18, 21]. Moreover, it has also been demonstrated that the degree of bone loss is lower in thalassemic patients receiving more blood transfusions [34].

Recently, Kenny et al. [19] have shown a direct association between testosterone levels and bone mass, hypothesizing beneficial effects of testosterone supple-

ment in men at risk of osteoporotic fractures. This relationship might be partially explained by the known association between anemia and low testosterone levels [43, 44] and confirmed by evidence that shows increased levels of hemoglobin due to the use of androgenic steroids [43].

It might be argued that our results might be caused by an underlying chronic inflammatory status. In fact, inflammation is independently associated with hemoglobin levels [11] and it has been suggested to play an important role in the bone resorption that occurs during aging [11, 28, 29]. Therefore, we re-performed the analyses, taking into account serum levels of IL-6 as a covariate in our adjusted models. Results did not differ from previous findings, which suggested that the relationships of hemoglobin levels and anemia with pQCT bone measures is independent of systemic inflammation.

The main limitation of the present study is its cross-sectional design, which does not allow us to assess the cause–effect mechanism. Therefore, we cannot exclude whether our findings are just the expression of a frailer health status of participants or are, indeed, a causative factor in the pathway leading from low hemoglobin levels to bone density loss. Longitudinal studies are needed to confirm our findings. The InCHIANTI study gives us the opportunity to adjust our analyses for many health and disease-related characteristics that are different between anemic and non-anemic persons, but there could be unmeasured confounders that we cannot adjust for. Clinical trials are necessary to assess whether treatments aimed at increasing hemoglobin levels are also able to provide improved bone quality. In our analyses we have not considered some medications able to interfere, positively or negatively, with bone density, such as hormone replacement therapy, calcium supplements or bisphosphonates, because of their extremely low use in our sample population. Finally, the bone density measurements considered in the present study are from tibial pQCT scans and, therefore, the generalization of our findings to the entire skeleton might be difficult.

In conclusion, our findings show that hemoglobin levels and anemia are negatively and independently associated with bone mass and density. The bone loss associated with hemoglobin levels occurs mainly in the cortical bone. Women with lower hemoglobin levels might present a higher bone loss than male counterparts. Further studies are needed to confirm our findings and to provide a better understanding of the pathophysiologic pathway that links anemia/hemoglobin levels with bone density and mass measures.

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