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Relationships between markers of inflammation and bone density: findings from the Hertfordshire Cohort Study

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Abstract

Purpose—The aim of this study was to examine the association between indices of inflammation and bone mineral density (BMD) in a population-based cohort of older adults in the United Kingdom.

Methods—Analyses were based on a sample of 194 men and 171 women of the Hertfordshire Cohort Study (community-living, older adults). Dual energy X-ray absorptiometry (DXA) was performed at the lumbar spine and proximal femur at baseline and repeated at a median of 4.5 years (inter-quartile range 3.6 to 5.2). Inflammatory markers (CRP, TNF, IL-1 β , IL-6, IL-8, IL-10, adiponectin and leptin) were ascertained at baseline using enzyme-linked immunosorbent assay (ELISA) techniques and Bio-Plex Pro Assays. Gender-adjusted linear regression was used to examine the associations between markers of inflammation and outcomes with and without adjustment for anthropometric and lifestyle factors.

Results—The mean (SD) ages at baseline were 64.4 (2.5) and 66.5 (2.7) years for men and women respectively. Higher levels of adiponectin and adiponectin:leptin ratios were each associated with lower baseline lumbar spine and femoral neck BMD in gender-adjusted ($p < 0.01$) and fully-adjusted ($p < 0.05$) analyses. Lower levels of IL-10 and TNF were each associated with accelerated decline in lumbar spine BMD in both gender-adjusted ($p = 0.05$) and fully-adjusted ($p < 0.05$) analyses.

Conclusions—In a cohort of older adults, high levels of adiponectin and adiponectin:leptin ratios were both associated with lower BMD at the lumbar spine and femoral neck at baseline, and

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Conflicts of interest

Nicholas Fuggle, Leo Westbury, Holly Syddall, Niharika Duggal, Sarah Shaw, Kate Maslin, Elaine Dennison, Janet Lord and Cyrus Cooper declare that they have no conflicts of interest.

lower IL-10 was associated with accelerated decline in BMD at the lumbar spine. This adds weight to the theory that bone health can be influenced by changes in immune phenotype and alterations in adipokine homeostasis.

Keywords

Inflammation; bone mineral density; adipokine; interleukin; CRP; DXA

Introduction

Osteoporosis is a condition characterised by demineralisation of bone with a subsequent increased risk of fracture and is a major global health concern [1]. It is particularly common in women, affecting an estimated 22 million European women over the age of 50 [2]. Risk factors for osteoporosis include older age, family history, low body mass index, smoking, excessive alcohol consumption and corticosteroid usage [3].

Although inconsistent, there is evidence that indolent, low-level inflammation leads to cellular senescence, impaired DNA repair and biological ageing. Studies have even related inflammation to indices of musculoskeletal health. Experimentally, these have demonstrated a role of pro-inflammatory mediators (including TNF, IL-1 β and IL-6) in the alteration of bone structure [4], via the inhibition of osteoblasts. With regard to clinical indices, population studies have demonstrated varying relationships between incident fracture and CRP [5–11], TNF [12,9,6], IL-6 [9,12,13,6] and IL-10 [6], and between bone mineral density (BMD) and CRP [6,10,14,7,15,16], TNF [6,16,17], IL-6 [6,16–18], IL-10 [6] and adipokines including adiponectin and leptin [19–22].

Adipokines, in addition to elements of the inflammatory cytokine cascade, have been shown to have marked immune homeostatic effects. Adiponectin manifests an anti-inflammatory state via inhibition of macrophage activation and subsequently lower levels of TNF and interferon- γ and increased levels of IL-10 and IL-1RA [23]. Leptin possesses a more pro-inflammatory profile, leading to higher levels of TNF, IL-6 and IL-12 [24] with these inflammatory mediators having effects on the musculoskeletal system downstream. Previous studies have investigated adipokine relationships with bone turnover markers [25], and bone mineral density (BMD) in pre- and post-menopausal women [21]. However, literature on the longitudinal relationships between inflammatory markers and BMD in older populations is lacking.

The Hertfordshire Cohort Study (HCS) provides an ideal opportunity to address this issue, in consort with examination of the longitudinal relationship between BMD and inflammatory markers, in a population-based cohort of community-dwelling older men and women in the United Kingdom (26).

The objective of this current analysis was to examine the associations between baseline markers of inflammation and level and change in total lumbar spine and femoral neck bone mineral density (BMD) among HCS participants.

Methods

The Hertfordshire Cohort Study

The HCS comprises 1579 men and 1418 women born in Hertfordshire between 1931 and 1939 and who still lived there in 1998 to 2004 when they completed a baseline home interview and research clinic for detailed characterisation of their socio-demographic, lifestyle and clinical characteristics; the study has been described in detail previously [26].

Smoking status, weekly alcohol consumption and customary level of physical activity (Dalloso questionnaire [27]) were ascertained by a nurse-administered questionnaire at the home interview. A 'prudent diet' score was derived from a food-frequency questionnaire using principal components analysis; higher scores reflect healthier diets [28]. Details of all prescription and over-the-counter medications currently taken were coded according to the British National Formulary; the number of systems medicated was used as a marker of co-morbidity.

Investigations conducted at the baseline clinic included measurement of height (using a Harpenden pocket stadiometer, Chasmors Ltd, London, UK) and weight (on a SECA floor scale, Chasmors Ltd, London, UK). Blood samples were taken and serum was aliquoted and stored at -80°C. Dual energy X-ray absorptiometry (DXA) at the lumbar spine and proximal femur was performed using a Hologic QDR 4500 instrument (Vertec Scientific, Reading, UK) to ascertain total lumbar spine and total femoral neck BMD. Measurement precision error of this device has been described previously [29]. In 2004-5, 642 men and women (73% of those invited) agreed to participate in a clinical follow-up study during which medication use was reassessed: DXA measurements were repeated for 568 participants.

Ascertainment of baseline inflammatory markers

Baseline posterity samples were available for 365 of the 568 participants. High sensitivity C-reactive protein (hs-CRP) levels were measured by ELISA using a commercial kit (IBL international, Hamburg, Germany) according to manufacturer's instructions. A multiplex based assay for the cytokines IL-1 β , IL-6, IL-10, IL-8 and TNF (Bio-Rad Laboratories, Munich, Germany) was performed according to manufacturer's instructions. Data acquisition and analysis was conducted using Bio-Plex Manager software version 6.0. Intra assay coefficients of variation (CV %) ranged from 7.15 to 13.89.

Serum adiponectin (serum diluted 1:5000) and leptin (serum diluted 1:2000) were assessed separately by solid phase sandwich ELISA (R&D Systems, Abingdon, UK). Data analysis was performed using GraphPad Prism software (GraphPad Software Ltd, USA).

The analysis sample for this paper comprised the 365 participants who had non-missing data for at least one of the inflammatory markers considered and who had complete data for lumbar spine and femoral neck BMD at baseline and follow-up.

The HCS study had ethical approval from the Hertfordshire and Bedfordshire Local Research Ethics Committee and all participants gave written informed consent to participate

in the study and for their health records to be accessed in the future. Investigations were conducted in accordance with the principles expressed in the Declaration of Helsinki.

Statistical methods

Height and weight were highly correlated ($r=0.46$, $p<0.001$ for men; $r=0.38$, $p<0.001$ for women); to avoid multi-collinearity problems, a sex-specific standardised residual of weight-adjusted-for-height was derived. Smoking status was categorised into ever smokers and never smokers. Conditional change in total lumbar spine and femoral neck BMD was characterised by the residuals obtained after estimating sex-specific linear regression models for measures at follow-up on measures at baseline with adjustment for individual follow-up duration; this measure of residual change is independent of baseline level.

Data were described using summary statistics. Apart from IL-8, all inflammatory markers were highly positively skewed and were log-transformed. Linear regression was used to examine the association between each baseline inflammatory marker and both baseline level and change in femoral neck and lumbar spine BMD. Gender-adjusted and fully-adjusted models, accounting for gender, baseline age, height, weight-for-height, smoking history, alcohol consumption, prudent diet score, physical activity and use of hormone replacement therapy were implemented. Models for conditional change in BMD were additionally adjusted for bisphosphonate use at follow-up (no participants used bisphosphonates at baseline).

To ensure comparability of effect sizes, sex-specific standard deviation scores were derived for inflammatory markers and outcomes and used in models. Owing to modest sample size, men and women were pooled and analyses were adjusted for gender (interaction effects between gender and the inflammatory markers were non-significant in gender-adjusted models); $p<0.05$ was regarded as statistically significant. Analyses were conducted using Stata, release 13.

Results

Participant characteristics

Characteristics of the 365 HCS participants who were included in the analysis sample are presented in Table 1. Mean (SD) age at HCS baseline was 64.4 (2.5) and 66.5 (2.7) years among men and women respectively. Median (inter-quartile range) time between bone scans was 5.2 (4.8, 5.6) years among men and 3.6 (3.1, 4.0) years among women. On average: men had higher BMD than women at the lumbar spine and femoral neck; both sexes gained BMD at the lumbar spine; and women lost BMD at the femoral neck but there was no change at this site for men.

The proportion of participants who were of manual social class did not differ significantly between the analysis sample and the 2632 participants who attended the HCS baseline clinic but were not included in the analysis sample.

Appendix 1 presents Pearson correlations between the markers of inflammation. TNF was moderately correlated with IL-1 β , IL-6 and IL-10 ($0.22 \leq r \leq 0.32$, $p<0.001$) and

negatively correlated with IL-8 ($r=-0.25$, $p<0.001$). IL-1 β was moderately correlated with IL-10 ($r=0.26$, $p<0.001$).

Relationships between markers of inflammation and lumbar spine and femoral neck BMD at baseline

The associations between each inflammatory marker and both level and change in lumbar spine and femoral neck BMD are presented in Table 2 and Figure 1. Lower levels of leptin and higher levels of IL-1 β , adiponectin and adiponectin:leptin ratios were each associated with lower baseline lumbar spine and femoral neck BMD; associations for adiponectin and adiponectin:leptin ratios were robust in fully-adjusted analyses. For example, an SD increase in adiponectin was associated with a mean reduction in baseline lumbar spine BMD of 0.11 (95% CI: 0.01, 0.21) SDs in fully-adjusted analyses; reductions regarding femoral neck BMD were similar for this marker. In gender-adjusted analyses, higher levels of TNF and lower levels of hs-CRP were also associated with lower femoral neck BMD but these were attenuated by full adjustment.

Longitudinal relationships between markers of inflammation and conditional change in lumbar spine and femoral neck BMD

Lower levels of TNF and IL-10 and higher levels of IL-8 were each associated with accelerated decline in lumbar spine BMD (Table 2); associations regarding TNF and IL-10 were robust in fully-adjusted analysis (Table 2 and Figure 1). For example, in fully-adjusted analyses, an SD decrease in IL-10 was associated with accelerated loss of lumbar spine BMD of 0.10 (95% CI: 0.00, 0.20) SD scores. No inflammatory markers were associated with change in femoral neck BMD.

Discussion

Using data from the Hertfordshire Cohort Study, we have examined associations between baseline inflammatory markers and level, and rate of loss, of lumbar spine and femoral neck BMD. Higher adiponectin and adiponectin:leptin ratios (a ratio which purports to assess the inflammatory balance of the adipokine profile [23,24]) were associated with lower baseline BMD at both the lumbar spine and femoral neck; determinants of accelerated decline in lumbar spine BMD included lower IL-10. Fully-adjusted associations were little altered when additionally adjusted for number of systems medicated (a marker of comorbidity). No baseline inflammatory markers were associated with change in femoral neck BMD.

Our study has many strengths. First, the HCS provides a detailed characterisation of community-dwelling older people in England and measurements were carried out according to strict protocols by trained fieldworkers. Moreover, the HCS database is managed by an experienced multi-disciplinary team. Second, we examined the associations between a wide panel of inflammatory markers and components of bone health, relationships which have not been previously investigated in an elderly, UK population.

Our study has some limitations. First, a healthy responder bias has, unsurprisingly, been identified in HCS [26]. However, HCS participant characteristics are nonetheless broadly comparable with those of participants in the nationally representative Health Survey for

England [26]. Furthermore, our analyses were internal so unless the associations of interest differed systematically among subgroups of the population, no major bias should have been introduced. Second, the inflammatory markers and outcomes contained missing values and to maximise statistical power, participants were included in the analysis sample if they had non-missing values for at least one inflammatory marker and bone outcome. Third, the measurement of serum measures of inflammation at a single time-point may be vulnerable to the influence of concurrent illness. However, due to the organisation of the research clinics, it is unlikely that participants would have attended if they were unwell. This is supported by the fact that the majority of inflammatory markers were within the normal range. Additionally, baseline inflammatory marker measurements have been successfully used to predict cardiovascular risk [30]. Fourthly, the sample size used for analysis was reasonably small and due to the number of statistical tests performed, we cannot exclude the possibility of chance findings. However, the results reported in this study are broadly consistent with the wider literature [20,31,16,6].

The immune system and inflammatory response changes across the lifecourse. This alteration is suspected to occur secondary to overloading of the immune system with activated, primed macrophages, lymphocytes and dendritic cells in response to continuous stress and inflammation. This results in a chronic, low-grade inflammatory state [32] with changes in the pro- and anti-inflammatory cytokine, chemokine landscape [33,34]. Ageing can also effect bone with an increasingly prevalent pro-osteoclastic environment leading to increased bone resorption and a subsequent propensity to fracture [35].

Our current analysis has identified associations between adiponectin to leptin ratio and baseline bone mineral density at both femora and vertebrae. Adipocytes and osteoblasts share a common progenitor, and thus, adipokine cross-talk between the two resultant tissues is not surprising. Previous studies have demonstrated an increased risk of incident fracture with increasing adiponectin level in women [31] and in an elderly US population of men [20]. The influence of adipokines on BMD has been investigated in a younger, female, Korean population by Haam and colleagues [21] who found a negative association between leptin concentration with non-vertebral BMD and a positive association between high-molecular weight (HMW) adiponectin and total hip BMD in premenopausal women but a negative association of HMW adiponectin with BMD at both femoral and vertebral sites in post-menopausal women. In addition, an association between higher adiponectin and accelerated loss of hip areal BMD has been reported previously [22]; in our study higher adiponectin was only associated with lower baseline lumbar spine and lower femoral neck BMD. Our study therefore contributes significantly to the adipokinetic literature as an investigation of a mixed-sex population of older adults, which uses BMD as the outcome measure and highlights the relevance of the adiponectin to leptin ratio, rather than considering the levels of each mediator in isolation. Our current study adds to previous work that we have published on the effect of adipose tissue on the bone [36].

Our study also suggested associations between inflammatory cytokines and baseline BMD, including associations between higher levels of IL-1 β and TNF, and lower levels of hs-CRP, in relation to lower BMD, although these results were not robust to adjustment. Interleukin-1 β is a profligate proinflammatory cytokine. Although short-term release of

IL-1 β is necessary for fracture healing, long term exposure can lead to bone loss and has been associated with osteoporosis [37]. In vitro studies have shown that IL-1 β causes osteoclastogenesis via induction of RANKL and inhibition of osteoprotegerin [38] and reduces osteoblast migration [39]. Our finding is therefore in keeping with the literature and supports evidence for the influence of the inflammatory cascade on bone minerality and structure.

The associations that we identified between hs-CRP and TNF and baseline DXA outcomes may reflect the heterogeneous relationships between both mediators and bone mineral density in previous investigations [19,6,15,7,14,16,17]. The ubiquity of these inflammatory markers, and their common release (and thus presence in sera) in response to inflammatory stimuli of any aetiology, is likely the cause. Further tissue-specific work in vivo is required to delineate associations with bone density.

In this current study, we identified relationships between the inflammatory markers IL-10, IL-8 and TNF and change in BMD but only at the lumbar spine and not the femoral neck. This may be explained by the greater degree of trabeculation in the lumbar spine, increased metabolic activity and thus, increased vulnerability to manipulation by inflammatory mediators. Interleukin-10 is primarily an anti-inflammatory cytokine which dampens T helper 1 cell activity and lipopolysaccharide-stimulated immune activation. Work in animal models has demonstrated that IL-10 deficiency results in adverse bone outcomes including the development of osteopenia, attenuated bone formation and phenotypically long, fragile bones [40]. This is likely through the inhibition of osteoclastogenesis and promotion of osteoblastic differentiation [41]. These cellular effects are supported by findings in human studies relating IL-10 haplotypes with reduced BMD in postmenopausal females [42].

Interleukin-8 plays a primary role in neutrophil chemotaxis as well as correlating positively with Receptor Activator of Nuclear kappa-B Ligand (RANKL). Our study suggested some association between higher levels of IL-8 and accelerated decline at the lumbar spine. This is in keeping with an adverse effect of inflammation on BMD, though this finding was not robust in the fully-adjusted model. It was surprising that lower levels of TNF were associated with accelerated decline in lumbar spine BMD in our study; indeed this is at odds with a previous finding in a smaller study of older adults in Australia which showed that higher levels of TNF predicted greater decline in BMD [16]. Therefore, we cannot rule out the possibility that this result is a chance finding in our study. However, another study in a group of elderly males in Sweden found no association between TNF level and BMD [6]. Further studies are required to clarify this relationship, though it may be explained by increased spinal degeneration and osteoarthritis; associations between higher TNF and accelerated loss of knee cartilage have been reported previously [43]. Osteoarthritis (OA) is associated with raised intra-articular TNF; thus, a serum increase in TNF may be representative of an increased burden of OA.

In conclusion, we have demonstrated an association between higher levels of adiponectin and adiponectin: leptin ratios and lower baseline bone mineral density. Lower levels of the anti-inflammatory cytokine IL-10 were associated with loss of BMD in longitudinal follow-

up. Further work, particularly tissue-specific, in vivo experimentation, is required to elucidate the role of inflammatory mediators at the bone-inflammation interface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Mini-abstract

Among 365 Hertfordshire Cohort Study participants (aged 59-71 years at baseline), higher adiponectin and adiponectin:leptin ratios were associated with lower baseline lumbar spine and femoral neck BMD. Lower IL-10 was associated with accelerated decline in lumbar spine BMD. This suggests that bone health can be influenced by changes in immune phenotype and alterations in adipokine homeostasis.

Figure 1. SD difference (95% CI) in level and conditional change in total lumbar spine and total femoral neck BMD per SD increase in inflammatory marker

Separate linear regression models for each inflammatory predictor were adjusted for the following baseline predictors: gender, age, height, weight-for-height residual, smoking history (ever vs never), alcohol consumption, diet quality, physical activity and use of hormone replacement therapy. Conditional change models were also adjusted for bisphosphonate use at follow-up. Adjusted p-values are presented.

Conditional change measures are independent of baseline level; a positive estimate illustrates that the inflammatory marker was associated with reduced loss of BMD over time and a negative estimate reflects accelerated loss

Ad:lep: Adiponectin: leptin ratio

Table 1
Participant characteristics at HCS baseline

Mean (SD)	Men (n=194)	Women (n=171)	Number of observations
Age at bone scan (years)	64.4 (2.5)	66.5 (2.7)	365
Time between bone scans (years) *	5.2 (4.8, 5.6)	3.6 (3.1, 4.0)	365
Height (cm)	174.8 (6.6)	161.8 (6.0)	365
Weight (kg)	80.8 (11.5)	69.3 (12.2)	365
BMI (kg/m ²)	26.5 (3.4)	26.4 (4.4)	365
Ever smoked **	115 (59.3%)	57 (33.3%)	365
Alcohol consumer (1 unit per week) **	172 (88.7%)	95 (55.6%)	365
Prudent diet score	-0.6 (2.0)	1.0 (1.8)	365
Hormone replacement therapy use **	0 (0.0%)	32 (18.7%)	365
Bisphosphonate use at follow-up **	3 (1.5%)	15 (8.8%)	365
Dallosso physical activity score	65.0 (13.7)	62.6 (14.5)	365
Number of systems medicated *	1 (0, 1)	1 (0, 2)	365
CRP (mg/L) *	0.8 (0.4, 2.0)	1.1 (0.5, 2.4)	350
TNF (pg/ml) *	7.0 (2.9, 11.6)	3.5 (2.9, 9.8)	354
IL-1 β (pg/ml) *	1.14 (0.95, 1.33)	1.04 (0.95, 1.14)	362
IL-6 (pg/ml) *	5.8 (3.4, 8.5)	5.8 (3.1, 7.6)	316
IL-8 (pg/ml) *	7.4 (5.4, 10.0)	6.9 (5.6, 9.3)	300
IL-10 (pg/ml) *	7.0 (5.9, 10.6)	7.1 (5.9, 10.0)	363
Adiponectin (ug/ml) *	3.4 (1.8, 6.7)	3.7 (2.5, 5.8)	358
Leptin (ng/ml) *	6.0 (3.6, 10.3)	18.2 (10.2, 28.7)	358
Adiponectin (ug/ml):Leptin (ng/ml) ratio *	0.5 (0.2, 1.4)	0.2 (0.1, 0.4)	351
<i>Total lumbar spine BMD</i>			
Baseline measure (g/cm ²)	1.06 (0.15)	0.95 (0.17)	365
Follow-up measure (g/cm ²)	1.10 (0.18)	0.96 (0.17)	365
Annual change from baseline to follow-up (g/cm ² /yr)	0.007 (0.009)	0.003 (0.014)	365
<i>Total femoral neck BMD</i>			
Baseline measure (g/cm ²)	1.03 (0.14)	0.89 (0.13)	365
Follow-up measure (g/cm ²)	1.03 (0.14)	0.87 (0.12)	365
Annual change from baseline to follow-up (g/cm ² /yr)	0.000 (0.006)	-0.005 (0.01)	365

* Median (lower quartile, upper quartile)

** n(%)

Table 2
SD difference in outcome (95% CI) per SD increase in inflammatory marker

Outcome	Inflammatory predictor	Gender-adjusted		Fully-adjusted*	
		Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Baseline total lumbar spine BMD	CRP	0.07 (-0.04,0.17)	0.202	-0.02 (-0.13,0.08)	0.681
	TNF	-0.09 (-0.20,0.01)	0.083	-0.07 (-0.18,0.03)	0.143
	IL-1 β	-0.11 (-0.21,-0.01)	0.038	-0.09 (-0.19,0.01)	0.079
	IL-6	-0.04 (-0.16,0.07)	0.450	-0.05 (-0.16,0.06)	0.341
	IL-8	-0.06 (-0.18,0.06)	0.322	-0.02 (-0.13,0.09)	0.732
	IL-10	-0.01 (-0.12,0.09)	0.803	-0.00 (-0.10,0.10)	0.968
	Adiponectin	-0.14 (-0.25,-0.04)	0.007	-0.11 (-0.21,-0.01)	0.029
	Leptin	0.16 (0.06,0.26)	0.002	0.05 (-0.06,0.16)	0.342
	Ad:lep ratio	-0.20 (-0.31,-0.10)	<0.001	-0.11 (-0.21,-0.00)	0.042
Baseline total femoral neck BMD	CRP	0.13 (0.02,0.23)	0.018	-0.01 (-0.11,0.09)	0.846
	TNF	-0.11 (-0.22,-0.01)	0.037	-0.09 (-0.18,0.01)	0.067
	IL-1 β	-0.11 (-0.22,-0.01)	0.034	-0.08 (-0.18,0.01)	0.070
	IL-6	-0.02 (-0.13,0.09)	0.727	-0.02 (-0.12,0.08)	0.674
	IL-8	-0.06 (-0.18,0.05)	0.275	-0.00 (-0.10,0.10)	0.997
	IL-10	-0.01 (-0.12,0.09)	0.801	0.00 (-0.09,0.09)	0.956
	Adiponectin	-0.15 (-0.26,-0.05)	0.004	-0.11 (-0.20,-0.02)	0.021
	Leptin	0.18 (0.08,0.28)	0.001	0.02 (-0.08,0.12)	0.756
	Ad:lep ratio	-0.24 (-0.34,-0.13)	<0.001	-0.10 (-0.20,-0.00)	0.044
Conditional change in total lumbar spine BMD	CRP	-0.09 (-0.20,0.02)	0.106	-0.10 (-0.20,0.01)	0.080
	TNF	0.13 (0.02,0.23)	0.018	0.10 (0.00,0.21)	0.045
	IL-1 β	0.03 (-0.08,0.13)	0.597	0.04 (-0.06,0.14)	0.428
	IL-6	0.02 (-0.10,0.13)	0.777	0.02 (-0.09,0.13)	0.751
	IL-8	-0.14 (-0.26,-0.03)	0.015	-0.11 (-0.22,0.01)	0.073
	IL-10	0.10 (-0.00,0.21)	0.050	0.10 (0.00,0.20)	0.042
	Adiponectin	0.09 (-0.01,0.20)	0.084	0.07 (-0.03,0.17)	0.174
	Leptin	0.03 (-0.08,0.13)	0.626	-0.00 (-0.11,0.11)	0.985
	Ad:lep ratio	0.04 (-0.06,0.15)	0.425	0.06 (-0.05,0.16)	0.311
Conditional change in total femoral neck BMD	CRP	-0.08 (-0.19,0.02)	0.117	-0.05 (-0.16,0.06)	0.351
	TNF	0.03 (-0.08,0.14)	0.585	0.01 (-0.09,0.12)	0.799
	IL-1 β	-0.02 (-0.13,0.08)	0.639	-0.03 (-0.13,0.08)	0.598
	IL-6	-0.02 (-0.14,0.09)	0.680	-0.02 (-0.13,0.09)	0.746
	IL-8	-0.04 (-0.15,0.08)	0.522	-0.00 (-0.12,0.11)	0.936
	IL-10	-0.01 (-0.12,0.09)	0.826	-0.02 (-0.12,0.09)	0.773
	Adiponectin	-0.01 (-0.12,0.09)	0.825	-0.01 (-0.12,0.09)	0.815
	Leptin	0.05 (-0.06,0.16)	0.350	0.07 (-0.04,0.19)	0.210
	Ad:lep ratio	-0.06 (-0.16,0.05)	0.300	-0.07 (-0.18,0.04)	0.217

Separate gender-adjusted and fully-adjusted linear regression models were fitted for each predictor.

* Fully-adjusted models accounted for the following characteristics at baseline: gender, age, height, weight-for-height residual, smoking history (ever vs never), alcohol consumption, diet quality, physical activity and use of hormone replacement therapy. Conditional change models were also adjusted for bisphosphonate use at follow-up.

Sex-specific SD scores were derived for all inflammatory predictors and bone outcomes.

Unadjusted estimates correspond to Pearson correlations between inflammatory markers and outcomes.

Ad:lep: Adiponectin: leptin ratio

Significant associations ($p < 0.05$) are highlighted in bold