Published in final edited form as:
J Bone Miner Res. 2012 May ; 27(5): 1167–1176. doi:10.1002/jbmr.1559.

Inflammatory Markers and the Risk of Hip Fracture: The Women's Health Initiative

Kamil E. Barbour, PhD[1], **Robert Boudreau, PhD**[1], **Michelle E. Danielson, PhD**[1], **Ada O. Youk, PHD**[1], **Jean Wactawski-Wende, PhD**[2], **Nancy C. Greep, MD**[3], **Andrea Z. LaCroix, MD**[4], **Rebecca D. Jackson, MD**[5], **Robert B. Wallace, MD**[6], **Douglas C. Bauer, MD**[4], **Matthew A. Allison, MD**[7], and **Jane A. Cauley, DrPH**[1]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA

[2]University at Buffalo, Buffalo, NY, USA

[3]John Wayne Cancer Institute, Santa Monica, CA, USA

[4]University of California San Francisco, San Francisco, CA, USA

[5]Ohio State University, Ohio, OH, USA

[6]University of Iowa, Department of Epidemiology, Iowa City, IA, USA

[7]University of California San Diego, La Jolla, CA,USA

Abstract

Cytokines play a major role in bone remodeling *in vitro* and in animal models, with evidence supporting the involvement of inflammatory markers in the pathogenesis of osteoporosis. However, less is known about the longitudinal association of inflammatory markers with hip fracture. We tested whether high receptor levels of pro-inflammatory cytokines are associated with an increased risk of hip fracture in older women. We used a nested case-control study design from the Women's Health Initiative Observational Study (WHI-OS) and selected 400 cases with physician adjudicated incident hip fractures and 400 age, race, and date of blood draw matched controls. Participants were chosen from 39,795 postmenopausal women without previous hip fractures, not using estrogens or other bone-active therapies. Incident hip fractures (median follow-up 7.1 years) were verified by review of radiographs and confirmed by blinded central adjudicators. Hip fractures with a pathological cause were excluded. In multivariable models, the risk of hip fracture for subjects with the highest levels of inflammatory markers (quartile 4) compared with those with lower levels (quartiles 1, 2, and 3) was 1.43 (95% CI, 0.98 to 2.07) for IL-6 SR and 1.41 (95% CI, 0.97 to 2.05) for TNF SR1 and 1.57 (95% CI, 1.09 to 2.25) for TNF

Corresponding Author: Jane A. Cauley, PhD, University of Pittsburgh, Dept of Epidemiology 130 DeSoto St, Crabtree A524, Pittsburgh, Pennsylvania 15261 JCauley@edc.pitt.edu; Telephone: 412-624-0218. Kamil Barbour: barbourk@edc.pitt.edu Robert Boudreau: boudreaur@edc.pitt.edu Michelle Danielson: Danielsonm@edc.pitt.edu Ada Youk: ayouk@pitt.edu Jean Wactawski-Wende: jww@buffalo.edu Nancy Greep: ncgreep@gmail.com Andrea LaCroix: alacroix@whi.org Rebecca Jackson: rebecca.jackson@osumc.edu Robert Wallace: Robert-Wallace@uiowa.edu Douglas Bauer: dbauer@psg.ucsf.edu Matthew Allison: Mallison@ucsd.edu.

Conflict of Interest

Drs. Allison, Barbour, Boudreau, Danielson, Greep, and Wactawski-Wende report no competing interests.

Author Roles': Study design: JAC, KEB, MED. Study conduct: JAC, MED. Data collection: JAC, MED. Data analysis: KEB, RB, AOY. Data interpretation: JAC, KEB, RB, MED, AOY, JWW, NCG, AZL, RDJ, RBW, DCB, MAA. Drafting manuscript: KEB, JAC.

Revising manuscript content: JAC, KEB, RB, MED, AOY, JWW, NCG, AZL, RDJ, RBW, DCB, MAA. Approving final version of manuscript: JAC, KEB, RB, MED, AOY, JWW, NCG, AZL, RDJ, RBW, DCB, MAA. KEB takes responsibility for the integrity of the data analysis.

SR2. In subjects with all three markers in the highest quartile, the risk ratio of fracture was 2.27 (95% CI to 1.04 to 4.93) in comparison with subjects with 0 or 1 elevated marker(s) (p trend = 0.042). Elevated levels of inflammatory markers for all 3 cytokine soluble receptors were associated with an increased risk of hip fractures in older women. Future clinical trials should test whether interventions to decrease inflammatory marker levels reduces hip fractures.

Keywords

Inflammation; hip fractures; cytokines; women; osteoporosis; nested case-control

Introduction

Elevated levels of pro-inflammatory markers (i.e., cytokines) have been shown to be associated with an increased risk of adverse outcomes, including type 2 diabetes(1) mortality(2), declines in both physical(3) and cognitive function(4), dementia(5) and cardiovascular disease (CVD).(6,7) Interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) are cytokines that play a major role in bone remodeling, with several *in vitro* and rodent studies showing the involvement of inflammatory markers in the pathogenesis of osteoporosis.(8,9) Pro-inflammatory markers have been shown to act on mesenchymal stem cells and osteoclast precursors to enhance osteoclast mediated bone resorption. In the first physiological pathway, these cytokines bind to stromal cells and increase the expression of Receptor Activated Nuclear Factor-kB ligand (RANKL), macrophage-colony stimulating factor (M-CSF) and decrease osteoprotegerin (OPG) production resulting in increased activation of osteoclasts.(10) In the second physiological pathway, estrogen deficiency results in cytokine mediated osteoclast activation.(11,12)

The association between pro-inflammatory markers and hip fractures is uncertain. A prior prospective study showed that elevated inflammatory markers are a risk factor for incident fractures.(13) However, this prior study included all non-traumatic fractures (N=156) and did not have enough power to assess this association for hip fractures (N=39). Hip fractures contribute the greatest morbidity and mortality among all other osteoporotic fractures.(14) The one-year mortality rate after a hip fracture in women is estimated to range from 17-22%.(15,16) We conducted a nested case-control study from the Women's Health Initiative Observational Study (WHI-OS) among 400 cases with physician adjudicated incident hip fractures and 400 age, race, and date of blood draw matched controls. We tested whether high receptor levels of pro-inflammatory cytokines are associated with an increased risk of hip fracture in older women. We focused specifically on the soluble receptors for inflammatory markers as opposed to the markers themselves for the following reasons. In our prior study (13) this association was particularly strong for the soluble receptors of TNF. In addition, increases in TNF-α and IL-6 are usually transient, whereas elevations of soluble receptors for these cytokines appear to be more constant.(17) Prior research suggests that antigens may induce shedding of soluble cytokine receptors in an attempt to weaken the inflammatory response. Thus, elevated levels of soluble receptors may represent a more prolonged or severe inflammatory state.(18,19)

Methods

Study Population

The WHI-OS is a prospective cohort study that enrolled 93,676 women ages 50-79 years from 1994-1998 at 40 US clinical centers.(20) Women were eligible if they were postmenopausal, unlikely to move or die within three years, not enrolled in the WHI Clinical Trials and not currently participating in any other clinical trial. The study was approved by

Human Subjects Review Committees at each participating institution, and all participants provided written informed consent.

Follow-up and Outcome Ascertainment

Women were sent questionnaires annually to report the occurrence of any hospitalization and a wide variety of outcomes including fractures. Follow-up time for hip fractures ranged from 0.7 - 9.3 years as of August, 2004 with a median duration of 7.1 years. At that time, 3.7% of participants had withdrawn or were lost to follow-up and 5.3% had died. Hip fractures were verified by review of radiology reports and confirmed by blinded central adjudicators.(21) Hip fractures with a possible or confirmed pathological cause resulting from bone tumors, Paget's disease, bone and joint prosthesis, or surgical manipulation were excluded.

Nested Case-Control Study Design

The present analyses use a nested case-control design within the prospective design of the WHIOS. Participants were excluded if they had a prior history of hip fracture at baseline, were currently taking hormones or had taken them up to one year prior to enrollment, or at baseline were taking androgens, selective estrogen receptor modulators, antiestrogens, or other osteoporosis treatments (bisphosphonates, calcitonin). Women without sufficient serum stored or with unknown ethnicity were also excluded leaving a final study group of 39,795 eligible participants. From the eligible women, a total of 404 incident hip fractures occurred. We randomly selected 400 incident hip fractures to comprise the case group. From the remaining without hip fractures, one control per case was selected with individual matching by age at screening (+/- one year), race/ethnicity, and date of blood draw (+/- 120 days).

Clinical Variables

Current use of prescription and over the counter medications was recorded by clinic interviewers by direct inspection of containers. Prescription names were entered into the WHI database and assigned drug codes using Medispan software.

Vitamin and mineral supplements, including usual current supplement doses of elemental calcium and Vitamin D preparations, taken at least twice weekly for the prior two weeks, were entered directly from information on container labels as described above. Dietary intakes of calcium and Vitamin D were also assessed using a semi-quantitative food frequency questionnaire(22). Total calcium and Vitamin D intake was defined as the sum of diet and supplements.

Questionnaires ascertained information on date of birth, race/ethnicity, history of fracture after age 55, parental history of hip fracture, diabetes treatment, rheumatoid arthritis (RA), smoking history, self-rated health status, alcohol consumption, corticosteroid use, NSAID use ≥2 years, and total number of falls during last follow-up. Physical activity was classified on the basis of frequency and duration of walking and mild, moderate and strenuous recreational activities in the prior week. Kilocalories of energy expended in a week was calculated (metabolic equivalent (MET), score=kcal hours/week/kg).(23) Physical function was measured using the 10-item Rand-36 physical function scale (0 to 100) with higher scores indicating better physical function.(24) We compared women with a score >90 versus ≤90, this cutoff corresponds to the median score. A frailty score was computed and included self-reported muscle weakness, impaired walking, exhaustion, low physical activity and unintended weight loss between baseline and three years of follow-up.(25)

Weight was measured on a balance beam scale with the participant dressed in indoor clothing without shoes. Height was measured using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg)/ height^2 (m^2).

Laboratory Procedures

A 12 hour fasting blood sample was obtained at the baseline visit, processed and stored at -80° C according to strict quality control procedures.(26) Stored serum samples were sent to testing laboratories where laboratory personnel were blinded to case-control status for all measurements. Soluble receptors of interleukin 6 (IL-6 SR) and tumor neurosis factor (TNF SR1 and TNF SR2) were measured in duplicate with ELISA kits (R&D Systems, Minneapolis, MN, USA) at the University of Vermont. The detectable limits for the IL-6 soluble receptor (using the DR600 kit), TNF soluble receptor I (using the DRT100kit), and TNF soluble receptor II (using the DRT200 kit) were 6.5 3, and 1 pg/ml, respectively. The interassay CVs of IL-6 SR, TNF SR1, and TNF SR2 were 12.5-14.8%, 6.7-10%, and 5.6-6.2%, respectively. Sex steroid hormones were measured at the Reproductive Endocrine Research Laboratory at USC, a WHI designated core laboratory. Estradiol and testosterone concentrations were quantified using sensitive and specific RIAs following organic solvent extraction and celite column partition chromatography.(27-30) For estradiol, the intraassay and interassay CVs were 7.9% and 8-12%, respectively and for testosterone, 6% and 10-12%, respectively. Bioavailable hormone concentrations were calculated using mass action equations.(31-33) Sex hormone binding globulin (SHBG) was measured using a solid phase two site chemiluminescent immunoassay.(34) The intraassay and interassay CVs were 4.1-7.7% and 5.8-13%, respectively. Serum levels of cystatin-C were measured with the Dade Behring BN-II nephelometer and Dade Behring reagents (Ramsey,MN) using a particle enhanced immunonepholometric assay at Medical Research Laboratories International in Highland Heights, Kentucky. Serum C-terminal telopeptide of Type 1 collagen (CTx) and aminoterminal procollagen extensions propeptide (PINP) were measured by immunoassay (Synarc Inc., Lyon, France). Serum 25-hydroxvitamin D [25(OH)D] was measured by using radioimmunoassay with DiaSorin reagents (Diasorin, Stillwater, Minnesota). The sensitivity of the 25(OH)D assay was 1.5 ng/ml. Interassay CVs were 11.7%, 10.5%, 8.6%, and 12.5% at 5.6, 22.7, 33.0, and 49 ng/ml of 25(OH)D.

Statistical Methods

Baseline characteristics were compared between hip fracture cases and matched controls, using McNemar's test for categorical variables and paired t-tests for continuous data. We reported the median and interquartile range (IQR) for variables that were not normally distributed, and performed non-parametric analyses using the Wilcoxon signed-rank test. We assigned cytokine soluble receptor concentrations to quartile categories based on the distribution within the controls. We hypothesized that the cases would be disproportionately in the group with higher cytokine levels. The reason for this is that controls provide the expected concentration of inflammatory markers in the population that gave rise to the cases. The complexity and interrelatedness of cytokines involved makes it is unlikely that one biomarker would capture all of the risk information. Therefore, a composite measure of inflammation which combines the number of soluble cytokine receptors in the highest quartile for IL-6 and TNF-α was used to determine hip fracture risk. High levels of two or more inflammatory markers more likely represent systemic inflammation than a high level of just one inflammatory marker.(35,36) This composite measure was predefined based on our prior manuscript.(13) To further assess the potential for confounding, participant characteristics were compared across number of inflammatory markers in the highest quartile. The dose-response associations for the number of high inflammatory markers and participants characteristics were evaluated using Jonckheere Terpstra and Cochrane-

Armitage tests of trend, and by treating number of high inflammatory markers as a continuous variable.

For multivariable models, the associations were assessed using conditional logistic regression models to account for the matched case-control design. The odds ratio was used as an approximation of the risk ratio, based on the relative rarity of the outcome incident hip fractures. To examine the impact of these biomarkers individually we compared women with the lowest cytokine receptor concentrations quartile 1 (Q1) to women with higher concentrations (quartiles 2, 3, and 4), and tested for dose-response relationships. Women in the top quartile (Q4) of cytokine soluble receptors appeared to be the most at risk for hip fracture; and thus were compared to all other women (Q123). Associations were then examined with adjustment for BMI, parental history of hip fracture, previous fractures, self-reported health, treated diabetes, RA, physical activity smoking, alcohol use, total calcium and vitamin D intake, NSAID use, and corticosteroid use. Further multivariable models compared women with 2 or 3 inflammatory markers in the highest quartile to women with ≤1 inflammatory marker in the highest quartile. To investigate mechanisms by which inflammatory markers might be associated with hip fractures, we added the following variables individually to the base model to determine if they mediated this association: frailty score, RAND-36 physical function scale, number of falls, sexsteroid hormones, cystatin-C, bone turnover (CTX and PINP), and 25(OH)D. We then adjusted for all variables simultaneously (except for frailty, which we hypothesized would be correlated with measures of physical function because both rely on the RAND Short Form-36 physical function scale). We also determined the associations between potential mediators and hip fracture adjusted for the base analysis and inflammatory marker levels. This analysis was performed to better understand the directionality (augmentation or attenuation) of potential mediation. The variance inflation factor (VIF) was used to assess multicollinearity in multivariable models. VIF values were <2.5 for all independent variables indicating that multicollinearity was likely not present in this study.

The vast majority of hip fractures occurred among whites. Thus, a secondary analysis examining our hypothesis was performed among whites only.

Results

Participant Characteristics

The mean age of the subjects was 71 ± 6.2 years and 95% were white, Table 1. Hip fracture cases had significantly lower BMI, and physical activity. They were more likely to report corticosteroid use and current smoking compared to controls. In addition, serum levels of 25(OH)D, bioavailable estradiol, and bioavailable testosterone were significantly lower among cases. Conversely, serum cystatin-C levels were significantly higher among cases. TNF SR2 (p=0.04) concentrations were significantly higher among cases versus controls. IL-6 SR (p=0.28) and TNF SR1 (p=0.07) concentrations did not differ between cases and controls.

Participant characteristics varied by number of high inflammatory markers in the controls only, Table 1. Whites were more likely to have a greater number of high inflammatory markers than other ethnicities. A higher number of high inflammatory markers was positively (p trend<0.05) associated with older age, higher BMI, and greater levels of bioavailable estradiol and serum cystatin-C. The positive association between bioavailable estradiol and number of high inflammatory markers was also independent of BMI (p trend=0.003) (data not shown). There was also an inverse association for number of high inflammatory markers with higher physical activity, and better self-reported health. SHBG levels decreased as the number of high inflammatory markers increased, however this

association was not significant (p trend=0.09). Bone resorption marker levels, serum 25(OH)D, and bioavailable testosterone levels did not vary by number of high inflammatory markers.

The Association of Quartiles of Inflammatory Markers with Hip Fractures

There was a lack of a dose-response relationship between increasing quartiles of soluble cytokine receptors and hip fracture risk, Table 2. In addition, women in Q4 of cytokine soluble receptor concentrations were compared to all other women in the cohort. In the unadjusted models, women in Q4 of IL-6 SR had 1.53 [95% CI: 1.10 to 2.14] times the risk of incident hip fracture compared to women in the lower IL-6 SR quartiles. This association was slightly attenuated and no longer significant in the multivariable model, RR=1.43 [95% CI: 0.98 to 2.07]. The association between TNF SR2 and hip fractures remained significant in the multivariable model [RR=1.56; 95% CI: 1.09 to 2.22]. There was no association between TNF SR1 and incident hip fractures in the multivariable model [RR=1.40; 95% CI: 0.97 to 2.03].

Number of High Inflammatory Markers and Hip Fracture

The risk of incident hip fracture was highest among women with 3 “high” levels (quartile 4) of inflammatory markers, Table 3. In the base analysis, women with 2 or 3 high inflammatory markers had 41% [95% CI: -11 to 124] and 161% [95% CI: 41 to 381] increased risk of incident hip fracture compared to women with 0 or 1 level (p trend=0.002), respectively. Adjustment for potential mediators one at a time resulted in small attenuations and some augmentations of the association between inflammatory markers and incident hip fractures. After adjusting for estradiol, the increased risk of fracture for women with 3 high inflammatory markers compared to women with 1 or 0 high inflammatory markers went from 161% to 200%. The most notable attenuation (decrease in 40 percentage points) in hip fracture risk occurred after adjusting for cystatin-C. Women with 3 high inflammatory markers had a 176% [95% CI: 22 to 525] increased risk of hip fracture compared to women with 0 or 1 high inflammatory marker(s) in the final summary model after adjusting for variables in the base model and all potential mediators. There was also a positive linear trend (p trend=0.018) between number of high inflammatory markers and hip fractures in this model.

Mediators and Hip Fracture

The associations between potential mediators and hip fracture incidence adjusted for the base analysis and inflammatory marker levels are shown in Table 4. SHBG and bioavailable testosterone concentrations were significantly associated with hip fracture [RR=1.41; 95% CI: 1.13 to 1.75 and RR=0.96; 95% CI: 0.94 to 0.99, respectively]. Frailty, physical function, falls, bioavailable estradiol, cystatin-C, bone turnover markers, and 25(OH)D were not significantly associated with hip fracture.

Secondary Analysis

Among whites only, inflammatory marker levels were a stronger predictor of hip fracture when compared to the entire cohort. Women with 2 or 3 high inflammatory marker levels had 220% [95% CI: 42 to 623] and 79% [95% CI: -1 to 222) increased risk of hip fracture compared to women with 0 or 1 high inflammatory marker(s) in the final summary model (p trend=0.003), respectively.

Discussion

In this prospective, nested-case-control study, we found that women in the highest quartile for all three of IL-6 SR, TNF SR1 and TNF SR2 had over 2 times the risk of incident hip fracture compared to women with 1 or 0 inflammatory makers in the highest quartile. This risk is roughly equivalent to the risk associated with a one standard deviation decrease in bone mineral density.(37) These associations were independent of BMI, self-reported health, physical activity, parental history of fracture, history of fracture, treated diabetes, RA, calcium and vitamin D intake, NSAID and corticosteroid use, frailty, physical function, falls, sex hormones, cystatin-C, bone turnover markers, and 25(OH)D. These findings extend our previous findings (13) on all clinical fractures to hip fractures, the most devastating consequence of osteoporosis.

Adjustment for potential mediators primarily augmented the association between number of inflammatory marker in the top quartile and incident hip fractures. We initially hypothesized that adjusting for bioavailable estradiol would attenuate this association because of data showing estrogens oppose the action of cytokines(38). However, in our cohort there was a positive association between bioavailable estradiol and number of inflammatory markers in the highest quartile independent of BMI. Similarly, estradiol levels have been shown to be positively correlated with pro inflammatory markers in older women. (39,40) However, contrary to the association of inflammatory makers with hip fracture; bioavailable estradiol was lower among those with hip fractures compared to controls. Thus, negative confounding(41) occurred after adjustment for bioavailable estradiol in consequence of the directionality of these associations. Conversely, cystatin-C (a marker for poor renal function) strongly attenuated the association between inflammatory markers and hip fracture. We observed a positive association between cystatin-C levels and number of inflammatory markers in the highest quartile. Several prospective cohort studies have identified an association between inflammatory makers and decline in kidney function. (42-44) Though the biological mechanism has not been established, several hypotheses exist. *In vivo* studies have shown that glomerular injury can be induced directly by TNF-α(45,46), or mediated by immune cells (i.e., monocytes and macrophages).(42) Conversely, reduced renal function may result in an increase of inflammatory markers in the blood.(47) In this scenario, cystatin-C would not be in the causal pathway, and thus would likely be a confounder of the association between inflammatory markers and fracture. Poor renal function has also been identified as a risk factor for hip fractures in older women.(48-50) In our study, cystatin-C concentrations were higher in cases versus controls. The directionality of these associations influenced the attenuation(41) as a result of adjusting for cystatin-C.

There is an increased understanding and recognition of the role of the immune system in the development of osteoporosis.(51) Multiple cytokines (pro-inflammatory and anti-inflammatory) and hormones interact to regulate osteoblast and osteoclast differentiation and activity. The balance in these systems plays an important role in the regulation of osteoblasts and osteoclasts. In addition, several longitudinal studies among older women have found an association between high levels of inflammatory makers and increased bone loss.(52-55) However, in our analyses of the Health ABC cohort, the association between inflammation and fractures was independent of BMD.(13)

TNF-α stimulates osteoclast differentiation in vitro and in vivo, (56,57). This can be accomplished indirectly through suppression of OPG expression and stimulation of RANK in mesenchymal cells.(10) TNF-α has also been shown to activate osteoclast precursors directly by acting synergistically with RANKL. This direct mechanism occurs as a result of estrogen deficiency leading to a marked increase of TNF-α.(11) In our study, the increased risk of hip fracture was greater for those in the highest quartile for TNF SR2 (56%) and TNF

SR1 (40%) compared to other participants. Our previous study found similar risks between soluble receptors for TNF-α and incident fractures.(34) This suggests that the role of these biological markers in fracture etiology may be similar for hip and other types of fractures.

IL-6 may influence bone loss and osteoporosis.(51) IL-6 is stimulated in response to PTH and other cytokines including TNF-α.(58) IL-6 SR may enhance biological activity of IL-6. In cell culture, IL-6 only stimulated osteoclastogenesis, in the presence of IL-6 SR.(59) Also, in transgenic mice, IL-6 SR may bind to IL-6 and increase its biological activity. In our study, the association between serum IL-6 SR and hip fractures was considerable, but not significant. The risk of hip fracture was 43% more likely among participants in the highest IL-6 SR quartile compared to other subjects, but it was not statistically significant.

Our study has a number of strengths. We examined multiple markers of inflammation in relation to incident hip fractures, the most serious consequence of osteoporosis. We also adjusted for many potential confounders, eliminated hormone users from analysis, and explored several mechanisms of potential mediation underlying this association in order to focus more carefully on this group. There were several limitations in our study. First, BMD was only measured in 3 WHI clinics, thus we were unable to account for it in our analysis. However, the association of inflammatory markers with incident fractures was independent of BMD in our previous analyses.(13) Also, hip fractures are a rare outcome in our study population affecting approximately 1.01% of women with an annual risk of about 0.14%. This may reflect their relatively young age (age 50-79 years) at enrollment. Among women in a top cytokine soluble receptor quartile, there was an estimated 50% increase in risk, compared to all other women. Therefore, the absolute risk may have only increased from approximately 0.14% per year to around 0.22% for women in a top inflammatory marker quartile. Third, the adverse effect of inflammation on bone resorption may be exacerbated in states of estrogen deficiency, for instance what is observed after menopause. Therefore, our results are primarily generalizable to postmenopausal Caucasian women and cannot be extrapolated to premenopausal women or men. Also, the design of our study was a prospective nested case control study. We identified all reported incident hip fracture cases that occurred over the follow up period. We then chose a control matched on age, race and blood draw. Because hip fractures are more common at advanced ages and among whites, the characteristics of the women differ slightly from the entire WHI cohort because the characteristics were driven by their positive hip fracture history. Furthermore, we measured cytokine soluble receptor concentrations in the serum; however these levels may differ in the bone microenvironment and over time. Serum assays may not reflect local cytokine soluble receptor levels. In addition, several covariates (i.e., physical activity and dietary and supplementary intake of calcium) were measured using self-report; therefore misclassification as a consequence of recall bias is possible. Moreover, as in most epidemiologic cohort studies, we initially relied on self-report of all hip fractures. Medical records were then obtained to confirm the hip fracture by central adjudication review at the WHI coordinating center. Hip fractures are serious events; it's doubtful a participant would forget to report it. Nonetheless, perhaps in the care of proxies, hip fractures may be unreported. This type of misclassification would likely be non-differential and bias our findings to the null. Finally, residual confounding due to unmeasured factors is a component of all observational studies. For instance, accounting for frailty or health status in an analysis may be limited by self-report.

In summary, elevated levels of inflammatory markers for all 3 cytokine soluble receptors were associated with an increased risk of hip fractures in older women. The association was strongest when we combined all inflammatory makers into a composite variable suggesting that inflammatory burden may be an important biologic factor. Future clinical trials should test whether interventions to decrease inflammatory marker levels reduces hip fractures.

Moreover, inhibition of RANKL with RANKL inhibitors could also potentially block the adverse effects of inflammation on bone resorption.

Acknowledgments

The sponsor (NHLBI) has played a role in design and analyses of WHI. We would also like to acknowledge the WHI Investigators. For a list of the WHI investigators, see the Appendix, available at www.annals.org. WHI study protocols are available at http://whiscience.org.

The WHI program is funded by the National Heart, Lung and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services. The sponsor played a role in the design and analysis of the WHI. Additional support for these analyses was provided by US Public Health Service Research grants: AR053105 and AR048919.

Dr. Youk has received research support and/or honoraria from Eli Lilly & Co., Amgen and GlaxoSmithKline. She has also received consulting fees from Merk & Co. Dr. Cauley has received research support and consulting fees from Novartis Pharmaceuticals. Dr LaCroix serves as a consultant to Procter & Gamble and receives research grant support from Pfizer and the Alliance for Better Bone Health. Dr. Jackson has received research support from and is on the speaker's bureau for Procter & Gamble Pharmaceuticals, has received research and conference support from Novartis, and has received an honorarium as a Continuing Medical Education speaker for Aventis/Alliance for Better Bone Health. Dr. Bauer has received research support from Novartis Pharmaceuticals, Amgen, Proctor & Gamble Pharmaceutical Co. and Merck & Co., Inc. Dr. Wallace serves on clinical trial safety monitoring boards for Novartis Pharmaceuticals and Merck & Co., Inc.

References

1. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes. 2004; 53(3):693–700. [PubMed: 14988254]
2. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH Jr. Heimovitz H, Cohen HJ, Wallace R. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med. 1999; 106(5):506–512. [PubMed: 10335721]
3. Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti MC, Cohen HJ, Penninx B, Pahor M, Wallace R, Havlik RJ. Serum IL-6 level and the development of disability in older persons. J Am Geriatr Soc. 1999; 47(6):639–646. [PubMed: 10366160]
4. Weaver JD, Huang MH, Albert M, Harris T, Rowe JW, Seeman TE. Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. Neurology. 2002; 59(3):371–378. [PubMed: 12177370]
5. McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging. 2001; 22(6):799–809. [PubMed: 11754986]
6. Kritchevsky SB, Cesari M, Pahor M. Inflammatory markers and cardiovascular health in older adults. Cardiovasc Res. 2005; 66(2):265–275. [PubMed: 15820195]
7. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004; 351(25):2599–2610. [PubMed: 15602020]
8. Kimble RB, Matayoshi AB, Vannice JL, Kung VT, Williams C, Pacifici R. Simultaneous block of interleukin-1 and tumor necrosis factor is required to completely prevent bone loss in the early postovariectomy period. Endocrinology. 1995; 136(7):3054–3061. [PubMed: 7789332]
9. Weitzmann MN, Cenci S, Rifas L, Haug J, Dipersio J, Pacifici R. T cell activation induces human osteoclast formation via receptor activator of nuclear factor kappaB ligand-dependent and -independent mechanisms. J Bone Miner Res. 2001; 16(2):328–337. [PubMed: 11204433]
10. Kostenuik PJ, Shalhoub V. Osteoprotegerin: a physiological and pharmacological inhibitor of bone resorption. Curr Pharm Des. 2001; 7(8):613–635. [PubMed: 11375772]
11. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP, Sierra O, Pacifici R. Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. Proc Natl Acad Sci U S A. 2003; 100(18):10405–10410. [PubMed: 12923292]

12. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science. 1992; 257(5066):88–91. [PubMed: 1621100]

13. Cauley JA, Danielson ME, Boudreau RM, Forrest KY, Zmuda JM, Pahor M, Tylavsky FA, Cummings SR, Harris TB, Newman AB. 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): 1088–1095. [PubMed: 17419681]

14. Khosla S, Amin S, Orwoll E. Osteoporosis in men. Endocr Rev. 2008; 29(4):441–464. [PubMed: 18451258]

15. Kiebzak GM, Beinart GA, Perser K, Ambrose CG, Siff SJ, Heggeness MH. Undertreatment of osteoporosis in men with hip fracture. Arch Intern Med. 2002; 162(19):2217–2222. [PubMed: 12390065]

16. Schurch MA, Rizzoli R, Mermillod B, Vasey H, Michel JP, Bonjour JP. A prospective study on socioeconomic aspects of fracture of the proximal femur. J Bone Miner Res. 1996; 11(12):1935–1942. [PubMed: 8970896]

17. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, Wallach D. Variation in serum levels of the soluble TNF receptors among healthy individuals. Lymphokine Cytokine Res. 1992; 11(3):157–159. [PubMed: 1327192]

18. Honda M, Yamamoto S, Cheng M, Yasukawa K, Suzuki H, Saito T, Osugi Y, Tokunaga T, Kishimoto T. Human soluble IL-6 receptor: its detection and enhanced release by HIV infection. J Immunol. 1992; 148(7):2175–2180. [PubMed: 1545125]

19. 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. Proc Natl Acad Sci U S A. 1992; 89(11):4845–4849. [PubMed: 1317575]

20. Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw JE. The Women's Health Initiative recruitment methods and results. Ann Epidemiol. 2003; 13(9 Suppl):S18–77. [PubMed: 14575939]

21. Curb JD, McTiernan A, Heckbert SR, Kooperberg C, Stanford J, Nevitt M, Johnson KC, Proulx-Burns L, Pastore L, Criqui M, Daugherty S. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. Ann Epidemiol. 2003; 13(9 Suppl):S122–128. [PubMed: 14575944]

22. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Ann Epidemiol. 1999; 9(3):178–187. [PubMed: 10192650]

23. Manson JE, Greenland P, LaCroix AZ, Stefanick ML, Mouton CP, Oberman A, Perri MG, Sheps DS, Pettinger MB, Siscovick DS. Walking compared with vigorous exercise for the prevention of cardiovascular events in women. N Engl J Med. 2002; 347(10):716–725. [PubMed: 12213942]

24. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. Health Econ. 1993; 2(3):217–227. [PubMed: 8275167]

25. Woods NF, LaCroix AZ, Gray SL, Aragaki A, Cochrane BB, Brunner RL, Masaki K, Murray A, Newman AB. Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. J Am Geriatr Soc. 2005; 53(8):1321–1330. [PubMed: 16078957]

26. Anderson GL, Manson J, Wallace R, Lund B, Hall D, Davis S, Shumaker S, Wang CY, Stein E, Prentice RL. Implementation of the Women's Health Initiative study design. Ann Epidemiol. 2003; 13(9 Suppl):S5–17. [PubMed: 14575938]

27. Freeman RK, Bateman BG, Goebelsmann U, Arce JJ, James J. Clinical experience with the amniotic fluid lecithin-sphingomyelin ratio. II. The L-S ratio in “stressed pregnancies”. Am J Obstet Gynecol. 1974; 119(2):239–242. [PubMed: 4823393]

28. Goebelsmann U, Arce JJ, Thorneycroft IH, Mishell DR Jr. Serum testosterone concentrations in women throughout the menstrual cycle and following HCG administration. Am J Obstet Gynecol. 1974; 119(4):445–452. [PubMed: 4842588]

29. Probst-Hensch NM, Ingles SA, Diep AT, Haile RW, Stanczyk FZ, Kolonel LN, Henderson BE. Aromatase and breast cancer susceptibility. Endocr Relat Cancer. 1999; 6(2):165–173. [PubMed: 10731105]
30. Stanczyk FZ, Shoupe D, Nunez V, Macias-Gonzales P, Vijod MA, Lobo RA. A randomized comparison of nonoral estradiol delivery in postmenopausal women. Am J Obstet Gynecol. 1988; 159(6):1540–1546. [PubMed: 3144919]
31. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, Shore RE, Riboli E, Toniolo P, Kaaks R. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev. 2002; 11(10 Pt 1):1065–1071. [PubMed: 12376508]
32. Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. J Steroid Biochem. 1982; 16(6):801–810. [PubMed: 7202083]
33. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999; 84(10):3666–3672. [PubMed: 10523012]
34. Cauley JA, Lacroix AZ, Wu L, Horwitz M, Danielson ME, Bauer DC, Lee JS, Jackson RD, Robbins JA, Wu C, Stanczyk FZ, LeBoff MS, Wactawski-Wende J, Sarto G, Ockene J, Cummings SR. Serum 25-hydroxyvitamin D concentrations and risk for hip fractures. Ann Intern Med. 2008; 149(4):242–250. [PubMed: 18711154]
35. Penninx BW, Kritchevsky SB, Newman AB, Nicklas BJ, Simonsick EM, Rubin S, Nevitt M, Visser M, Harris T, Pahor M. Inflammatory markers and incident mobility limitation in the elderly. J Am Geriatr Soc. 2004; 52(7):1105–1113. [PubMed: 15209648]
36. Penninx BW, Geerlings SW, Deeg DJ, van Eijk JT, van Tilburg W, Beekman AT. Minor and major depression and the risk of death in older persons. Arch Gen Psychiatry. 1999; 56(10):889–895. [PubMed: 10530630]
37. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet. 1993; 341(8837):72–75. [PubMed: 8093403]
38. Pacifici R. Estrogen deficiency, T cells and bone loss. Cell Immunol. 2008; 252(1-2):68–80. [PubMed: 17888417]
39. Maggio M, Ceda GP, Lauretani F, Bandinelli S, Corsi AM, Giallauria F, Guralnik JM, Zuliani G, Cattabiani C, Parrino S, Ablondi F, Dall'aglio E, Ceresini G, Basaria S, Ferrucci L. SHBG, sex hormones, and inflammatory markers in older women. J Clin Endocrinol Metab. 2011; 96(4): 1053–1059. [PubMed: 21239514]
40. Stork S, Bots ML, Grobbee DE, van der Schouw YT. Endogenous sex hormones and C-reactive protein in healthy postmenopausal women. J Intern Med. 2008; 264(3):245–253. [PubMed: 18341528]
41. Mehio-Sibai A, Feinleib M, Sibai TA, Armenian HK. A positive or a negative confounding variable? A simple teaching aid for clinicians and students. Ann Epidemiol. 2005; 15(6):421–423. [PubMed: 15967387]
42. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. Kidney Int. 2005; 68(1):237–245. [PubMed: 15954913]
43. Bash LD, Erlinger TP, Coresh J, Marsh-Manzi J, Folsom AR, Astor BC. Inflammation, hemostasis, and the risk of kidney function decline in the Atherosclerosis Risk in Communities (ARIC) Study. Am J Kidney Dis. 2009; 53(4):596–605. [PubMed: 19110358]
44. Fried L, Solomon C, Shlipak M, Seliger S, Stehman-Breen C, Bleyer AJ, Chaves P, Furberg C, Kuller L, Newman A. Inflammatory and prothrombotic markers and the progression of renal disease in elderly individuals. J Am Soc Nephrol. 2004; 15(12):3184–3191. [PubMed: 15579522]
45. Bertani T, Abbate M, Zoja C, Corna D, Perico N, Ghezzi P, Remuzzi G. Tumor necrosis factor induces glomerular damage in the rabbit. Am J Pathol. 1989; 134(2):419–430. [PubMed: 2916653]
46. Le Hir M, Haas C, Marino M, Ryffel B. Prevention of crescentic glomerulonephritis induced by anti-glomerular membrane antibody in tumor necrosis factor-deficient mice. Lab Invest. 1998; 78(12):1625–1631. [PubMed: 9881962]

47. Keller CR, Odden MC, Fried LF, Newman AB, Angleman S, Green CA, Cummings SR, Harris TB, Shlipak MG. Kidney function and markers of inflammation in elderly persons without chronic kidney disease: the health, aging, and body composition study. Kidney Int. 2007; 71(3):239–244. [PubMed: 17183246]
48. Ensrud KE, Lui LY, Taylor BC, Ishani A, Shlipak MG, Stone KL, Cauley JA, Jamal SA, Antoniucci DM, Cummings SR. Renal function and risk of hip and vertebral fractures in older women. Arch Intern Med. 2007; 167(2):133–139. [PubMed: 17242313]
49. LaCroix AZ, Lee JS, Wu L, Cauley JA, Shlipak MG, Ott SM, Robbins J, Curb JD, Leboff M, Bauer DC, Jackson RD, Kooperberg CL, Cummings SR. Cystatin-C, renal function, and incidence of hip fracture in postmenopausal women. J Am Geriatr Soc. 2008; 56(8):1434–1441. [PubMed: 18662213]
50. Fried LF, Biggs ML, Shlipak MG, Seliger S, Kestenbaum B, Stehman-Breen C, Sarnak M, Siscovick D, Harris T, Cauley J, Newman AB, Robbins J. Association of kidney function with incident hip fracture in older adults. J Am Soc Nephrol. 2007; 18(1):282–286. [PubMed: 17167115]
51. Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. Immunol Rev. 2005; 208:207–227. [PubMed: 16313351]
52. Abrahamsen B, Bonnevie-Nielsen V, Ebbesen EN, Gram J, Beck-Nielsen H. 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. 2000; 15(8):1545–1554. [PubMed: 10934653]
53. 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):1952–1958. [PubMed: 18285417]
54. Gertz ER, Silverman NE, Wise KS, Hanson KB, Alekel DL, Stewart JW, Perry CD, Bhupathiraju SN, Kohut ML, Van Loan MD. Contribution of serum inflammatory markers to changes in bone mineral content and density in postmenopausal women: a 1-year investigation. J Clin Densitom. 2010; 13(3):277–282. [PubMed: 20605499]
55. Scheidt-Nave C, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, Pfeilschifter J. 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):2032–2042. [PubMed: 11344203]
56. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature. 1986; 319(6053):516–518. [PubMed: 3511389]
57. Johnson RA, Boyce BF, Mundy GR, Roodman GD. Tumors producing human tumor necrosis factor induced hypercalcemia and osteoclastic bone resorption in nude mice. Endocrinology. 1989; 124(3):1424–1427. [PubMed: 2917519]
58. Jilka RL. Cytokines, bone remodeling, and estrogen deficiency: a 1998 update. Bone. 1998; 23(2): 75–81. [PubMed: 9701464]
59. Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, Koishihara Y, Ohsugi Y, Kumaki K, Taga T, et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc Natl Acad Sci U S A. 1993; 90(24):11924–11928. [PubMed: 8265649]

J Bone Miner Res. Author manuscript; available in PMC 2013 May 01.

Table 1

Characteristics by case control status and across number of high[7] inflammatory markers in the control group.

	Controls N (%) Mean ± Std	Hip Fractures N (%) Mean ± Std	P-value[+]	0 (N=211) N(%) Mean ± Std	1 (N=101) N (%) Mean ± Std	2 (N=63) N (%) Mean ± Std	3 (N=24) N (%) Mean ± Std	P-Trend
White	380 (95.0)	380 (95.0)	>0.99	196 (92.9)	98 (97.0)	61 (96.8)	24 (100)	0.58
Age at baseline >70 years	132 (33.0)	132 (33.0)	>0.99	129 (61.1)	68 (67.3)	50 (79.4)	20 (80.3)	<0.01
BMI, kg/m^2			<0.01					
<25	144 (36.1)	193 (48.6)		87 (41.4)	36 (35.6)	14 (22.2)	6 (25.0)	<0.01
25-30	150 (37.6)	127 (32.0)		80 (38.1)	38 (37.6)	24 (38.1)	8 (33.3)	
≥30	105 (26.3)	77 (19.4)		43 (20.5)	27 (26.7)	25 (39.7)	10 (41.7)	
Corticosteroid use	4 (1.0)	16 (4.0)	0.01	2 (1.0)	0 (0.0)	2 (3.2)	0 (0.0)	0.59
NSAID use ≥2 years	81 (21.7)	83 (20.7)	0.73	43 (20.4)	22 (21.8)	18 (28.6)	4 (16.7)	0.44
RAND 36 – Physical functioning >90	117 (30.1)	84 (21.8)	0.01	70 (34.3)	30 (30.0)	12 (19.7)	4 (17.4)	0.02
Frailty	66 (16.5)	89 (22.3)	0.04	30 (14.2)	16 (15.8)	12 (19.1)	8 (33.3)	0.07
General health status, fair/poor	42 (10.6)	61 (15.3)	0.05	17 (8.3)	13 (13.0)	8 (12.9)	4 (17.4)	<0.01
Treated diabetes	19 (4.8)	24 (6.0)	0.43	6 (2.8)	5 (5.0)	5 (7.9)	3 (12.5)	0.02
RA	23 (5.8)	28 (7.0)	0.47	12 (5.7)	4 (4.0)	6 (9.5)	1 (4.2)	0.68
Alcohol lifetime consumption			0.61					0.09
Non drinker	70 (17.6)	58 (14.6)		34 (16.2)	13 (12.9)	17 (27.9)	6 (25.0)	
Past drinker	80 (20.2)	89 (22.4)		44 (21.0)	17 (16.8)	15 (24.6)	4 (16.7)	
<1 drink per day	205 (51.6)	212 (53.3)		107 (51.0)	56 (55.5)	28 (45.9)	13 (54.2)	
≥1 drinks per day	42 (10.6)	39 (9.8)		25 (11.9)	15 (14.8)	1 (1.6)	1(4.2)	
Current smoker	10 (2.5)	36 (9.1)	<0.01	8 (3.9)	1 (1.0)	0 (0)	1 (4.2)	0.51
History of fracture age ≥55 years	82 (20.5)	96 (24.0)	0.24	44 (20.9)	19 (18.8)	12 (19.1)	7 (29.2)	0.73
Parental history of hip fracture	64 (16.0)	80 (20.0)	0.14	31 (14.7)	18 (17.8)	13 (20.6)	2 (8.3)	0.76
Physical activity, MET – hrs/wk[*]	11 (4–19)	7 (2–15)	<0.01	12 (4–20)	11 (4–23)	5 (1–15)	5 (1–11)	<0.01
Total number of falls at follow-up[*]	2 (0–4)	1 (1–3)	0.82	1 (0–4)	2 (1–4)	2 (0–4)	1.5 (0.5–3)	0.89
Total vitamin D intake, IU/day	321 (127–561)	310 (120–541)	0.47	284 (112–560)	452 (140–561)	427 (188–656)	104 (172–454)	0.29
Serum 25(OH)D, ng/ml	23.9 ± 7.2	22.4 ± 8.1	0.01	23.8 ± 6.9	24.2 ± 6.3	24.4 ± 7.5	21.3 ± 8.2	0.51
Total calcium Intake, mg/day	1167 ± 684	1072 ± 694	0.05	1167 ± 718	1197 ± 667	1216 ± 620	925 ± 600	0.51
TNF SR1, pg/ml[*]	1567 (1370–1838)	1623 (1376–1956)	0.07	—	—	—	—	—

	Controls N (%) Mean ± Std	Hip Fractures N (%) Mean ± Std	P-value[+]	0 (N=211) N(%) Mean ± Std	1 (N=101) N (%) Mean ± Std	2 (N=63) N (%) Mean ± Std	3 (N=24) N (%) Mean ± Std	P-Trend
TNF SR2, pg/ml[*]	2490 (2114–2844)	2551 (2158–3062)	0.04	—	—	—	—	—
IL-6 SR, pg/ml[*]	39223 (31013–47714)	40046 (30625–50302)	0.28	—	—	—	—	—
Bioavailable estradiol, pg/ml	7.5 ± 4.5	6.6 ± 4.3	<0.01	7.1 ± 4.3	6.8 ± 3.4	9.1 ± 5.2	11.1 ± 6.7	<0.01
Bioavailable testosterone, pg/ml	12.6 ± 7.0	10.9 ± 6.3	<0.01	12.4 ± 7.0	13.0 ± 7.8	11.8 ± 4.9	14.6 ± 7.5	0.52
SHBG, μg/dl	1.6 ± 0.8	1.8 ± 0.9	<0.01	1.6 ± 0.7	1.6 ± 0.9	1.5 ± 0.7	1.4 ± 0.6	0.09
Cystatin-C, mg/L	1.06 ± 0.2	1.10 ± 0.3	0.02	0.97 ± 0.14	1.03 ± 0.17	1.29 ± 0.36	1.34 ± 0.30	<0.01
PINP, ng/ml	49.6 ± 23.7	51.0 ± 23.0	0.42	49.1 ± 25.9	48.4 ± 17.3	53.1 ± 25.8	49.4 ± 21.1	0.49
CTx, ng/ml	0.41 ± 0.19	0.45 ± 0.22	0.02	0.41 ± 0.19	0.40 ± 0.15	0.44 ± 0.23	0.42 ± 0.20	0.47

[*] Medians and IQR

[+] P-values from McNemar's test for categorical data, and paired t-tests and the Wilcoxon signed-rank test for continuous data

[¶] Number of inflammatory markers in the top quartile according to the distribution of cytokine soluble receptor concentrations among the controls.

Table 2

WHI: Risk ratios (95% CI's) of hip fracture[+] according to quartiles of cytokine soluble receptor concentrations among the controls

	Q1	Q2	Q3	Q4	P-trend	Q4 vs. Q123[*]
IL-6 SR						
Unadjusted (N pairs=399)	1.00	0.74 (0.48, 1.15)	0.75 (0.50, 1.11)	1.26 (0.84, 1.89)	0.282	1.53 (1.10, 2.14)
MV model[¶] (N pairs=363)	1.00	0.62 (0.37, 1.02)	0.64 (0.41, 1.01)	1.04 (0.65, 1.66)	0.812	1.43 (0.98, 2.07)
TNF SR1						
Unadjusted (N pairs=396)	1.00	0.94 (0.54, 1.35)	1.08 (0.72, 1.61)	1.31 (0.88, 1.94)	0.088	1.33 (0.98, 2.09)
MV model[¶] (N pairs=360)	1.00	1.00 (0.63, 1.59)	1.14 (0.72, 1.81)	1.48 (0.91, 2.40)	0.085	1.40 (0.97, 2.03)
TNF SR2						
Unadjusted (N pairs=398)	1.00	1.09 (0.73, 1.64)	1.00 (0.66, 1.51)	1.57 (1.06, 2.33)	0.033	1.53 (1.12, 2.09)
MV model[¶] (N pairs=362)	1.00	1.09 (0.69, 1.74)	0.96 (0.60, 1.56)	1.57 (0.98, 2.54)	0.076	1.56 (1.09, 2.22)

IL-6_SR quartile cut points (pg/ml) are 31007.98, 39222.27 and ≥47713.73

TNF SR1 quartile cut points (pg/ml) are 1373.34, 1566.66, and ≥1842.70

TNF SR2 quartile cut points (pg/ml) are 2113.50, 2489.66, and ≥2848.40

[+] Hip fractures and controls selection matched on age, ethnicity and blood draw date.

[¶] Multivariable adjustment includes BMI, self-reported health, physical activity, parental history of hip fracture, history of fracture, smoking, alcohol use, NSAID use, treated diabetes, RA, corticosteroid use, and total calcium and vitamin D intake.

[*] Reference group (quartiles 1, 2, and 3 combined)

Table 3

Risk ratios (95% CIs) of hip fracture, according to number of high[¶] inflammatory markers

	0,1 (N=588)	2 (N=131)	3 (N=75)	P trend
Crude analysis (N pairs=394)	1.00 (ref)	1.25 (0.84–1.84)	2.42 (1.43–4.09)	0.001
Base analysis[†] (N pairs= 358)	1.00 (ref)	1.41 (0.89–2.24)	2.61 (1.41–4.81)	0.001
Base analysis[†] + frailty score (N pairs=358)	1.00 (ref)	1.44 (0.90–2.30)	2.55 (1.38–4.71)	0.002
Base analysis[†] + RAND 36 Physical Functioning (N pairs = 344)	1.00 (ref)	1.40 (0.87–2.23)	2.62 (1.41–4.85)	0.002
Base analysis[†] + total number of falls at follow-up (N pairs = 333)	1.00 (ref)	1.44 (0.89–2.32)	2.79 (1.46–5.31)	0.001
Base analysis[†] + bioavailable estradiol (N pairs=348)	1.00 (ref)	1.53 (0.95–2.48)	3.00 (1.59-–5.67)	<0.001
Base analysis + bioavailable testosterone (N pairs=355)	1.00 (ref)	1.37 (0.86–2.20)	2.86 (1.51–5.41)	0.001
Base analysis[†] + SHBG (N pairs=356)	1.00 (ref)	1.40 (0.87–2.24)	2.81 (1.48–5.35)	0.002
Base analysis[†] + cystatin-C (N pairs=353)	1.00 (ref)	1.31 (0.77–2.20)	2.21 (1.12–4.36)	0.024
Base analysis[†] + PINP (N pairs=345)	1.00 (ref)	1.48 (0.92–2.40)	2.80 (1.49–5.27)	0.001
Base analysis[†] + CTx (N pairs=348)	1.00 (ref)	1.40 (0.87–2.26)	2.44 (1.32–4.53)	0.003
Base analysis[†] + 25(OH)D (N pairs=357)	1.00 (ref)	1.41 (0.88–2.25)	2.61 (1.41–4.83)	0.002
Summary multivariable model[§] (N pairs =290)	1.00 (ref)	1.36 (0.74–2.52)	2.76 (1.22–6.25)	0.018

N indicates number of case-control pairs included in the analysis.

[¶]Number of inflammatory markers in the top quartile according to the distribution of cytokine soluble receptor concentrations among the controls.

[†]Base analysis was matched on age, ethnicity, blood draw date, controlled for BMI, self-reported health, physical activity, parental history of hip fracture, history of fracture, smoking, alcohol use, NSAID use, treated diabetes, RA, corticosteroid use, and total calcium and vitamin D intake.

[§]Controlled for base analysis, physical function, total number of falls, bioavailable estradiol and testosterone, SHBG, Cystatin-C, PINP, CTx, and 25(OH)D.

Table 4

The association between potential mediators and hip fracture adjusted for the base analysis[†] and number of high[¶] inflammatory markers

	Risk Ratios	95% CIs
Frailty (N pairs=358)	1.36	0.86–2.16
RAND 36 Physical Functioning >90 (N pairs = 344)	0.65	0.43–1.01
Total number of falls at follow-up (N pairs = 333)	0.97	0.92–1.02
Bioavailable estradiol, pg/ml (N pairs=348)	0.96	0.92–1.00
Bioavailable testosterone, pg/ml (N pairs=355)	0.97	0.94–0.99
SHBG, μg/dl (N pairs=356)	1.41	1.13–1.75
Cystatin-C, ng/ml (N pairs=353)	1.49	0.63–3.54
PINP, ng/ml (N pairs=345)	1.00	0.99–1.01
CTx, ng/ml (N pairs=348)	1.48	0.63–3.50
25(OH)D, ng/ml (N pairs=357)	0.97	0.95–1.00

N indicates number of case-control pairs included in the analysis.

[†]Base analysis was matched on age, ethnicity, blood draw date, controlled for BMI, self-reported health, physical activity, parental history of hip fracture, history of fracture, smoking, alcohol use, NSAID use, treated diabetes, RA, corticosteroid use, and total calcium and vitamin D intake.

[¶]Number of inflammatory markers in the top quartile according to the distribution of cytokine soluble receptor concentrations among the controls.