

Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis

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Abstract Factors involved in inflammation are linked with those critical for bone remodeling. We examined the association between serum high sensitivity C-reactive protein (hsCRP) levels and bone mineral density (BMD) in healthy women. Serum concentrations of hsCRP and total alkaline phosphatase (ALP) were measured in premenopausal ($n = 3,662$) and postmenopausal ($n = 1,031$) women aged 30 years or older. BMD was measured at the femoral neck and lumbar spine using dual energy X-ray absorptiometry. According to the WHO definition, osteopenia was diagnosed at $-2.5 < T\text{-score} < -1.0$ SD, and osteoporosis was diagnosed at $T\text{-score} \leq -2.5$ SD at any sites. Compared with normal subjects, log-transformed serum hsCRP levels were higher in osteopenic and osteoporotic subjects (all, $P < 0.001$) with linearity (P for trend < 0.001), after adjustment for age, BMI and menopausal status. Menopausal status did not have a significant interaction on the association ($P = 0.457$). In both premenopausal and postmenopausal women, serum total ALP levels were higher in the subjects with higher hsCRP quintiles than those with the lowest quintile (all, P for trend < 0.001). Multivariate-adjusted odds ratio (OR) for osteoporosis and osteopenia were 1.35 (95% CI, 1.08 to 1.68) in the highest hsCRP quintile of pre-

menopausal women, and OR for osteoporosis was 1.54 (95% CI, 1.10 to 2.53) in the highest hsCRP quintile of postmenopausal women. These findings suggest that subclinical systemic inflammation may be associated with bone turnover rate and bone mass in healthy women.

Keywords Bone density · Bone turnover · C-reactive protein · Inflammation · Osteoporosis

Introduction

Osteoporosis is considered an imbalance of the remodeling process, in which bone resorption exceeds bone formation. Growing understanding of this process has shown that factors involved in inflammation are linked with those critical for bone physiology and remodeling [1, 2]. For example, pro-inflammatory cytokines, which are critical mediators of inflammatory responses, have also been found to regulate bone metabolism, even in individuals without immunological diseases [3]. One of the most important of these cytokines is interleukin (IL)-6, which is produced by osteoblasts, monocytes and T-cells and has been implicated in the pathogenesis of various metabolic bone diseases, including postmenopausal osteoporosis [4], Paget's disease [5], polyostotic fibrous dysplasia [6], osteoporosis associated with hematologic malignancy [7] and Gorham-Stout disease [8]. The IL-6 has been shown to promote osteoclast differentiation and activation [9]. IL-1 is another potent stimulator of bone resorption [10] that has been linked to the accelerated bone resorption seen in idiopathic [11] and postmenopausal osteoporosis [12]. Tumor necrosis factor (TNF)- α was shown to be involved in tumor-induced bone resorption [13] and non-tumor-induced osteopenia [14], as well as to stimulate bone resorption and to inhibit bone formation [14]. In osteoblasts and bone marrow stromal cells, these pro-inflammatory cytokines have been

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observed to up-regulate the receptor activator of nuclear factor- κ B ligand (RANKL) [15], leading to increased osteoclastogenesis and bone resorption. Activated T lymphocytes are also an important source of RANKL [16], supporting the concept that an inflammatory state can contribute to pathologic bone loss.

C-reactive protein (CRP), a member of the pentraxin family of innate immune recognition proteins, is regarded as a sensitive marker of systemic inflammation [17]. CRP is predominantly produced in the liver, and IL-1, IL-6 and TNF- α have been identified as regulators of CRP production [18, 19]. Recently, more sensitive immunoassays for CRP (high sensitivity CRP, hsCRP) have become available, making possible the measurement and comparison of low CRP levels in blood. These sensitive assays have revealed the relationship between hsCRP levels and the development and progression of coronary heart disease [20, 21] and osteoarthritis [22]. In this study, we investigated the association between hsCRP levels and bone mineral density (BMD) in healthy pre- and postmenopausal women.

Subjects and methods

Subjects

The study population consisted of 11,732 Korean women aged 30 years or older in whom BMD was measured consecutively at the Health Promotion Center of Asan Medical Center (Seoul, Korea) between January 2002 and July 2003. A specially trained nurse administered a questionnaire to all subjects to obtain information on their smoking and drinking habits, leisure time physical exercise (more/less than two times per week), medication history, history of previous medical or surgical diseases and reproductive history. The height and weight of each subject were measured while the subject was dressed in light clothing and without shoes. Oral temperature was measured, and a physician examined each patient. Routine laboratory and imaging examinations included complete blood counts; liver, renal and thyroid function tests; serum concentrations of glucose, calcium, phosphorus, alkaline phosphatase (ALP), rheumatoid factor, FSH and hsCRP; urinalysis; stool occult blood test; simple chest X-ray; duodenofibroscopy; sigmoidoscopy; abdominal ultrasonography; mammography and Pap smear.

Women were excluded if any information on menopausal history was missing or if the subject had undergone hysterectomy prior to natural menopause. Women who had taken drugs, including vitamin D, which might affect bone metabolism or hsCRP level for more than 6 months or within the previous 12 months were also excluded. Subjects were excluded if they had suffered from any disease that might affect bone metabolism or hsCRP level. Women who had had a stroke or dementia were also excluded because of concerns related to their limited physical activity. Also excluded were women with fever

(oral temperature $\geq 38.0^{\circ}\text{C}$) or any abnormal findings on routine laboratory and imaging examinations, including abnormal leukocyte count (< 4.0 or $> 10.0 \times 10^9/\text{l}$); elevated serum aspartate aminotransferase (≥ 40 U/l), alanine aminotransferase (≥ 40 U/l), or total ALP (> 120 U/l) concentration; abnormal serum calcium (< 8.3 mg/dl, < 2.1 mmol/l, or > 10.0 mg/dl, > 2.5 mmol/l) or phosphorus (< 2.5 mg/dl, < 0.8 mmol/l; or > 4.5 mg/dl, > 1.4 mmol/l) concentration; decreased serum albumin concentration (< 3.0 g/dl < 30 g/l); elevated serum creatinine (≥ 1.5 mg/dl, ≥ 133 $\mu\text{mol/l}$) or fasting glucose (≥ 126 mg/dl, ≥ 7.0 mmol/l) concentration; abnormal serum free T4 (≤ 0.8 ng/dl, ≤ 10 pmol/l, or ≥ 1.9 ng/dl, ≥ 24 pmol/l) or TSH (≤ 0.5 or ≥ 5.0 mU/l) concentration, or serum rheumatoid factor ≥ 20 IU/ml. Finally, in order to exclude the subjects with infectious or immune diseases, 26 subjects with hsCRP levels > 10 mg/l were excluded [21]. Of the initial 11,732 women, 4,693 were deemed eligible for study, of whom 3,662 were premenopausal and 1,031 were postmenopausal. Postmenopausal status was defined as cessation of menses for at least 1 year, which was confirmed by a serum FSH concentration > 30 IU/l. This study was approved by the Ethics Committee of Asan Medical Center.

Biochemical measurements

Serum hsCRP concentration was measured by a particle-enhanced immunoturbidometric method using a Cobas Integra 700 (Roche Diagnostic System, Basel, Switzerland). According to the EP5-A proposal of the National Committee for Clinical Laboratory Standards, the intra- and inter-assay coefficients of variations (CVs) were 1.3 and 3.0%, respectively. The median hsCRP concentration in 1,399 healthy persons was 0.64 mg/l, and the 25th, 75th, and 95th percentiles were 0.34, 1.23, and 4.01 mg/l, respectively [23].

Serum ALP concentration was measured by the Bowers and McComb method at 37°C with AMP buffer using a Toshiba 200 FR Autoanalyzer (Toshiba Medical System Co., Ltd., Tokyo). The intra- and inter-assay CVs were 0.7 and 1.3%, respectively, and the reference interval was 40–120 U/l.

BMD measurements

Areal BMD (g/cm^2) was measured at the non-dominant femoral neck and the anterior-posterior lumbar spine (L2–L4) using dual energy X-ray absorptiometry (QDR 4500-A, Hologic, Inc., Waltham, Mass.), software version 4.84. The in vivo precision of the machine was 1.2% for the femoral neck and 0.85% for the lumbar spine. Normal *T*-score was calculated using the installed software of the apparatus, in which mean \pm SD BMD was established from those of the northeastern Asian young women [24]. According to the WHO definition, osteopenia was diagnosed at $-2.5 < T\text{-score} < -1.0$ SD, and

osteoporosis was diagnosed at T -score ≤ -2.5 SD at any sites.

Analyses

Bivariate associations between menopausal status and clinical and non-clinical variables were determined using the chi-square test for categorical variables and the t test with a normal distribution for continuous variables. Serum hsCRP concentration, which showed a positively skewed distribution, was compared using the Wilcoxon rank-sum test, and was categorized into five levels at the nearest quintile points in other analyses. Analysis of variance (ANOVA) test and chi-square test were used to compare clinical and behavioral characteristics according to quintiles for serum hsCRP level. The Tukey method was used for multiple comparisons in the ANOVA test. After adjustments for age and body mass index (BMI), and, for postmenopausal women, years since menopause, the means and 95% confidence intervals of log-transformed hsCRP concentrations were compared by analysis of covariance (ANCOVA) among subjects' BMD status (normal vs. osteopenia vs. osteoporosis). Logarithmic transformation was used for serum hsCRP concentrations in the ANCOVA analyses because of its positively skewed distribution. To examine the linearity of the log-transformed hsCRP levels according to subjects' BMD status, the significance test for linearity (P for trend test) was performed. Multiple regression analyses were used to explore the effect of hsCRP concentration on BMD and serum ALP after adjustment for covariates. We first considered several variables deemed of interest (hsCRP quintile levels, age, BMI, years since menopause), independent of their

statistical significance. Health behavior variables (smoking habits, drinking habits, leisure time physical exercise) were included with the stepwise method, retaining only those that were statistically significant. In addition, the association between serum hsCRP levels and osteoporosis and/or osteopenia was determined by logistic regression analyses. Adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated. Age, BMI and years since menopause (for menopausal women) were included as covariates in this logistic regression. To examine the relationship between serum ALP levels and BMD, partial correlation coefficients were computed after adjustment for confounders, including age and BMI, as well as years since menopause in postmenopausal women. All statistical analyses were performed with SAS statistical software, with a P value of 0.05 considered statistically significant.

Results

Baseline clinical characteristics by menopausal status

The characteristics of the study subjects are shown in Table 1. The mean \pm SD age of the premenopausal women was 42.6 ± 5.1 years (range, 30–55 years), and the mean \pm SD age of the postmenopausal women was 57.6 ± 5.3 years (range, 41–76 years), with the mean \pm SD years since menopause in the latter group being 7.7 ± 5.7 years (range 1–31 years). The median hsCRP concentration in the premenopausal women was 0.7 mg/l, with the 25th, 75th and 95th percentiles being 0.5, 1.0 and 2.7 mg/l, respectively. In the postmenopausal women, the median hsCRP concentration was 1.0 mg/l, and the 25th, 75th and 95th percentiles were 0.6, 1.5

Table 1 Baseline characteristics of subjects by menopausal status. *BMI* body mass index; *ALP* alkaline phosphatase; *hsCRP* high sensitivity C-reactive protein. SI conversion factors: to convert mg/dl to mmol/l for calcium, multiply values by 0.2495; to convert mg/dl to mmol/l for phosphorus, multiply values by 0.3229. Values are mean \pm SD unless otherwise specified

Variables	Premenopausal ($n = 3,662$)	Postmenopausal ($n = 1,031$)	P value
Age (years)	42.6 ± 5.1	57.6 ± 5.3	< 0.001
Height (cm)	158.1 ± 4.9	154.5 ± 5.4	< 0.001
Weight (kg)	56.4 ± 7.1	57.8 ± 7.5	< 0.001
BMI (kg/m^2)	22.5 ± 2.7	24.2 ± 2.9	< 0.001
Years since menopause	-	7.7 ± 5.7	
Smoking habits, no. (%)			0.003
Never	1,939 (53.0%)	532 (51.6%)	
Current or past	1,279 (34.9%)	406 (39.4%)	
Unknown	444 (12.1%)	93 (9.0%)	
Drinking habits, no. (%)			0.003
No	2,957 (80.8%)	800 (77.6%)	
Yes	659 (18.0%)	204 (19.8%)	
Unknown	46 (1.3%)	27 (2.6%)	
Leisure physical exercise, no. (%)			< 0.001
No	3,204 (87.5%)	847 (82.2%)	
Yes	412 (11.3%)	157 (15.2%)	
Unknown	46 (1.3%)	27 (2.6%)	
Serum total ALP concentration (U/l)	50.2 ± 11.3	73.2 ± 17.2	< 0.001
Serum calcium concentration (mg/dl)	9.0 ± 0.4	9.2 ± 0.4	< 0.001
Serum phosphorus concentration (mg/dl)	3.7 ± 0.5	4.1 ± 0.5	< 0.001
Serum hsCRP concentration (mg/l) (median, interquartile range)	0.7 (0.5–1.0)	1.0 (0.6–1.5)	< 0.001

and 3.4 mg/l, respectively. These findings were similar to those previously observed in 1,399 healthy Korean women [23]. The concentrations of serum hsCRP, total ALP, calcium and phosphorus were significantly higher in the postmenopausal than in the premenopausal women (Table 1). Of the premenopausal women, 1,177 (32.1%) and 23 (0.6%) were osteopenic and osteoporotic, respectively. The number of subjects with osteoporosis was too small for separate analysis, thus osteopenic and osteoporotic groups were combined in the following analyses. Of the postmenopausal women, 594 (57.6%) and 232 (22.5%) were osteopenic and osteoporotic, respectively.

Relationship between serum hsCRP concentration and BMD status

Median serum hsCRP concentrations (interquartile range) were 0.6 (0.4–0.9), 0.8 (0.5–1.3) and 1.1 (0.7–1.8) in normal, osteopenic and osteoporotic subjects, respectively. Using non-parametric test, there was a significant difference in the levels among the BMD status ($P < 0.001$), showing a tendency to increase in normal to osteoporotic subjects. We compared logarithmically transformed serum hsCRP concentrations with BMD status after adjustment for age, BMI and menopausal status because the hsCRP level was significantly correlated with age ($\gamma = 0.200$, $P < 0.001$) and BMI ($\gamma = 0.291$, $P < 0.001$). The log-transformed hsCRP levels were significantly higher in osteoporotic and osteopenic subjects than in normal subjects ($F = 13.861$, $P < 0.001$), and showed linearity (P for trend < 0.001) (Fig. 1a). Menopausal status did not have a significant interaction on the association ($P = 0.457$).

By menopausal status, we also compared logarithmically transformed serum hsCRP concentrations according to the BMD status. In premenopausal women, the log-transformed hsCRP levels were significantly higher in the subjects with osteopenia/osteoporosis than in normal subjects after adjustment for age and BMI ($F = 5.534$, $P = 0.021$) (Fig. 1b). In postmenopausal women, there was a significant difference in hsCRP level relative to BMD status ($F = 3.097$, $P = 0.046$) with linearity (P for trend = 0.008) after adjustments for age, BMI and years since menopause (Fig. 1c). The log-transformed hsCRP levels were significantly higher in osteoporotic than in normal subjects ($P = 0.014$).

Associations of serum hsCRP quintiles with BMD

When we categorized the subjects into five levels according to serum hsCRP concentration (Table 2), we found that the percentages of current or past smokers, drinkers and those who participated in leisure time physical exercise were similar in the hsCRP quintiles for both pre- and postmenopausal women. We found, however, that age, weight and BMI were higher in the

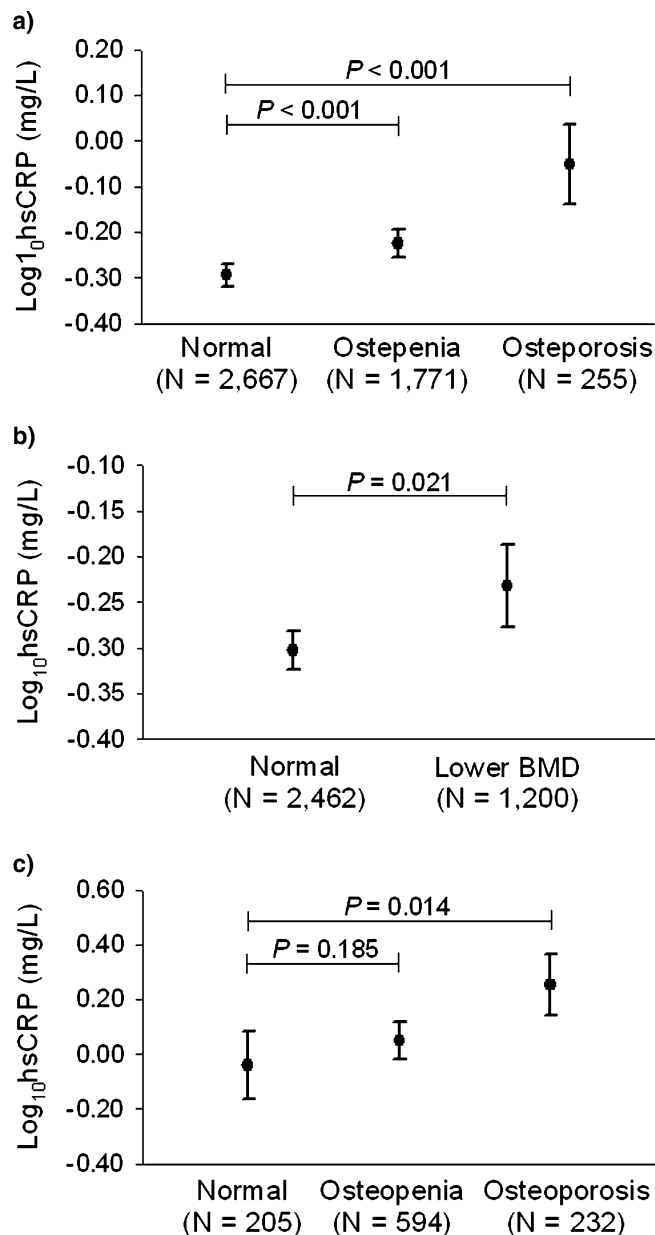


Fig. 1 Serum hsCRP concentrations among normal, osteopenic and osteoporotic subjects. Osteopenia and osteoporosis were defined according to the WHO classification ($-2.5 < T$ -score ≤ -1.0 SD and T -score ≤ -2.5 SD, respectively). Serum hsCRP concentrations were logarithmically transformed because of its right-skewed deviation, and data were given as estimated mean \pm 95% confidence intervals in all subjects after adjustment for age, body mass index and menopausal status (a), in premenopausal women after adjustment for age and body mass index (b), and in postmenopausal women after adjustment for age, body mass index and duration of menopause (c). In premenopausal women, the number of subjects with osteoporosis ($n = 23$) was too small for separate analysis, thus osteopenic and osteoporotic groups were combined (“lower BMD”). There were significant differences in hsCRP levels in all subjects ($F = 13.861$, $P < 0.001$), premenopausal women ($F = 5.534$, $P = 0.021$), and postmenopausal women ($F = 3.097$, $P = 0.046$).

higher hsCRP quintiles than in the lowest quintile for both groups of women (P for trend < 0.001). In postmenopausal women, height was lower in the 4th and 5th

Table 2 Clinical characteristics according to serum hsCRP concentration quintiles. *BMI* body mass index; *ALP* alkaline phosphatase; *hsCRP* high sensitivity C-reactive protein. SI conversion factors: to convert mg/dl to mmol/l for calcium, multiply values by 0.2495; to convert mg/dl to mmol/l for phosphorus, multiply values by 0.3229. Values are mean \pm SD unless otherwise specified

hsCRP quintile	Premenopausal (<i>n</i> = 3,662)					<i>P</i> value*
	Q1 (<i>n</i> = 905)	Q2 (<i>n</i> = 473)	Q3 (<i>n</i> = 772)	Q4 (<i>n</i> = 767)	Q5 (<i>n</i> = 745)	
Serum hsCRP concentration (mg/l)	≤ 0.4	0.5	0.6~0.7	0.8~1.1	≥ 1.2	
Age (years)	42.0 \pm 5.0	42.6 \pm 4.9	42.5 \pm 4.9	43.2 \pm 5.0†	43.0 \pm 5.4†	< 0.001
Height (cm)	158.2 \pm 4.8	158.2 \pm 5.2	158.1 \pm 4.8	158.0 \pm 5.0	157.9 \pm 5.1	NS‡
Weight (kg)	54.5 \pm 6.4	55.8 \pm 6.2†	55.4 \pm 6.7	57.1 \pm 7.3†	59.2 \pm 7.9†	< 0.001
BMI (kg/m ²)	21.7 \pm 2.4	22.3 \pm 2.3†	22.1 \pm 2.5†	22.9 \pm 2.8†	23.7 \pm 3.0†	< 0.001
Smokers, no. (%)	312 (34.5%)	168 (35.5%)	264 (34.2%)	261 (34.0%)	274 (36.8%)	NS
Drinkers, no. (%)	175 (19.3%)	92 (19.5%)	132 (17.1%)	124 (16.2%)	136 (18.3%)	NS
Leisure physical exercise, no. (%)	97 (10.7%)	57 (12.1%)	82 (10.6%)	81 (10.6%)	95 (12.8%)	NS
Serum total ALP concentration (U/l)	47.6 \pm 10.1	48.1 \pm 10.3	49.1 \pm 11.2†	51.6 \pm 11.0†	54.4 \pm 12.4†	< 0.001
Serum calcium concentration (mg/dl)	8.9 \pm 0.3	9.0 \pm 0.3	9.0 \pm 0.3	9.0 \pm 0.4	9.0 \pm 0.4	NS
Serum phosphorus concentration (mg/dl)	3.7 \pm 0.6	3.7 \pm 0.4	3.7 \pm 0.5	3.7 \pm 0.5	3.7 \pm 0.5	NS
hsCRP quintile	Postmenopausal (<i>n</i> = 1,031)					<i>P</i> value*
	Q1 <i>n</i> = 201	Q2 <i>n</i> = 222	Q3 <i>n</i> = 203	Q4 <i>n</i> = 207	Q5 <i>n</i> = 198	
Serum hsCRP concentration (mg/l)	≤ 0.5	0.6~0.8	0.9~1.1	1.2~1.7	≥ 1.8	
Age (years)	56.6 \pm 5.5	56.9 \pm 4.9	58.0 \pm 5.3	58.2 \pm 5.3†	58.3 \pm 5.3†	0.002
Height (cm)	155.8 \pm 6.1	154.5 \pm 5.3	154.9 \pm 5.6	154.0 \pm 5.1†	153.2 \pm 4.8†	< 0.001
Weight (kg)	54.5 \pm 6.4	55.8 \pm 6.2†	55.4 \pm 6.7†	57.1 \pm 7.3†	59.2 \pm 7.9†	< 0.001
BMI (kg/m ²)	22.7 \pm 2.6	24.0 \pm 2.7†	24.1 \pm 2.9†	24.9 \pm 2.8†	25.1 \pm 2.7†	< 0.001
Years since menopause (years)	6.9 \pm 5.7	7.1 \pm 5.6	7.7 \pm 5.3	8.1 \pm 5.6	8.6 \pm 6.1†	0.014
Smokers, no. (%)	81 (40.3%)	86 (38.7%)	72 (35.5%)	82 (39.6%)	85 (42.9%)	NS
Drinkers, no. (%)	43 (21.4%)	48 (21.6%)	35 (17.2%)	44 (21.3%)	34 (17.2%)	NS
Leisure physical exercise, no. (%)	36 (17.9%)	35 (15.8%)	36 (17.7%)	21 (10.1%)	29 (14.6%)	NS
Serum total ALP concentration (U/l)	70.4 \pm 17.9	71.0 \pm 16.6	74.9 \pm 17.6†	72.3 \pm 16.2	77.6 \pm 16.8†	< 0.001
Serum calcium concentration (mg/dl)	9.1 \pm 0.4	9.2 \pm 0.4	9.3 \pm 0.4	9.2 \pm 0.4	9.2 \pm 0.4	NS
Serum phosphorus concentration (mg/dl)	4.1 \pm 0.5	4.1 \pm 0.5	4.1 \pm 0.5	4.1 \pm 0.6	4.1 \pm 0.5	NS

*By *F* test (ANOVA test) for continuous variables and chi-square test for categorical variables. †*P* < 0.05 vs Q1 using Tukey method (for multiple comparisons) to compare clinical characteristics among hsCRP quintiles. ‡NS not significant

hsCRP quintiles than in the lowest quintile (*P* for trend < 0.001), and years since menopause was significantly longer in the highest hsCRP quintile than in the lowest quintile (*P* for trend = 0.001). Although there were no differences in serum calcium and phosphorus concentrations, serum total ALP levels tended to be higher in the higher quintiles in both pre- and postmenopausal women (*P* for trend < 0.001). Compared with those in the lowest quintile, premenopausal women in the 3rd to 5th quintiles, and postmenopausal women in the 3rd and 5th quintiles, had significantly higher serum total ALP concentrations. These differences in serum total ALP levels were persistently higher in both groups even after adjustments for confounding variables using multiple regression analysis (Table 3).

The multiple regression analysis also showed independent associations between each of these variables and BMD (g/cm²) (Table 3). In premenopausal women, age was negatively, and BMI was positively, correlated with BMD at both sites. In postmenopausal women, BMI was positively, and years since menopause was negatively, correlated with BMD. In the latter group, age was significantly correlated with BMD at the femoral neck, but not at the lumbar spine. In both groups, however, neither drinking habits nor leisure time physical exercise

was significantly correlated with BMD at either site. Smoking habits were negatively associated with BMD only at the lumbar spine in postmenopausal women ($\beta = -0.025$, *P* = 0.002). Importantly, compared with the lowest quintile, the 5th hsCRP quintile was significantly associated with lower BMD at the femoral neck in both pre- (*P* = 0.003) and postmenopausal (*P* < 0.001) women. At the lumbar spine, the 5th hsCRP quintile tended to be associated with lower BMD in both pre- (*P* = 0.060) and postmenopausal (*P* = 0.071) women. Smoking, which can influence the production of IL-6 [25], did not have a significant interaction on their associations of the hsCRP levels with serum ALP levels and BMD at all sites in both premenopausal and postmenopausal women (*P* = 0.086 to 0.856).

Partial correlation analysis revealed that serum ALP concentrations were negatively correlated with BMD at the femoral neck and lumbar spine both in premenopausal ($\gamma = -0.159$, *P* < 0.001, and $\gamma = -0.162$, *P* < 0.001, respectively) and postmenopausal women ($\gamma = -0.242$, *P* < 0.001, and $\gamma = -0.264$, *P* < 0.001, respectively). Therefore, we analyzed the association of the hsCRP levels with BMD values after additional adjustment for serum ALP concentrations. At the femoral neck, the 5th hsCRP quintile showed lower BMD in postmenopausal

Table 3 Multiple regression analyses of bone mineral density (*BMD*) (g/cm^2) and serum alkaline phosphatase (*ALP*) levels in premenopausal and postmenopausal women. *BMD* bone mineral density; *ALP* alkaline phosphatase; *hsCRP*, high sensitivity C-reactive protein; *BMI*, body mass index. A first block of variables considered of interest (*hsCRP* quintile levels, age, *BMI*, years since

menopause) was included in this analysis, independently of their statistical significance. Health behavior variables (smoking habits, drinking habits, leisure physical exercise) were then included with the stepwise method, retaining only those that were statistically significant. * *NS* not significant

Variables	Premenopausal (<i>n</i> = 3,662)						Postmenopausal (<i>n</i> = 1,031)					
	Serum total ALP		Femoral neck BMD		Spine BMD		Serum total ALP		Femoral neck BMD		Spine BMD	
	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value
Quintiles for <i>hsCRP</i>												
Q1 (lowest)	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Q2	0.191	NS*	0.001	NS	-0.002	NS	-0.303	NS	-0.008	NS	0.001	NS
Q3	1.266	0.019	0.001	NS	-0.001	NS	3.795	0.028	-0.009	NS	-0.011	NS
Q4	3.388	<0.001	-0.001	NS	0.003	NS	1.232	NS	-0.006	NS	-0.009	NS
Q5 (highest)	6.000	<0.001	-0.014	0.003	-0.011	0.060	6.378	<0.001	-0.030	<0.001	-0.024	0.071
Age	0.165	<0.001	-0.002	<0.001	-0.001	0.001	0.013	NS	-0.003	<0.001	-0.002	NS
<i>BMI</i>	0.321	<0.001	0.012	<0.001	0.011	<0.001	0.110	NS	0.010	<0.001	0.011	<0.001
Years since menopause	-		-		-		0.197	NS	-0.004	<0.001	-0.008	<0.001
Smoking history												
No	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Yes	-		-		-		-		-		-0.025	0.002
Unknown	-		-		-		-		-		-0.001	NS
Alcohol consumption												
No	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Yes	-		-		-		-		-		-	
Unknown	-		-		-		-		-		-	
Regular exercise												
No	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Yes	-		-		-		-		-		-	
Unknown	-		-		-		-		-		-	

women ($\beta = -0.025$, $P = 0.008$), although the significance disappeared in premenopausal women ($\beta = -0.007$, $P = 0.166$).

Odds ratios (ORs) of the *hsCRP* quintiles for osteoporosis and/or osteopenia

Figure 2 presented the results of multiple logistic regression analyses with osteoporosis and/or osteopenia as the dependent variable. In premenopausal women, the OR for osteopenia/osteoporosis was significantly higher in the 5th *hsCRP* quintile than in the lowest quintile (OR = 1.35, 95% CI = 1.08 to 1.68) (Fig. 2a). In postmenopausal women, the OR for osteoporosis was 1.64 (95% CI = 1.10 to 2.53) in the highest *hsCRP* quintile (Fig. 2b). After the additional adjustment for serum ALP concentrations, the significantly elevated ORs for osteoporosis and/or osteopenia disappeared in premenopausal (OR = 1.17, 95% CI = 0.93 to 1.46) and postmenopausal women (OR = 1.31, 95% CI = 0.78 to 2.20).

Discussion

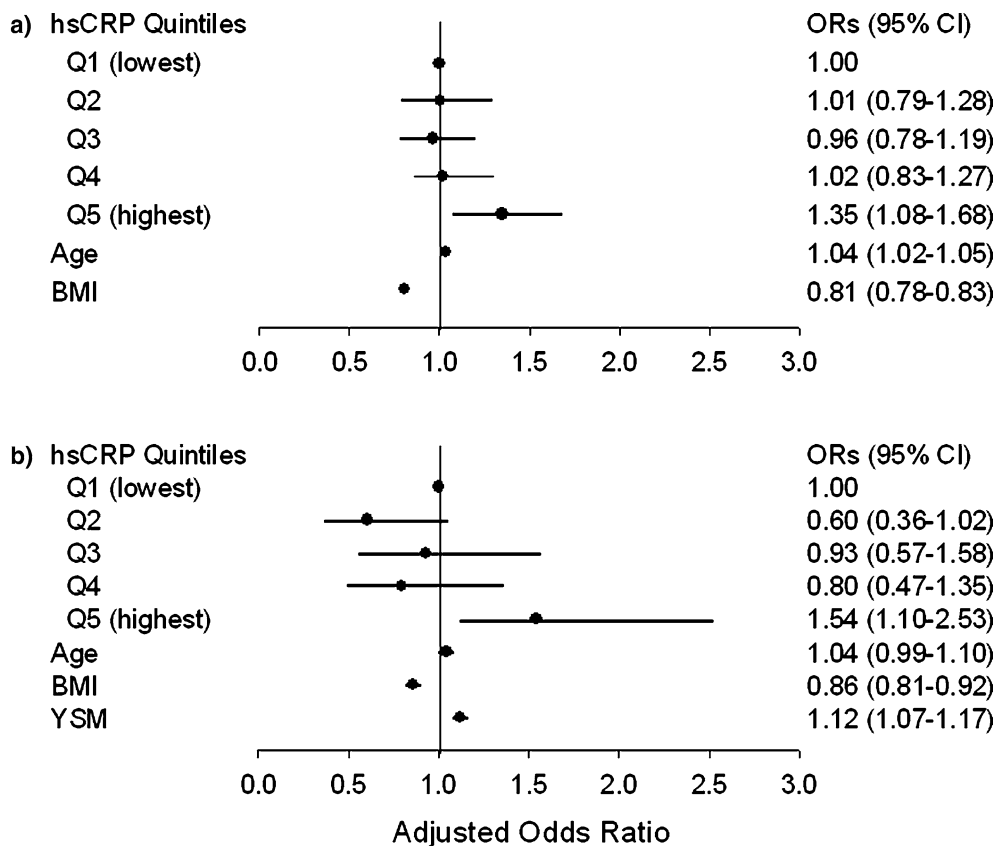
An association between circulating *hsCRP* level and *BMD* or biochemical bone turnover markers has been observed in several immune and inflammatory diseases.

For example, in patients with rheumatoid arthritis, higher *CRP* was associated with lower *BMD* [26] and higher bone turnover, as measured by urinary excretion of pyridinoline and deoxypyridinoline collagen cross-linking [27]. In addition, elevated *CRP* was associated with increased urinary excretion of pyridinium cross-links in patients with ankylosing spondylitis [28] and seronegative spondyloarthropathy [29]. To our knowledge, however, the *hsCRP* assay has not been used previously to determine the relationship between sub-clinical systemic inflammation and osteoporosis in healthy individuals without immune and inflammatory diseases.

We have shown here that pre- and postmenopausal women with osteopenia and/or osteoporosis had higher serum *hsCRP* levels than those with normal *BMD*, and that *hsCRP* levels higher than 1.2 mg/l and 1.8 mg/l were significantly associated with osteoporosis and/or osteopenia in pre- and postmenopausal women, respectively. Furthermore, *hsCRP* levels were positively correlated with total serum ALP levels. These findings suggest that higher *hsCRP* levels are associated with higher bone turnover rate, resulting in lower bone mass even in healthy women with normal *hsCRP* levels, and that a subclinical inflammatory process plays an important role in bone metabolism.

The mechanisms linking *hsCRP* and bone metabolism are not clear, but activated inflammatory cytokines

Fig. 2 Adjusted odds ratios and 95% confidence intervals for osteoporosis and/or osteopenia relative to hsCRP quintile in premenopausal (a) and postmenopausal women (b). Logistic regression analyses were performed to determine the odds ratio of osteopenia/osteoporosis (in premenopausal women) or osteoporosis (in postmenopausal women) according to hsCRP quintile. Adjusted odds ratios were calculated along with 95% CI. The lowest quintile (quintile I) was the reference



are likely to be involved. Inflammatory processes can up-regulate many cytokines, such as IL-1, IL-6 and TNF- α , which strongly stimulate CRP production from the liver [18, 19] as well as induce bone resorption [3, 4, 10, 14], and increased bone resorption may result in increased bone turnover and decreased BMD. In support of this hypothesis, the production of IL-1, IL-6 and/or TNF- α by peripheral blood monocytes was positively correlated with bone resorption or spinal bone loss in healthy pre- [30] and postmenopausal [31] women, and serum IL-6 concentrations predicted femoral bone loss in healthy postmenopausal women [32]. In addition, serum concentrations of IL-6 and TNF- α could be positively correlated with serum hsCRP levels in healthy subjects [33] and those with myocardial infarction [34]. Another possibility is that the association between higher hsCRP levels and lower BMD comes from vitamin D deficiency [35]. In this study, however, the subjects were limited to those with normal serum levels of calcium, phosphorus and alkaline phosphatase in order to minimize any confounding effects of concomitant hyperparathyroidism or vitamin D deficiency. In addition, the serum levels of calcium and phosphorus did not differ among the hsCRP quintiles in pre- and postmenopausal women.

In the present study, we noted that height was significantly reduced in the 4th and 5th hsCRP quintiles, raising the question of vertebral fractures. However, we did not have a chance to examine spine X-ray for the

presence of such fractures. In addition, we found that the association of hsCRP levels with BMD at the lumbar spine was weaker than at the femoral neck, especially in postmenopausal women (Table 3). The reason for this is not entirely clear, especially when it is considered that the spine is metabolically more active than the femoral neck due to its higher surface-to-volume ratio (largely trabecular) [36]. Osteoarthritis, which occurs more often at the spine in the elderly, may have falsely increased BMD measured by dual energy X-ray absorptiometry [37]. Further, subjects with osteoarthritis have been reported to have significantly higher hsCRP levels [22]. Thus, it is possible that osteoarthritic changes associated with aging may have ameliorated the association between hsCRP levels and BMD at the spine.

We excluded 26 subjects with the hsCRP levels > 10 mg/l (10.1 to 26.5 mg/l) in order to eliminate the possibility of including subjects who may have had infectious or immune diseases [21]. Twenty were premenopausal and six were postmenopausal women. Of the 26 subjects, 21 were obese, 11 were hypertensive, 5 showed dyslipidemia, and 3 showed impaired fasting glucose. Addition of these subjects in the analyses strengthened the associations between serum hsCRP levels and BMD in premenopausal women. Compared with the lowest quintile, the highest hsCRP quintile showed lower BMD at the femoral neck ($\beta = -0.016$, $P < 0.001$) and lumbar spine ($\beta = -0.015$, $P = 0.001$), and the OR for osteopenia/osteoporosis in the highest quintile was 1.42

(95% CI = 1.21 to 1.83). In postmenopausal women, the results were not changed by the addition of six subjects with high hsCRP levels (data not shown).

Although associations between atherosclerosis and osteopenia have been well documented [38, 39, 40], it is not clear whether one is the result of the other or whether they develop through common mechanisms. Recently, several lines of evidence have suggested that common or related mechanisms control both of these conditions from their early stages [41, 42]. The results shown here indicate that the risk of lower BMD is independently correlated with hsCRP concentration, a known marker for the risk of atherosclerosis [20]. Thus, it is feasible that a systemic inflammatory process may, at least partially, be a common mechanism for the development for lower bone mass and atherogenesis.

There were several potential limitations to this study. First, the study population was comprised of women who visited a health promotion center, and may not have been representative of the general population residing in a community, thus possibly resulting in selection bias. To minimize this, we intentionally applied strict exclusion criteria based on medical history and routine laboratory findings. Second, because this was a cross-sectional study, we cannot determine if there is a causal relationship between hsCRP levels and BMD values. Further prospective studies are necessary to determine whether this correlation is reflective of a causal relationship.

In conclusion, we have shown that increased serum hsCRP concentrations are associated with lower BMD and higher bone turnover rate in a large population of healthy women, and that hsCRP concentrations greater than 1.2 mg/l for premenopausal women and greater than 1.8 mg/l for postmenopausal women are significantly associated with osteoporosis and/or osteopenia. These findings suggest that subclinical systemic inflammation may be an important factor in bone turnover rate and bone mass.

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