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Published in:
Bone

DOI:
[10.1016/j.bone.2015.05.035](https://doi.org/10.1016/j.bone.2015.05.035)

2015

[Link to publication](#)

Citation for published version (APA):

Herlin, M., McGuigan, F., Luthman, H., & Åkesson, K. (2015). Polymorphisms in inflammation associated genes ALOX15 and IL-6 are associated with bone properties in young women and fracture in elderly. *Bone*, 79, 105-109. <https://doi.org/10.1016/j.bone.2015.05.035>

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Polymorphisms in inflammation associated genes ALOX15 and IL-6 are associated with bone properties in young women and fracture in elderly

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Conflict of Interest

Maria Herlin, Fiona E McGuigan, Holger Luthman, Kristina Åkesson declare that they have no conflict of interest.

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Abstract

Purpose: *ALOX12* and *ALOX15* encode arachidonate lipoxygenases which produce lipid metabolites involved in inflammatory processes. Metabolites generated by *ALOX12* and *ALOX15* can activate the expression of the potent pro-inflammatory cytokine IL-6, and produce endogenous ligands for *PPARG*. In this study, polymorphisms in *ALOX12*, *ALOX15*, *IL6* and *PPARG* were investigated for association with bone properties in young and elderly Swedish women.

Methods: Three SNPs in *ALOX12*, five in *ALOX15*, one each in *IL6* and *PPARG* were genotyped in the cohorts PEAK-25 (n=1061 women; all 25y) and OPRA (n=1044 women; all 75y). Bone mineral density (BMD) and quantitative ultrasound (QUS) were analyzed in both cohorts; trabecular bone score (TBS) in PEAK-25; bone loss, fracture incidence and serum C-reactive protein (CRP) were assessed in OPRA.

Results: In the elderly women *ALOX15* (rs2619112) was associated with CRP levels (p=0.004) and incident fracture of any type (p=0.014), although not with BMD or ultrasound. In young women, carrying the common T allele (*ALOX 15* rs748694) was associated with lower QUS values (p=0.002-0.006). The *IL6* SNP was associated with lower BMD in PEAK-25 (femoral neck p=0.034; hip p=0.012). TBS was not associated with variation in any gene. Variants in the *ALOX12* and *PPARG* were not associated with BMD in either cohort.

Conclusions: This study suggests that variation in inflammation related genes *ALOX15* and *IL6* were associated with bone microarchitecture and density in young adult women, but appear to be less important in the elderly, despite an observed association with CRP as a marker of inflammation and incident fracture.

Keywords

Polymorphism, *ALOX12*, *ALOX15*, *IL-6*, *PPARG*, bone, inflammation

Introduction

Evidence suggests that inflammation influences bone turnover and hence may contribute to the aetiopathogenesis of osteoporosis [1], a disease characterized by reduced bone mineral density (BMD) and disruption of bone microarchitecture, resulting in increased risk of fracture.

There is a close relationship between the immune and skeletal systems [2] and epidemiological studies have demonstrated an increased risk of developing osteoporosis in inflammatory conditions such as ankylosing spondylitis [3], rheumatoid arthritis [4] and inflammatory bowel disease [5]. In addition, pro-inflammatory cytokines have been associated with osteoblast and osteoclast regulation, and with bone resorption [6]. However, inflammation is also a part of the aging process, possibly due to the increased production of pro-inflammatory cytokines mediated through estrogen-regulated pathways, consistent with observations that bone loss at menopause is associated with low grade inflammation [7]. Although the mechanisms are not fully understood, systemic inflammation can affect bone remodeling, leading to adverse effects on bone structure and strength [8].

Based on the hypothesis that age related low-grade inflammation influences bone phenotypes, we have evaluated candidate genes with a putative role in inflammation-induced modulation of bone tissue, skeletal integrity and fracture risk.

The 17p13 region of the human genome contains quantitative trait loci regulating BMD [9]. This region, which was also identified in an early meta-analysis of genome wide association studies (GWAS) for femoral neck BMD [10], includes the genes encoding arachidonate 12-lipoxygenase (*ALOX12*) and arachidonate 15-lipoxygenase (*ALOX15*). Lipid metabolites are produced from the polyunsaturated arachidonic acid [11], some of which have pro-inflammatory effects [12] and have been suggested to play role in chronic inflammation [13]. In experimental studies *ALOX15*-knockout mice have higher BMD and bone strength compared to wild type mice, suggesting *ALOX15* to be a negative regulator of BMD [14]. Epidemiological studies have reported associations, albeit inconsistent, between genetic variation in both the *ALOX12* and *ALOX15* genes and bone properties [15-20], and with body

composition and fat mass [21,22]. Strengthening their putative role as candidate genes for osteoporosis, ALOX12/15 metabolites can act as ligands for peroxisome proliferator-activated receptor gamma (*PPAR* γ) [23]. Activation of *PPAR* γ -dependent pathways promotes adipogenesis at the expense of osteoblastogenesis, promotes osteoclast differentiation [24] and has a proinflammatory role [25]. Metabolites generated by ALOX12/15 also directly activate expression of the proinflammatory cytokine interleukin 6 (*IL*-6) [12], whose levels are elevated in inflammatory states [26]. Polymorphisms in *IL*6 and *PPARG* have been associated with osteoporosis [27-31].

This study investigates polymorphisms in *ALOX12*, *ALOX15*, *IL*6 and *PPARG* genes in relation to osteoporosis associated phenotypes. Reflecting the temporal aspect of age-related inflammation, the study has been performed in young and elderly Swedish women. The phenotypes investigated comprehensively reflect bone microstructure and strength. In the elderly women we also investigated the relationship between these genes and the most commonly used biomarker of inflammation, the acute phase reactant C-reactive protein (CRP).

Materials and methods

Subjects

Two population based cohorts of Swedish women were studied. The Malmö Osteoporotic Prospective Risk Assessment (OPRA) cohort consists of 1044 elderly women all aged 75 years and prospectively followed for 10 years, and the PEAK-25 cohort consists of 1061 women all aged 25 years. Details of these two cohorts have been published previously [32,33]. Participants gave written informed consent and the study was approved by the Regional Ethical Review Board in Lund and performed according to the principles of the Helsinki agreement.

Measurement of bone phenotypes and body composition

BMD was measured with dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy for PEAK-25 and Lunar DPX-L for OPRA, (Lunar Corporation, Madison, WI, USA) at femoral neck, lumbar

spine, total hip and total body. Fat and lean mass for total body was also measured with the same instruments. Calibrations were performed daily using a manufacturer supplied phantom. The precision error (coefficient of variation) was 0.94%, 1.45%, 4.01% for total body, lumbar spine and femoral neck, respectively in the OPRA cohort [34], and 0.90% and 0.65% for femoral neck and lumbar spine, respectively in the PEAK-25 cohort [35]. Bone loss was calculated in the OPRA cohort as BMD at 5 year follow-up minus BMD at baseline.

Quantitative ultrasound measurements (QUS), i.e. speed of sound (SoS), broadband ultrasound attenuation (BUA) and stiffness index (SI), were performed on the right calcaneus using the Lunar Achilles® system (Lunar Corporation, Madison, WI, USA). Precision was 1.5 for BUA and SoS [36]. Calibrations were performed daily.

Trabecular bone score (TBS) index is a novel approach applied to DXA images to estimate bone microstructure. Due to technical limitations TBS could not be calculated from the Lunar DPX-L, therefore spine acquisitions were available only for the PEAK-25 cohort. These were analyzed using the manufacture's provided software (Encore 2004; GE medical-Lunar, Madison, WI) and a standardized protocol was followed for assessment of TBS indexing [37]. TBS was calculated as the mean value of the individual measurements for each vertebra (L1 to L4).

Incident Fractures

For the OPRA cohort, information on fractures was continuously registered through the X-rays files at the Radiology Department, Malmö, Skåne University Hospital. Incident fracture data was collected until October 31st 2012, providing a maximum follow-up for fracture of 17.2 years (mean 13.1 years). In this paper we analyse 'any incident fracture' which includes hip, distal radius, vertebra, shoulder, pelvis and proximal tibia. The majority of fractures recorded were attributable to low energy trauma [38]. Excluded were fractures of the face, hands and feet and fractures resulting from pathology and high energy trauma.

Analysis of C-reactive protein

CRP measurements were available only in the OPRA cohort. Blood samples were centrifuged and stored at -80°C. CRP was analyzed by routine methods using Roche Diagnostics (Cobas).

The lowest detectable limit was 0.6 mg/L and coefficient of variation (CV) 3.6 – 4.1. CRP levels >10 mg/ml was assumed to result from acute inflammation and subsequently 77 women were excluded from the analysis. Since the lowest detectable limit for CRP was 0.6 mg/L, missing (undetectable) values were imputed [39].

Genotyping

Total genomic DNA was isolated from blood using the QIAamp 96 DNA blood kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

Three single nucleotide polymorphisms (SNPs) in *ALOX12*, five SNPs in *ALOX15* and one SNP each in *IL-6* and *PPARG* were genotyped in both cohorts (Table 1) although *PPAR γ* was available only for a proportion of PEAK-25 (n=472). The rationale for SNP selection at the time when genotyping was performed is outlined below. SNPs for *ALOX12* were selected from the literature as having been previously investigated for BMD [16,18,20]. SNP rs312466 is located in the promoter, while rs1126667 and rs2292350 are NCBI tagging SNPs located in an LD block [16]. For *ALOX15*, with the exception of rs8074545 upstream of the coding region (which is intergenic and therefore has potential functionality) all selected SNPs were tagging SNPs previously investigated for BMD (rs748694; rs9894225; rs916055; rs2619112) [20]. The *IL-6* tagging SNP rs10242595, located downstream of the coding region was selected for its previous association with decreased fat mass [40] while *PPAR γ* rs1801282 (Pro12Ala) is the most extensively examined in a variety of diseases including osteoporosis [31].

Genotyping of the Pro12Ala *PPAR γ* SNP used restriction enzyme analysis of PCR amplified DNA and gel electrophoresis. All other genotyping was performed using iPLEX (Sequenom, San Diego, CA, USA) or TaqMan SNP genotyping Assay (Applied Biosystems, Foster City, CA, USA). Approximately 3% of the samples from each cohort were genotyped in duplicate with 100% concordance.

Genotype and allele frequencies were similar in both cohorts. Minor allele frequencies (MAF) of all the polymorphisms were comparable with European populations reported in

HapMap (Table 1). All SNPs, except for rs1801282_*PPAR* γ in the OPRA cohort ($p=0.013$) due to a slightly higher than predicted rare allele frequency, conformed to Hardy Weinberg Equilibrium.

Statistical analysis

Statistical analyses were performed using SPSS version 22 (IBM Corp., NY, USA) and associations with a p -value of <0.05 were considered nominally significant. The phenotypes and several of the markers studied are not independent (i.e. are correlated or in linkage disequilibrium), therefore applying a Bonferroni correction would be over-stringent. We therefore report the uncorrected p -values and acknowledge the fact that multiple tests were performed.

Using a co-dominant model (comparing the three genotypes, under the assumption that neither of the alleles is dominant), genotype specific differences between the phenotypes were analyzed by ANOVA. Association adjusting for confounding factors (body weight, height, smoking) was performed using regression analysis. To facilitate comparison with other published studies, the rs748694_*ALOX15* was also analysed as carriage of the common T allele (i.e. TT, TC versus CC). The χ^2 test was used to analyze genotype differences in fracture incidence.

Results

Characteristics of the participants from the two cohorts are shown in Table S1. As expected, the young PEAK-25 women had higher BMD and QUS values compared to the elderly women in the OPRA cohort, while total body fat was higher in the OPRA cohort.

In neither of the two cohorts were ***ALOX12*** SNPs associated with BMD, bone microarchitecture or fracture (data not shown). In PEAK-25, a nominal association with spine BMD did not withstand adjustment for weight, height and smoking.

The promoter SNP rs748694_***ALOX15*** was associated with calcaneal microarchitecture in the PEAK-25 cohort (unadjusted p -values: BUA $p=0.037$, SoS $p=0.021$ and stiffness $p=0.023$).

Carriers of the common 'T' allele displayed lower BUA (unadjusted $p = 0.006$), SoS (unadjusted $p = 0.006$) and stiffness (unadjusted $p = 0.002$). The association withstood adjustment for weight, height, smoking and femoral neck BMD (Table 2), suggesting that the relationship with calcaneal microarchitecture is independent of bone density. No association was seen with trabecular architecture (TBS) however (Table 2). While there was a trend towards lower bone density among those carrying at least one copy of the 'T' allele, this was weak (total body BMD, $p=0.046$) or non-significant (Table 2).

In the elderly OPRA participants, **ALOX15** SNPs were not associated with QUS or BMD. Women with 1 or 2 copies of the rare G allele of rs2619112_ **ALOX15** had a trend towards higher incidence of fractures of any type sustained after the age of 75. However, this was not mirrored in the relationship with bone density or ultrasound phenotypes (data not shown). Excluding women receiving treatment for osteoporosis (estrogen or bisphosphonates ($n=48$)) did not appreciably alter the results.

The variant allele of **IL6**_rs10242595 was associated with lower BMD in the PEAK-25 but not the OPRA cohort and there were no associations with other bone phenotypes in either the young or elderly women (Table 3).

Variation in **PPARG** was not associated with bone phenotypes in either cohort (data not shown).

Bone loss, only calculated in the elderly women, was not associated with any of the analyzed variants in **ALOX12**, **ALOX15**, **IL6** or **PPARG** (Table S2).

Body composition i.e. fat and lean mass, did not differ according to **ALOX12**, **IL6** or **PPARG** genotype in either of the cohorts. For **ALOX15** no association was observed with body composition in the PEAK-25 cohort, while in OPRA, an association was seen for rs8074545_ **ALOX15** with total body fat (25536 ± 7811 / 26983 ± 7956 / 25550 ± 7294 ; $p = 0.026$), and remained significant after adjustment for height (data not shown).

Serum levels of CRP showed a allele dependent increase with the highest levels in homozygotes for the common A allele of rs2619112_ **ALOX15** (baseline $p = 0.004$; 5-year

follow up ($p=0.035$) (Table 4). Polymorphisms in *ALOX12*, *IL-6* or *PPAR γ* were not associated with levels of CRP (data not shown).

Discussion

In this study, we analysed the association between polymorphisms in *ALOX12*, *ALOX15*, *IL6* and *PPARG* with bone phenotypes and with the inflammatory biomarker CRP. We used the candidate gene approach since GWAS is not definitive in identifying all the genetic variation contributing to complex disease phenotypes. There is ample evidence of a role for the selected genes in regulation of inflammation and bone remodeling.

The results from our study suggest an association, albeit weak, between genetic variation in the *ALOX15* gene and altered serum levels of CRP in 75-year old women. This appears to support the hypothesis of a pro-inflammatory role of the *ALOX15* pathway in humans, although in our cohorts, polymorphisms in *ALOX15* do not appear to play a direct role in skeletal integrity in older women, while apparently influencing bone microarchitecture in young women.

While other studies have investigated the association between *ALOX15* and bone in pre- and post-menopausal women these were in cohorts of different ethnicities and with a focus only on BMD [15,19]. In the present study we aimed at investigating the role of *ALOX15* polymorphisms using cohorts with a homogeneous genetic background and investigating phenotypes reflecting skeletal integrity and fracture risk. A variant in *ALOX15*, located in the promoter and thus potentially functional was associated, independent of bone density, with higher quantitative ultrasound values in the young women. This indicates a role for *ALOX15* in skeletal microarchitecture, although this was not confirmed by the spine trabecular bone score data. We can only speculate the extent to which differential loading patterns at these skeletal sites may have contributed to these observations. In the elderly women the results appear contradictory; no appreciable genotype related differences on BMD or QUS yet a tendency towards higher fracture incidence despite lower CRP levels. We interpret these observations to support a role for *ALOX15* in modulating inflammatory cytokines [12] while suggesting that fractures are not directly related to increased inflammatory activity, at least not as reflected by serum CRP levels [39]. Unfortunately, other inflammatory biomarkers were not available and neither fracture nor CRP was available in PEAK-25, which is an undoubted limitation of the study.

We found that *ALOX12* polymorphisms were associated with BMD only in the young but not the elderly women, contradicting the low BMD and increased vertebral fracture risk among post-menopausal women observed in another study [18]. The much higher age at inclusion

of the women in the OPRA cohort leads us to conjecture whether *ALOX12* may be most important during critical periods of high bone turnover, for example during bone accrual and menopause.

In PEAK-25 *IL6* was associated with lower femoral neck and spine BMD, which is supported by previous studies reporting association with BMD, ultrasound and fractures in women and men [27-30]. The same SNP has also been associated with decreased fat mass in men [40]. In the present study, there was no association between *IL6* and bone properties in the elderly women, although previously a functional promoter polymorphism (rs1800795), which increased *IL-6* promoter activity and plasma IL-6 levels, was weakly correlated with lower QUS values and fracture [30].

Adipose tissue is a source of inflammatory cytokines and *PPARG* is widely associated with type II diabetes and concomitant obesity. Association between *PPARG* and lower BMD has previously been reported in pre- and post-menopausal women [31,41], however our results suggest *PPARG* variation is not a major player in the regulation of bone strength, which may reflect ethnic differences between Japanese and Swedish populations as well as the analysis of different SNPs.

The low grade inflammatory state characteristic of aging was the background on which we hoped to better understand the role of inflammation associated genes in bone regulation. Our results only partially realize this ambition and require cautious interpretation as the observed associations are modest. Disappointingly, we conclude that the analysed SNPs in *ALOX12*, *ALOX15*, *IL-6* or *PPARG* do not appear to make a major contribution to bone phenotypes at very advanced ages, while in young adult women *ALOX15* and *IL6* were associated with bone density and microarchitecture. Potential associations could be masked by gene-gene interactions (which were out with the scope of this study to address) or by the fact that both pro- and anti-inflammatory metabolites are generated by *ALOX12/15* pathways and could modulate bone phenotypes [42].

The present study has its limitations, among them the fact that the SNPs were not identified from GWAS, but rather selected from the literature. On the other hand, SNP based GWAS do not identify all disease risk variants. Many variants conferring a small risk are overlooked and many SNPs falling below the accepted threshold of statistical significance may be relevant when investigated through pathway analyses [43,44]. Another limitation is that not all phenotypes were available in both cohorts, which in the case of the TBS data, introduces some uncertainty as to what extent bone microarchitecture rather than bone density is influenced. To better understand the contribution of inflammation related genes during the life-course it would be interesting to compare the very elderly women with women in early post-menopause as it may be that potential associations are masked, due to the long time since estrogen withdrawal or by underlying comorbidities.

In summary, we found that variation in inflammation related genes *ALOX15* and *IL6* was associated with bone density and microarchitecture in young adult women although the role of these genes in the pathogenesis of osteoporosis in the very elderly is less easy to interpret.

Acknowledgements

Thanks are extended to Didier Hans (Center of Bone diseases, Lausanne University Hospital, Switzerland); Leif Group (Diabetes and Endocrinology, Lund University, Sweden); the research nurses at the Clinical and Molecular Osteoporosis Research Unit, Malmö; Åsa Almgren and Siv Braun for data management and to all the women who kindly participated in the study. This work was supported by grants from the Swedish Research Council (K2012-52X-14691-10-3), FAS (2007-2125), Greta and Johan Kock Foundation, A. Pålsson Foundation, A. Osterlund Foundation, the H Järnhardt foundation, King Gustav V and Queen Victoria Foundation, Åke Wiberg Foundation, Thelma Zoegas Foundation, The Swedish Rheumatism Association, Skåne University Hospital Research Fund, Research and Development Council of Region Skåne, Sweden, Tissues in Motion Programme at Lund University.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Table 1. SNP information and genotype distribution in the cohorts

				OPRA					PEAK-25				
SNP	Chr	Location	Major/minor allele	Common homozygote	Heterozygote	Variant homozygote	MAF		Common homozygote	Heterozygote	Variant homozygote	MAF	European MAF
<i>ALOX12</i>													
rs1126667	17	Exon 6	G/A	302	508	179	0.44		331	471	190	0.43	0.42
rs2292350	17	Intron 2	G/A	368	475	147	0.39		347	479	164	0.41	0.40
rs312466	17	Promoter	G/A	299	466	175	0.43		309	461	178	0.43	0.42
<i>ALOX15</i>													
rs8074545	17	Upstream	C/G	542	392	62	0.26		545	379	71	0.26	0.21
rs748694	17	Promoter	A/G	284	504	202	0.46		289	490	212	0.46	0.48
rs9894225	17	Promoter	A/G	532	377	59	0.24		549	360	74	0.26	0.26
rs2619112	17	Intron 12	A/G	279	498	223	0.44		269	497	237	0.48	0.41
rs916055	17	3'UTR	C/T	434	438	129	0.35		415	465	116	0.35	0.32
rs10242595_IL-6	7	Downstream	A/G	557	370	72	0.26		543	397	61	0.26	0.31
rs1801282_PPARGy	3	Exon 4	C/G	709	201	26	0.14		343	114	15	0.15	0.09

Table 2. Association between rs748694_ALOX15 with BMD, QUS and TBS in the PEAK-25 cohort

	TT (n=289)	TC (n=490)	CC (n=212)	Genotypic p (adj)*	T carriers	CC	Allelic p (adj)*
Total Body BMD	1.169 (0.074)	1.173 (0.070)	1.183 (0.077)	0.054	1.171 (0.072)	1.183 (0.077)	0.046
Femoral Neck BMD	1.057 (0.123)	1.049 (0.124)	1.061 (0.124)	0.889	1.053 (0.124)	1.061 (0.124)	0.415
Total Hip BMD	1.056 (0.136)	1.057 (0.123)	1.063 (0.161)	0.866	1.058 (0.122)	1.074 (0.122)	0.164
Lumbar spine BMD	1.242 (0.132)	1.236 (0.129)	1.242 (0.138)	0.911	1.238 (0.139)	1.242 (0.138)	0.696
QUS-BUA	117.3 (9.8)	117.0 (10.7)	119.4 (11.0)	0.075	117.1 (10.4)	119.4 (11.0)	0.034
QUS-SOS	1573.5 (31.6)	1573.1 (33.0)	1580.7 (31.8)	0.021	1573.2 (32.5)	1580.7 (31.8)	0.017
QUS-SI	98.6 (13.7)	98.3 (15.1)	102.0 (14.7)	0.023	98.4 (14.6)	102.0 (14.6)	0.012
Trabecular Bone Score	1.424 (0.070)	1.422 (0.065)	1.424 (0.069)	0.442	1.424 (0.070)	1.423 (0.065)	0.212

Values are mean (SD). BMD (g/cm²), BUA (dB/MHz), SoS (m/s)

*Adjusted for weight, height and smoking. QUS phenotypes additionally corrected for FN-BMD

Table 3. Association of rs10242595_IL-6 with BMD and ultrasound in the PEAK-25 cohort

Phenotype	TT		TC		CC		p-value	p-value (adjusted*)
	Mean	±SD	Mean	±SD	Mean	±SD		
Total body BMD	1.175	0.072	1.175	0.073	1.161	0.081	0.353	0.271
Femural neck BMD	1.060	0.125	1.051	0.121	1.028	0.126	0.118	0.034
Total hip BMD	1.240	0.132	1.239	0.132	1.227	0.127	0.745	0.583
Lumbar spine BMD	1.069	0.121	1.056	0.121	1.033	0.129	0.05	0.012
BUA	117.8	11.0	117.6	10.1	115.0	8.8	0.2	0.374
SoS	1574.9	32.3	1575.9	32.5	1565.9	32.1	0.12	0.939
Stiffness	99.3	14.9	99.5	14.4	95.0	13.8	0.11	0.624
Trabecular bone score	1.425	0.068	1.425	0.069	1.409	0.075	0.245	0.294

Values are mean (SD)

*Adjusted for weight, height and smoking. QUS phenotypes additionally corrected for FN-BMD

Table 4. Association of rs2419112_ALOX15 with serum levels of CRP in the OPRA cohort

	AA		AG		GG		p-value	p-value (adjusted*)
	Mean	±SD	Mean	±SD	Mean	±SD		
CRP (age 75)	0.785	1.148	0.665	1.071	0.512	1.118	0.025	0.004
CRP (age 80)	0.816	1.158	0.654	1.102	0.569	1.095	0.101	0.035
CRP (age 85)	0.542	1.033	0.595	1.169	0.342	1.128	0.279	0.192

Values are mean (SD)

*Adjusted for weight

Table S1. Baseline characteristics of the cohorts

Variable	OPRA			PEAK-25		
	Mean	± SD	Range	Mean	± SD	Range
Age (years)	75.2	0.1	75.0 - 75.9	25.5	0.2	24.7 - 25.9
Weight (kg)	67.8	11.7	34.0 - 110	64.6	11.1	40 - 135
Height (cm)	160.5	5.7	140 - 180	167.6	6.1	150 - 187
BMD (g/cm²)						
Total body	1.006	0.098	0.718 - 1.422	1.174	0.073	0.969 - 1.478
Femoral neck	0.765	0.138	0.328 - 1.318	1.055	0.124	0.746 - 1.604
Total hip	0.848	0.149	0.498 - 1.416	1.062	0.121	0.742 - 1.593
Lumbar spine				1.239	0.132	0.842 - 1.885
Ultrasound						
BUA (dB/MHz)	102	10	56 - 136	118	11	59 - 149
SoS (m/s)	1523	27	1425 - 1643	1575	32	1497 - 1706
Stiffness	71	13	31 - 112	99	15	42 - 151
Fat mass						
Total body fat mass (g)	26087	7902	2969 - 57452	21045	8202	5506 - 69521
Percentage fat mass (%)	39.1	6.9	7.6 - 56.5	31.7	7.2	12.2 - 58.1

Table S2. Association of *ALOX15*, *ALOX12*, *IL6* and *PPARG* with bone loss in the OPRA cohort

SNP	Total body bone loss		Bone loss at femoral neck		Bone loss at total hip	
	p-value*	p-value**	p-value*	p-value**	p-value*	p-value**
rs8074545_ALOX15	0.713	0.714	0.799	0.893	0.736	0.792
rs748694_ALOX15	0.829	0.815	0.738	0.727	0.322	0.276
rs9894225_ALOX15	0.608	0.596	0.770	0.847	0.843	0.887
rs2619112_ALOX15	0.532	0.555	0.575	0.355	0.932	0.812
rs916055_ALOX15	0.595	0.567	0.984	0.633	0.605	0.363
rs1126667_ALOX12	0.846	0.824	0.758	0.507	0.599	0.346
rs2292350_ALOX12	0.970	0.997	0.884	0.943	0.997	0.876
rs312466_ALOX12	0.744	0.721	0.946	0.669	0.757	0.456
rs10242595_IL-6	0.249	0.231	0.132	0.371	0.139	0.340
rs1801282_PPARG	0.730	0.721	0.227	0.300	0.333	0.270

* adjusted for weight change

** adjusted for weight change and BMD at baseline

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