**Lower leg arterial calcification assessed by high-resolution peripheral quantitative computed tomography is associated with bone microstructure abnormalities in women**

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Mini Abstract

In older women, the presence of lower leg arterial calcification assessed by high-resolution peripheral quantitative computed tomography is associated with relevant bone microstructure abnormalities at the distal tibia and distal radius.

**Abstract**

Purpose: Here we report the relationships of bone geometry, volumetric BMD, and bone microarchitecture with lower leg arterial calcification (LLAC) as assessed by HR-pQCT.

Methods: We utilised the Hertfordshire Cohort Study (HCS), where we were able to study associations between measures obtained from HR-pQCT of the distal radius and distal tibia in 341 participants with or without LLAC. Statistical analyses were performed separately for women and men. We used linear regression models to investigate the cross-sectional relationships between LLAC and bone parameters.

Results: The mean (SD) age of participants was 76.4 (2.6) and 76.1 (2.5) years in women and men, respectively. One hundred and eleven of 341 participants (32.6%) had LLAC that were visible and quantifiable by HR-pQCT. The prevalence of LLAC was higher in men than in women (46.4% (n=83) vs. 17.3% (n=28), p<0.001). After adjustment for confounding factors, we found that women with LLAC had substantially lower Ct.area (*β*=-0.33, p=0.016), lower Tb.N (*β*=-0.54, p=0.013) and higher Tb.Sp (*β*=0.54, p=0.012) at the distal tibia and lower Tb.Th (*β*=-0.49, p=0.027) at the distal radius compared with participants without LLAC. Distal radial or tibial bone parameters analyses in men according to their LLAC status revealed no significant differences with the exception of Tb.N (*β*=0.27, p=0.035) at the distal tibia.

Conclusion: In the HCS, the presence of LLAC assessed by HR-pQCT was associated with relevant bone microstructure abnormalities in women. These findings need to be replicated and further research should study possible pathophysiological links between vascular calcification and osteoporosis.

**Introduction**

Atherosclerosis and osteoporosis are common in elderly individuals, and have previously been regarded as independent age-related disorders [1, 2]. However, some studies have suggested overlap in the etiological mechanisms of these diseases [3, 4]. For example, bone metabolism and vascular physiology share several regulatory factors and the process of vascular calcification, as a marker of atherosclerosis, in many ways resembles that of bone formation [5, 6].

Several studies suggest that vascular calcification (mainly abdominal aortic calcification (AAC) and coronary artery calcification (CAC)) is associated with decreased areal bone mineral density (aBMD). Moreover, vascular calcification (AAC and/or AAC) is associated with a higher risk of hip and vertebral fractures [7]. Furthermore, a few quantitative computed tomography (QCT) studies have focussed on vascular calcification and bone health [8, 9]. Lower trabecular volumetric bone mineral density (Tb.vBMD) was related to high AAC, but not to CAC, in a biracial (black and white) cohort of healthy middle-aged women independent of age and shared risk factors between osteoporosis and cardiovascular disease (CVD) [8]. In a sub-study of the Multi-Ethnic Study of Atherosclerosis (MESA), lower Tb.vBMD was related to high AAC in men and CAC in women [9]. In the Framingham study, lower Tb.vBMD, but not cortical volumetric bone mineral density (Ct.vBMD), was related to greater severity of AAC and CAC both in men and women [10]. Furthermore, in experimental studies on uremic rats, an association has also been demonstrated between impaired bone metabolism or restoration of bone metabolism and the development of vascular calcifications; i.e. the so-called calcification paradox [11, 12].

More recently, high resolution peripheral quantitative computed tomography (HR-pQCT) was used to compare 66 patients with end stage renal disease on chronic haemodialysis with and without CAC. It was found that those with CAC had lower Ct.vBMD and trabecular bone volume than those without [13].

Currently, a comprehensive body of literature exists to describe calcification in different vascular beds (mainly AAC and CAC) and the relationship with bone density (areal and/or volumetric) and fractures. However, little is known about potential links between bone microstructure and vascular calcification. Furthermore, there is a paucity of information specifically related to the lower leg arterial calcification (LLAC) whereas the evaluation of this vascular bed may help to study the osteo-vascular interactions.

The development of HR-pQCT has enabled us to investigate bone in greater detail and could be a promising tool to characterize LLAC. Quantification of LLAC by HR-pQCT has been described by Patsch *et al* [14], who reported that LLAC was correlated with CAC assessed by multidetector computed tomography scan in patients on chronic haemodialysis [14].The aim of this study was therefore to investigate relationships of bone geometry, volumetric BMD, and bone microarchitecture with LLAC in a well phenotyped cohort of older men and women from Hertfordshire.

**Methods**

**Study population**

The Hertfordshire Cohort Study (HCS) is a population-based UK cohort which was designed to examine the relationships between growth in infancy and the subsequent risk of adult diseases, such as osteoporosis. Study design and recruitment have been described in detail previously [15]. HCS participants were generally comparable with those in the nationally representative Health Survey for England [15]. In brief, in conjunction with the National Health Service Central Registry and the Hertfordshire Family Health Service Association, we traced men and women who were born between 1931 and 1939 in Hertfordshire and still lived there during the period 1998–2003. A nurse-administered questionnaire and clinic visit were done at this time. In 2011-2012, 592 men and women from the geographical area of East Hertfordshire were invited to take part in the study and a home visit which included a structured interview was conducted in 443 patients. Of these, 350 agreed to have a HR-pQCT scan (distal radius and distal tibia) performed between 0.8 and 18 months later, with a mean (SD) gap of 9.2 (3.2) months. The East and North Hertfordshire Ethical Committees granted ethical approval for the study and all participants gave written informed consent in accordance with the Declaration of Helsinki [16].

**Demographic and clinical assessment**

A structured interview was performed during a home visit in 2011-2012. Specifically, the following characteristics were recorded: age, alcohol consumption, smoking status, physical activity (activity time in last 2 weeks (min/day)), current use of bisphosphonates. Assessment of smoking habits included questions concerning smoker status (never, ex or current). Alcohol consumption (unit per week) was also recorded [17]. History of diabetes mellitus, high blood pressure (HBP), stroke, ischaemic heart disease (heart attack, angina or heart failure), or peripheral arterial disease (claudication) was obtained through self-report. Height was measured to the nearest 0.1 cm using a wall-mounted SECA stadiometer on the day of scanning, and weight using electronic scales (make, model) to the nearest 0.1 kg. Body Mass Index (BMI) was calculated as weight/height² (kg/m²).

Details regarding dietary calcium intake, dietary vitamin D intake (mg) and socioeconomic status were available from the nurse-administered questionnaire conducted in 1998-2003 [15]. Dietary calcium intake was assessed using a food frequency questionnaire [18]. Socioeconomic status was determined using own current or most recent occupation of the participant in men and single women, and of the husband in ever-married women based on the Office of Population Censuses and Surveys Standard Occupational Classification Scheme for occupation and social class.

**High-resolution peripheral quantitative computed tomography**

Each participant had measurements of the non-dominant distal radius using HR-pQCT (XtremeCT, Scanco Medical AG, Switzerland) except when it had previously been fractured in which case the dominant side was scanned. This allowed acquisition of a stack of parallel CT slices using a two-dimensional detector array. A total of 110 slices were obtained which represented a volume of bone 9mm in axial length with a nominal resolution (voxel size) of 82μm. The scanned limb was immobilized during the examination in a carbon fiber cast. Antero-posterior 2D scout views were performed to determine the region to be scanned. All scans were acquired by one of two trained technicians using standard positioning techniques. These were in keeping with the manufacturer’s guidelines and as described by Boutroy *et al.* [19]. Each scan was assessed for motion artefact, and if present a second scan was performed. The quality of the measurements was assessed by using a 5-point scale recommended by the manufacturer (1, excellent; 2, good; 3, acceptable; 4, poor; 5, unacceptable). Only examinations with quality grades 1 through 3 were included in the study. For this reason 42 radius scans and 8 tibia scans were excluded.

Image analysis was carried out using the standard manufacturer’s method which has been described in detail previously [20, 21]. In brief, we used a semi-automated, hand-drawn contouring system to delineate the periosteal surface. A threshold-based algorithm then separated cortical from trabecular compartments. The threshold used to discriminate cortical from trabecular bone was set to one-third of the apparent cortical bone density value. Standard morphologic analysis produced total (Tt.vBMD, mg/cm3) and trabecular BMD (Tb.vBMD, mg/cm³). Trabecular number (Tb.N, per cm) was determined using the ridge-extraction method [22]. Trabecular thickness (Tb.Th, µm) and separation (Tb.Sp, µm) were calculated from trabecular density and trabecular number according to standard morphologic relationships [23]. Each measure has been validated against micro-CT imaging [24].

Further analysis was performed using an automated segmentation algorithm [25]. Assessments were made of total cross-sectional area (Tt.area, mm²), cortical area (Ct.area, mm²), and cortical density (Ct.vBMD, mg/cm³). Cortical density was determined as the average mineral density in the region of auto-segmentation cortical bone mask. Using Image Processing Language (IPL, Version 6.1, ScancoMedical), cortical porosity (Ct.Po, %) was derived from the number of void voxels in each thresholded cortex image divided by the number of voxels in the cortex. Cortical thickness (Ct.Th, µm) was determined from the threshold cortex image using a distance transform after removal of intracortical pores [26].

A calibration phantom (Scanco Medical AG, Bruttisellen, Switzerland) was used which included 5 cylinders containing a mixture of hydroxyapatite and resin. The mineral concentrations of these cylinders were 0, 100, 200, 400 and 800 mg HA/cm³. The value of 0 mg HA/cm³ equates to a soft tissue background devoid of mineral. Quality control testing was performed on a weekly basis and quality assurance on a daily basis. Short term precision values (% CV) for cortical and trabecular BMD have been shown to range from 0.3 to 1.2 [27]. The effective dose to the subject during each scan was < 3μSv.

**Quantification of lower leg arterial calcification (LLAC)**

Based on the specific aim of providing a software tool for the quantification of lower leg arterial calcifications captured by HR-pQCT imaging, a dedicated semi-automated computer algorithm implemented in IPL (Image Processing Language) has been previously developed [13]. Prior to software-based evaluations, images were visually inspected to confirm an image quality better than motion grade 4 [28] and to determine the presence or absence of LLAC. After inspection, 9 patients were excluded because image quality was lower than expected and 341 participants remained in our LLAC analysis which has been described in detail previously [14]. Lower leg arterial calcifications were defined as linear or tubular hyperdensity zones of circular, semi-circular, or crescent-like shape. We required that such calcifications corresponded to the anatomical territory of the anterior tibial artery (ATA), the posterior tibial artery (PTA), interosseous branches or smaller intramuscular or subcutaneous arterioles (defined as “others”) (Figure 1). Cutaneous calcifications (typically spot-like) or other non-vascular (e.g. post-traumatic) soft tissue calcifications that did not meet these criteria were not included. The algorithm was only applied to scans in which vascular calcifications were identified [29]. As we did for bone, each 110 slices were analysed for the presence or absence of LLAC representing a volume of 9mm in axial length. In the absence of vascular calcification, LLAC was recorded as zero mg HA. Prevalence was defined as a LLAC score >1 to rule out false positives. Typical post processing time per LLAC assessment ranged between 10 and 20 min, depending on the need for manual adjustments.

**Statistical analysis**

Statistical analyses were performed using STATA version 13.1. Variables were assessed for normality and transformed if necessary. Descriptive statistics for continuous variables were expressed as mean, standard deviation or median, interquartile range (IQR); and categorical variables were expressed as frequency and percentage. Differences in continuous variables between participants with and without LLAC and between genders were assessed using Student’s t-tests or Mann-Whitney tests and in categorical variables using Pearson’s Χ² test or Fisher’s exact test. To test interreader agreement of the LLAC measure, the analysis run of the first operator (JP) was compared with a run (n=14) of a second operator (CM) and expressed by the interreader agreement (ICC). The overlaps between ATA, PTA and “Others” were visualized by Venn diagram. Statistical significance was defined as a p-value of ≤ 0.05. HRpQCT variables were transformed using the Fisher-Yates rank-based inverse normal transformation to create z-scores.

There were differences in the values of bone and LLAC variables in men and women, and the relationships between them did show gender-interactions for Tb.N (p=0.011) and Tb.Sp (p=0.013) at the distal tibia and for Tb.vBMD (p=0.010), and Tb.Th (p=0.004) at the distal radius. As a result, we analysed and present results separately for the two genders. Primary analysis used linear regression to examine the associations between LLAC and HR-pQCT bone parameters in women in the distal tibia and radius. This analysis was undertaken with and without adjustment for a priori confounders: age, height, weight, smoking status, alcohol intake, diabetes, daily calcium intake, daily vitamin D intake, physical activity, social class and current use of bisphosphonates. Secondary analysis used linear regression to examine the associations between LLAC and HR-pQCT bone parameters in men in the distal tibia and radius. This analysis was undertaken with and without adjustment for a priori confounders: age, height, weight, smoking status, alcohol intake, diabetes, daily calcium intake, daily vitamin D intake, physical activity, social class and current use of bisphosphonates.

**Results**

One hundred and eleven of 341 participants (32.6%) had LLAC (score >1) that were visible and quantifiable by HR-pQCT. The prevalence of LLAC was higher in men than in women (46.4% (n=83) vs. 17.3% (n=28), p<0.001). As described above, it was required that LLAC corresponded to the anatomical territory of the ATA, PTA, or others. Eighty-three (24.3%) and eighty-six (25.2%) of 341 participants had respectively ATA and PTA that were visible and quantifiable by HR-pQCT. However, there was an overlap between the prevalence of calcification in these vascular beds (Figure 2). Moreover, the ICC was 0.807 (95% CI 0.494, 0.934).

The mean (SD) age of participants was 76.4 (2.6) and 76.1 (2.5) years in women and men, respectively. Characteristics of study participants were compared in the two sexes by LLAC status (Table 1). Among women, participants with LLAC (n=28) were older than those without LLAC (n=134) (p<0.001). Among men, participants with LLAC (n=83) differed significantly in terms of socioeconomic status from those without LLAC (n=96) (p=0.043). The two groups did not differ significantly in terms of smoking status, alcohol consumption, high blood pressure and diabetes among women or men. A total of 72 (21.1%) participants (46 (25.7%) men and 26 (16.1%) women) were classed as having IHD. Among women, participants with LLAC differed significantly in terms of IHD from those without LLAC (n=8 (28.6%) versus n=18 (13.4%), p=0.047). Peripheral arterial disease was only reported in 4 individuals (2 men and 2 women).

Distal radial and tibial bone parameters in participants with and without LLAC are shown in Table 2 and Figure 3 for women. Analyses in women revealed that Ct.area and Ct.Th were lower in participants with LLAC (*β*=-0.37, p=0.004 and *β*=-0.40, p=0.014, respectively) at the distal tibia. Adjustment for confounding factors did not materially affect the relationship described for Ct.area (*β*=-0.33, p=0.016) but differences in Ct.Th were attenuated (*β*=-0.30, p=0.083). Regarding trabecular parameters, analyses in women revealed that Tb.vBMD and Tb.N were lower in participants with LLAC (*β*=-0.49, p=0.019 and *β*=-0.50, p=0.013, respectively) at the distal tibia while Tb.Sp was higher (*β*=0.53, p=0.008). Adjustment for confounding factors did not materially affect the relationship described for Tb.N (*β*=-0.54, p=0.013) and Tb.Sp (*β*=0.54, p=0.012) but differences in Tb.vBMD at the distal tibia were attenuated (*β*=-0.38, p=0.087). Similar results were found for Tb.vBMD (*β*=-0.55, p=0.004), Tb.N (*β*=-0.45, p=0.027) and Tb.Sp (*β*=0.48, p=0.016) at the distal radius with a lower Tb.Th (*β*=-0.53, p=0.009) also observed but only Tb.Th (*β*=-0.49, p=0.027) remained significant after adjustment for confounding factors.

With the exception of Tb.N at the distal tibia when adjusted (*β*=0.27, p=0.035), distal radial or tibial bone parameters analyses in men according to their LLAC status revealed no significant differences (results not shown).

**Discussion**

In this study, we utilized HR-pQCT to investigate geometric, volumetric and microstructural parameters at the distal radius and tibia in participants with and without LLAC in a cohort of elderly men and women from Hertfordshire. After adjustment for confounding factors, we found that women with LLAC had substantially lower Ct.area, lower Tb.N and higher Tb.Sp at the distal tibia and lower Tb.Th at the distal radius compared with participants without LLAC. Distal radial or tibial bone parameters analyses in men according to their LLAC status revealed no significant differences with the exception of Tb.N at the distal tibia.

In previous studies, arterial disease of the lower limbs was associated to bone abnormalities [30, 31] and a surrogate marker of arterial disease such as vascular calcification appears to be linked with poor bone health. Researchers have found associations between CAC, AAC, lower areal BMD [32, 33], and greater bone loss over time [34, 35] and major osteoporotic fractures such as vertebral and hip fractures [7, 36, 37]. However, other studies did not find a relationship between bone parameters and vascular calcification [38-40].

Central QCT studies have focused on vascular calcification and bone health [8-10, 41, 42], with results being inconclusive. Lower spine vBMD, a mostly trabecular site, was related to high AAC, but not to CAC, in a biracial (black and white) cohort of 490 healthy middle-aged women (45-58 years of age, 61% white, 64% perimenopausal) independent of age and shared risk factors between osteoporosis and CVD [8]. In a sub-study of the Multi-Ethnic Study of Atherosclerosis (MESA), they also used QCT to assess vBMD and the presence and extent of CAC and AAC among 946 women (mean age = 65.5 years) and 963 men (mean age = 64.1 years). Lower vBMD was related to high AAC in men and CAC in women [9]. An inverse association has also been demonstrated between vBMD (lumbar and thoracic) and vascular calcification (AAC, CAC and carotid artery calcified plaques) in African-American men and women with type 2 diabetes mellitus [41]. In the Framingham study, lower Tb.vBMD, but not Ct.vBMD, was related to greater severity of AAC and CAC both in men and women [10]. Lastly, in a cross-sectional study of 278 Afro-Caribbean men (mean age 56) with BMD assessed using peripheral QCT of the radius and tibia, Ct.vBMD, but not Tb.vBMD, was inversely associated AAC presence [42].

There are very few studies that have completed similar analyses using HR-pQCT to determine microarchitectural changes. The study most similar to ours that utilized HR-pQCT compared 66 patients with end stage renal disease on chronic haemodialysis with and without CAC. They found that those with CAC had lower Ct.vBMD and trabecular bone volume than those without [13]. In another study performed by Pelletier, they compared 53 patients on chronic haemodialysis with and without AAC and found that those with AAC had lower Ct.Th and higher sclerostin serum level [43]. Another population-based study with 693 subjects (men<50 years, men≥50yrs, premenopausal women and postmenopausal women) investigated the association between bone microarchitecture assessed by HR-pQCT at the distal radius and AAC [44]. Chow *et al.* found that Tb.N and Tb.Sp were inversely associated with AAC in men over 50 years (after adjustment for age, body mass index and cigarette smoking status), but not in younger men, in premenopausal and postmenopausal women.

The former finding, regarding trabecular microstructure abnormalities, is in keeping with our results but we found these abnormalities at the distal tibia in postmenopausal women with LLAC. It remains unclear why there seems to be such a sharp contrast between the sexes. Intriguingly, women with LLAC, but not men, differed significantly in terms of IHD from those without LLAC. We speculate that LLAC in women, but not in men, might be associated with CAC as previously demonstrated in patients on chronic haemodialysis [14]. Association between bone microstructure abnormalities and LLAC could be highlighted only if there is also an association between LLAC and CAC.

Many factors have been implicated in the concurrent development of vascular calcification and impaired bone metabolism (i.e. the so-called calcification paradox), including chronic inflammation, hypovitaminosis D, oxidative stress and oestrogen deficiency [45-49]. Any or a combination of these factors might in part explain the link between vascular calcification and bone metabolism. However, the molecular mechanisms responsible for both vascular calcification and impaired bone metabolism are not fully understood. The mediators and pathways involved in the calcification paradox are centered around the transdifferentiation of VSMCs to bone-like cells, including cells with an osteoblastic and chondrocytic phenotype. Differences in OPG/RANKL and Pi/PPI ratios, along with differences in the response to TGF-β in both tissues, have been implicated in tuning the many parallels between bone mineralization and ectopic vessel calcification into an opposite net result [48]. With respect to the OPG/RANK/RANKL system, a recent study found no evidence to suggest that the RANKL inhibitor denosumab contributed to the progression of vascular calcification or to an increased risk of cardiovascular adverse events in 60- to 90-year-old women with osteoporosis and a high cardiovascular risk [50]. Concerning the Wnt/β-catenin signalling pathway, association between serum sclerostin level and AAC in patients with CKD remains to be clarified [43, 51]. Moreover, lower serum DKK1 levels were associated with severe AAC in older men regardless of age and other potential confounders [52].

The strengths of our study include that this is a well phenotyped large cohort of elderly men and women, enabling adjustment for multiple confounders. However this study also has some potential limitations. Based on the cross-sectional nature of this study design, causality cannot be established because we are unable to determine temporal relationships between the variables. Another limitation of our study is the relatively small number of individuals with LLAC. This limits the power of the study, particularly when the group is divided into men and women for analyses. The short scan length (9mm stack) is also a limitation. Finally, HR-pQCT data is restricted to the peripheral skeleton and does not provide a direct measure of bone quality at axial sites such as hip and vertebrae which are more common sites of fragility fracture.

In summary, this study finds that the presence of LLAC is assessable by HR-pQCT and associated with relevant bone microstructure abnormalities at the distal tibia and distal radius in women. However, these results need to be replicated in other cohorts and prospective studies are needed to certify the association between LLAC and bone microstructure differences. Further research is needed to explore the pathophysiological links between the two conditions and the potential for common preventive and therapeutic interventions.

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**Conflict of interest statement**

Professor Cooper has received consultancy fees/honoraria from Servier; Eli Lilly; Merck; Amgen; Alliance; Novartis; Medtronic; GSK; Roche.

Julien Paccou, Mark Edwards, Janina Patsch, Kate Ward, Karen Jameson, Charlotte Moss, and Elaine Dennison declare that they have no conflict of interest.

**References**

[1] Dennison E, Mohamed MA, Cooper C. Epidemiology of osteoporosis. Rheum Dis Clin North Am. 2006;32:617-629.

[2] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:e442.

[3] Tanko LB, Christiansen C, Cox DA, [Geiger MJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Geiger%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=16234963), [McNabb MA](http://www.ncbi.nlm.nih.gov/pubmed/?term=McNabb%20MA%5BAuthor%5D&cauthor=true&cauthor_uid=16234963), [Cummings SR](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cummings%20SR%5BAuthor%5D&cauthor=true&cauthor_uid=16234963). Relationship between osteoporosis and cardiovascular disease in postmenopausal women. J Bone Miner Res. 2005;20:1912-1920.

[4] Pennisi P, Signorelli SS, Riccobene S, Celotta G, Di Pino L, La Malfa T, Fiore CE. Low bone density and abnormal bone turnover in patients with atherosclerosis of peripheral vessels. Osteoporos Int 2004;15:389–395.

[5] Massy ZA, Drueke TB. Vascular calcification. Curr Opin Nephrol Hypertens 2013;22:405-12.

[6] Demer LL, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. Arterioscler Thromb Vasc Biol. 2014;34:715-23.

[7] Szulc P, Blackwell T, Schousboe JT, [Bauer DC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bauer%20DC%5BAuthor%5D&cauthor=true&cauthor_uid=23983224), [Cawthon P](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cawthon%20P%5BAuthor%5D&cauthor=true&cauthor_uid=23983224), [Lane NE](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lane%20NE%5BAuthor%5D&cauthor=true&cauthor_uid=23983224), Cummings SR, Orwoll ES, Black DM, Ensrud KE. High hip fracture risk in men with severe aortic calcification: MrOS study. J Bone Miner Res. 2014;29:968-75.

[8] Farhat GN , Cauley JA , Matthews KA , Newman AB , Johnston J , Mackey R, Edmundowicz D, Sutton-Tyrrell K. Volumetric BMD and vascular calcification in middle-aged women: the Study of Women’s Health Across the Nation. J Bone Miner Res 2006;21:1839-46.

[9] Hyder JA, Allison MA, Wong N, Papa A, Lang TF, Sirlin C, Gapstur SM, Ouyang P, Carr JJ, Criqui MH. Association of coronary artery and aortic calcium with lumbar bone density: the MESA Abdominal Aortic Calcium Study. Am J Epidemiol. 2009;169:186-94.

[10] [Chan JJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chan%20JJ%5BAuthor%5D&cauthor=true&cauthor_uid=25871790), [Cupples LA](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cupples%20LA%5BAuthor%5D&cauthor=true&cauthor_uid=25871790), [Kiel DP](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kiel%20DP%5BAuthor%5D&cauthor=true&cauthor_uid=25871790), [O'Donnell CJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=O'Donnell%20CJ%5BAuthor%5D&cauthor=true&cauthor_uid=25871790), [Hoffmann U](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hoffmann%20U%5BAuthor%5D&cauthor=true&cauthor_uid=25871790), [Samelson EJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Samelson%20EJ%5BAuthor%5D&cauthor=true&cauthor_uid=25871790). QCT Volumetric Bone Mineral Density and Vascular and Valvular Calcification: The Framingham Study. [J Bone Miner Res.](http://www.ncbi.nlm.nih.gov/pubmed/25871790##) 2015;30:1767-74.

[11] De Schutter TM, Behets GJ, Jung S, Neven E, D'Haese PC, Querfeld U. Restoration of bone mineralization by cinacalcet is associated with a significant reduction in calcitriol-induced vascular calcification in uremic rats. Calcif Tissue Int. 2012;91:307-15.

[12] De Schutter TM, Neven E, Persy VP, Behets GJ, Postnov AA, De Clerck NM, D'Haese PC. Vascular calcification is associated with cortical bone loss in chronic renal failure rats with and without ovariectomy: the calcification paradox. Am J Nephrol. 2011;34: 356-66.

[13] Cejka D, Weber M, Diarra D, Reiter T, Kainberger F, Haas M. Inverse association between bone microarchitecture assessed by HR-pQCT and coronary artery calcification in patients with end-stage renal disease. Bone. 2014;64:33-38.

[14] Patsch JM, Zulliger MA, Vilayphou N, Samelson EJ, Cejka D, Diarra D, Berzaczy G, Burghardt AJ, Link TM, Weber M, Loewe C. Quantification of lower leg arterial calcifications by high-resolution peripheral quantitative computed tomography. Bone. 2014;58:42-7.

[15] Syddall HE, Aihie SA, Dennison EM, Martin HJ, Barker DJ, Cooper C. Cohort profile: the Hertfordshire cohort study. Int J Epidemiol 2005;34:1234-42.

[16] Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc 2009;107:403-5.

[17] NHS Choices. [www.nhs.uk/livewell/alcohol/Pages/Alcoholhome.aspx](http://www.nhs.uk/livewell/alcohol/Pages/Alcoholhome.aspx)

[18] Robinson SM, Jameson KA, Batelaan SF, Martin HJ, Syddall HE, Dennison EM, Cooper C, Sayer AA; Hertfordshire Cohort Study Group. Diet and its relationship with grip strength in community-dwelling older men and women: the Hertfordshire cohort study. J Am Geriatr Soc 2008;56:84-90.

[19] Boutroy S, Bouxsein ML, Munoz F, Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. J Clin Endocrinol Metab 2005;90:6508-15.

[20] Laib A, Hauselmann HJ, Ruegsegger P. In vivo high resolution 3D-QCT of the human forearm. Technol Health Care 1998;6:329-37.

[21] Khosla S, Riggs BL, Atkinson EJ, Oberg AL, McDaniel LJ, Holets M, Peterson JM, Melton LJ 3rd. Effects of sex and age on bone microstructure at the ultradistal radius: a population-based non-invasive in vivo assessment. J Bone Miner Res 2006;21:124-31.

[22] Hildebrand T, Ruegsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. J Microsc 1997;185:67-75.

[23] Parfitt AM, Mathews CH, Villanueva AR, Kleerekoper M, Frame B, Rao DS. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest 1983;72:1396-409.

[24] MacNeil JA, Boyd SK. Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality. Med Eng Phys 2007;29:1096-105.

[25] Buie HR, Campbell GM, Klinck RJ, MacNeil JA, Boyd SK. Automatic segmentation of cortical and trabecular compartments based on a dual threshold technique for in vivo micro-CT bone analysis. Bone 2007;41:505-15.

[26] Burghardt AJ, Kazakia GJ, Ramachandran S, Link TM, Majumdar S. Age- and gender-related differences in the geometric properties and biomechanical significance of intracortical porosity in the distal radius and tibia. J Bone Miner Res 2010;25:983-93.

[27] Paggiosi MA, Eastell R, Walsh JS. Precision of High-Resolution Peripheral Quantitative Computed Tomography Measurement Variables: Influence of Gender, Examination Site, and Age. Calcif Tissue Int. 2014;94:191-201.

[28] Pialat JB, Burghardt AJ, Sode M, Link TM, Majumdar S. Visual grading of motion induced image degradation in high resolution peripheral computed tomography: impact of image quality on measures of bone density and micro-architecture Bone. 2012;50:111–118.

[29] Burghardt AJ, Kazakia GJ, Majumdar S. A local adaptive threshold strategy for high resolution peripheral quantitative computed tomography of trabecular bone. Ann. Biomed. Eng. 2007;35:1678–1686.

[30] Laroche M, Pouilles JM, Ribot C, Bendayan P, Bernard J, Boccalon H, Mazieres B. Comparison of the bone mineral content of the lower limbs in men with ischaemic atherosclerotic disease; Clin Rheumatol. 1994;13:611-614.

[31] London GM, Marchais SJ, Guérin AP, de Vernejoul MC. Ankle-brachial index and bone turnover in patients on dialysis - J Am Soc Nephrol 2016; 26:476-483.

[32] Figueiredo CP, Rajamannan NM, Lopes JB, Caparbo VF, Takayama L, Kuroishi ME, Oliveira IS, Menezes PR, Scazufca M, Bonfá E, Pereira RM. Serum phosphate and hip bone mineral density as additional factors for high vascular calcification scores in a community-dwelling: the Sao Paulo Ageing & Health Study (SPAH) Bone 2013;52:354–359.

[33] Schulz E, Arfai K, Liu X, Sayre J, Gilsanz V. Aortic calcification and the risk of osteoporosis and fractures. J Clin Endocrinol Metab. 2004;89:4246-53.

[34] Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham heart study. Calcif Tissue Int. 2001;68:271-6.

[35] Naves M, Rodriguez-Garcia M, Diaz-Lopez JB, Gomez-Alonso C, Cannata-Andia JB. Progression of vascular calcifications is associated with greater bone loss and increased bone fractures. Osteoporos. Int. 2008;19:1161-66.

[36] Szulc P, Kiel DP, Delmas PD. Calcifications in the abdominal aorta predict fractures in men: MINOS study. J Bone Miner Res 2008;23:95-102.

[37] Szulc P, Samelson EJ, Sornay-Rendu E, Chapurlat R, Kiel RP. Severity of aortic calcification is positively associated with vertebral fracture in older men-a densitometry study in the STRAMBO cohort. Osteoporos Int. 2013;24:1177-84.

[38] Sinnott B, Syed I, Sevrukov A, Barengolts E. Coronary calcification and osteoporosis in men and postmenopausal women are independent processes associated with aging. Calcif Tissue Int. 2006,78:195–202.

[39] Shen H, Bielak LF, Streeten EA, Ryan KA, Rumberger JA, Sheedy PF, Shuldiner AR, Peyser PA, Mitchell BD. Relationship between vascular calcification and bone mineral density in the old-order Amish. Calcif Tissue Int. 2007;80:244–250.

[40] Kim KI, Suh JW, Choi SY, Chang HJ, Choi DJ, Kim CH, Oh BH. Is reduced bone mineral density independently associated with coronary artery calcification in subjects older than 50 years? J Bone Miner Metab. 2011;29:369–376.

[41] [Divers J](http://www.ncbi.nlm.nih.gov/pubmed/?term=Divers%20J%5BAuthor%5D&cauthor=true&cauthor_uid=21437982), [Register TC](http://www.ncbi.nlm.nih.gov/pubmed/?term=Register%20TC%5BAuthor%5D&cauthor=true&cauthor_uid=21437982), [Langefeld CD](http://www.ncbi.nlm.nih.gov/pubmed/?term=Langefeld%20CD%5BAuthor%5D&cauthor=true&cauthor_uid=21437982), [Wagenknecht LE](http://www.ncbi.nlm.nih.gov/pubmed/?term=Wagenknecht%20LE%5BAuthor%5D&cauthor=true&cauthor_uid=21437982), [Bowden DW](http://www.ncbi.nlm.nih.gov/pubmed/?term=Bowden%20DW%5BAuthor%5D&cauthor=true&cauthor_uid=21437982), [Carr JJ](http://www.ncbi.nlm.nih.gov/pubmed/?term=Carr%20JJ%5BAuthor%5D&cauthor=true&cauthor_uid=21437982), Hightower RC, Xu J, Hruska KA, Freedman BI. Relationships between calcified atherosclerotic plaque and bone mineral density in African Americans with type 2 diabetes. [J Bone Miner Res.](http://www.ncbi.nlm.nih.gov/pubmed/?term=vascular+calcification+(AAC%2C+CAC+and+carotid+artery+calcified+plaques)+in+African-American+men+and+women+with+type+2+diabetes##) 2011;26:1554-60.

[42] Kuipers AL, Zmuda JM, Carr JJ, [Terry JG](http://www.ncbi.nlm.nih.gov/pubmed/?term=Terry%20JG%5BAuthor%5D&cauthor=true&cauthor_uid=23974859), [Patrick AL](http://www.ncbi.nlm.nih.gov/pubmed/?term=Patrick%20AL%5BAuthor%5D&cauthor=true&cauthor_uid=23974859), [Ge Y](http://www.ncbi.nlm.nih.gov/pubmed/?term=Ge%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=23974859), Hightower RC, Bunker CH, Miljkovic I.. Association of volumetric bone mineral density with abdominal aortic calcification in African ancestry men. [Osteoporos Int.](http://www.ncbi.nlm.nih.gov/pubmed/23974859##) 2014;25:1063-9.

[43] Pelletier S, Confavreux CB, Haesebaert J, Guebre-Egziabher F, Bacchetta J, Carlier MC, Chardon L, Laville M, Chapurlat R, London GM, Lafage-Proust MH, Fouque D. Serum sclerostin: the missing link in the bone-vessel cross-talk in hemodialysis patients? Osteoporosis Int 2015; 26:2165-2174.

[44] Chow JT, Khosla S, Melton LJ 3rd, Atkinson EJ, Camp JJ, Kearns AE. Abdominal Aortic Calcification, BMD, and Bone Microstructure: A Population-Based Study. J Bone Miner Res. 2008;23:1601-12.

[45] Eriksson AL, Movérare-Skrtic S, Ljunggren Ö, Karlsson M, Mellström D, Ohlsson C.. High-sensitivity CRP is an independent risk factor for all fractures and vertebral fractures in elderly men: the MrOS Sweden study. J Bone Miner Res. 2014;29:418-23.

[46] Norman PE, Powell JT. Vitamin D and cardiovascular disease. Circ Res. 2014;114:379-93.

[47] Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. Circulation. 2008;117:2938–2948.

[48] Persy V, D'Haese P. Vascular calcification and bone disease: the calcification paradox. Trends Mol Med. 2009;15:405-16.

[49] Hjortnaes J, Butcher J, Figueiredo JL, Riccio M, Kohler RH, Kozloff KM, Weissleder R, Aikawa E. Arterial and aortic valves calcifications inversely correlate with osteoporotic bone remodelling: A role for inflammation. Europ Heart J 2010;31:1975-1984.

[50] Samelson EJ, Miller PD, Christiansen C, Daizadeh NS, Grazette L, Anthony MS, Egbuna O, Wang A, Siddhanti SR, Cheung AM, Franchimont N, Kiel DP. RANKL inhibition with denosumab does not influence 3-year progression of aortic calcification or incidence of adverse cardiovascular events in postmenopausal women with osteoporosis and high cardiovascular risk. J Bone Miner Res. 2014;29:450-7.

[51] Claes KJ, Viaene L, Heye S, Meijers B, d'Haese P, Evenepoel P. Sclerostin: Another Vascular Calcification Inhibitor? J Clin Endocrinol Metab 2013;98:3221-3228.

[52] Szulc P, Schoppet M, Rachner TD, Chapurlat R, Hofbauer LC. Severe abdominal aortic calcification in older men is negatively associated with DKK1 serum levels: the STRAMBO study. J Clin Endocrinol Metab. 2014;99:617-24.

**Table 1 Characteristics of study participants**

|  |  |  |
| --- | --- | --- |
|  | Men (n=179) | Women (n=162) |
| **Variables** | **LLAC ≤1** **(n=96)** | **LLAC >1** **(n=83)** | **P-value** | **LLAC ≤1** **(n=134)** | **LLAC >1** **(n=28)** | **P-value** |
| Age, year | 76.0 ± 2.5 | 76.2 ± 2.6 | 0.614 | 76.0 ± 2.5 | 78.2 ± 2.3 | **<0.001** |
| Body weight, kg | 82.8 ± 12.2 | 82.3 ± 12.2 | 0.800 | 71.6 ± 12.9 | 72.0 ± 12.9 | 0.882 |
| Body height, cm | 174 ± 6.1 | 173 ± 6.8 | 0.829 | 159.6 ± 5.7 | 161.4 ± 5.9 | 0.128 |
| BMI (kg/m²) | 27.5 ± 3.7 | 27.4 ± 3.8 | 0.898 | 28.1 ± 4.7 | 27.7 ± 5.0 | 0.662 |
| Ever smoked, n (%) | 54 (56.3) | 52 (62.7) | 0.385 | 54 (40.3) | 6 (21.4) | 0.084 |
| Alcohol consumption, unit per weekMedian [IQR] | 6.7 [1.8-12.6] | 7.1 [1.8-16.6] | 0.731 | 0.5 [0-4.6] | 0.4 [0.1-4.8] | 0.884 |
| Social status¹  - I-IIINM - IIIM-V | 44 (49.4)45 (50.6) | 28 (34.1)54 (65.9) | **0.043** | 60 (44.8)74 (55.2) | 12 (42.9)16 (57.1) | 0.853 |
| Activity time in last 2 weeks (min/day | 192 [130-286] | 180 [116-268] | 0.612 | 208 [150-284] | 174 [104-221]  | 0,071 |
| Daily calcium intake (mg) | 1234 ± 291 | 1227 ± 307 | 0.880 | 1134 ± 403 | 1100 ± 303 | 0.672 |
| HBP, n (%) | 44 (45.8) | 46 (55.4) | 0.201 | 58 (43.3) | 17 (60.7) | 0.093 |
| Diabetes, n (%) | 10 (10.4) | 14 (16.9) | 0.207 | 16 (11.9) | 4 (14.3) | 0.753 |
| IHD, n (%) | 23 (24.0) | 23 (27.7) | 0.567 | 18 (13.4) | 8 (28.6) | **0.047** |
| Bisphosphonates, n (%)  | 3 (3.1) | 6 (7.2) | 0.306 | 19 (14.2) | 7 (25.0) | 0.164 |

Values are the mean ± SD (significant results are indicated in bold). ¹I-IIINM (I to III non-manual), IIIM-V (III manual to V)

LLAC: lower leg arterial calcification; BMI: body mass index; HBP: high blood pressure; IHD: ischemic heart disease (heart attack, angina or heart failure)