C-reactive protein, bone loss, fracture, and mortality in elderly women: a longitudinal study in the OPRA cohort.

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C-reactive protein; bone loss, fracture and mortality in elderly women: a longitudinal study in the OPRA cohort

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Conflict of Interest

Sofia Berglundh, Linnea Malmgren, Holger Luthman, Fiona McGuigan and Kristina Åkesson declare that they have no conflict of interest.

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ABSTRACT

Purpose: Inflammation may contribute to the pathophysiology underlying impaired bone metabolism. This study investigates the association between C-reactive protein (CRP), bone mineral density (BMD), bone loss, fracture risk and mortality in women aged 75 and above.

Methods: This longitudinal study is based on 1044 women, all age 75 at inclusion, reassessed at age 80 and 85, with a mean follow-up time of 11.6 years (maximum 16.9 years).

Results: Women in the lowest CRP quartile (mean 0.63 mg/L) had lower BMD compared to the highest (mean 5.74 mg/L) at total hip (TH) (0.809 vs. 0.871 g/cm^2, p<0.001) and femoral neck (FN) (0.737 vs. 0.778 g/cm^2, p=0.007). A single measurement of CRP was not associated with bone loss, however, women with persistently elevated CRP i.e. ≥3mg/L at age 75 and 80 had significantly higher bone loss compared to women with CRP <3mg/L (TH: -0.125 vs. -0.085 g/cm^2, p=0.018 and FN: -0.127 vs. -0.078 g/cm^2, p=0.005) during 10 years of follow-up. Women in the highest CRP quartile had a lower risk of osteoporotic fractures (HR 0.76 (95 % CI 0.52-0.98)) compared to the lowest, even after adjusting for weight and BMD. Mortality risk was only increased among women with the highest CRP levels.

Conclusion: CRP was not an indicator for low BMD, bone loss or fracture in elderly women in this study. Persistently elevated CRP however seemed to be detrimental to bone health and may be associated with a higher rate of bone loss. Only the highest CRP levels were associated with mortality.

Keywords: CRP, fracture, bone density, inflammation, elderly

MINI ABSTRACT

This longitudinal study investigates the association between CRP, osteoporosis, fractures and mortality in 1044 elderly women. CRP was not an indicator for low BMD, bone loss or fracture in elderly women, however women with elevated CRP levels over a prolonged period lost more bone over the ten year follow-up, although fracture risk was not increased.

INTRODUCTION

Osteoporosis and the associated risk of fracture is a major public health concern in the aging population and causes immense morbidity [1] and mortality [2]. In Sweden, approximately 21% of women and 6% of men in the age range 50-84 are classified as having osteoporosis [3] and for middle-aged women the lifetime risk for an osteoporotic fracture is estimated at 50% [4].

There is growing evidence that low grade inflammation may make an important contribution to the development and progression of a number of age related diseases [5-9], and to the pathophysiology behind impaired bone metabolism [10, 11]. It is known that systemic inflammation affects bone mineral density (BMD) negatively in patients with autoimmune chronic diseases such as rheumatoid arthritis, regardless of corticosteroid use [12, 13]. Furthermore, a number of studies suggest that pro-inflammatory cytokines may mediate the bone loss associated with aging and estrogen depletion [14].

One of the most commonly used biomarkers of inflammation, the acute phase reactant C-reactive protein (CRP), increases with age and several large population based studies have demonstrated an inverse correlation between increasing levels of CRP and BMD [15, 16] or change in BMD [17]. An association between low-grade inflammation and increased risk of fractures has also been reported [18-24] which may be independent of BMD [21, 22, 24].To date, most studies are cross sectional and although primarily performed in postmenopausal women, typically ranging between 50y and 75y, little research has been done on very elderly women and the few prospective studies have only investigated bone loss over a short time period [15-17].

The aim of this study was to investigate our hypotheses that serum concentration of CRP is associated with low BMD, increased fracture risk and mortality in 75-year-old women. Furthermore we hypothesised that bone loss would be higher in elderly women who had elevated CRP levels over a prolonged period.

MATERIALS and METHODS

Subjects

This study was performed in 1044 women, all 75 years old at inclusion, who constitute the Malmö Osteoporosis Prospective Risk Assessment (OPRA) cohort which has been described in detail previously [25]. The participants were selected at random from the Malmö population files between 1995 and 1999 and invited by letter a week after their seventy-fifth birthday. No exclusion criteria were applied. Of the 1604 women initially invited, 1044 agreed to participate in the baseline (BL) examination. The following reasons were given for not participating: 376 were unwilling, 139 were too ill to attend, 13 died shortly after invitation and 32 were not reached. The women were followed-up at 5 and 10 years after the first visit i.e. at age 80 and 85, with a total follow-up time of 16.9 yrs (mean 11.6 yrs). CRP was available in 1004, 672 and 329 women at baseline, 5 and 10 years respectively.

Participants gave written informed consent and the Regional Ethical Review Board in Lund approved the study, which was performed according to the principles of the Helsinki declaration.

Bone mineral density

In this study we report BMD (g/cm^2) measured at total hip (TH) and femoral neck (FN) which was assessed at all time-points using dual energy X-ray absorptiometry (DXA-L, Lunar, Madison, WI, USA). Precision coefficients (CVs) were 3.26% for TH and 4.01% for FN and assessed in the cohort by repeated measurements after repositioning [26].

Bone loss, calculated as BMD at follow-up minus BMD at baseline, was analyzed between age 75-80; 75-85 as well as between age 80 and 85.

Other variables

Data on potential covariates were collected at all visits. Body weight and height were measured using standardized methods and body mass index (BMI) calculated (kg/m^2). Fat and lean mass (g) were measured at total body and trunk using DXA as described previously.

The participants completed questionnaires to obtain information on health status, smoking (non-smoker, current, previous), alcohol habits, co-morbidities, medications and self-assessed physical activity [27].

Fractures and mortality

Information on prospectively sustained fractures was obtained through questionnaires at 1, 3, 5 and 10 years after baseline examination, answering if a fracture had been sustained since the last visit. Fractures were ascertained against the files of the Department of Radiology at the Malmö University Hospital, which is the only hospital serving the city. Fracture information was updated until October 31st 2012 providing a mean follow-up for fracture of 11.2 years (maximum 16.9 years). Fractures at the hip, distal radius, spine, pelvis and proximal humerus were classified as major osteoporotic fractures. Fractures resulting from pathology and high energy were excluded.

Data on mortality after baseline inclusion until 2012 was obtained from the Swedish national population register.

Sampling procedures & blood biochemistry

Blood samples were non-fasting and drawn between 8 am and 1 pm, before centrifugation and storage at -80°C.

C-reactive protein was analyzed by routine methods using Roche Diagnostics (Cobas) at the Department of Clinical Chemistry, Malmö, Skåne University Hospital. The lowest detectable limit was 0.6 mg/L and coefficient of variation (CV) 3.6 – 4.1. Alkaline phosphatase (ALP), calcium, creatinine and albumin were analyzed using Beckman synchron LX20–4 auto-analyzer at BL, for continuous analysis at the 5 yr follow-up and for all the 10 yr follow-up samples. COBAS autoanalyzer (Roche) was used to analyze those 5-yrs samples that were not sent directly for analysis when collected (n= 435) and were analyzed as a batch in 2011. Adjusted values have been calculated after method updates. Serum concentration of 25-hydroxy vitamin D (25(OH)D) was measured with liquid chromatography-mass SPEC linked to a HPLC-system with inter-assay co-efficient of variation (CV) of 3-6%. Serum parathyroid hormone (PTH) was analysed with Elecsys PTH immunoassay (Roche Diagnostics) at BL and with Immunometric sandwich assay with ALP-enzyme labeling at the 5 and 10 yr follow-up (Immulite 2000 Immunoassay Systems, DPC-Diagnostic Products Corporation). Baseline values were adjusted to the new method.

Other indicators of inflammation (only available at the 5 and 10 yr follow-up) included erythrocyte sedimentation rate (ESR) and white blood cell count (WBC), and were measured using routine methods.

Statistics

Data was checked for normality however even after log transformation CRP distribution remained right-skewed. Since the lowest detectable limit for CRP was 0.6 mg/L, missing (undetectable) values were imputed. This imputation method does not bias the assumed log-normally distributed population attributes (i.e. the mean, SD and distribution properties are unaffected by the insertion of these randomly generated "quasi-values") [28]. CRP values were used as continuous variables and additionally, categorised into quartiles. With the purpose of specifically investigating low grade inflammation, women with CRP levels >10mg/ml (n=72; (7.2%)) were excluded from the statistical analysis when comparing quartiles of CRP. The correlation between CRP and BMD, bone loss, anthropometric and biochemistry phenotypes was examined using Pearson's or Spearman's correlation as appropriate. To determine the effect size of CRP on BMD and bone loss we used multiple regression under using two models to adjust for potential confounders (model 1: weight and model 2: weight, current smoking habit, vitamin D). Possible confounders were selected *a priori* based on clinical relevance and previous studies.

For comparing characteristics between quartiles of CRP (or quintiles when women with assumed active inflammation i.e. CRP levels >10 mg/L were included in the analyses (mortality)) we used analysis of variance (ANOVA) or Kruskal Wallis test for comparing means and Chi-square or Fisher's exact test for the distribution of categorical variables.

Since a single CRP measurement may not reflect the effect of inflammation over a prolonged time period, we also classified women, who had attended the two visits at age 75 and 80, according to their CRP levels at BOTH time points on the assumption that they had maintained that status throughout the 5 year period between visits. The established clinical cutoff of ≥3mg/L as a moderate to high level was used as follows: Group 1 CRP <3mg/L at *75y AND* at *80y;* Group 2 CRP ≥3mg/L at *75y OR* at *80y;* Group 3 CRP ≥3mg/L at *75y AND at 80y.* To compare differences in BMD between these categories we used ANOVA and analysis of covariance (ANCOVA) to adjust for possible confounders (e.g. weight and baseline BMD).

To evaluate the risk of fracture between quartiles of CRP, Cox proportional hazard regression analyses (HRs, with 95% confidence intervals (CI)) were performed. Fracture rates per 1000 person years were calculated. Risk of major osteoporotic and hip fractures, during three time intervals was investigated: Fractures occurring between (i) 75y-80y (ii) 75y-end of study and (iii) from 80y- end of study. CRP levels measured at age 75y were used for the first two intervals while CRP at age 80y was used for the final interval.

For mortality we used Kaplan Meier survival curves for quartiles (only participants with CRP≤10mg/L) or quintiles (all participants) of CRP.

All statistics were performed using IBM SPSS Statistics (v18-22 IBM Corp., NY, USA) and a p-value <0.05 was considered nominally significant.

RESULTS

Characteristics of the study subjects

Characteristics of the women from the OPRA cohort at age 75, 80 and 85 are presented in table 1 and supplementary table 1. At 75y the mean CRP level was 3.9 (median 1.9) mg/L, but after exclusion of the women with CRP >10mg/L (i.e. assumed active inflammation) the mean was 2.5 (median 1.7) mg/L. At age 80 and 85 the mean CRP levels were 3.7 (median 1.9) and 3.4 (median 1.7) mg/L respectively.

CRP was positively correlated to ALP (r=0.263), ESR (r=0.444) both p<0.001; WBC (r=0.198, p<0.002) PTH (r=0.087, p=0.006) and inversely to albumin (r= -0.299), calcium (r= -0.125) both p<0.001, and vitamin D (r= -0.080, p=0.011).

CRP was strongly and positively associated with adipose tissue as total body fat (r=0.284, p<0.001), weight and BMI (r=0.265 – 0.284, p<0.001). Similar correlations were observed at 80 and 85 y (r=0.227 – 0.252, p<0.001 and r=0.219 – 0.263, p<0.001). Table 2 shows that these phenotypes change incrementally from lowest to highest CRP quartile. The association between CRP and total body fat remained even after adjustment for smoking, lean mass and albumin (r=0.286, p <0.001). Women with the highest CRP levels had higher PTH, ALP, WBC and ESR levels and steroid use.

Association between CRP, BMD and bone loss

BMD was lower in the lowest CRP quartile compared to the highest at total hip (0.809 vs. 0.871 g/cm^2, p<0.001) and femoral neck (0.737 vs. 0.778 g/cm^2, p=0.007) (table 2). A similar non-significant trend was observed at age 80 but not at 85. In the unadjusted analyses CRP was positively and significantly associated with femoral neck BMD, however, after adjusting for body weight CRP was inversely and no longer significantly associated with BMD (β= -0.015, p=0.615) (Supplementary table 2A). The results did not differ substantially with exclusion of women using systemic steroids, HRT, bisphosphonates or women with the lowest levels of physical activity. Excluding participants with CRP levels above 10 mg/l did not significantly alter the results (data not shown).

Absolute bone loss (g/cm^2) was not incremental across the quartiles although women with the highest CRP levels had a higher bone loss over 5 and 10 years at the hip and femoral neck compared to those in the lowest quartile (table 2). The contribution of CRP to bone loss at the hip was non-significant after adjustment (β= -0.044 , p= 0.284; Supplementary table 2B).

When CRP is measured at each visit, we get a snap-shot of that individual's inflammatory status, without knowing whether this status is transient or stable. Making the assumption that an individual's CRP status has been stable over a prolonged time period if a similar value is recorded at two consecutive measurements 5-years apart, we compared short and longer term bone loss depending on whether CRP was clinically low (<3mg/L) or moderately high (>3mg/L) at both 75 and 80 years of age. Women with persistently elevated CRP values over a 5 year period had significantly higher 10 year bone loss compared to women with persistently low CRP levels (total hip -0.125 vs. -0.085, p=0.018; Fem Neck -0.127 vs. -0.078, p=0.005) (Table 3, fig 1). The result remained significant for FN (p=0.039) after adjusting for weight, baseline BMD and after exclusion of steroid users. There were no differences between the groups regarding smoking habits, vitamin D levels or use of HRT and bisphosphonates and therefore not adjusted for.

CRP and risk of fracture

For the cohort overall, fracture incidence per 1000 person years was 19 (hip) and 73 (major osteoporotic fractures) and did not differ statistically between CRP quartiles (Appendix Fig 1).

In keeping with the generally higher BMD observed in women with higher CRP levels at age 75y, the risk of sustaining a major osteoporotic fracture was significantly lower in the highest CRP quartile compared to the lowest reference (Q1) category both over 5 years and over the total follow-up. In table 4 hazard ratio's (HR) for short and long-term fracture risk are presented adjusted for smoking, weight, total hip BMD and previous fractures sustained prior to baseline. Hip fracture risk over any of the time intervals studied was not associated with CRP, however the risk for major osteoporotic fracture over the total follow-up was lower for the highest CRP quartile (HR 0.72, 95 % CI 0.52-0.98). The association between CRP and fracture risk was affected by the exclusion of participants using steroids (see table 4 legend for details).

CRP and risk of mortality

Low grade inflammation was not associated with all-cause mortality, and there was no substantial differences between women in the CRP quartiles, during the 5 year period 75-80y (data not shown) or over the complete follow-up period (fig. 2A).

To more fully explore the effect of inflammation, on short and long-term mortality we also analysed the data including women with CRP levels >10mg/L in the analyses and used quintiles rather than quartiles. Women in the highest CRP quintile at age 75 (mean 12.58 mg/L) had an increased risk of mortality during the following 5-years (unadjusted HR 2.19 (95% CI 1.18-4.06); adjusted HR 1.91 (0.99-3.71)) (*compared to Q1*). Over the complete follow-up period, women in the highest CRP quintile had an increased risk of mortality (HR 1.36, 95% CI 1.06-1.75) (fig 2B). However, after adjustment for factors with a potential influence on mortality (*smoking, weight, diabetes and serum creatinine*) significance was lost. Analysing CRP as a continuous variable, the adjusted mortality HR was 1.31 (1.08-1.59) at BL to 5 yrs and 1.11 (1.03-1.21) at BL to end of follow-up.

DISCUSSION

In this study of elderly Swedish women we have investigated the relationship between CRP and BMD, bone loss over time and the risk of fracture and mortality. Contrary to our expectations we did not find an inverse association between CRP and BMD, rather, that women in the highest CRP quartile had the highest BMD and a lower risk of major osteoporotic fractures. However, among those women with higher CRP levels sustained over 5 years, bone loss was greater and among those with the highest CRP levels mortality was two-fold higher.

Studies of the relationship between CRP and BMD have produced conflicting results. Koh et al., reported that CRP levels are higher in osteopenic and osteoporotic women [16] as did Ganesan et al., although significance was lost after adjusting for ethnicity, age, BMI, HRT and immobility [29]. Furthermore, Sponholtz et al., found that CRP was positively and significantly associated with femoral neck BMD in women shortly after menopause among hormone therapy users (mean age 59y) [30]. In terms of fracture risk, several studies have also reported no association between elevated CRP and low BMD, despite a higher fracture risk [21, 22, 24, 31].

In our cohort of elderly women, CRP levels were strongly correlated with high BMI and total body fat at all ages from 75 to 85. This is in line with previous findings [32] and might explain why CRP was found to be a positive predictor of BMD i.e. CRP may have functioned as a surrogate marker of body weight. High BMI is considered to be BMD protective due to its mechanical load on weight bearing bones. Moreover, adipose tissue induces higher levels of estrogen via aromatase in postmenopausal women, which also have a positive effect on bone metabolism [33]. On the other hand, it is known that adipose tissue functions as an endocrine organ, releasing adipokines and inducing systemic inflammation [9]. Hence, the complex relationship between fat mass and inflammation may compromise the use of CRP as a possible indicator for osteoporosis and fracture risk. In line with this, we did not find any association between CRP and BMD after adjusting for weight.

One of the advantages of our study is the focus on elderly women (above 75 y) while most other studies have examined a younger population (mean 42-65 y). We found that a single elevated serum CRP was not associated with low bone density among aged women, whereas it was less clear for bone loss at total hip and femoral neck (lumbar spine and total body BMD were not evaluated since these sites have been proven less reliable sites for assessing bone loss in the elderly [34]). Women in the highest quartile of CRP tended to have a higher bone loss compared to the lowest, but the difference was not statistically significant. This might be explained by the inter-variance of bone loss between participants and the margin of error of the DXA measurement, precluding identification of small effects. Another possible explanation is that some women lose a greater amount of bone mass

earlier in life, in particular around menopause, meaning that inflammation might have an effect on bone loss earlier in life, not detected in our cohort because of the high age of the participants.

To further understand long term but low grade inflammation, our cohort offered the possibility to combine measurements five years apart. Interestingly, women with elevated CRP at both occasions had a higher rate of bone loss, even after adjustment. Although the constantly high CRP group was relatively small, this suggests that prolonged low grade inflammatory activity might have a negative impact on bone metabolism which is consistent with what is known from clinical inflammatory conditions. Nevertheless, the correlations between CRP, weight and BMD also complicates the interpretation on possible causality, therefore our findings require further investigation, ideally with a larger number of similarly aged participants.

Inflammation might negatively affect aspects of bone beyond density such as bone quality and propensity for fracture. Previous studies have demonstrated an inverse association between CRP and femoral neck composite strength index [22] while others have reported that CRP is associated with changes in trabecular bone in older men [31]. In neither study was BMD associated with CRP. With fracture being the ultimate qualitative parameter, we found discrepant risk estimates and no dose-dependent relationship between levels of CRP and the risk of fracture. On the contrary, the risk of osteoporotic fracture was lower and significantly so in CRP quartiles two and four using the baseline measurement. This was not explained by the higher body weight among women in the highest CRP quartile, but remained after correcting for weight and BMD. Hip fracture risk was not associated with CRP. Our results differ from previous studies reporting that the risk of fracture increases with higher CRP [20, 22, 23, 31]. Schett et al. found a higher relative risk for non-traumatic fractures in the highest vs. the lowest tertile of CRP in a middle aged population [24] and similarly, Eriksson et al. found a higher risk for the highest tertile of CRP, compared to the lowest and medium tertile combined [21]. Although reports suggests a dose-dependent relationship between fracture risk and CRP, a recent study including 18,586 men and women showed an U-shaped relationship between CRP [18]. This supports our findings in the elderly indicating a more complex relationship between fracture risk associated with CRP. We can speculate on the role of non-bone related factors and the changing material properties of aging bone.

We also investigated if the old age of our cohort introduced a bias due to mortality; however, even though women in the highest quartile tended to have a slightly higher mortality rate, this was not significant and cannot explain the lower risk of fracture. Furthermore, the higher risk of mortality was only seen in women with the highest CRP including those with levels suggesting more active inflammation.

There are some limitations to this study. Firstly, a high sensitivity CRP (hs-CRP) assay was not available and consequently the possibility to detect effects in the lower CRP range (<0.6 mg/L) may have been missed. However, since BMD increased almost linearly with increasing CRP and while fracture risk decreased, it is unlikely that using hs-CRP would have altered the findings. Additionally, we addressed this problem by imputation of values in the undetectable lower range. Secondly, other markers of inflammation, such as IL-6 and TNF-alpha, which might have provided useful additional information, were not available**.** It was outwith the remit of this present study, to define specific diseases, medications and the effect they might have on CRP levels.

A key strength of this study is that this cohort consists of women with the exact same age, all post-menopausal and of the same background, therefore avoiding confounding from age, ethnicity, menopausal status or sex in the analyses. The prospective design with long follow-up and consecutive measurements of BMD and biomarkers is an advantage. This makes the study of particular value being the first to allow investigation of bone loss associated with low grade inflammatory response and fracture risk in elderly and very old women.

In conclusion, this study suggests that serum CRP may not be useful as an indicator for low BMD, bone loss or fracture risk in elderly women. However persistently elevated CRP may contribute to increased age-associated bone loss. Only the highest CRP levels were associated with increased mortality in this cohort.

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Table 1. Characteristics of OPRA after EXCLUSION of participants with CRP > 10 mg/L

	75y (n=932)	80y (n=629)	85y (n=309)
Age	75.2 (0.1)	80.2 (0.2)	85.2 (0.1)
Weight	68 (11)	66 (11)	64 (11)
Height	161 (6)	159 (6)	159 (6)
BMI (kg/m2)	26 (4)	26 (4)	25 (4)
Current smoker	131 (14%)	64 (10%)	20 (7%)
Lowest PAL**	15 (2%)	12 (2%)	6 (2%)
Fat mass - total body (g)	26044 (7819)	25942 (8109)	24700 (8293)
Fat mass - trunk (g)	12551 (3911)	12887 (4101)	12043 (4075)
BMD - Fem Neck (g/cm^2)	0.769 (0.137)	0.716 (0.128)	0.696 (0.141)
BMD - Total Hip (g/cm^2)	0.848 (0.148)	0.802 (0.139)	0.775 (0.142)
CRP (mg/L)*	1.7 (2.6)	1.8 (2.9)	1.6 (2.3)
Vitamin D (nmol/L)	63 (19)	78 (29)	78 (26)
ALP (ukat/L)*	1.36 (0.43)	1.25 (0.44)	1.1 (0.3)
WBC (10^9/L)*	not measured	6.4 (2.1)	6.3 (2.1)
ESR (mm/h)*	not measured	12 (11)	15 (12)
Calcium (mmol/L)	2.41 (0.07)	2.42 (0.12)	2.34 (0.09)
PTH (pmol/L)*	4.2 (2.3)	3.8 (3.3)	4.2 (3.2)
Creatinine (umol/L)	69 (17)	75 (20)	82 (21)
P-Albumin (g/L)	41 (2)	40 (3)	40 (2)
Bisphosphonate use	27 (3%)	43 (7%)	36 (12%)
Calcium use	60 (6%)	154 (24%)	133 (43%)
Vitamin D use	56 (6%)	96 (15%)	82 (27%)
HRT use	17 (2%)	9 (1%)	13 (4%)
Steroid use	21 (2%)	27 (4%)	12 (4%)

n=72 women with CRP levels >10mg/ml and excluded from the analyses

Numbers are mean (SD) or count (%). *Median (Inter quartile range (IQR)).

**Lowest Physical activity level (PAL) is Bedbound/walking aid indoors

ALP (alkaline phosphatase), WBC (white blood cell count), ESR (erythrocyte sedimentation rate)

Table 2. Clinical characteristics according to quartiles of CRP at age 75

	0.60-0.82 mg/L Q1 (Lowest)		0.83-1.70 mg/L Q2		1.71-3.40 mg/L Q3		3.41-10.00 mg/L Q4 (Highest)		p-value
	n= 231		n= 240		n= 229		n= 227		
Weight (kg)	62	(10)	68	(10)	69	(11)	72	(13)	<0.001
Height (cm)	161	(6)	161	(6)	161	(6)	160	(6)	0.303
BMI (kg/m2)	24.0	(3.5)	26.1	(3.5)	26.9	(3.8)	27.9	(4.4)	<0.001
Current smoker	38	(17%)	25	(10%)	33	(14%)	33	(15%)	0.307
BMD & body composition									
BMD-Fem Neck (g/cm^2)	0.737	(0.138)	0.769	(0.129)	0.774	(0.143)	0.778	(0.135)	0.007
BMD-Total Hip (g/cm^2)	0.809	(0.150)	0.856	(0.142)	0.859	(0.154)	0.871	(0.139)	<0.001
Fat mass - total body (g)	21689	(6992)	26403	(6992)	27902	(7065)	28675	(7473)	<0.001
Fat mass - trunk (g)	10253	(3605)	12798	(3559)	13419	(3628)	13980	(3862)	<0.001
Biochemistry									
P-Albumin (g/L)	41.4	(2.2)	41.3	(2.0)	40.8	(2.1)	40.6	(2.4)	<0.001
P-ALP (ukat/L)*	1.26	(0.41)	1.36	(0.46)	1.32	(0.37)	1.54	(0.54)	<0.001
P-Calcium (mmol/L)	2.41	(0.08)	2.41	(0.06)	2.40	(0.07)	2.40	(0.08)	0.371
P-Creatinine (umol/L)	66	(11)	69	(14)	70	(16)	73	(23)	<0.001
S-PTH (pmol/L)*	4.0	(2.1)	4.1	(2.0)	4.3	(2.3)	4.5	(2.8)	0.006
S-25 (OH)D3 (nmol/L)	62	(21)	64	(18)	63	(18)	62	(19)	0.796
B-SR, @ 80yrs (mm/h)*	12	(11)	10	(8)	12	(13)	18	(15)	0.027
WBC @ 80yrs (10^9/L)*	6.3	(2.6)	5.8	(1.9)	6.55	(2.5)	6.9	(2.2)	0.005
Bone loss									
Bone loss FN (75y-80y)	-0.055	(0.096)	-0.067	(0.079)	-0.048	(0.086)	-0.072	(0.071)	0.061
Bone loss FN (75y-85y)	-0.080	(0.113)	-0.084	(0.095)	-0.076	(0.121)	-0.117	(0.090)	0.100
Bone loss FN (80y-85y)	-0.032	(0.101)	-0.012	(0.084)	-0.029	(0.102)	-0.039	(0.077)	0.311
Bone loss TH (75y-80y)	-0.051	(0.089)	-0.056	(0.073)	-0.048	(0.083)	-0.068	(0.069)	0.146
Bone loss TH (75y-85y)	-0.085	(0.099)	-0.085	(0.090)	-0.089	(0.108)	-0.115	(0.082)	0.219
Bone loss TH (80y-85y)	-0.039	(0.093)	-0.024	(0.072)	-0.039	(0.08)	-0.048	(0.076)	0.322

Numbers are mean (SD) or count (%). *Median (IQR).

TH=Total Hip, FN= Femoral Neck, PAL=Physical activity level

P Comparison between quartiles (ANOVA or Kruskal Wallis for means; $\chi 2$ / Fisher's exact test for categorical data).

Table 3. Change in BMD and the association between persistently HIGH or LOW CRP level

	n=351 CRP <3mg/L 75y **and** 80y	**n= 150** CRP ≥3mg/L 75y **or** 80y	**n= 151** CRP ≥3mg/L 75y **and** 80y	P^1	P^2
5-year bone loss (75y-80y)					
Fem Neck	-0.056 *(n=330)*	-0.063 *(n=139)*	-0.073 *(n=132)*	0.158	ns
Total Hip	-0.051 *(n=322)*	-0.062 *(n=135)*	-0.068 *(n=130)*	0.088	ns
5-year bone loss (80y-85y)					
Fem Neck	-0.022 *(n=178)*	-0.027 *(n=75)*	-0.046 *(n=64)*	0.199	ns
Total Hip	-0.033 *(n=177)*	-0.032 *(n=76)*	-0.049 *(n=64)*	0.385	ns
10-year bone loss (75y-85y)					
Fem Neck	-0.078 *(n=175)*	-0.085 *(n=73)*	-0.127 *(n=62)*	0.006	0.005
Total Hip	-0.085 *(n=173)*	-0.087 *(n=73)*	-0.125 *(n=62)*	0.019	0.018

Change in BMD (g/cm2) is based on CRP values measured at both 75y and 80y

P^1 Comparison between all three groups using ANOVA

P^2 Post hoc analysis (Hochberg) between CRP <3mg/L and ≥3mg/L

Number of participants in parenthesis

Table 4. Association between CRP quartile and Major Osteoporotic and Hip fracture

Fracture Risk Period		Major Osteoporotic fracture			Hip fracture		
		HR[#]	95 % CI	P-value	HR[#]	95 % CI	P-value
Single CRP measure at 75y and **Short-term fracture risk [a] (75y – 80y)**	**Q 1 *(Lowest)***	1			1		
	Q 2	0.62	0.39-0.99	0 .04*	0.91	0.36-2.31	0.84
	Q 3	0.74	0.46-1.17	0.19	1.24	0.51-3.00	0.63
	Q 4 *(Highest)*	0.62	0.37-1.02	0 .06**	0.93	0.35-2.49	0.89
Single CRP measure at 75y and **Long-term fracture risk [b] (75y – end of follow-up)**	**Q 1 *(Lowest)***	1			1		
	Q 2	0.84	0.64-1.11	0.22	1.00	0.64-1.56	0.99
	Q 3	0.75	0.56-1.00	0.05	0.91	0.57-1.45	0.68
	Q 4 *(Highest)*	0.72	0.52-0.98	0.04	1.04	0.63-1.70	0.89
Single CRP measure at 80y and **Short-term fracture risk [c] (80y – end of follow-up)**	**Q 1 *(Lowest)***	1			1		
	Q 2	0.80	0.56-1.14	0.22	1.18	0.69-2.03	0.55
	Q 3	0.85	0.59-1.23	0.40	0.92	0.51-1.64	0.77
	Q 4 *(Highest)*	0.79	0.54-1.17	0.25	0.75	0.40-1.40	0.36

[#]HR adjusted for previous adult fracture, smoking, TH-BMD, physical activity, weight

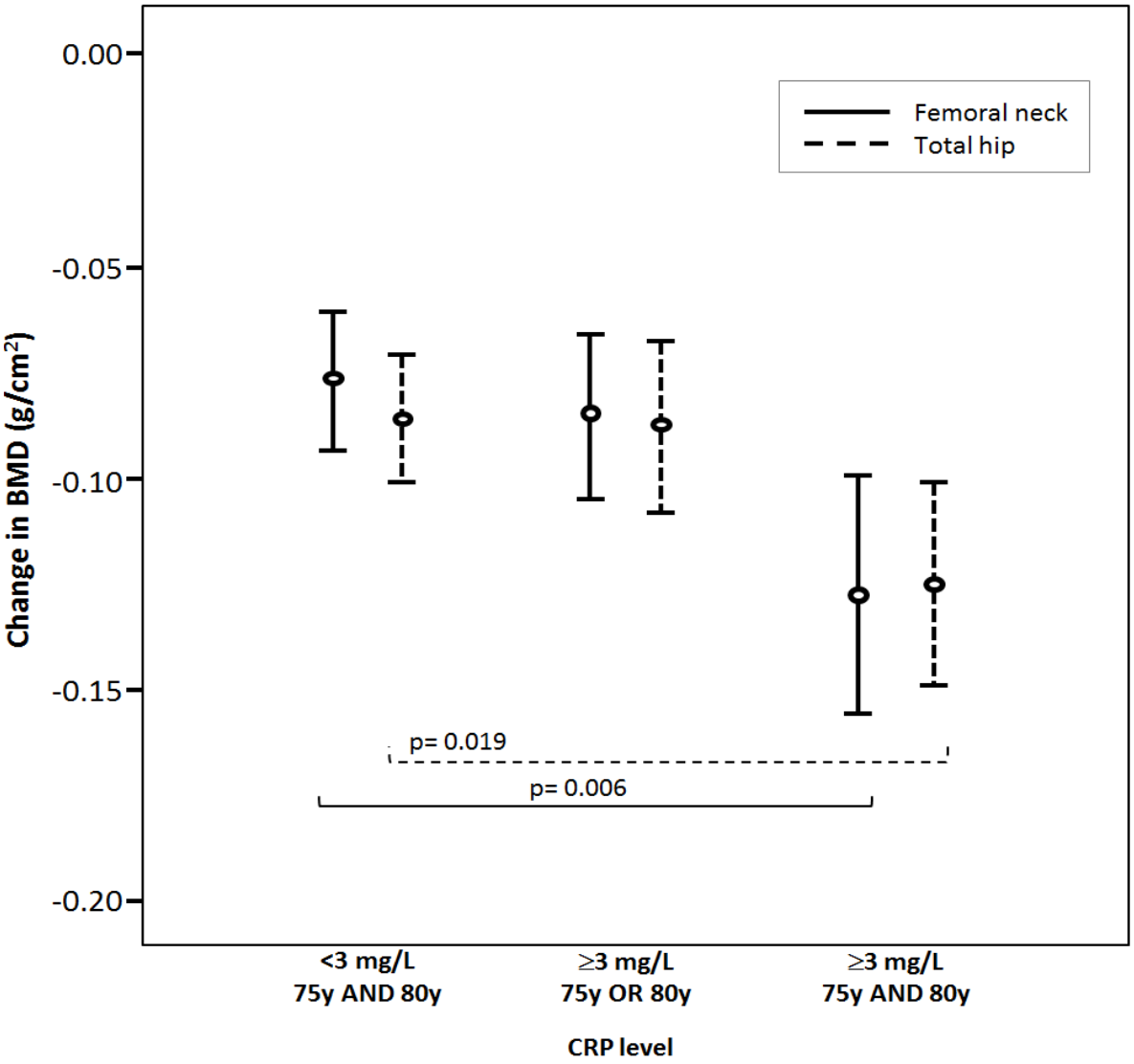
Mean follow-up times: [a]4.98 yrs (osteoporotic); 4.99 yrs (hip); [b]8.94 yrs (osteoporotic); 10.78 yrs (hip); [c]5.98 yrs (osteoporotic); 7.01 yrs (hip)

Reported p-values from Cox regression analysis

[*]Non-significant when steroid users excluded

[**]Significant when steroid users excluded

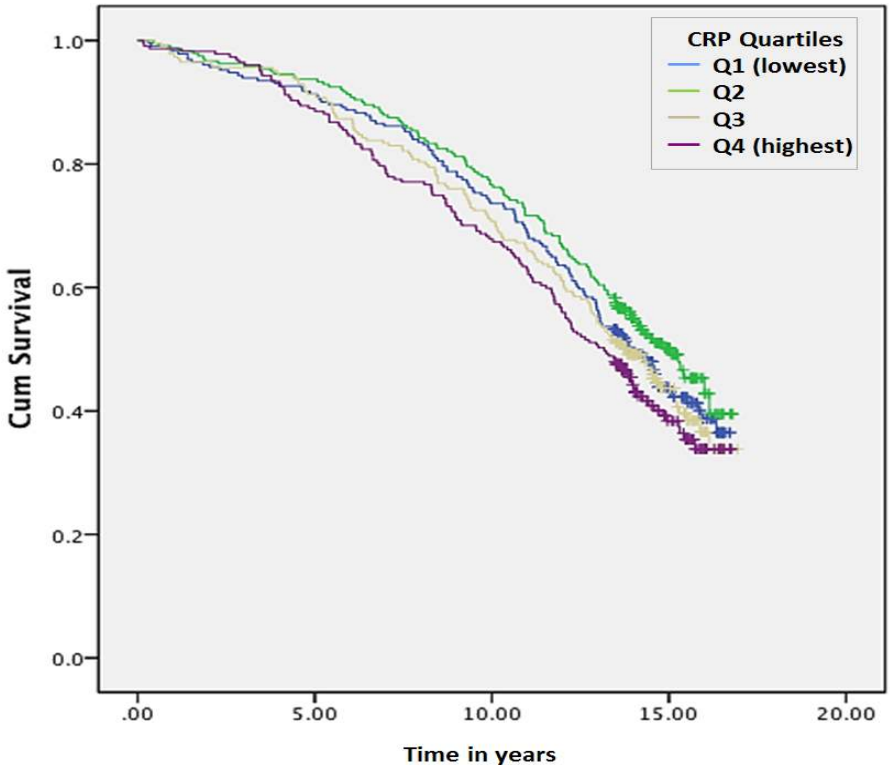
Fig. 1 Association between persistently HIGH or LOW CRP levels & change in BMD from age 75 to 85



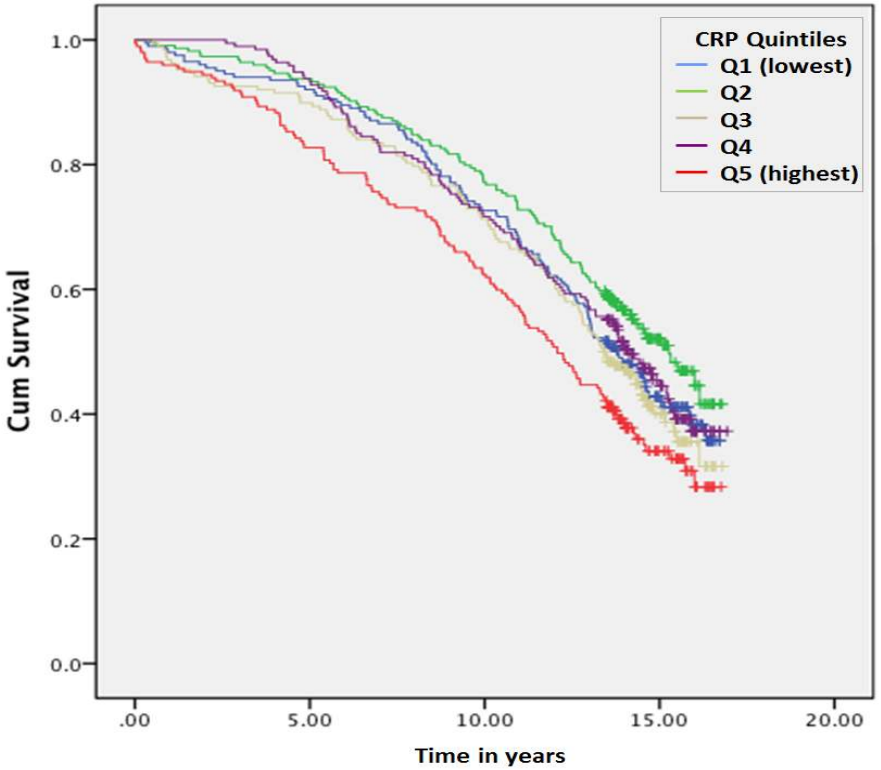
To determine the relationship between persistently elevated or low levels of CRP on bone loss women were categorised according to their CRP level at BOTH time-points (i.e. age 75 and age 80) using the established clinical cut-off of ≥3 as a moderate to high CRP level

Figure 2. Survival from baseline to end of study in relationship to CRP category A) excluding and B) including women with assumed active inflammation

A)



B.



In A) women with assumed active inflammation (i.e. CRP >10mg/ml) are excluded from the analyses, and quartiles are used (Q4: 3.41 – 10.00)
In B) women with CRP >10mg/ml are included and analysed using quintiles to capture the upper range of CRP values (Q5: 5.00 – 82.00)

Supplementary On-line material

Supplementary Table 1. Characteristics of the OPRA cohort PRIOR TO EXCLUSION of women with CRP levels above 10 mg/L

	75y (n=1044)		80y (n=715)		85y (n=382)	
Age	75.2	(0.2)	80.2	(0.2)	85.2	(0.1)
Weight	68	(12)	66	(11.5)	64	(11)
Height	161	(6)	159	(6)	158	(6)
BMI (kg/m2)	26	(4)	26	(4)	25	(4)
Current smoker	145	(14%)	76	(11%)	22	(6%)
Lowest PAL**	21	(2%)	16	(2%)	8	(2%)
Fat mass - total body (g)	26087	(7902)	25914	(8196)	24682	(8348)
Fat mass - trunk (g)	12571	(3932)	12879	(4161)	12013	(4085)
BMD - Fem Neck (g/cm^2)	0.765	(0.138)	0.713	(0.129)	0.690	(0.136)
BMD - Total Hip (g/cm^2)	0.848	(0.149)	0.800	(0.142)	0.768	(0.138)
CRP (mg/L)*	1.9	(3.2)	1.9	(3.3)	1.7	(2.7)
Vitamin D (nmol/L)	62	(19)	78	(30)	79	(26)
ALP (ukat/L)*	1.36	(0.46)	1.26	(0.43)	1.10	(0.30)
WBC (10^9/L)*	Not measured		6.4	(2.2)	6.4	(2.2)
ESR (mm/h)*	Not measured		12	(11)	15	(11)
Calcium (mmol/L)	2.40	(0.07)	2.41	(0.13)	2.34	(0.09)
PTH (pmol/L)*	4.2	(2.4)	3.9	(3.4)	4.3	(3.2)
Creatinine (umol/L)	70	(19)	74	(20)	82	(23)
P-Albumin (g/L)	41	(3)	40	(3)	40	(2)
Bisphosphonate use	33	(3%)	50	(7%)	44	(12%)
Calcium use	69	(7%)	181	(25%)	161	(42%)
Vitamin D use	65	(6%)	113	(16%)	97	(25%)
HRT use	18	(2%)	9	(1%)	14	(4%)
Steroid use	29	(3%)	35	(5%)	17	(5%)

Numbers are mean (SD) or count (%). *Median (Inter quartile range (IQR)).

**Lowest Physical activity level (PAL) is Bedbound/walking aid indoors

ALP (alkaline phosphatase), WBC (white blood cell count), ESR (erythrocyte sedimentation rate)

Supplementary Table 2. Contribution (effect size) of CRP at 75y, 80y and 85y to bone density and bone loss

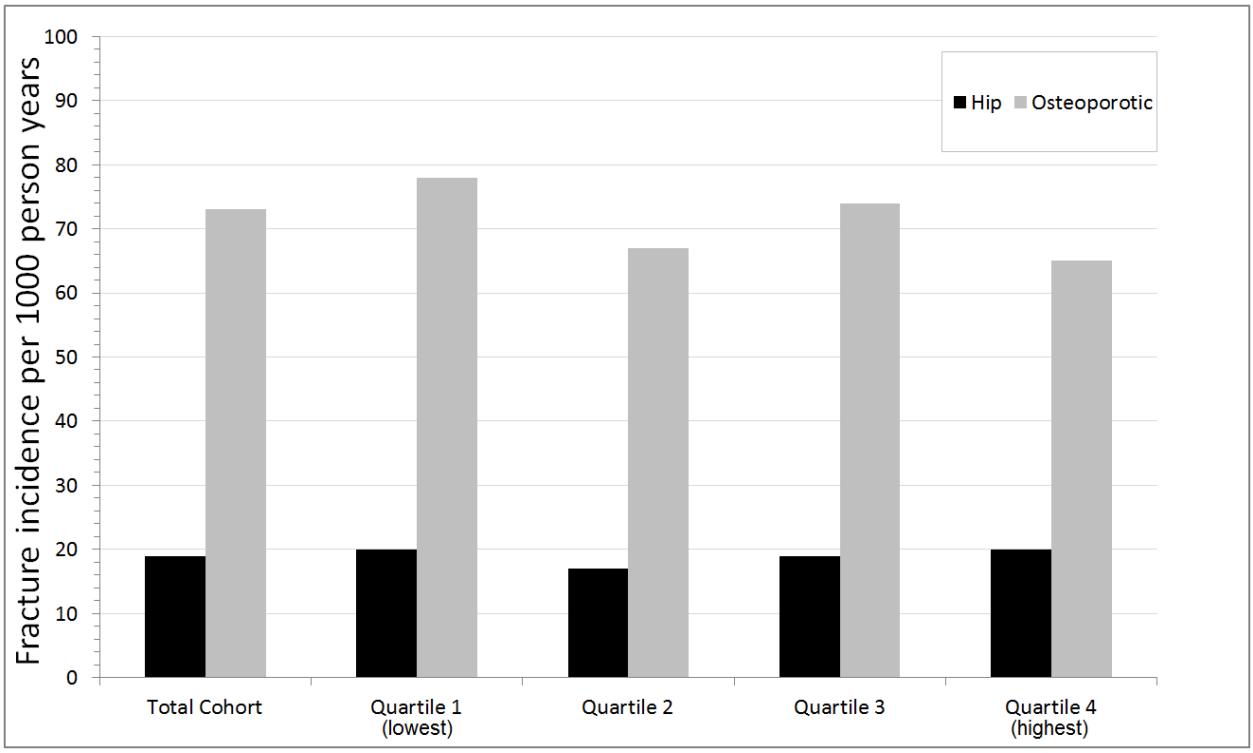
A) Bone Density						
	75y		**80y**		**85y**	
Femoral Neck	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
Unadjusted	0.106	0.001	0.068	0.087	0.033	0.561
Model 1	-0.015	0.615	-0.029	0.434	-0.049	0.369
Model 2	-0.011	0.726	-0.024	0.538	-0.054	0.328
Total Hip						
Unadjusted	0.145	<0.001	0.094	0.018	0.077	0.176
Model 1	0.017	0.571	-0.015	0.679	-0.015	0.774
Model 2	0.024	0.418	-0.003	0.926	-0.026	0.628
B) Bone loss						
Femoral Neck	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
Unadjusted	-0.046	0.243	-0.099	0.064	-0.061	0.271
Model 1*	-0.009	0.823	-0.060	0.281	-0.072	0.212
Model 2°	-0.014	0.736	-0.071	0.206	-0.053	0.394
Total Hip						
Unadjusted	-0.089	0.027	-0.100	0.064	-0.012	0.824
Model 1*	-0.044	0.284	-0.044	0.425	-0.006	0.915
Model 2°	-0.049	0.229	-0.054	0.327	0.002	0.975

Model 1: Adjusted for weight

Model 2: Adjusted for weight, Vitamin D level and smoking habit

Exclusion of participants using HRT/Steroids/Bisphosphonates or participants with very low physical activity level did not significantly change the result. β values are standardised; P-values are from multi-linear regression

Supplementary Figure 1. Incidence of hip and osteoporotic fractures per 1000 person years for total cohort and for quartiles of CRP



Fracture incidence is based on prospective fractures from age 75 to end of follow-up