

**INFLAMMATORY MARKERS AND THE RISK OF HIP FRACTURE: THE WOMEN'S  
HEALTH INITIATIVE**

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**University of Pittsburgh, 2011**

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, the most devastating consequence of osteoporosis. The public health significance of hip fractures is substantial; the one-year mortality rate after a hip fracture in women is estimated to range from 17-22%. Thus, preventing hip fractures is a public health imperative. We tested whether high receptor levels of pro-inflammatory cytokines are associated with an increased risk of hip fracture in older women. The Women's Health Initiative Observational Study (WHI-OS) is a nested case-control study among 400 cases with physician adjudicated incident hip fractures and 400 age, race, and date of blood draw matched controls. Subjects were selected from 39,795 postmenopausal women without previous hip fractures, not using estrogens or other bone-active therapies. Conditional logistic regression was used to estimate the risk ratios for hip fracture. Incident hip fractures (median follow-up 7.1 years) were verified by review of radiological, magnetic resonance imaging, or operative reports and confirmed by blinded central adjudicators. Hip fractures with a pathological cause were excluded. In multivariate models, the risk ratio (95% CIs) of hip fracture for subjects with the highest inflammatory markers (quartile 4) compared with those with lower inflammatory markers (quartiles 1, 2, and 3) was 1.43 (0.98, 2.07) for interleukin-6 receptor (IL-6 SR) and 1.41 (0.97, 2.05) for tumor necrosis factor receptor

1 (TNF SR1) and 1.57 (1.09-2.25) for tumor necrosis factor receptor 2 (TNF SR2). In subjects with all three high inflammatory markers, the risk ratio of fracture was 2.27 (1.04, 4.93) in comparison with subjects with 0 or 1 elevated marker(s) (p trend = 0.042). Inflammatory markers may be one of the strongest risk factors of hip fracture to date. Future clinical trials should test whether interventions to decrease inflammatory marker levels reduces hip fractures.

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## 1.0 INTRODUCTION

Elevated interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) have been shown to be associated with an increased risk of adverse outcomes, including type II diabetes(1) mortality(2), declines in both physical(3) and cognitive function(4), dementia(5) and cardiovascular disease (CVD).(6, 7) Cytokines also 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 cytokines (i.e., IL-6 and TNF $\alpha$ ) act on mesenchymal stem cells and osteoclast precursors to enhance osteoclast mediated bone resorption. In the first physiological pathway, IL-6 and TNF- $\alpha$  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) only. 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%.(14)

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- $\alpha$  and IL-6 are usually transient, whereas elevations of soluble receptors for these cytokines appear to be more constant.(15) 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.(16, 17)

## **2.0 METHODS**

### **2.1 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.(18) 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.

### **2.2 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 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.(19) 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.

### **2.3 NESTED CASE-CONTROL DESIGN**

The present analyses use a nested case-control design within the prospective design of the WHI-OS. 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).

### **2.4 CLINICAL VARIABLES**

Current use of personal 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(20). Total calcium and Vitamin D intake was defined as the sum of diet and supplements.

Questionnaires ascertained information on date of birth, race/ethnicity, age at menopause, history of personal fracture after age 55, parental history of hip fracture, diagnosed diabetes and if under treatment, current and past smoking amount and duration, self-rated health status and lifetime alcohol consumption, corticosteroid use, use of NSAIDs  $\geq 2$  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= $\text{kcal hours/week/kg}$ ).(21) Physical function was measured using the 10-item Rand-36 physical function scale.(22) The scale is scored from 0 to 100, with higher scores indicating better physical function. We compared women with a score of  $>90$  versus  $\leq 90$ ; 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.(23)

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  $\text{weight (kg)/height}^2 \text{ (m}^2\text{)}$ .

## **2.5 LABORATORY METHODS**

A 12 hour fasting blood sample was obtained at the baseline visit, processed and stored at  $-80^\circ\text{C}$  according to strict quality control procedures.(24) 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 necrosis factor (TNF SR1 and TNF SR2) were measured with ELISA (CV% <16%) at the University of Vermont. 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.(25-28) For estradiol, the intra-assay and inter-assay 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.(29-31) Sex hormone binding globulin (SHBG) was measured using a solid phase two site chemiluminescent immunoassay.(32) The intra-assay and inter-assay 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 immunonephelometric 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-hydroxyvitamin 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.

## **2.6 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. The variables IL-

6 SR, TNF SR1 and TNF SR2 were not normally distributed; therefore all these variables were log transformed and subsequently back-transformed to generate geometric means which were reported. Geometric means and standard deviations reported are likely to be smaller than normal calculations involving untransformed data. The reason for this is that the natural log transformation reduces the influence of extreme observations and outliers resulting in exponentiated geometric values that inherit the reduced influence of extreme observations. We assigned cytokine soluble receptor concentrations to quartile categories based on the distribution within the controls. 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 $\alpha$  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.(33, 34) 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 Cochran-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, 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  $\leq 1$  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, sex-steroid hormones, cystatin-C, bone turnover, and serum 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). Adding only potential mediators (without confounders) to the crude model (not shown in tables to avoid redundancy) strengthened the association among those with 2 inflammatory markers in the highest quartile by an estimated 40 percentage points and attenuated the association for those with 3 inflammatory markers in the highest quartile by 10 to 20 percentage points. The variance inflation factor (VIF) was used to test for multicollinearity in multivariable models. VIF values were  $< 2.5$  for all covariates indicating that multicollinearity was likely not present in this study.

## 3.0 RESULTS

### 3.1 PARTICIPANT CHARACTERISTICS

The mean age of the subjects was  $71 \pm 6.2$  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. The geometric mean inflammatory marker levels of TNF SR1 ( $p=0.039$ ) and TNF SR2 ( $p=0.038$ ) were higher among cases versus controls. Also, IL-6 SR was higher for cases compared to controls; however this association was not significant ( $p=0.280$ ).

Participant characteristics varied by number of high inflammatory markers, Table 2. 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 ( $p \text{ trend} < 0.05$ ) associated with older age, higher BMI, and greater levels of bioavailable estradiol and serum cystatin-C. 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.

### **3.2 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, OR=1.43 [95% CI: 0.98 to 2.07]. The association between TNF SR2 and hip fractures remained significant in the multivariable model [OR=1.57; 95% CI: 1.09 to 2.25]. There was no association between TNF SR1 and incident hip fractures in the multivariable model [OR=1.41; 95% CI: 0.97 to 2.05].

### **3.3 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 43% [95% CI: -6, 127] and 166% [95% CI: 43, 392] increased risk of incident hip fracture compared with those with 0 or 1 level (p trend=0.001), 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 markers went from 166% to 209%. 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 127% [95% CI: 4, 394] increased risk of hip fracture compared to women with 0 or 1 high inflammatory marker in the final summary model after adjusting for variables in the base model and all potential mediators. There was also a positive linear trend ( $p_{\text{trend}}=0.042$ ) between number of high inflammatory markers and hip fractures in this model.

#### 4.0 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 >2 times the risk of 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.(35) These associations were independent of BMI, self-reported health status, physical activity, parental history of fracture, history of fracture, treated diabetes, 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 all potential mediators except one 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(36). 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. (37, 38) However, contrary to the association of inflammatory makers with hip fracture; bioavailable estradiol was negatively associated with hip fractures. Thus, negative confounding(39) occurred

after adjustment for bioavailable estradiol in consequence of the directionality of these associations. Conversely, cystatin-C (a marker for poor renal function) was the lone biomarker which strongly attenuated the association between inflammatory markers and hip fracture. We observed a positive correlation 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.(40-42) 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- $\alpha$ (43, 44), or mediated by immune cells (i.e., monocytes and macrophages).(40) Poor renal function has also been identified as a risk factor for hip fractures in older women.(45-47) In our study, cystatin-C concentrations were significantly higher in cases versus controls. The directionality of these associations influenced the attenuation or positive confounding(39) 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.(48) 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.(49-52) However, in our analyses of the Health ABC cohort, the association between inflammation and fractures was independent of BMD.(13)

TNF $\alpha$  stimulates osteoclast bone resorption *in vitro* and *in vivo*, (53, 54) possibly increasing bone resorption through the differentiation of osteoclast precursors. This can be accomplished indirectly through suppression of OPG expression and stimulation of RANK in

cells.(10) TNF $\alpha$  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 $\alpha$ .(11) The increased risk of hip fracture was greater for those in the highest quartile for TNF SR2 (57%) and TNF SR1 (41%) compared to other participants. Our previous study found similar risks between soluble receptors for TNF $\alpha$  and incident fractures.(32) 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 remodeling and osteoporosis.(48) IL-6 is stimulated in response to PTH and other cytokines including TNF $\alpha$ .(55) 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.(56) 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. 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. Our results are primarily generalizable to older Caucasian women. 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. 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. Finally, residual confounding due to unmeasured factors is a component of all observational studies. 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.

Future clinical trials should test whether interventions to decrease inflammatory marker levels reduces hip fractures.

## 5.0 TABLES

**Table 1. Baseline characteristics among 400 matched hip fracture cases and controls**

	Controls N (%) Mean ± Std	Hip Fractures N (%) Mean ± Std	P-value <sup>+</sup>
Ethnicity			>0.99
White	380 (95.0)	380 (95.0)	
Black	10 (2.5)	10 (2.5)	
Hispanic	2 (0.5)	2 (0.5)	
American Indian	3 (0.8)	3 (0.8)	
Asian/Pacific Islander	5 (1.3)	5 (1.3)	
Age at baseline >70 years	132 (33.0)	132 (33.0)	>0.99
Body mass index (BMI), kg/m <sup>2</sup>			<0.01
<25	144 (36.1)	193 (48.6)	
25-30	150 (37.6)	127 (32.0)	
≥30	105 (26.3)	77 (19.4)	
Hormone therapy usage status			0.80
Never used	302 (75.5)	305 (76.3)	
Past user	98 (24.5)	95 (23.8)	
Corticosteroid use	4 (1.0)	16 (4.0)	0.01
NSAID use ≥2 years	21.7	20.7	0.73
RAND 36 – Physical functioning >90	117 (30.1)	84 (21.8)	0.01
Frailty	16.5	22.3	0.04
General health status, fair/poor	42 (10.6)	61 (15.3)	0.05
Treated diabetes	4.8	6.0	0.43

**Table 1 Continued**

Alcohol			0.61
Non drinker	70 (17.6)	58 (14.6)	
Past drinker	80 (20.2)	89 (22.4)	
<1 drink per day	205 (51.6)	212 (53.3)	
≥1 drinks per day	42 (10.6)	39 (9.8)	
Smoking			<0.01
Never smoked	215 (54.3)	214 (54.3)	
Past smoker	171 (43.2)	144 (36.6)	
Current smoker	10 (2.5)	36 (9.1)	
History of fracture age ≥55 years	82 (20.5)	96 (24.0)	0.24
Parental history of hip fracture	64 (16.0)	80 (20.0)	0.14
Total expend phys act, MET – hrs/week	13.9 ± 15.3	10.7 ± 12.7	<0.01
Total number of falls at follow-up	2.8 ± 3.2	2.4 ± 2.7	0.09
Total vitamin D intake, IU/day	373 ± 276	383.0 ± 396	0.71
Serum 25(OH)D, ng/ml	23.9 ± 7.2	22.4 ± 8.1	0.01
Total calcium Intake , mg/day	1167 ± 684	1072 ± 694	0.05
TNF SR1, pg/ml*	1595.1 ± 1.2	1702.2 ± 1.3	0.04
TNF SR2, pg/ml*	2489.2 ± 1.3	2583.2 ± 1.3	0.04
IL-6 SR, pg/ml*	37384.1 ± 1.5	38561.1 ± 1.5	0.28
Bioavailable estradiol, pg/ml	7.5 ± 4.5	6.6 ± 4.3	<0.01
Bioavailable testosterone, pg/ml	12.6 ± 7.0	10.9 ± 6.3	<0.01
SHBG, µg/dl	1.6 ± 0.8	1.8 ± 0.9	<0.01
Cystatin-C, mg/L	1.06 ± 0.2	1.10 ± 0.3	0.02
PINP, ng/ml	49.6 ± 23.7	51.0 ± 23.0	0.42
CTx, ng/ml	0.41 ± 0.19	0.45 ± 0.22	0.02

\*Geometric means and standard deviations

†P-values from McNemar's test for categorical data and paired t-tests for continuous data

**Table 2: Baseline characteristics by number of high inflammatory markers in the control group.**

	0 (N=211)	1 (N=101)	2 (N=63)	3 (N=24)	P trend
	N (%)	N (%)	N (%)	N (%)	
	Mean ± Std	Mean ± Std	Mean ± Std	Mean ± Std	
Ethnicity					0.58
Whites	196 (92.9)	98 (97.0)	61 (96.8)	24 (100)	
Blacks	7 (3.3)	1 (0.98)	2 (3.2)	0 (0.0)	
Hispanic/American Indian/Asian	8 (3.8)	2 (1.96)	0 (0.0)	0 (0.0)	
Age at baseline >70 years	129 (61.1)	68 (67.3)	50 (79.4)	20 (80.3)	<0.01
Body mass index (BMI), kg/m <sup>2</sup>					
<25	87 (41.4)	36 (35.6)	14 (22.2)	6 (25.0)	<0.01
25-30	80 (38.1)	38 (37.6)	24 (38.1)	8 (33.3)	
≥30	43 (20.5)	27 (26.7)	25 (39.7)	10 (41.7)	
Hormone therapy usage status					0.46
Never used	165 (78.2)	69 (68.3)	46 (73.0)	21 (87.5)	
Past user	46 (21.8)	32 (31.7)	17 (27.0)	3 (12.5)	
Corticosteroid use	2 (1.0)	0 (0.0)	2 (3.2)	0 (0.0)	0.59
NSAID use ≥2 years	43 (20.4)	22 (21.8)	18 (28.6)	4 (16.7)	0.44
RAND 36– Physical functioning >90	70 (34.3)	30 (30.0)	12 (19.7)	4 (17.4)	0.02
Frailty	30 (14.2)	16 (15.8)	12 (19.1)	8 (33.3)	0.07
General health status, fair/poor	17 (8.3)	13 (13.0)	8 (12.9)	4 (17.4)	<0.01
Treated diabetes	6 (2.8)	5 (5.0)	5 (7.9)	3 (12.5)	0.02
Alcohol					0.09

**Table 2 Continued**

Non drinker	34 (16.2)	13 (12.9)	17 (27.9)	6 (25.0)	
Past drinker	44 (21.0)	17 (16.8)	15 (24.6)	4 (16.7)	
<1 drink per day	107 (51.0)	56 (55.5)	28 (45.9)	13 (54.2)	
≥1 drinks per day	25 (11.9)	15 (14.8)	1 (1.6)	1(4.2)	
Smoking					0.51
Never smoked	112 (53.9)	50 (49.5)	41 (66.1)	11 (45.8)	
Past smoker	88 (42.3)	50 (49.5)	21 (33.9)	12 (50.0)	
Current smoker	8 (3.9)	1 (1.0)	0 (0)	1 (4.2)	
History of fracture age ≥55 years	44 (20.9)	19 (18.8)	12 (19.1)	7 (29.2)	0.73
Parental history of hip	31 (14.7)	18 (17.8)	13 (20.6)	2 (8.3)	0.76
Total expend phys act, MET–hrs/week	15.2 ± 16.2	13.7 ± 13.3	11.5 ± 16.2	8.5 ± 10.7	0.02
Total number of falls at follow-up	2.8 ± 3.5	3.0 ± 3.6	2.7 ± 3.6	2.6± 3.5	0.83
Total vitamin D intake, IU/day	357.2 ± 278.4	398.0 ± 268.4	426.4 ± 279.2	263.6 ± 224	0.78
25(OH)D, ng/ml	23.8 ± 6.9	24.2 ± 6.3	24.4 ± 7.5	21.3 ± 8.2	0.51
Total calcium intake, mg/day	1166.6 ± 718.4	1196.6 ± 666.7	1216.4 ± 619.6	925.1 ± 599.6	0.51
Bioavailable estradiol, pg/ml	7.1 ± 4.3	6.8 ± 3.4	9.1 ± 5.2	11.1 ± 6.7	<0.01
Bioavailable testosterone , ng/dl	12.4 ± 7.0	13.0 ± 7.8	11.8 ± 4.9	14.6 ± 7.5	0.52
SHBG, µg/dl	1.6 ± 0.7	1.6 ± 0.9	1.5 ± 0.7	1.4 ± 0.6	0.09
Cystatin-C, mg/L	0.97 ± 0.14	1.03 ± 0.17	1.29 ± 0.36	1.34 ± 0.30	<0.01
PINP, ng/ml	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	0.40 ± 0.15	0.44 ± 0.23	0.42 ± 0.20	0.47

**Table 3: WHI: Risk ratios (95% CI's) of hip fracture<sup>†</sup> according to quartiles of cytokine soluble receptor concentrations among the controls**

	Q1	Q2	Q3	Q4	P-trend	Q4 vs. Q123*
<b>IL-6 SR</b>						
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 <sup>‡</sup> (N pairs=363)	1.00	0.60 (0.36,1.01)	0.64 (0.40, 1.02)	1.06 (0.65, 1.71)	0.760	1.43 (0.98, 2.07)
<b>TNF SR1</b>						
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 <sup>‡</sup> (N pairs=360)	1.00	1.07 (0.67, 1.73)	1.11 (0.68, 1.79)	1.49 (0.90, 2.47)	0.125	1.41 (0.97, 2.05)
<b>TNF SR2</b>						
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 <sup>‡</sup> (N pairs=362)	1.00	1.09 (0.68, 1.73)	0.96 (0.59, 1.55)	1.57 (0.97, 2.53)	0.105	1.57 (1.09, 2.25)

<sup>†</sup>Hip fractures and controls selection matched on age, ethnicity and blood draw date.

<sup>‡</sup>Multivariable adjustment includes BMI, self-reported health, physical activity, parental history of hip fracture, history of fracture, smoking, alcohol use, NSAID use, treated diabetes, corticosteroid use, and total calcium and vitamin D intake.

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

IL-6 SR quartile cut points (pg/ml) are 31007.98, 39222.27 and  $\geq 47713.73$

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

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

**Table 4. 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
P-value		0.267	0.001	
Base analysis <sup>†</sup> (N pairs= 358)	1.00 (ref)	1.43 (0.94 – 2.27)	2.66 (1.43 – 4.92)	0.001
P-value		0.136	0.002	
Base analysis <sup>†</sup> + frailty score (N pairs=358)	1.00 (ref)	1.45 (0.91 – 2.31)	2.59 (1.40 – 4.81)	0.002
P-value		0.070	0.002	
Base analysis <sup>†</sup> + RAND 36 Physical Functioning (N pairs = 344)	1.00 (ref)	1.39 (0.88 – 2.18)	2.70 (1.47 – 4.91)	<0.001
P-value		0.160	0.001	
Base analysis <sup>†</sup> + total number of falls at follow-up (N pairs = 333)	1.00 (ref)	1.45 (0.90 – 2.34)	2.81 (1.47 – 5.39)	0.002
P-value		0.131	0.002	
Base analysis <sup>†</sup> + bioavailable estradiol (N pairs=348)	1.00 (ref)	1.55 (0.96 – 2.50)	3.09 (1.63 – 5.87)	<0.001
P-value		0.075	0.001	
Base analysis + bioavailable testosterone (N pairs=355)	1.00 (ref)	1.39 (0.87 – 2.23)	2.92 (1.53 – 5.55)	<0.001
P-value		0.085	<0.001	
Base analysis <sup>†</sup> + SHBG (N pairs=356)	1.00 (ref)	1.40 (0.87 – 2.24)	2.67 (1.43 – 4.99)	0.002
P-value		0.166	0.002	
Base analysis <sup>†</sup> + cystatin-C (N pairs=353)	1.00 (ref)	1.32 (0.78–2.23)	2.26 (1.14 –4.48)	0.021
P-value		0.299	0.020	
Base analysis <sup>†</sup> + PINP (N pairs=345)	1.00 (ref)	1.49 (0.92–2.41)	2.81 (1.49–5.31)	0.001
P-value		0.108	0.001	
Base analysis <sup>†</sup> + CTx (N pairs=348)	1.00 (ref)	1.42 (0.88 – 2.30)	2.50 (1.34 – 4.66)	0.003

**Table 4 Continued**

P-value		0.155	0.004	
Base analysis <sup>†</sup> + 25(OH)D (N pairs=357)	1.00 (ref)	1.42 (0.89 – 2.28)	2.65 (1.43 – 4.92)	<0.001
P-value		0.146	0.002	
Summary multivariate model <sup>§</sup> (N pairs =301)	1.00 (ref)	1.33 (0.74-2.39)	2.27 (1.04-4.93)	0.042
P-value		0.345	0.039	

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

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

<sup>§</sup>Controlled for base analysis, physical function, total number of falls, bioavailable estradiol and testosterone, SHBG, cystatin-C, PINP, CTx, and 25(OH)D.

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