Circulating Levels of Inflammatory Markers Predict Change in Bone Mineral Density and Resorption in Older Adults: A Longitudinal Study

Changhai Ding, Venkat Parameswaran, Ray Udayan, John Burgess, and Graeme Jones

Menzies Research Institute (C.D., G.J.), University of Tasmania, Hobart, Tasmania 7000, Australia; and Diabetes and Endocrine Services (V.P., J.B.) and Department of Clinical Chemistry (R.U.), Royal Hobart Hospital, Hobart, Tasmania 7001, Australia

Context: IL-1, IL-6, and TNF-α play an important role in the pathogenesis of osteoporosis in animals; however, evidence that these play a similar role in bone loss in human studies is limited.

Objective: Our objective was to determine the associations between serum markers of inflammation and changes in bone mineral density (BMD) and urinary pyridinoline (PYR) to creatinine (Cr) ratio over 2.9 yr in older adults.

Methods: A total of 168 randomly selected subjects (mean 63 yr, range 52–78, 48% female) was studied. BMD was measured by dual-energy x-ray absorptiometry at baseline (mean T score: -0.18 to -0.61) and 2.9 yr later. Serum high-sensitivity (hs) C-reactive protein (CRP), IL-6, TNF-α, and the urinary PYR/Cr ratio were measured on both occasions.

Results: The mean annual loss of BMD was 0.15, 0.15, and 0.34% at total body, spine, and hip, respectively. Change in total body BMD was associated with baseline hs-CRP, IL-6, and TNF-α, as well as change in hs-CRP (β: -0.41%/U, 95% confidence interval -0.68%, -0.15%) and IL-6 (β: -0.62%/U, 95% confidence interval -1.01%, -0.23%). If these markers were put in the same predictive model, only IL-6 remained largely unchanged. Changes in other BMD sites were significantly predicted by IL-6 (hip and spine) and TNF-α (spine only). Finally, change in the PYR/Cr ratio was positively associated baseline IL-6, hs-CRP, and their changes (all $P < 0.05$) in women, but not men.

Conclusions: Variation within the low levels of inflammatory markers observed in this study, especially IL-6, predicts bone loss and resorption, suggesting that targeted antiinflammatory therapy has potential for the prevention of osteoporosis. **(*J Clin Endocrinol Metab* 93: 1952–1958, 2008)**

Osteoporosis, characterized by the loss of bone mass and deterioration of bone microarchitecture with a resulting increase in bone fragility and, therefore, susceptibility to fracture in both women and men, is now a major public health issue (1, 2). A number of risk factors for osteoporosis are well recognized, including age, female sex, smoking, physical inactivity, and vitamin D and estrogen deficiency. In recent years, inflammation has been also implicated. Experimental studies in animals have provided substantial evidence suggesting that certain inflammatory cytokines, including IL-1, IL-6, and TNF-α, play an important role in the pathogenesis of osteoporosis (3–7). However, evidence that these inflammatory cytokines play a similar role in human studies is limited. Preliminary studies on the associations between the circulating cytokine levels and bone mineral density (BMD) (8–11) or bone loss (12, 13) in postmenopausal or older women have provided inconsistent or even contradictory results. These inconsistencies may reflect that these circulating cytokines do not accurately reflect what is happening in the local bone microenvironment or that there exist natural antagonists in the serum, which may interfere with assay interpretation. In addition, the associations between circulating inflammatory cytokines and bone loss in older men have not been reported.

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2007-2325 Received October 18, 2007. Accepted February 13, 2008.

First Published Online February 19, 2008

Abbreviations: BMD, Bone mineral density; BMI, body mass index; Cr, creatinine; CRP, C-reactive protein; CV, coefficient of variation; HRT, hormone replacement therapy; hs, high-sensitivity; PYR, pyridinoline; RA, rheumatoid arthritis.

High-sensitivity (hs) C-reactive protein (CRP), a circulating marker of systemic inflammation, has been identified as an independent risk factor for cardiovascular events in healthy postmenopausal women (14). Hepatic CRP production is stimulated primarily by IL-6 and IL-1-like cytokines (15), and higher serum levels of hs-CRP are associated with lower BMD (16), higher levels of bone turnover markers (17), and, recently, greater risk of fracture (18, 19). However, this association is unlikely to be causal, and there are no reports documenting the associations between circulating hs-CRP and bone loss in older women and men.

Therefore, the aim of this study was to determine the associations between serum markers of inflammation and changes in BMD and the urinary pyridinoline (PYR) to creatinine (Cr) ratio over 2.9 yr in randomly selected older women and men.

Subjects and Methods

Subjects

The study was performed in southern Tasmania from March until August 2002. The follow-up study was conducted approximately 2.9 yr later (range 2.6–3.3 yr). Subjects between ages 50 and 79 yr were selected randomly from the roll of electors in southern Tasmania (population 229,000) with an equal number of men and women. Institutionalized persons were excluded. This study was conducted as part of the Tasmanian Older Adult Cohort Study, an ongoing, prospective, population-based study in 1100 subjects aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of osteoarthritis and osteoporosis (the overall response rate was 57% at baseline, and 82% for follow-up). We selected the first 193 subjects to perform the serum inflammatory markers measurements at baseline and follow-up. At follow-up, these measurements were not performed in 25 subjects due to insufficient serum sample, leaving 168 subjects. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants. Self-report of rheumatoid arthritis (RA), asthma, cardiovascular disease, diabetes, and medication usage [including nonsteroidal antiinflammatory drugs, cyclooxygenase-2 inhibitors, prednisolone, and hormone replacement therapy (HRT)] were recorded by questionnaire. Smoking, physical activity (no/mild, moderate, and vigorous), and menopause status were also recorded.

Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Model 707; Seca Delta, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI) [weight (kg)/height (m^2)] was also calculated.

BMD measurement

Bone mass was measured using dual-energy x-ray absorptiometry at the total body, lumbar spine, and total hip at baseline and follow-up. The instrument used was a Hologic Delphi densitometer on array setting (Hologic, Inc., Waltham, MA). The software program was not altered during the study time frame. Bone mass was examined as areal BMD (g/cm^2), which is calculated by dividing the bone mineral content by the area measured. Precision estimates *in vivo* are 2–3% in our hands. The longitudinal coefficient of variation (CV) for our instrument between 2002 and 2007 using daily measurements of a spine phantom was 0.39% for areal BMD. Rates of change in BMD were calculated as: percent change per annum = [100 (follow-up BMD − baseline BMD/baseline BMD)/time between two scans in years].

Bone resorption assessment

Bone resorption was assessed on two occasions by measuring the urinary PYR/Cr ratio by a chemiluminescent competitive immunoassay on 20-ml aliquot taken from a timed overnight urine collection, protected from light by a black bag, and stored at −20 C before analysis (Pyrilinks-D; Diagnostic Products Corp., Los Angeles, CA). The CV in our hands was of the order of 7.5% (20). Change in the PYR/Cr ratio was calculated as: change per annum = (follow-up PYR/Cr ratio − baseline PYR/Cr ratio)/time between two scans in years.

Serum inflammatory markers measurement

Serum was isolated and refrigerated overnight in plastic tubes, at which time aliquots were prepared and stored at −80 C. The IL-1β, IL-6, and TNF-α were measured at baseline and then at 2.9 yr with a solid-phase, two-site chemiluminescent enzyme immunometric assay method using IMMULITE IL-1β, IMMULITE IL-6, and IMMULITE TNF-α (all from EURO/DPC Llanberis, Gwynedd, UK). Samples with undetectable cytokine concentrations were assigned a value corresponding to the lower limit of detection of the assay: 1.5 pg/ml for IL-1β, 2 pg/ml for IL-6, and 1.7 pg/ml for TNF-α. The CVs in our hands were 3% for IL-1β, 8% for IL-6, and 6% for TNF-α (in-house data).

High-sensitivity (hs) testing for CRP was performed using the CRP-Latex (II) immunoturbidimetric assay (Architect c8000; Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL). The lower detection limit of the assay is 0.01 mg/liter. The CV in our hands was of the order of 4.8% (in-house data). Changes in these markers were calculated as: change per annum = (follow-up value − baseline value)/time between two scans in years.

Data analysis

Univariable and multivariable linear regression analyses were used to examine the associations between annual percent change in BMD and hs-CRP (or IL-6/TNF-α), and its change before and after adjustment for age, sex, weight, height, smoking status, other disease status (RA, cardiovascular disease, asthma, and diabetes), and IL-6/TNF-α (or hs-CRP). Logistic regression analyses were used to examine the associations between change in the PYR/Cr ratio (top quartile *vs.* other quartiles) and inflammatory markers after adjustment for the aforementioned factors. Standard diagnostic checks of model fit and residuals were routinely made, and data points with large residuals and/or high influence were investigated for data errors. Inflammatory markers and their changes were not normally distributed, but no transformations (log, square, and cubic) provided better results, so we kept the data untransformed as independent variables in the regression analyses. Partial correlation coefficients from these models were also obtained. Quartiles of baseline and changes in all inflammatory markers were calculated, and the associations between these quartiles and annual percent change in BMD were also determined by linear regression analyses.

Due to significant sex differences in changes in BMD, interactions between sex and inflammatory markers were investigated by regressing the change in BMD (or change in the PYR/Cr ratio) on inflammatory markers (or changes) within sex strata (zero = male and one = female), and assessed by testing the statistical significance of the coefficient of a [sex × serum level of an inflammatory marker (or its change)] product term after adjustment for confounders. Interactions between years since menopause (zero = less than 10 yr since menopause, one = 10 or more than 10 yr since menopause) and inflammatory biomarkers were also investigated.

A P value less than 0.05 (two tailed) or a 95% confidence interval not including the null point was regarded as statistically significant. All statistical analyses were performed on SPSS version 12.0 for Windows (SPSS, Inc., Chicago, IL).

Results

A total of 193 subjects (48% female) aged between 52 and 78 yr (mean 63) participated, and 168 had complete data at follow-up. There were no significant differences in demographical factors and BMD between the current cohort and the subjects who did not have serum markers measured (data not shown). Characteristics of the subjects are presented in Table 1. The T scores at total body, lumbar spine, and hip were generally normal with 3.1, 7.8, and 0.5% subjects having a T score of less than −2.5 SD at each of the three sites, respectively. The serum levels of inflammatory markers were generally low with 98.4% of IL-1β undetectable in this sample, so this was not further analyzed. Over 2.9 yr, whereas serum levels of hs-CRP and IL-6 remained largely unchanged (all $P > 0.05$), the serum level of TNF-α decreased ($P = 0.01$). Change in IL-6 was positively associated with change in TNF-α ($P = 0.001$), change in hs-CRP ($P < 0.001$), and age ($P < 0.001$), but not BMI ($P = 0.80$). Change in TNF-α was positively associated with BMI ($P = 0.01$), but not change in hs-CRP ($P = 0.71$) or age ($P = 0.45$). Change in hs-CRP was not associated with age ($P = 0.16$) or BMI ($P = 0.86$). The significant associations between age and change in hip BMD ($\beta = -0.05\%$/yr; $P = 0.004$) decreased in magnitude (20% change in coefficient) after adjustment for IL-6, but not hs-CRP or TNF-α.

In the whole sample, both baseline hs-CRP and change in hs-CRP were associated with change in total body BMD after adjustment for most covariates, but these associations decreased in magnitude or became nonsignificant after further adjustment for IL-6 (Table 2) but not for TNF-α. The associations between hs-CRP, change in hs-CRP and change in total body BMD in women who were more than or equal to 10 yr past menopause appeared more evident ($r = -0.58$, $P = 0.001$; and $r = -0.48$, $P = 0.01$, respectively). With regard to bone turnover, hs-CRP and their changes were not significantly associated with change in the PYR/Cr ratio in the whole sample (data not shown); however, they were significantly associated with change in the PYR/Cr ratio in women after further adjustment for years since menopause (Table 3).

The associations between baseline IL-6, change in IL-6, and change in total body and spine BMD were all significant after adjustment for all covariates (Table 2 and Fig. 1). After further adjustment for hs-CRP and TNF-α, these associations remained largely unchanged and even became significant at the hip (Table 2). No significant interactions between sex or years since menopause and IL-6 for changes in BMD were determined, although the associations between IL-6, change in IL-6, and change in total body BMD again appeared more evident in women more than or

TABLE 1. Characteristics of participants

	Total	Males	Females
No. of baseline variables	193	100	93
Age (yr)	62.6 (7.1)	63.3 (7.2)	61.9 (6.9)
Height (cm)	167.6 (9.2)	174.2 (6.3)	160.6 (6.2)
Weight (kg)	77.5 (14.1)	82.7 (13.7)	72.0 (12.5)
BMI (kg/m^2)	27.6 (4.4)	27.2 (3.9)	28.0 (4.9)
Current smokers (%)	15	17	13
RA (%)	10.9	8.0	14.4
Asthma (%)	14.8	9.2	20.9
Cardiovascular diseases (%)	4.2	7.1	1.1
Diabetes (%)	5.8	3.1	8.8
Occupation-related vigorous PA (%)	48.6	55.6	41.2
Recreational vigorous PA (%)	39.6	32.9	46.4
Use of COX-2 inhibitors (%)	9.9	10.0	9.8
Use of NSAIDs (%)	7.3	5.0	9.8
Use of prednisolone (%)	2.1	2.0	2.2
Menopause (%)			95
Total body BMD	1.08 (0.17)	1.15 (0.19)	1.02 (0.11)
Spine BMD	1.02 (0.20)	1.07 (0.22)	0.96 (0.14)
Hip BMD	0.97 (0.18)	1.03 (0.18)	0.89 (0.16)
Total body T score	−0.61 (1.12)	0.02 (0.12)	−0.93 (1.26)
Spine T score	−0.43 (1.63)	−0.11 (1.83)	−0.77 (1.30)
Hip T score	−0.18 (1.16)	−0.01 (1.24)	−0.35 (1.04)
PYR/Cr ratio (μmol/mmol)	3.96 (3.05,5.09)	3.36 (2.67,4.14)	4.65 (3.60,5.81)
Hs-CRP (mg/liter)	2.25 (1.10,4.24)	1.89 (1.02,3.64)	2.50 (1.12,4.85)
IL-6 (pg/ml)	2.89 (2.08,4.00)	3.06 (2.10,4.05)	2.68 (2.06,3.98)
TNF (pg/ml)	7.20 (5.05,11.62)	6.95 (4.70,11.65)	7.35 (5.55,11.18)
No. of changes over 2.9 yr, pa	168	88	80
Change in total body BMD (%)	−0.15 (2.04)	0.004 (2.35)	−0.31 (1.66)
Change in spine BMD (%)	−0.15 (1.53)	0.09 (1.38)	−0.40 (1.65)
Change in hip BMD (%)	−0.34 (1.44)	−0.44 (1.40)	−0.23 (1.47)
Change in PYR/Cr (μmol/mmol)	0.27 (−0.06,0.27)	0.23 (−0.01,0.50)	0.32 (−0.09,0.92)
Change in hs-CRP (mg/liter)	−0.003 (−0.51,0.15)	−0.00 (−0.30,0.24)	−0.03 (−0.82,0.13)
Change in IL-6 (pg/ml)	+0.03 (−0.29,0.31)	0.03 (−0.33,0.34)	0.02 (−0.24,0.29)
Change in TNF-α (pg/ml)	−0.16 (−1.26,0.76)	−0.16 (−1.55,0.76)	−0.16 (−1.20,0.65)

Mean (SD) or median (interquartile range) except for percentages. COX, Cyclooxygenase; NSAIDs, nonsteroidal antiinflammatory drugs; PA, physical activity; pa, per annum.

TABLE 2. Associations between inflammatory markers and change in BMD

	Multivariable[a] β (95% CI) (% pa)	Multivariable[b] β (95% CI) (% pa)	Multivariable[c] β (95% CI) (% pa)
Total body BMD change			
Baseline hs-CRP	**−0.15 (−0.26, −0.04)**	**−0.17 (−0.28, −0.06)**	−0.12 (−0.24, +0.004)[d]
Change in hs-CRP	**−0.36 (−0.63, −0.11)**	**−0.41 (−0.68, −0.15)**	**−0.30 (−0.59, −0.02)**[d]
Baseline IL-6	**−0.22 (−0.37, −0.06)**	**−0.25 (−0.41, −0.09)**	**−0.23 (−0.42, −0.05)**[e]
Change in IL-6	**−0.55 (−0.93, −0.16)**	**−0.62 (−1.01, −0.23)**	**−0.48 (−0.93, −0.03)**[e]
TNF-α	−0.07 (−0.14, +0.01)	−0.06 (−0.14, +0.01)	−0.03 (−0.11, +0.05)[d]
Change in TNF- α	−0.13 (−0.35, +0.09)	−0.11 (−0.32, +0.11)	−0.02 (−0.24, +0.20)[d]
Spine BMD change			
Baseline hs-CRP	−0.002 (−0.11, +0.10)	−0.01 (−0.12, +0.10)	+0.04 (−0.07, +0.15)[d]
Change in hs-CRP	−0.12 (−0.36, +0.12)	−0.15 (−0.40, +0.10)	+0.02 (−0.25, +0.28)[d]
Baseline IL-6	−0.10 (−0.24, +0.04)	**−0.16 (−0.31, −0.01)**	−0.15 (−0.33, +0.03)[e]
Change in IL-6	**−0.51 (−0.86, −0.17)**	**−0.64 (−1.00, −0.28)**	**−0.69 (−1.12, −0.25)**[e]
TNF-α	**−0.07 (−0.14, −0.00)**	−0.05 (−0.12, +0.02)	−0.03 (−0.10, +0.04)[d]
Change in TNF- α	−0.04 (−0.23, +0.15)	−0.004 (−0.20, +0.19)	+0.11 (−0.09, +0.30)[d]
Hip BMD change			
Baseline hs-CRP	+0.07 (−0.03, +0.17)	+0.03 (−0.07, +0.14)	+0.07 (−0.04, +0.18)[d]
Change in hs-CRP	+0.07 (−0.16, +0.30)	−0.04 (−0.28, +0.20)	+0.02 (−0.25, +0.28)[d]
Baseline IL-6	−0.08 (−0.23, +0.07)	−0.13 (−0.29, +0.05)	**−0.19 (−0.37, −0.01)**[e]
Change in IL-6	−0.12 (−0.48, +0.24)	−0.32 (−0.68, +0.05)	−0.37 (−0.82, +0.07)[e]
TNF-α	−0.04 (−0.11, +0.03)	−0.02 (−0.09, +0.05)	-0.004 (−0.08, +0.07)[d]
Change in TNF- α	−0.01(−0.21, +0.18)	+0.04 (−0.15, +0.24)	+0.10 (−0.11, +0.30)[d]

Dependent variable: percentage change in BMD per annum (pa). Independent variable: an inflammatory marker or its change per unit. *Bold* denotes statistically significant result. CI, Confidence interval.

[a] Adjusted for sex, baseline age, body weight, height, smoking status, BMD, and the baseline marker or change in the marker where appropriate.

[b] Further adjusted for RA, asthma, cardiovascular disease, and diabetes.

[c] Further adjusted for: [d] IL-6 and its change; [e] hs-CRP, TNF-α , and their changes.

equal to 10 yr past menopause (r = −0.53, $P = 0.003$; and r = −0.50, $P = 0.002$, respectively). With regard to bone turnover, IL-6 and its change were not significantly associated with change in the PYR/Cr ratio in the whole sample (data not shown); however, both were significantly associated with change in the PYR/Cr ratio in women after further adjustment for years since menopause (Table 3). The change in odds ratio after further adjustment is due to the small number of subjects with diabetes (n = 7) who were well away from menopause.

The associations between baseline TNF-α and change in total body or spine BMD were of borderline significance (Table 2), but when quartiles of baseline TNF-α were used for analysis, it was significantly associated with change in total body BMD (β = −0.31% per quartile; $P = 0.035$) and change in spine BMD (β = −0.29% per quartile; $P = 0.024$). These associations became of borderline significance after further adjustment for IL-6 ($P = 0.102$ and $P = 0.078$, respectively) but remained unchanged after adjustment for hs-CRP. There was a significant interaction between sex and baseline TNF-α for change in spine BMD (men had greater bone loss; $P = 0.039$), but not total body BMD. Furthermore, we found that TNF-α and its change were not significantly associated with change in the PYR/Cr ratio in men (data not shown) and women (Table 3).

If data were considered as quartiles, then the highest quartile of baseline CRP (>4.2 mg/liter) or IL-6 (>4 pg/ml) was associated with a significantly greater loss of BMD compared with the lowest three quartiles (data not shown). Results remained largely unchanged if subjects with RA, coronary heart diseases, diabetes,

TABLE 3. Associations between inflammatory markers and change in the PYR/Cr ratio in women

	Multivariable[a] OR (95% CI)	Multivariable[b] OR (95% CI)	Multivariable[c] OR (95% CI)
Baseline hs-CRP	1.19 (0.95, 1.49)	1.27 (0.98, 1.64)	**1.66 (1.11, 2.47)**
Change in hs-CRP	1.25 (0.79, 1.98)	1.38 (0.81, 2.36)	**2.12 (1.00, 4.50)**
Baseline IL-6	**1.81 (1.15, 2.84)**	**1.88 (1.14, 3.09)**	**2.80 (1.27, 6.19)**
Change in IL-6	3.01 (0.89, 10.16)	3.84 (0.94, 15.66)	**13.87 (1.32, 145.75)**
Baseline TNF-α	1.02 (0.88, 1.19)	1.04 (0.89, 1.21)	1.08 (0.91, 1.29)
Change in TNF- α	1.14 (0.73, 1.79)	1.20 (0.76, 1.89)	1.32 (0.76, 2.29)

Dependent variable: change in the PYR/Cr ratio (the highest quartile *vs*. other three quartiles). Independent variable: an inflammatory marker or its change per unit. *Bold* denotes statistically significant result. CI, Confidence interval; OR, odds ratio.

[a] Adjusted for baseline age, body weight, height, smoking status, the PYR/Cr ratio, and the baseline marker or change in the marker where appropriate.

[b] Further adjusted for RA, asthma, cardiovascular disease, and diabetes.

[c] Further adjustment for years since menopause.

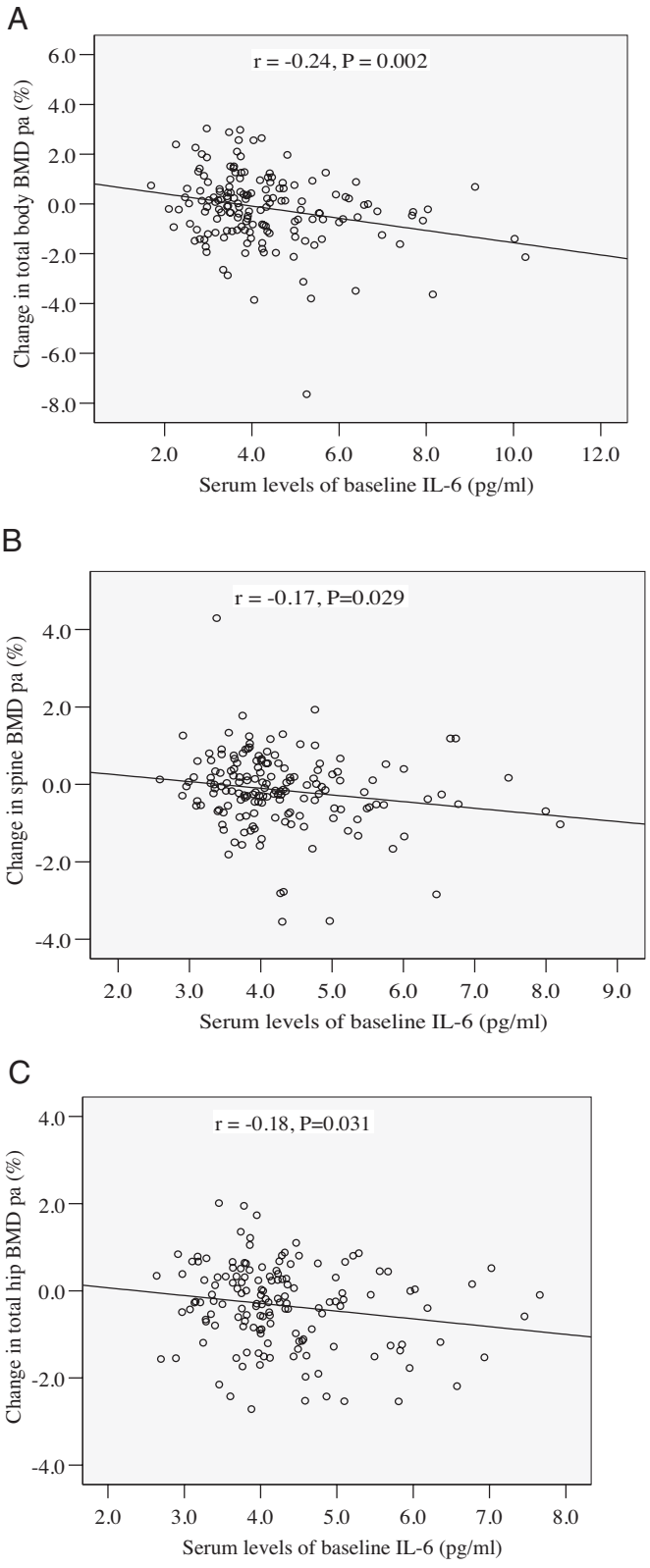

FIG. 1. Associations between IL-6 at baseline and change in total body (*top*), spine (*middle*), and total hip (*bottom*) BMD over 2.9 yr in older subjects. Residuals from regression of baseline IL-6 or change in BMD on factors (including gender, age, body weight, height, smoking status, BMD, change in IL-6, disease status, and hs-CRP for change in hip BMD) were added to the mean baseline IL-6 or change in BMD, and then the "adjusted" baseline IL-6 against "adjusted" change in BMD was plotted. pa, Per annum.

or asthma were excluded for analyses (data not shown), except that the interaction between sex and change in IL-6 for change in total body BMD became significant ($P = 0.042$). They also remained largely unchanged after further adjustment for medication uses, urinary Cr levels, and physical activity (data not shown). HRT use and years since menopause were not significantly associated with IL-6 and its change. The associations between bone loss and inflammatory markers remained largely unchanged after adjustment for HRT use and years since menopause.

Discussion

This longitudinal study is the first to document comprehensively the associations between baseline inflammatory markers, change in inflammatory markers, and bone loss/resorption in older adults. The most consistent associations were for IL-6 in both older women (especially those greater than 10 yr postmenopausal) and men, with much less consistency for hs-CRP and TNF-α, especially after adjustment for IL-6, suggesting that IL-6 is the most relevant inflammatory marker and may have a causal association with bone loss.

It is well demonstrated by animal and *in vitro* studies that IL-6 is responsible for osteoclastogenesis and increased trabecular bone resorption after the loss of sex steroids due to menopause or andropause (3, 4, 6). In this longitudinal study of a randomly selected older population with low-grade systemic inflammation and low prevalence of osteoporosis and other diseases, IL-6 was consistently associated with markers of bone health (change in BMD at all sites and bone resorption). In turn, IL-6 was associated with age consistent with other studies (4) but not other factors of interest such as BMI, and aging related hip bone loss appeared at least partially mediated by an increase in IL-6. Larger studies will be required to confirm these results for fracture. So far, the literature on the role of IL-6 in bone loss and osteoporosis in humans is conflicting. A cross-sectional study reported that serum IL-6 was negatively associated with BMD at radius ultradistal in postmenopausal women in an unadjusted analysis (10), whereas other cross-sectional studies have reported no significant associations in women (8, 9, 11). Longitudinally, a German group has reported that serum IL-6 was a significant predictor of femoral (not lumbar spine) bone loss in women in the early postmenopausal period (13), whereas a Danish group reported that serum IL-6 was associated with an increase in lumbar spine BMD over 5 yr in perimenopausal women (12). In contrast, we measured serum IL-6 levels on two occasions, and found that both baseline IL-6 and change in IL-6 were consistently associated with bone loss at all sites.

Like IL-6, data derived from animal and *in vitro* studies also support a role for TNF-α as a skeletal catabolic agent that stimulates osteoclastogenesis while simultaneously inhibiting osteoblast function after the loss of gonadal function (5, 7). However, there are few data to document the similar role of TNF-α in humans. Although Zheng *et al.* (21) reported that TNF-α produced by stimulated whole blood cells was negatively associated with lumbar spine BMD in postmenopausal women, Salamone

et al. (22) reported that TNF-α produced by peripheral blood mononuclear cells was not associated with change in BMD at the lumbar spine and femoral neck in premenopausal women. So far, there are no data to show associations between serum TNF-α and bone loss in women or men, though Scheidt-Nave *et al.* (13) reported that serum TNF-α was not associated with change in BMD in postmenopausal women. In this study we found that serum levels of TNF-α were associated with bone loss (total body in the whole sample and lumbar spine in men). However, change in TNF-α was associated with change in IL-6, and the associations between TNF-α and bone loss decreased in magnitude after adjustment for IL-6, suggesting that the effects of TNF-α are not independent of IL-6. However, we found that TNF-α was not associated with bone resorption.

In this sample, change in hs-CRP correlated significantly with change in IL-6, but not TNF-α, consistent with CRP production being stimulated by IL-6 (23). We further determined the significant associations between hs-CRP, bone loss, and bone resorption (PYR/Cr ratio) (the latter mainly in late postmenopausal women). These associations paralleled with those between IL-6 and bone measures, but not those between TNF-α and bone measures. They also decreased in magnitude after further adjustment for IL-6. These suggest that IL-6 both increases hs-CRP and influences bone loss/resorption, and that hs-CRP has no independent effect. They may also be related to other factors because change of hs-CRP was still significantly associated with change in total body BMD after adjustment for IL-6. It is unknown if hs-CRP itself is a pivotal mediator of bone loss, similar to its role in atherosclerosis (23)

This study has important implications. First, it provides direct evidence that bone loss in older adults involves a low-grade inflammatory process. Second, a CRP (>4.2 mg/liter) and IL-6 (>4 pg/ml) may be used to identify the groups that have rapid bone loss in older people. Finally, given that HRT is not recommended for the prevention and treatment of osteoporosis in older women due to cardiovascular side effects (24, 25), and bisphosphonates do not prevent fracture in subjects with normal bone density (26), targeted antiinflammatory therapies particularly anti-IL-6 therapy (27) may be suitable alternative interventions. So far, anti-TNF-α therapy has been beneficial to arrest bone loss in patients with RA (28), spondyloarthropathy (29), and Crohn's disease (30), but it is unclear if this is due to an effect on the disease or directly on bone.

The strengths of the present study lie in the measurements of inflammatory markers on two occasions, which allowed us to assess the variation in these markers over time. Variation in inflammatory markers may be less affected by natural antagonists in the serum than cross-sectional levels of these markers. We assessed bone loss at not only total hip and lumbar spine, but also total body, and consistent associations were found for IL-6. Our study has several potential limitations. First, the sample size was small, which does not allow examination of weak associations for bone markers and does not provide a large enough sample for fracture. Previous studies reported that serum hs-CRP level was a significant predictor of osteoporotic fracture (18, 19). A recent study suggested that higher levels of serum IL-6 and TNF-α may be associated (not significant) with an increased risk of fracture in older men and women (31). Second, the response rate at baseline was 57%, possibly due to an extensive protocol that takes 3 h at each visit. This did leave the possibility open for selection bias, which might be a reason for the high prevalence of RA. However, whereas the sample contained subjects with some diseases, the results were largely unchanged when the analyses were adjusted for disease status, or these subjects were excluded. We also tend to have high rates of retention (82%) to offset this. Third, we did not measure the bone formation markers and serum estrogen levels, so we cannot determine the role of bone formation and estrogen in the links between inflammatory markers and bone loss in this study. Fourth, the period of 2.9-yr follow-up is not a long time, but this study is currently in phase 3 (5-yr follow-up), thus data will be available over a longer period of time to confirm these associations. Finally, measurement error may influence results. However, all measures were highly reproducible, suggesting that this is unlikely.

In conclusion, this longitudinal study documents that variation within the low levels of inflammatory markers observed in this study, especially IL-6, predicts bone loss and resorption, suggesting that targeted antiinflammatory therapy may have potential for the prevention of osteoporosis.

Acknowledgments

We thank the subjects who made this study possible, Catrina Boon and Pip Boon for their role in collecting the data, and Dr. Steve Quinn for statistical support.

Address all correspondence and requests for reprints to: Dr. Changhai Ding, Menzies Research Institute, Private Bag 23, Hobart, Tasmania 7000, Australia. E-mail: changhai.ding@utas.edu.au.

This work was supported by the National Health and Medical Research Council of Australia, Tasmanian Community Fund, Arthritis Foundation of Australia, and University of Tasmania Grant-Institutional Research Scheme.

C.D. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure Information: The authors have nothing to declare.

References

1. **Sambrook P, Cooper C** 2006 Osteoporosis. Lancet 367:2010–2018
2. **Raisz LG** 2005 Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 115:3318–3325
3. **Manolagas SC, Jilka RL** 1995 Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 332:305–311
4. **Ershler WB, Keller ET** 2000 Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 51:245–270
5. **Nanes MS** 2003 Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. Gene 321:1–15
6. **Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC** 1992 Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257:88–91
7. **Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR** 1986 Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature 319:516–518
8. **Khosla S, Peterson JM, Egan K, Jones JD, Riggs BL** 1994 Circulating cytokine levels in osteoporotic and normal women. J Clin Endocrinol Metab 79:707–711
9. **Kania DM, Binkley N, Checovich M, Havighurst T, Schilling M, Ershler WB**

1995 Elevated plasma levels of interleukin-6 in postmenopausal women do not correlate with bone density. J Am Geriatr Soc 43:236–239
10. **Papadopoulos NG, Georganas K, Skoutellas V, Konstantellos E, Lyritis GP** 1997 Correlation of interleukin-6 serum levels with bone density in postmenopausal women. Clin Rheumatol 16:162–165
11. **McKane WR, Khosla S, Peterson JM, Egan K, Riggs BL** 1994 Circulating levels of cytokines that modulate bone resorption: effects of age and menopause in women. J Bone Miner Res 9:1313–1318
12. **Abrahamsen B, Bonnevie-Nielsen V, Ebbesen EN, Gram J, Beck-Nielsen H** 2000 Cytokines and bone loss in a 5-year longitudinal study–hormone replacement therapy suppresses serum soluble interleukin-6 receptor and increases interleukin-1-receptor antagonist: the Danish Osteoporosis Prevention Study. J Bone Miner Res 15:1545–1554
13. **Scheidt-Nave C, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, Pfeilschifter J** 2001 Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab 86:2032–2042
14. **Ridker PM, Hennekens CH, Buring JE, Rifai N** 2000 C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836–843
15. **Moshage H** 1997 Cytokines and the hepatic acute phase response. J Pathol 181:257–266
16. **Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, Chung YE, Kim GS** 2005 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. Osteoporos Int 16:1263–1271
17. **Kim BJ, Yu YM, Kim EN, Chung YE, Koh JM, Kim GS** 2007 Relationship between serum hsCRP concentration and biochemical bone turnover markers in healthy pre- and postmenopausal women. Clin Endocrinol (Oxf) 67:152–158
18. **Pasco JA, Kotowicz MA, Henry MJ, Nicholson GC, Spilsbury HJ, Box JD, Schneider HG** 2006 High-sensitivity C-reactive protein and fracture risk in elderly women. JAMA 296:1353–1355
19. **Schett G, Kiechl S, Weger S, Pederiva A, Mayr A, Petrangeli M, Oberhollenzer F, Lorenzini R, Redlich K, Axmann R, Zwerina J, Willeit J** 2006 High-sensitivity C-reactive protein and risk of nontraumatic fractures in the Bruneck study. Arch Intern Med 166:2495–2501
20. **Jones G, Dwyer T, Hynes KL, Parameswaran V, Greenaway TM** 2005 Vitamin D insufficiency in adolescent males in Southern Tasmania: prevalence, determinants, and relationship to bone turnover markers. Osteoporos Int 16:636–641
21. **Zheng SX, Vrindts Y, Lopez M, De Groote D, Zangerle PF, Collette J, Franchimont N, Geenen V, Albert A, Reginster JY** 1997 Increase in cytokine production (IL-1 beta, IL-6, TNF-alpha but not IFN-gamma, GM-CSF or LIF) by stimulated whole blood cells in postmenopausal osteoporosis. Maturitas 26:63–71
22. **Salamone LM, Whiteside T, Friberg D, Epstein RS, Kuller LH, Cauley JA** 1998 Cytokine production and bone mineral density at the lumbar spine and femoral neck in premenopausal women. Calcif Tissue Int 63:466–470
23. **Labarrere CA, Zaloga GP** 2004 C-reactive protein: from innocent bystander to pivotal mediator of atherosclerosis. Am J Med 117:499–507
24. **Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R, Strickland OL, Wong ND, Crouse JR, Stein E, Cushman M, Women's Health Initiative Investigators** 2003 Estrogen plus progestin and the risk of coronary heart disease. N Engl J Med 349:523–534
25. **Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB, Women's Health Initiative Investigators** 2003 Effects of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA 290:1729–1738
26. **McClung MR, Geusens P, Miller PD, Zippel H, Bensen WG, Roux C, Adami S, Fogelman I, Diamond T, Eastell R, Meunier PJ, Reginster JY, Hip Intervention Program Study Group** 2001 Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. N Engl J Med 344:333–340
27. **Ding C, Jones G** 2006 Anti-interleukin-6 receptor antibody treatment in inflammatory autoimmune diseases. Rev Rec Clin Trials 1:193–200
28. **Vis M, Havaardsholm EA, Haugeberg G, Uhlig T, Voskuyl AE, van de Stadt RJ, Dijkmans BA, Woolf AD, Kvien TK, Lems WF** 2006 Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFkappaB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis. Ann Rheum Dis 65:1495–1499
29. **Allali F, Breban M, Porcher R, Maillefert JF, Dougados M, Roux C** 2003 Increase in bone mineral density of patients with spondyloarthropathy treated with anti-tumour necrosis factor alpha. Ann Rheum Dis 62:347–349
30. **Bernstein M, Irwin S, Greenberg GR** 2005 Maintenance infliximab treatment is associated with improved bone mineral density in Crohn's disease. Am J Gastroenterol 100:2031–2035
31. **Cauley JA, Danielson ME, Boudreau RM, Forrest KY, Zmuda JM, Pahor M, Tylavsky FA, Cummings SR, Harris TB, Newman AB, the Health ABC Study** 2007 Inflammatory markers and incident fracture risk in older men and women: the health aging and body composition study. J Bone Miner Res 22:1088–1095