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Association between Inflammatory Biomarkers and Bone Mineral Density in a Community-based Cohort of Men and Women: The Framingham Osteoporosis Study

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Abstract

OBJECTIVE—Based upon evidence in animal and in-vitro studies, we tested the hypothesis that higher serum concentrations of the cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and the inflammatory marker C-reactive protein (CRP) would be inversely associated with BMD in a community-based cohort of men and women, with the strongest associations among post-menopausal women not using menopausal hormone therapy (MHT).

METHODS—We ascertained fasting serum concentrations of IL-6, TNF-α, and CRP and measured BMD at the femoral neck, trochanter, total femur, and spine (L2-L4) using dual energy X-ray absorptiometry in 2,915 members of the Framingham Offspring cohort (1996 to 2001). We used multivariable linear regression to estimate the difference (β) in BMD at each bone site associated with a one-unit increase in log-transformed serum concentrations of IL-6, TNF-α, and CRP separately for men (n=1,293), pre-menopausal women (n=231), post-menopausal women using MHT (n=498), and post-menopausal women not using MHT (n=893).

RESULTS—Inflammatory biomarkers were not associated with BMD in men. Among premenopausal women, there were statistically significant, modest inverse associations between IL-6 and trochanter BMD (β=−0.030, p<0.01), and between CRP and femoral neck (β=−0.015, p=0.05) and trochanter BMD (β=−0.014, p=0.04). TNF-α, was positively associated with spine BMD (β=0.043, p=0.01). In post-menopausal MHT users, CRP was positively associated with

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femoral neck BMD (β=0.011, p=0.04). There were no associations among post-menopausal women not using MHT.

CONCLUSIONS—The lack of consistency in our results suggests that elevated circulating concentration of inflammatory biomarkers may not be a risk factor for low BMD.

Introduction

Osteoporosis, characterized by the progressive loss of bone mass leading to increased fracture risk, is among the most common rheumatic diseases. The lifetime risk for an osteoporotic fracture in the US is estimated at 39.7% for women and 13.1% for men (1). Osteoporosis-related fracture is associated with considerable morbidity (2) and mortality (3) in older adults. Given the anticipated increase in the proportion of older adults in the population over the next several decades, it is critical to identify modifiable risk factors to aid in the prevention and treatment of osteoporosis.

Animal and *in vitro* studies support the hypothesis that pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) influence the age- and estrogen-related decrease in bone mineral density (BMD). These immune factors have been shown to promote the proliferation, activity, and survival of osteoclasts while inhibiting survival of osteoblasts (4-7). IL-6 (8) and C-reactive protein (CRP) (9), an acute-phase protein used as a general marker of inflammation, are present at low levels in the blood of young adults, but rise with increasing age as BMD concurrently declines. Furthermore, estrogen status is an important determinant of inflammation among women. The decline of estrogen at menopause is associated with rapid increases in serum inflammatory markers and decreases in BMD (4, 6), while menopausal hormone therapy (MHT), promotes maintenance of BMD (10-11) and decreases levels of several inflammatory biomarkers (12). Thus, cytokines could be important mediators of the decline in BMD associated both with aging and estrogen deficiency.

In spite of this, epidemiologic studies of pro-inflammatory cytokines and BMD have produced mixed results. Associations with BMD have been reported for soluble IL-6 receptor (8) and CRP (13), however many other studies have not observed cross-sectional associations between serum concentrations of IL-6, TNF-α, or CRP and BMD (8, 14-17). Most of the studies conducted to this point have included only women (8, 13-15, 17-20), with particular focus on postmenopausal women (17-20). Thus, relatively little is known about associations between inflammatory markers and BMD in men or premenopausal women. We therefore investigated the associations of circulating concentrations of IL-6, TNF-α, and CRP with BMD of the hip and spine among middle-aged and older men and women of the community-based Framingham Offspring study. We hypothesized that serum concentrations of these biomarkers would be inversely associated with BMD. Because estrogen status may influence cytokine production, we further posited that the association would be strongest among those with the lowest estrogen status, particularly post-menopausal women not using menopausal hormone therapy.

Methods

Study population

The Framingham Offspring Study began in 1971 with the main objective of investigating the role of familial risk factors for coronary artery disease among the children of the Framingham Study Original Cohort and their spouses (21). The 5,124 Offspring participants who enrolled at baseline have returned at approximately 4-year intervals for extensive physical examinations, comprehensive questionnaires, anthropometric measurements, blood chemistries, and assessment of cardiovascular and other risk factors by trained clinical personnel. The current study is comprised of the 2,915 Offspring men and women who had BMD measures of the hip and spine obtained as part of the Framingham Osteoporosis Study from 1996 to 2001, and at least one of the following serum inflammatory biomarkers measured from 1998 to 2001: IL-6, TNF-α, high-sensitivity CRP (CRP). This study was approved by the institutional review boards at Hebrew SeniorLife and Boston University and informed consent was obtained from all participants.

Inflammatory Biomarkers

Fasting morning serum samples were collected and stored at or below −70°C. Serum concentrations of total (free+bound) IL-6 (pg/mL) and high-sensitivity total TNF-α (pg/mL) were measured by using commercially available enzyme-linked immunoassay kits (R&D Systems, Minneapolis, Minnesota). The averages of duplicate samples were used in the analysis. Serum CRP (mg/L) was measured once using a high-sensitivity assay (BN100 nephelometer, Dade Behring, Deerfield, Illinois). The intra-assay coefficients of variation (CV) for IL-6, TNF-α and CRP were 3.1%, 6.6%, and 3.2%, respectively. TNF-α concentrations were available only among a subset of 76% of participants.

Bone mineral density

BMD of the proximal right femur (femoral neck, trochanter, total femur) and the lumbar spine (average BMD of L2–L4) was measured in grams per square centimeter (g/cm^2) by a Lunar DPX-L (Lunar Radiation Corporation, Madison, WI, U.S.A.). Using standard positioning as recommended by the manufacturer, the right femur was scanned unless there was a history of previous fracture or hip joint replacement, in which case the left side was scanned. The CVs for the DPX-L were 1.7%, 2.5% and 0.9% for the femoral neck, trochanter and lumbar spine, respectively (22).

Other variables

Information on covariates was ascertained from measures collected at the time of BMD assessment and included sex, age (years), height (inches), body mass index (BMI, kg/m^2), physical activity level, current smoking status (yes/no), and, in women, current use of menopausal hormone therapy (yes/no) and menopause status (post-menopausal: yes/no). Height without shoes was measured to the nearest quarter inch with a stadiometer. Weight was measured to the nearest pound using a standard balance beam scale with participants in light clothing and no shoes. BMI was calculated as weight in kg divided by the square of height in meters. Physical activity level was assessed using the Physical Activity Scale for

the Elderly (PASE), a validated questionnaire of self-reported activity over the past seven days (23). Participants were classified as a current smoker if they reported regularly smoking cigarettes in the year prior to BMD measurement. Women were considered current menopausal hormone therapy users if they reported use (oral, patch or cream) at the time of the BMD assessment. Post-menopausal status at the time of BMD measurement was defined as having no menstrual periods for more than one year or current use of menopausal hormone therapy.

Statistical analysis

Distributions of IL-6, TNF-α, and CRP were right skewed and therefore logarithmically transformed prior to analyses. Because associations of IL-6, TNF-α, and CRP with BMD may be non-linear, inflammatory biomarkers were also categorized into quartiles. CRP was also categorized according to established clinical cut-points: low (<1 mg/L), borderline (1-3 mg/L), moderately high (3.01-10 mg/L), and markedly high concentrations (>10 mg/L) (24-26).

Analyses were first conducted within the whole cohort, and then separately within the following four groups of participants based on sex, menopause status and menopausal hormone therapy use: 1) men, 2) pre-menopausal women, 3) post-menopausal women using menopausal hormone therapy, and 4) post-menopausal women not using menopausal hormone therapy. For continuous biomarkers, multivariable linear regression was used to calculate the difference in BMD at each bone site (femoral neck, trochanter, total femur, lumbar spine) associated with a one unit increase in the log-transformed inflammatory biomarker. For quartiles and clinical CRP categories, least squares-adjusted mean BMD was compared among categories and a test for linear trend was calculated. Results of continuous, quartile and CRP clinical cut-point analyses indicated similar associations, thus only results using the continuous, log-transformed biomarkers are presented. Models were adjusted for covariates known to be important determinants of both BMD and biomarker concentration: sex (for whole cohort analyses only), age, height, BMI, physical activity and current smoking status. All statistical analyses were conducted using SAS/STAT software version 9.2 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the 2,915 men and women at the time of BMD assessment are described in Table 1. The mean age of the study population was 61 and ranged from 29 to 86. Among the 1,622 women, 1,391 (86%) were post-menopausal, and 36% of post-menopausal women were current users of menopausal hormone therapy. Post-menopausal women using menopausal hormone therapy were younger and more physically active than those not using menopausal hormone therapy. Median serum concentrations of IL-6 and TNF-α were greater in men compared to pre-menopausal women and post-menopausal women using menopausal hormone therapy, but similar to post-menopausal women not using menopausal hormone therapy. CRP was highest in post-menopausal women using menopausal hormone therapy, likely due to the known increase in serum CRP concentrations with oral menopausal hormone therapy use (27-28). As expected, BMD at all sites was highest among

men and decreased successively for pre-menopausal women, post-menopausal women using menopausal hormone therapy, and post-menopausal women not using menopausal hormone therapy.

In the whole study population, there were no statistically significant associations between log-transformed serum concentrations of IL-6, TNF-α and CRP and BMD at any site (data not shown). Among men, serum biomarkers were not associated with BMD at either the hip or spine as all regression coefficients were not significantly different from zero (Table 2). Among premenopausal women (Table 3), each unit increase in log-transformed serum IL-6 was associated with 0.030 g/cm^2 lower trochanter BMD ($p<0.01$). Similar associations were observed for the femoral neck (β=-0.023, p=0.07) and total femur (β=−0.19, p=0.12), yet they were not statistically significant. CRP was also negatively associated with trochanter BMD in premenopausal women (β-0.014, P=0.04), and there were borderline statistically significant associations with BMD at the femoral neck (β=−0.015, P=0.05) and total femur (β=−0.011, p=0.14). Neither IL-6 nor CRP was associated with lumbar spine BMD in pre-menopausal women. TNF-α, however, was positively associated with lumbar spine BMD (β=0.043, p=0.01) among pre-menopausal women, though not with hip BMD.

IL-6 and TNF-α were not associated with BMD at either the hip or the spine for post-menopausal women, regardless of menopausal hormone therapy use (Table 4). Among post-menopausal women not using menopausal hormone therapy, there was no association between CRP and BMD at any site. CRP was, however, positively associated with femoral neck BMD among those using menopausal hormone therapy (β=0.011, p=0.04). Positive associations were also seen for CRP at the trochanter and total femur, although they were not statistically significant.

Discussion

In this study of middle-aged and older men and women of the Framingham Offspring cohort, we found inverse relations of serum concentrations of IL-6 and CRP with hip BMD only in pre-menopausal, though the magnitudes of these associations were small. A one standard deviation decrease in BMD is associated with roughly double the risk of fracture, depending upon the BMD site and the type of fracture (29-31). The differences in BMD we observed in association with serum concentrations of inflammatory biomarkers represent small fractions of a standard deviation, and thus would be expected to have a negligible impact on fracture risk. Furthermore, we detected a positive association between TNF-α and lumbar spine BMD in premenopausal women, and between CRP and femoral neck BMD in post-menopausal women using menopausal hormone therapy. Therefore, our findings indicate that elevated circulating concentration of inflammatory biomarkers may not be an important risk factor for low BMD.

Associations between inflammatory biomarkers and BMD in prior epidemiologic studies have been inconsistent. Papadapoulos et al. found positive correlations between serum IL-6 levels and BMD at five of six bone sites measured in the study (18). Zheng et al. reported correlations between secretion of IL-1β, IL-6, and TNF-α by stimulated peripheral monocyte blood cells and lumbar spine BMD (32). Recently, de Pablo et al. found that BMD of the

total body, as well as sub-regions, decreased with increasing quintiles of serum CRP concentration (33). However, as in our study, several other analyses of markers of inflammation and BMD have failed to find any associations (8, 14-17). There are several possible reasons for these inconsistencies. First, signaling from cytokines such as IL-6 can have both inflammatory and anti-inflammatory effects (34-35). The effects of IL-6, TNF-α, and CRP on bone remodeling *in vivo* may therefore depend on the influence of other factors, such as other cytokines, receptors, and hormones not measured in these studies (7, 36). For example, both IL-6 (37) and TNF-α (38) signaling is influenced by the presence of soluble receptors. The soluble receptors for TNF-α have a longer half-life in serum than TNF-α itself (39), and their presence may relate to inflammation severity (38). Furthermore, soluble IL-6 receptor increases the half-life of IL-6, and studies in mice indicate that it may be required for the effect of IL-6 on osteoclastogenesis (40). Thus, the circulating concentrations of soluble receptors may be more relevant to bone than IL-6 and TNF-α themselves. Unfortunately, these measures were not available in our study.

Additionally, it may be that the local action of cytokines in the bone microenvironment, which is not easily measured in large epidemiologic studies, are more important for BMD than circulating, systemic concentrations. Nevertheless, serum and plasma measures of the cytokines examined in the current study have been linked to outcomes in several disease states, including rheumatoid arthritis (41-42), cardiovascular disease (43), and dementia (44), as well as in aging (45). Finally, sufficient variability in a single BMD measurement could obscure any effect of chronic, low level inflammation on bone mass, particularly if such an effect were to act slowly over a long period of time. Thus, chronic inflammation may be more relevant to the rate of bone loss than the absolute BMD value at a single time point. This is supported by two previous longitudinal studies. Ding et al. found direct relationships between IL-6, CRP, and TNF-α levels and three-year decreases in BMD, and also showed that IL-6 was the primary biomarker predictor of BMD in models containing all three cytokines (46). Furthermore, Scheidt-Nave et al. found that IL-6 predicted three-year decreases in total hip BMD in the first 10 years of menopause, but not thereafter (19). Additional longitudinal studies are required to help elucidate the relation between circulating concentrations of inflammatory cytokines and loss of BMD.

In the present study, there were statistically significant, though modest, inverse associations of IL-6 and CRP concentrations with BMD at the femoral neck and trochanter in pre-menopausal women only. This finding runs counter to our hypothesis that this association would be strongest in post-menopausal women not undergoing hormone replacement, who would be expected to have the lowest estrogen status. The reason for this discrepancy is unclear. However, Scheidt-Nave found that bone loss was greatest at the beginning of menopause, and that the association between IL-6 and bone loss was only apparent in the first decade after menopause. In addition, Slemenda et al. found that late peri-menopausal women (distinguished by irregular menses and elevated follicle stimulating hormone levels) had decreased estrogen concentrations compared to early peri-menopausal women, and were beginning to lose bone mass at the mid-radius (47). If our pre-menopausal group included a substantial number of perimenopausal women, these effects during the menopausal transition could explain our findings in this subgroup. Unfortunately our limited information

on classifying menopausal status precludes exploration of this possibility. Along the same lines, since our definition of post-menopausal women not taking estrogen did not account for time since menopause, the mixing of early and late post-menopausal women could have obscured an association between IL-6 and BMD in this group. We have no explanation for the observed positive association between TNF-α concentration and lumbar spine BMD in pre-menopausal women. However, we are, to the best of our knowledge, the first to examine associations between circulating inflammatory cytokine levels in a large group of pre-menopausal women, and our observations require confirmation in other studies.

Also contrary to our hypothesis, we observed a modest yet positive association between CRP concentration and femoral neck BMD in post-menopausal women using menopausal hormone therapy. There were similar, though not significant, associations between CRP and the other hip sites. CRP is an acute-phase protein produced in the liver and regulated, in part, by IL-6, and TNF-α (48). Although often used as a marker for systemic inflammation, low-level increases in CRP may be seen with non-inflammatory conditions (49) and with exogenous estrogen treatments (27-28). In contrast, menopausal hormone therapy has been reported to decrease TNF-α levels, though its relation with IL-6 is not clear (12). Indeed, in our study post-menopausal women taking menopausal hormone therapy had the highest median concentration of CRP. Since menopausal hormone therapy is associated with reduced bone loss, our finding of a positive association between CRP and BMD in this group is not surprising. Our study is, to our knowledge, among the first to examine the relation of circulating inflammatory biomarkers with BMD among men. While men have generally higher BMD than women, the incidence of osteoporosis in men is not trivial (50). Thus, it is important to find factors that may be useful for identifying men at the highest risk for low BMD. Although women have a higher overall inflammatory burden, this gap narrows with aging as men tend to have an accelerated rate of increase in inflammation (51). While inflammatory biomarkers are a potential tool for distinguishing men at high risk for low BMD, we did not find evidence to support this. If such a relation exists, it may be more easily identified longitudinally than in a cross-sectional study such as ours.

Among the limitations of this study is its cross-sectional design, which precludes the ability to establish causal relationships between serum inflammatory biomarker levels and BMD. We also measured serum biomarkers at a single point in time. Such measurements may not accurately reflect the long-term level of inflammation in an individual. Nevertheless, studies of the variability of repeated CRP measurements over time have concluded that they are relatively stable and comparable to measures of total cholesterol (52-54). Repeated measurements of TNF-α and IL-6 are likewise reported to be stable (52, 55), although one group concluded a single measurement of IL-6 was not a sufficiently accurate measure of an individual's inflammatory status (55). Our observations about the effect of estrogen were based on groups that would be expected to differ by estrogen status, rather than on specific biomarkers of sex hormones. We cannot exclude the possibility that unmeasured confounding impacted our results. A major strength of this study is the use of data from the Framingham Offspring Cohort. This well-characterized cohort allowed us the opportunity to observe associations between inflammatory biomarkers and BMD in substantial numbers of middle-aged and older adults expected to differ by estrogen status. We also had information on numerous potential confounders of the association of interest. Yet because the

Framingham Offspring cohort is primarily Caucasian, the generalizability of our results to other racial and ethnic groups is limited.

In conclusion, our results suggest that circulating concentrations of the inflammatory biomarkers IL-6, TNF-α and CRP may not be useful for identifying individuals at risk for low BMD. Despite our findings, there remains strong biological evidence that inflammation contributes to poor bone health, and circulating biomarkers have been associated with increased fracture risk in cohort studies (56-57). Further large, epidemiologic studies, preferably longitudinal and with direct measurement of estrogen, are needed to establish a definitive relation between inflammatory biomarkers and bone health, and whether bone loss is the causal link between inflammation and fracture. Other bone parameters that determine bone strength, such as bone structure and microarchitecture, that are not captured by two-dimensional BMD measures should also be explored. Additionally, more work is needed to develop tools that can assess inflammation at the bone microenvironment level and that are practical for implementation in large clinical studies. Confirmation of the hypothesized relation between inflammation and bone health could have important implications for the both the prediction and treatment of low bone mass, as such findings would potentially support the use of inflammatory biomarkers as tools to identify those at risk for low BMD, and espouse the development of anti-inflammatory interventions as a means to maintain bone health.

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References

1. Johnell O, Kanis J. Epidemiology of osteoporotic fractures. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2005; 16(Suppl 2):S3–7.
2. Johnell O, Kanis JA. An estimate of the worldwide prevalence, mortality and disability associated with hip fracture. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2004; 15(11):897–902.
3. Johnell O, Kanis JA, Oden A, Sernbo I, Redlund-Johnell I, Petterson C, et al. Mortality after osteoporotic fractures. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2004; 15(1):38–42.
4. Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. J Bone Miner Res. 1996; 11(8):1043–51. [PubMed: 8854239]

5. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine reviews. 2000; 21(2):115–37. [PubMed: 10782361]
6. Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. Endocrine reviews. 2002; 23(3):279–302. [PubMed: 12050121]
7. Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. Immunol Rev. 2005; 208:207–27. [PubMed: 16313351]
8. Giuliani N, Sansoni P, Girasole G, Vescovini R, Passeri G, Passeri M, et al. Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Exp Gerontol. 2001; 36(3):547–57. [PubMed: 11250125]
9. Woloshin S, Schwartz LM. Distribution of C-reactive protein values in the United States. N Engl J Med. 2005; 352(15):1611–3. [PubMed: 15829550]
10. Prince RL, Smith M, Dick IM, Price RI, Webb PG, Henderson NK, et al. Prevention of postmenopausal osteoporosis. A comparative study of exercise, calcium supplementation, and hormone-replacement therapy. N Engl J Med. 1991; 325(17):1189–95. [PubMed: 1922205]
11. Hasling C, Charles P, Jensen FT, Mosekilde L. A comparison of the effects of oestrogen/progestogen, high-dose oral calcium, intermittent cyclic etidronate and an ADFR regime on calcium kinetics and bone mass in postmenopausal women with spinal osteoporosis. Osteoporos Int. 1994; 4(4):191–203. [PubMed: 7949749]
12. Miller AP, Chen YF, Xing D, Feng W, Oparil S. Hormone replacement therapy and inflammation: interactions in cardiovascular disease. Hypertension. 2003; 42(4):657–63. [PubMed: 12913055]
13. Koh JM, Khang YH, Jung CH, Bae S, Kim DJ, Chung YE, et al. 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. 2005; 16(10):1263–71. [PubMed: 15702263]
14. Khosla S, Peterson JM, Egan K, Jones JD, Riggs BL. Circulating cytokine levels in osteoporotic and normal women. J Clin Endocrinol Metab. 1994; 79(3):707–11. [PubMed: 8077350]
15. McKane WR, Khosla S, Peterson JM, Egan K, Riggs BL. Circulating levels of cytokines that modulate bone resorption: effects of age and menopause in women. J Bone Miner Res. 1994; 9(8):1313–8. [PubMed: 7976512]
16. Kania DM, Binkley N, Checovich M, Havighurst T, Schilling M, Ershler WB. Elevated plasma levels of interleukin-6 in postmenopausal women do not correlate with bone density. J Am Geriatr Soc. 1995; 43(3):236–9. [PubMed: 7884109]
17. Ganesan K, Teklehaimanot S, Tran TH, Asuncion M, Norris K. Relationship of C-reactive protein and bone mineral density in community-dwelling elderly females. J Natl Med Assoc. 2005; 97(3):329–33. [PubMed: 15779496]
18. Papadopoulos NG, Georganas K, Skoutellas V, Konstantellos E, Lyritis GP. Correlation of interleukin-6 serum levels with bone density in postmenopausal women. Clin Rheumatol. 1997; 16(2):162–5. [PubMed: 9093798]
19. Scheidt-Nave C, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, et al. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab. 2001; 86(5):2032–42. [PubMed: 11344203]
20. Pasco JA, Kotowicz MA, Henry MJ, Nicholson GC, Spilsbury HJ, Box JD, et al. High-sensitivity C-reactive protein and fracture risk in elderly women. JAMA. 2006; 296(11):1353–5. [PubMed: 16985226]
21. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol. 1979; 110(3):281–90. [PubMed: 474565]
22. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PW, et al. Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. 2000; 15(4):710–20. [PubMed: 10780863]
23. Washburn RA, McAuley E, Katula J, Mihalko SL, Boileau RA. The physical activity scale for the elderly (PASE): evidence for validity. J Clin Epidemiol. 1999; 52(7):643–51. [PubMed: 10391658]

24. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003; 107(3):499–511. [PubMed: 12551878]
25. Ridker PM, Cook N. Clinical usefulness of very high and very low levels of C-reactive protein across the full range of Framingham Risk Scores. Circulation. 2004; 109(16):1955–9. [PubMed: 15051634]
26. Dhingra R, Gona P, Nam BH, D'Agostino RB Sr. Wilson PW, Benjamin EJ, et al. C-reactive protein, inflammatory conditions, and cardiovascular disease risk. Am J Med. 2007; 120(12): 1054–62. [PubMed: 18060926]
27. Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE. Hormone replacement therapy and increased plasma concentration of C-reactive protein. Circulation. 1999; 100(7):713–6. [PubMed: 10449692]
28. Cushman M, Legault C, Barrett-Connor E, Stefanick ML, Kessler C, Judd HL, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/ Progestin Interventions (PEPI) Study. Circulation. 1999; 100(7):717–22. [PubMed: 10449693]
29. Riis BJ, Hansen MA, Jensen AM, Overgaard K, Christiansen C. Low bone mass and fast rate of bone loss at menopause: equal risk factors for future fracture: a 15-year follow-up study. Bone. 1996; 19(1):9–12. [PubMed: 8830981]
30. Nguyen TV, Center JR, Eisman JA. Femoral neck bone loss predicts fracture risk independent of baseline BMD. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2005; 20(7):1195–201.
31. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2005; 20(7):1185–94.
32. Zheng SX, Vrindts Y, Lopez M, De Groote D, Zangerle PF, Collette J, et al. 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. 1997; 26(1):63–71. [PubMed: 9032749]
33. de Pablo P, Cooper MS, Buckley CD. Association between bone mineral density and C-reactive protein in a large population-based sample. Arthritis Rheum. 2012; 64(8):2624–31. [PubMed: 22487938]
34. Fattori E, Cappelletti M, Costa P, Sellitto C, Cantoni L, Carelli M, et al. Defective inflammatory response in interleukin 6-deficient mice. The Journal of experimental medicine. 1994; 180(4): 1243–50. [PubMed: 7931061]
35. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF, et al. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. The Journal of clinical investigation. 1998; 101(2):311–20. [PubMed: 9435302]
36. Jilka RL. Cytokines, bone remodeling, and estrogen deficiency: a 1998 update. Bone. 1998; 23(2): 75–81. [PubMed: 9701464]
37. Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. Journal of immunology. 2005; 175(6):3463–8.
38. Van Zee KJ, Kohno T, Fischer E, Rock CS, Moldawer LL, Lowry SF. Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor alpha in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89(11):4845–9. [PubMed: 1317575]
39. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, et al. Variation in serum levels of the soluble TNF receptors among healthy individuals. Lymphokine and cytokine research. 1992; 11(3):157–9. [PubMed: 1327192]
40. Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90(24):11924–8. [PubMed: 8265649]

41. Altomonte L, Zoli A, Mirone L, Scolieri P, Magaro M. Serum levels of interleukin-1b, tumour necrosis factor-a and interleukin-2 in rheumatoid arthritis. Correlation with disease activity. Clinical rheumatology. 1992; 11(2):202–5. [PubMed: 1617893]
42. Madhok R, Crilly A, Watson J, Capell HA. Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. Annals of the rheumatic diseases. 1993; 52(3):232–4. [PubMed: 8484679]
43. Vasan RS, Sullivan LM, Roubenoff R, Dinarello CA, Harris T, Benjamin EJ, et al. Inflammatory markers and risk of heart failure in elderly subjects without prior myocardial infarction: the Framingham Heart Study. Circulation. 2003; 107(11):1486–91. [PubMed: 12654604]
44. Paganelli R, Di Iorio A, Patricelli L, Ripani F, Sparvieri E, Faricelli R, et al. Proinflammatory cytokines in sera of elderly patients with dementia: levels in vascular injury are higher than those of mild-moderate Alzheimer's disease patients. Experimental gerontology. 2002; 37(2-3):257–63. [PubMed: 11772511]
45. Roubenoff R, Harris TB, Abad LW, Wilson PW, Dallal GE, Dinarello CA. Monocyte cytokine production in an elderly population: effect of age and inflammation. The journals of gerontology Series A, Biological sciences and medical sciences. 1998; 53(1):M20–6.
46. Ding C, Parameswaran V, Udayan R, Burgess J, Jones G. Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. J Clin Endocrinol Metab. 2008; 93(5):1952–8. [PubMed: 18285417]
47. Slemenda C, Hui SL, Longcope C, Johnston CC. Sex steroids and bone mass. A study of changes about the time of menopause. J Clin Invest. 1987; 80(5):1261–9. [PubMed: 3500182]
48. Moshage H. Cytokines and the hepatic acute phase response. J Pathol. 1997; 181(3):257–66. [PubMed: 9155709]
49. Kushner I, Rzewnicki D, Samols D. What does minor elevation of C-reactive protein signify? Am J Med. 2006; 119(2):166, e17–28. [PubMed: 16443421]
50. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2007; 22(3):465–75.
51. Yang Y, Kozloski M. Sex differences in age trajectories of physiological dysregulation: inflammation, metabolic syndrome, and allostatic load. J Gerontol A Biol Sci Med Sci. 2011; 66(5):493–500. [PubMed: 21350248]
52. Lee SA, Kallianpur A, Xiang YB, Wen W, Cai Q, Liu D, et al. Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. Cancer Epidemiol Biomarkers Prev. 2007; 16(11):2464–70. [PubMed: 18006938]
53. Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem. 2001; 47(3):444–50. [PubMed: 11238295]
54. Chen TH, Gona P, Sutherland PA, Benjamin EJ, Wilson PW, Larson MG, et al. Long-term C-reactive protein variability and prediction of metabolic risk. Am J Med. 2009; 122(1):53–61. [PubMed: 19114172]
55. Cava F, Gonzalez C, Pascual MJ, Navajo JA, Gonzalez-Buitrago JM. Biological variation of interleukin 6 (IL-6) and soluble interleukin 2 receptor (sIL2R) in serum of healthy individuals. Cytokine. 2000; 12(9):1423–5. [PubMed: 10976007]
56. Cauley JA, Danielson ME, Boudreau RM, Forrest KY, Zmuda JM, Pahor M, et al. Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study. J Bone Miner Res. 2007; 22(7):1088–95. [PubMed: 17419681]
57. Barbour KE, Boudreau R, Danielson ME, Youk AO, Wactawski-Wende J, Greep NC, et al. Inflammatory markers and the risk of hip fracture: the Women's Health Initiative. J Bone Miner Res. 2012; 27(5):1167–76. [PubMed: 22392817]

Significance and Innovation

- Increased activity of pro-inflammatory cytokines may be a risk factor for low bone mineral density (BMD) in the general community, yet epidemiologic evidence is inconsistent.
- While prior studies of the relation between circulating inflammatory biomarkers and bone mineral density (BMD) focused primarily on post-menopausal women, we examined different subgroups of estrogen status defined by menopause and use of menopausal hormone therapy (MHT), as well as men.
- Serum concentrations of inflammatory biomarkers were inversely related to hip BMD in premenopausal women, and directly related to spine BMD in premenopausal women and hip BMD in post-menopausal women using MHT, though associations were weak, and no relations were observed for men or postmenopausal women using MHT.
- Findings from this cross-sectional study indicate that serum inflammatory biomarkers may not be useful for identifying those at risk for low BMD, though this should be confirmed with longitudinal studies of bone loss.

Table 1

Descriptive characteristics of men and women in the Framingham Offspring Study with inflammatory biomarkers (1998-2001) and hip or spine BMD (1996-2001).

Characteristic*	Men	Premenopausal women	Post-menopausal women	
			Using menopausal hormone therapy	Not using menopausal hormone therapy
N	1293	231	498	893
Age (years)	61± 9	48 ± 4	59 ± 7	64 ± 8
Height (in)	68.9 ± 2.7	64.7 ± 2.4	63.7 ± 2.3	63.0 ± 2.4
BMI (kg/m^2)	28.8 ± 4.5	27.2 ± 6.5	26.9 ± 5.3	27.9 ± 5.6
Physical activity (PASE)	157 ± 87	162 ± 77	138 ± 72	125 ± 67
% current cigarette smoker	12	13	13	13
IL-6 (pg/mL)				
Median (inter-quartile range)	2.85 (1.85, 4.57)	2.09 (1.43, 3.04)	2.44 (1.71, 3.80)	2.91 (1.95, 4.54)
TNF-α (pg/mL)				
Median (inter-quartile range)	1.23 (0.97, 1.62)	1.03 (0.77, 1.69)	1.14 (0.83, 1.53)	1.29 (0.98, 1.67)
CRP (mg/L)				
Median (inter-quartile range)	1.92 (0.95, 4.36)	1.4 (0.59, 3.95)	3.89 (1.50, 7.77)	2.34 (1.06, 5.08)
Bone mineral density (g/cm^2)				
Femoral neck	0.973 ± 0.138	0.969 ± 0.133	0.894 ± 0.129	0.829 ± 0.137
Trochanter	0.884 ± 0.140	0.785 ± 0.127	0.738 ± 0.126	0.683 ± 0.134
Total femur	1.042 ± 0.145	1.007 ± 0.133	0.938 ± 0.135	0.874 ± 0.147
Lumbar spine	1.317 ± 0.204	1.264 ± 0.156	1.202 ± 0.193	1.098 ± 0.195

*Mean ± SD, unless otherwise indicated

Table 2

Multivariable-adjusted[a] linear regression coefficients (β) for the association of log-transformed IL-6, TNF-α and CRP with BMD of the hip and spine among *men* of the Framingham Offspring cohort.

	Neck		Trochanter		Total Femur		Lumbar spine	
	β[b]	P-value	β	P-value	β	P-value	β	P-value
IL-6 (pg/mL)	0.003	0.57	−0.002	0.67	0.001	0.86	0.009	0.28
TNF-α (pg/mL)	−0.004	0.64	−0.012	0.22	−0.009	0.35	−0.014	0.33
CRP (mg/L)	−0.006	0.11	−0.005	0.20	−0.005	0.22	−0.007	0.24

[a] Models adjusted for age, BMI, height, physical activity (PASE) and smoking

[b] Difference in BMD for each 1 unit increase in log-transformed IL-6, TNF-α or CRP

Table 3

Multivariable-adjusted[a] linear regression coefficients (β) for the association of log-transformed IL-6, TNF-α and CRP with BMD of the hip and spine among *pre-menopausal women* of the Framingham Offspring cohort.

	Neck		Trochanter		Total Femur		Lumbar spine	
	β[b]	P-value	β	P-value	β	P-value	β	P-value
IL-6 (pg/mL)	−0.023	0.07	−0.030	<0.01	−0.019	0.12	−0.001	0.94
TNF-α (pg/mL)	0.014	0.31	0.007	0.55	0.012	0.36	0.043	0.01
CRP (mg/L)	−0.015	0.05	−0.014	0.04	−0.011	0.14	0.001	0.90

[a] Models adjusted for age, BMI, height, physical activity (PASE) and smoking

[b] Difference in BMD for each 1 unit increase in log-transformed IL-6, TNF-α or CRP

Table 4

Multivariable-adjusted[a] linear regression coefficients (β) for the association of log-transformed IL-6, TNF-α and CRP with BMD of the hip and spine among *post-menopausal women* of the Framingham Offspring cohort.

	Neck		Trochanter		Total Femur		Lumbar spine	
	β[b]	P-value	β	P-value	β	P-value	β	P-value
Using menopausal hormone therapy								
IL-6 (pg/mL)	0.011	0.20	0.009	0.25	0.010	0.24	0.010	0.43
TNF-α (pg/mL)	−0.005	0.69	−0.004	0.74	−0.008	0.50	−0.025	0.20
CRP (mg/L)	0.011	0.04	0.007	0.16	0.009	0.10	0.008	0.35
Not using menopausal hormone therapy								
IL-6 (pg/mL)	−0.007	0.22	−0.009	0.10	−0.007	0.27	−0.005	0.55
TNF-α (pg/mL)	−0.008	0.38	0.001	0.89	−0.005	0.61	−0.006	0.64
CRP (mg/L)	0.004	0.28	0.002	0.56	0.004	0.28	0.006	0.30

[a] Models adjusted for age, BMI, height, physical activity (PASE) and smoking

[b] Difference in BMD for each 1 unit increase in log-transformed IL-6, TNF-α or CRP