

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

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## Abstract

**Summary** 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.

**Introduction** Here, we report the relationships of bone geometry, volumetric bone mineral density (BMD) and bone microarchitecture with lower leg arterial calcification (LLAC) as assessed by high-resolution peripheral quantitative computed tomography (HR-pQCT).

**Methods** We utilized 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 ( $\beta = -0.33$ ,  $p = 0.016$ ), lower Tb.N ( $\beta = -0.54$ ,  $p = 0.013$ ) and higher Tb.Sp ( $\beta = 0.54$ ,  $p = 0.012$ ) at the distal tibia and lower Tb.Th ( $\beta = -0.49$ ,  $p = 0.027$ ) at the distal radius compared with participants without LLAC. Distal radial or tibial bone parameter analyses in men according to their LLAC status revealed no significant differences with the exception of Tb.N ( $\beta = 0.27$ ,  $p = 0.035$ ) 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.

**Keywords** Bone microstructure · High-resolution peripheral quantitative computed tomography · Lower leg arterial calcification · Volumetric bone mineral density

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## 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 CAC) 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 the last 2 weeks (min/day)) and 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  $\text{weight/height}^2$  ( $\text{kg/m}^2$ ).

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 9 mm in axial length with a nominal resolution (voxel size) of 82  $\mu\text{m}$ . The scanned limb was immobilized during the examination in a carbon fibre 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,  $\text{mg}/\text{cm}^3$ ) and trabecular BMD (Tb.vBMD,  $\text{mg}/\text{cm}^3$ ). Trabecular number (Tb.N, per cm) was determined using the ridge-extraction method [22]. Trabecular thickness (Tb.Th,  $\mu\text{m}$ ) and separation (Tb.Sp,  $\mu\text{m}$ ) 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,  $\text{mm}^2$ ), cortical area (Ct.area,  $\text{mm}^2$ ) and cortical density (Ct.vBMD,  $\text{mg}/\text{cm}^3$ ). 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,  $\mu\text{m}$ ) was

determined from the threshold cortex image using a distance transform after removal of intracortical pores [26].

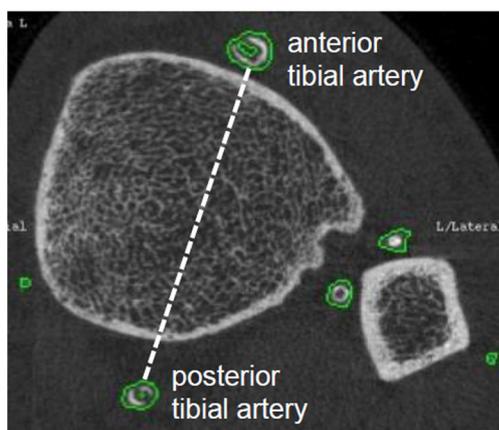
A calibration phantom (Scanco Medical AG, Bruttisellen, Switzerland) was used which included five cylinders containing a mixture of hydroxyapatite and resin. The mineral concentrations of these cylinders were 0, 100, 200, 400 and 800  $\text{mg HA}/\text{cm}^3$ . The value of 0  $\text{mg HA}/\text{cm}^3$  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  $<3 \mu\text{Sv}$ .

### Quantification of 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 Image Processing Language (IPL) 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") (Fig. 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 9 mm in axial length. In the absence of vascular calcification, LLAC was recorded as 0 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



**Fig. 1** Presence of lower leg arterial calcification. The anterior and posterior tibial artery (ATA and PTA respectively) are the most frequently affected sites. Interosseous branches are also visible on this figure

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  $\chi^2$  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  $\leq 0.05$ . 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

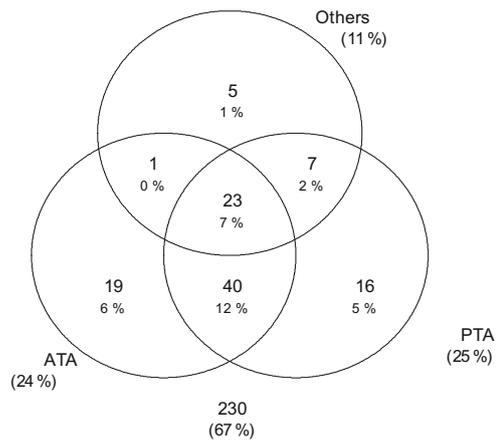
One hundred and eleven of 341 participants (32.6 %) had LLAC (score  $> 1$ ) that were visible and quantifiable by HR-pQCT. The prevalence of LLAC was higher in men than in women (46.4 % ( $n = 83$ ) vs. 17.3 % ( $n = 28$ ),  $p < 0.001$ ). As described above, it was required that LLAC corresponded to 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 (Fig. 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 %) vs.  $n = 18$  (13.4 %),  $p = 0.047$ ). Peripheral arterial disease was only reported in four individuals (two men and two women).

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

With the exception of Tb.N at the distal tibia when adjusted ( $\beta = 0.27$ ,  $p = 0.035$ ), distal radial or tibial bone parameter

N = 341



**Fig. 2** Prevalence of the different vascular beds involved in lower leg arterial calcifications (LLAC). Others included interosseous branches or smaller intramuscular or subcutaneous arterioles. ATA anterior tibial artery, PTA posterior tibial artery

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 parameter 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

**Table 1** Characteristics of study participants

Variables	Men (N=179)			Women (N=162)		
	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/m <sup>2</sup> )	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 week median [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 status <sup>a</sup>						
I-III-NM	44 (49.4)	28 (34.1)		60 (44.8)	12 (42.9)	
IIIM-V	45 (50.6)	54 (65.9)	0.043	74 (55.2)	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 italics)

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

<sup>a</sup> I-III-NM (I to III non-manual), IIIM-V (III manual to V)

**Table 2** Bone density and microstructure of distal radius and tibia in women with LLAC >1 vs. controls (LLAC ≤1)

Variables	Tibia				Radius			
	Unadjusted		Adjusted <sup>a</sup>		Unadjusted		Adjusted <sup>a</sup>	
	$\beta$ (95 % CI)	<i>p</i> value	$\beta$ (95 % CI)	<i>p</i> value	$\beta$ (95 % CI)	<i>p</i> value	$\beta$ (95 % CI)	<i>p</i> value
Tt.area Z-score	0.11 (-0.20, 0.41)	0.500	-0.05 (-0.33, 0.23)	0.731	-0.02 (-0.29, 0.25)	0.881	-0.10 (-0.36, 0.15)	0.417
Ct.area Z-score	<i>-0.37 (-0.62, -0.12)</i>	<i>0.004</i>	<i>-0.33 (-0.60, -0.06)</i>	<i>0.016</i>	-0.25 (-0.53, 0.02)	0.071	-0.19 (-0.48, 0.10)	0.203
Tb.area Z-score	0.16 (-0.17, 0.50)	0.337	-0.01 (-0.32, 0.31)	0.972	0.01 (-0.28, 0.30)	0.945	-0.06 (-0.35, 0.23)	0.680
Tb.vBMD Z-score	<i>-0.49 (-0.89, -0.08)</i>	<i>0.019</i>	-0.38 (-0.81, 0.06)	0.087	<i>-0.55 (-0.92, -0.17)</i>	<i>0.004</i>	-0.35 (-0.76, 0.06)	0.098
Tb.N Z-score	<i>-0.50 (-0.90, -0.11)</i>	<i>0.013</i>	<i>-0.54 (-0.96, -0.11)</i>	<i>0.013</i>	<i>-0.45 (-0.86, -0.05)</i>	<i>0.027</i>	-0.19 (-0.63, 0.25)	0.398
Tb.Th Z-score	-0.19 (-0.64, 0.25)	0.384	-0.01 (-0.50, 0.48)	0.973	<i>-0.53 (-0.92, -0.13)</i>	<i>0.009</i>	<i>-0.49 (-0.92, 0.06)</i>	<i>0.027</i>
Tb.Sp Z-score	<i>0.53 (0.14, 0.93)</i>	<i>0.008</i>	<i>0.54 (0.12, 0.95)</i>	<i>0.012</i>	<i>0.48 (0.09, 0.87)</i>	<i>0.016</i>	0.24 (-0.19, 0.67)	0.265
Ct.vBMD Z-score	-0.18 (-0.53, 0.17)	0.315	0.00 (-0.39, 0.39)	0.992	-0.08 (-0.53, 0.38)	0.732	0.13 (-0.65, 0.39)	0.624
Ct.Th Z-score	<i>-0.40 (-0.71, -0.08)</i>	<i>0.014</i>	-0.30 (-0.64, 0.04)	0.083	-0.22 (-0.60, 0.16)	0.246	-0.14 (-0.57, 0.29)	0.520
Ct.Po Z-score	-0.16 (-0.54, 0.21)	0.395	-0.30 (-0.75, 0.14)	0.179	-0.24 (-0.67, 0.20)	0.286	-0.23 (-0.66, 0.36)	0.554

HRpQCT variables were transformed using the Fisher-Yates rank-based inverse normal transformation to create Z-scores

*Tt.area* total area, *Ct.area* cortical area, *Tb.area* trabecular area, *Tb.vBMD* trabecular volumetric bone mineral density, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Tb.Sp* trabecular separation, *Ct.vBMD* cortical volumetric bone mineral density, *Ct.Th* cortical thickness, *Ct.Po* cortical porosity

<sup>a</sup> Adjusted for age, height, weight, smoking status, alcohol intake, diabetes, daily calcium intake, daily vitamin D intake, physical activity, social class and current use of bisphosphonates

Significant results are indicated in italics

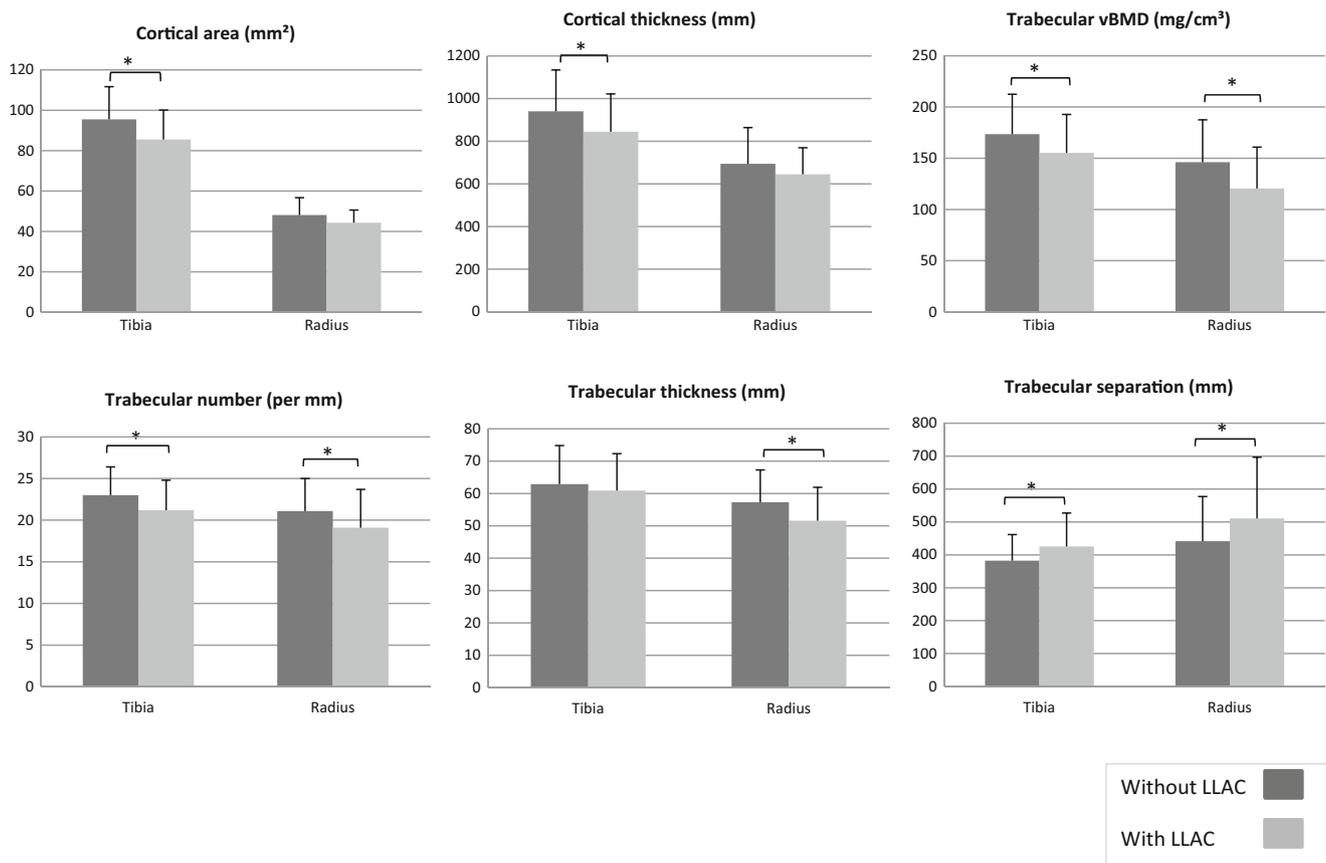
(MESA), they also used QCT to assess vBMD and the presence and extent of CAC and AAC among 946 women (mean age = 65.5 years) and 963 men (mean age = 64.1 years). Lower vBMD was related to high AAC in men and CAC in women [9]. An inverse association has also been demonstrated between vBMD (lumbar and 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 to 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 ≥ 50 years, 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 centred around the transdifferentiation of



**Fig. 3** Comparative analysis of bone density and microstructure of distal tibia and radius in women with LLAC (score >1) and without LLAC (score ≤1). Disease-specific differences of bone microstructure and geometry at the distal tibia and radius in women with and without LLAC are shown. *Ct.area* cortical area, *Ct.Th* cortical thickness,

*Tb.vBMD* trabecular volumetric bone mineral density, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *Tb.Sp* trabecular separation. Asterisks indicate significant differences between women with and without LLAC ( $p < 0.05$ ) before adjustment for confounding factors

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- $\beta$  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/ $\beta$ -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 (9 mm 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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**Compliance with ethical standards** 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.

**Conflicts of interest** 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.

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