Tracking changes of individual cortical pores over 1 year via HR-pQCT in a small cohort of 60-year-old females

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1. Introduction

High resolution peripheral quantitative computed tomography (HR-pQCT) has revolutionized 3D clinical visualization and assessment of trabecular and cortical bone microarchitecture at peripheral skeletal sites. Although the technology employs the same principles as conventional computed tomography (CT), HR-pQCT can achieve extremely high spatial resolution (typically 61–82 μm isotropic voxel size) with nominal radiation cost (~0.025 mSv) making it attractive to resolve micro-scale features of the peripheral skeleton (Lee et al., 2015). The high spatial resolution of HR-pQCT has provided researchers a unique insight into dynamic skeletal changes in several disease states including post-menopausal osteoporosis and osteopenia (Boutroy et al., 2005; Nishiyama et al., 2010), type 2 diabetes (Burghardt et al., 2010), chronic kidney disease (CKD) (Ghasem-Zadeh et al., 2022; Nishiyama et al., 2015), and rheumatoid arthritis (Perez et al., 2022), and has permitted in vivo examination of cortical porosity (Cooper et al., 2016; Hepp et al., 2022).

The cortical bone contains numerous pores including central canals (Haversian/Volkmann), and lacunar-canalicular pores occupied by the osteocyte and its processes. While these porosities are present throughout the cortex in both healthy and diseased states, bone can develop pathological pores in the cortex. Pathological cortical porosity is a common characteristic of cortical bone loss, along with cortical

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ABSTRACT

Introduction: High-resolution peripheral quantitative computed tomography (HR-pQCT) is a powerful tool that has revolutionized 3D longitudinal assessment of bone microarchitecture. However, cortical porosity, a common characteristic of cortical bone loss, is still often determined by static evaluation of overall porosity at one timepoint. Therefore, we sought to 1) describe a technique to evaluate individual cortical pore dynamics in aging females over one year using HR-pQCT imaging and 2) determine whether formation and expansion of pores would exceed contraction and infilling of pores.

Methods: HR-pQCT (60.7 μm resolution) images were acquired one year apart at the distal tibia and distal radius in seven female volunteers (60–72 years of age). Baseline and one-year images were registered at each bone site and a custom software was used to quantify dynamic activity of individual cortical pores using the following categories: developed, infilled, expanded, contracted, and static.

Results: Over the one-year period, cortical pores actively developed, contracted, expanded, and infilled. More pores expanded and developed vs. infilled or contracted leading to increased pore area in both tibial and radial sites (p = 0.0034 and p = 0.0474, respectively). Closed pores in the tibia, those that were not connected to the endosteal or periosteal surfaces, were the most dynamic of any pores type (open/closed) at either bone site.

Conclusion: This study demonstrates an approach to longitudinally track individual cortical pore activity in tibial and radial sites. These data expand conventional parameters for assessing cortical porosity and show increased porosity in one year of aging is caused by newly developed pores and expansion of existing pores.

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thinning and have long been understood to have negative ramifications on bone strength and mechanical integrity (Augat and Schorlemmer, 2006; Schaffler and Burr, 1988). They can range from tens- to hundreds-of microns in diameter (Cooper et al., 2016; Parfitt, 1994), and are a common cortical bone phenotype present in a range of clinical scenarios including aging, chronic kidney disease (CKD), and diabetes (Nickolas et al., 2013; Nicks et al., 2012; Samelson et al., 2018). The severity of cortical porosity is determined by the combination of the number and size of pores. Furthering our understanding of cortical porosity is important because the presence of pores can account for nearly 70% of the bone’s elastic modulus (stiffness) and even small increases in porosity can significantly reduce a bone’s ability to withstand forces (Dong and Guo, 2004; Schaffler and Burr, 1988; Turner, 2002). Elevated porosity correlates with reduced fracture toughness (Yeni et al., 1997) and clinical data support that higher porosity is associated with increased fracture risk (Bain et al., 2014).

Cortical porosity has traditionally been measured by assessing total porosity, reported as the percentage of void space relative to cortical area within a volume of interest (VOI) (Turner, 2002). While this is a useful parameter, it may overlook subtle yet important differences in pore dynamics such as how individual pores expand or contract over time. For example, consider two bones: bone 1 with many small pores and bone 2 with a few large pores – it is feasible that these bones could have dramatically different mechanical properties (Wachter et al., 2002). Consider that over time bone 1 fills in several small pores, but also forms a new pore, while bone 2 completely fills in one large pore while another similar size pore develops. This result could be no net change in total porosity; therefore, the total measure of cortical porosity would not truly reveal the dynamic nature of cortical activity. With longitudinal study designs using HR-pQCT being increasingly employed, the ability to capture the impact of disease and/or treatment on cortical bone microstructure is now possible in vivo. Thus, defining an approach capable of longitudinally tracking, and quantifying, individual pore dynamics would help to understand the contribution of cortical porosity in health and disease.

Our lab has previously demonstrated individual cortical pore dynamics could be quantified longitudinally using pre-clinical micro computed tomography (μCT) (Swallow et al., 2022). Using basic pore relationship categorizations (e.g. developed, infilled, expanded, contracted, or static), our preclinical study demonstrated that the expansion/progression of individual cortical pores could be mitigated, and the development of new pores suppressed with treatment, underscoring the importance of dynamic pore analysis (Swallow et al., 2022).

The current study aimed to broaden the application of individual pore tracking methodology first utilized in rat bone to human bone using HR-pQCT imaging. Utilizing HR-pQCT scans from aged volunteers as a proof of concept, we tested the hypotheses that: 1) individual pore dynamics can be tracked over time (1 year) using HR-pQCT imaging at two peripheral scan sites (tibia and radius diaphysis) and 2) over the course of a year, the formation and expansion of pores would exceed contraction and infilling of pores resulting in an overall increase in pore number and pore area occupied in the cortex.

2. Methods
2.1. Participants and experimental design

HR-pQCT scans were acquired using an XtremeCT II scanner (SCANCO Medical AG, Bruttisellen, Switzerland) within the Musculoskeletal Function, Imaging and Tissue (MSK-FIT) Resource Core of the Indiana Center for Musculoskeletal Health’s Clinical Research Center (Indiana University, Indianapolis, IN). The MSK-FIT core has Institutional Review Board approval from Indiana University to assess all-comers who provide written informed consent. To be eligible for inclusion in the current dataset, individuals were required to be 60 years of age or older, female, and have no self-reported disease with the potential to impact bone health, including diabetes, liver, or kidney disease, past or present history of cancer, thyroid disorder or cystic fibrosis (van den Bergh et al., 2021). They were also required to not be taking bone active therapies and have had two HR-pQCT imaging sessions approximately one year apart to generate repeat scans of the tibial and radius diaphyses. Prior to imaging, the individual’s height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg) were measured with a calibrated stadiometer (Seca 264; Seca GmbH & Co., Hamburg, Germany) and scale (MS140-300; Brecknell, Fairmont, MN). A whole-body dual-energy x-ray absorptiometry (DXA) (Norland Elite; Norland at Swissray, Fort Atkinson, WI) was used to measure appendicular skeletal muscle mass relative to height (ASM/height²; kg/m²) and total hip and spine areal bone mineral density (aBMD).

HR-pQCT images were acquired of the individual’s non-dominant arm and leg, as previously described (Warden et al., 2021). The non-dominant leg was defined as the leg contralateral to the participants’ non-dominant arm, as per the concept of crossed symmetry (Warden et al., 2021). Bone lengths were measured in triplicate at baseline using a segmometer (Realmert Flexible Segmometer, NutriActiva, Minneapolis, MN). Tibial length was measured as the distance from the distal tip of the medial malleolus to the medial joint line of the knee. Per convention, ulna length from the end of the olecranon process to the tip of its styloid process was measured as a surrogate for radial length. The bone length measured at baseline was used for follow-up scans as individuals were skeletally mature (Whittier et al., 2020b).

Scans were acquired with individuals in supine and the limb to be imaged immobilized using a padded, anatomically formed carbon fiber cast. After performance of scout scans, reference lines were placed at the medial edge of the distal radius articular surface and center of the tibia joint surface for radius and tibia scans, respectively (Whittier et al., 2020b). Scans were scored for motion artifact at the time of acquisition using a scale of 1 (no motion) to 5 (significant motion artifact causing substantial blurring). Scans graded three or higher were repeated. Scanner stability was confirmed throughout data collection by scanning phantoms with inserts of known density and volume.

2.2. Image processing

Baseline and one year follow-up images were reconstructed to an ISQ image file type according to the HR-pQCT manufacturer’s standard protocol and using their provided algorithm. ISQ files were converted to TIF image file type using the HKhs Scanco microCT ISQ FileReader Image J plugin (http://www.kunzelmann.de/4/software-imajeg-import-export-utilities.html) in order for compatibility across image processing programs utilized in this study. Before analyzing the images, the signal to noise ratio (SNR) was calculated for each baseline and endpoint grayscale image as 1) a quality control measure between repeat scans 1 year apart to evaluate tibial and radial HR-pQCT signal efficiency throughout the longitudinal study and 2) identify any scans in which noise may impede cortical porosity tracking results. Using the axial view, mean SNR was calculated on the anterior portion of the cortical bone as the mean signal intensity of a region of cortical bone divided by the standard deviation of noise (air):

\[
\text{SNR} = \frac{\text{SI mean}}{\text{SD noise}}
\]

where SI mean is the mean signal in the cortical ROI and SD noise is the standard deviation of the background noise in the image. Location and ROI size were kept standard across the study. Finally, Because of standardization of subject placement at the time of acquisition, our images did not require any rotation. It should be noted that bones off-center, angled, or ‘obliqued’ would require that scans undergo processing to align the long axis of the bone along the z-axis prior to further analysis.
The registration of the radii was localized to the landmarks of the radii, follow-up scans were assigned as reference and target, respectively. Reliability using intraclass correlation coefficients. Baseline and 1-year registered a subset (planes (sagittal, transaxial, and coronal) using a series of rotation steps and shifts in all three directions (settings: shift step = 1, rotation step = 0.5)). Quality of the registration was manually checked by toggling between baseline and follow-up greyscale images for cross over in cortical and trabecular landmarks. Upon complete registration, a volume of interest was selected to isolate the tibia (exclude the fibula) and maximize the volume in the z-plane. Baseline and follow-up scans were saved out as BMP image file types. Saving preferences included saving the resolution as loaded/no resizing and original data dynamic range. Radius diaphysis registration followed the same series of steps as described above except for the volume of interest which isolated the radius, excluding the ulna.

Following registration, a singular baseline slice and the corresponding follow-up slice (from the midpoint of the 168 slice volume of interest using the baseline image) was utilized for analysis. We chose this as a standard region for our analysis because the center slice was less susceptible to imaging offsets meaning that the slice was not impacted during registration (loss of most proximal and distal slices). For each individual, a threshold for the bone was determined for every baseline and follow-up slice using the Otsu method within ImageJ. Threshold values were recorded for later entry into the MATLAB analysis script. Using the baseline image, the cortex was isolated by a single trained individual using a semi-automatic segmentation approach with manual correction to create a region of interest (ROI) in ImageJ on the follow-up images (Whittier et al., 2020b). Manual correction was only needed on the endosteal surface in cases of numerous pores open to the marrow space and/or severe trabecularization of the endosteal surface. Segmented cortex ROI’s were saved by generating a 1-bit BMP masked image.

To track the dynamic actions of individual cortical pores we employed an individual pore tracking methodology using a custom MATLAB script (PorosityTrack_V4.4.m) as previously published in rodent models of chronic kidney disease (CKD) (Metzger et al., 2021; Swallow et al., 2022). The pore tracking script requires 3 files per individual: registered baseline BMP (greyscale), corresponding registered follow-up BMP (greyscale), segmented cortex ROI BMP (1-bit binary) from the baseline image.

The pore tracking MATLAB analysis process follows the following workflow outline with more specific details outlined in the paragraph below:

1. Validate the image inputs and their naming convention
2. Confirm voxel resolution of images (60.7 μm)
3. Input threshold for baseline image
4. Input threshold for follow-up image
5. Filter pixels to remove noise for conservative estimate of pores

During steps 3 and 4, the threshold for baseline and follow-up images from ImageJ were inserted into the program for continuity. To account for image artifacts, step 5 removed any non-bone regions within the cortex ROI that contained less than five connected pixels (<303.5 μm² area in 2D). This was an arbitrary size to exclude but was chosen to increase the rigor of assessment for assessing dynamic changes.

Prior to pore analysis, the MATLAB script executes a median filter on the 2D slices using the ‘medfilt2’ function. The MATLAB script fuses the thresholded, and therefore binarized, images, to create an overlay between follow-up and baseline images to compare borders and changes in morphology between timepoints. This permits the code to identify pores or bone in three scenarios: 1) present only in baseline, 2) only in follow-up, 3) present in both images (shared). Tissue area is defined as all area (bone and void) between the periosteal and endosteal surfaces. The image undergoes morphological operations to close gaps followed by a pseudo-shrink wrap that is applied to the cortex boundary (cortex). The program next classifies the pore as ‘closed’ (pore perimeter is fully contained within the cortex) or if a pore is ‘open’ (pore perimeter is partially opened on the endosteal or periosteal surface). Closed pores are determined by inverting the image and subtracting the background and marrow space (Fig. 1A). Subsequently, open pores are defined as All Pores – Closed Pores = Open Pores.

Pore dynamics are then determined by 1) indexing baseline pores 2) determining if there are any pixels present in a baseline pore AND a follow-up pore 3) finally indexing any unclassified follow-up pores after steps 1 and 2 are complete. Totalling up the number of pores identified permits pore numbers in both before and after images to be recorded. Pore relationships are then characterized based on a matrix as follows: if they are 1) filled, 2) static, 3) developed, 4) contracted or 5) expanded (Fig. 1B & 1C). Filled pores are those pores which are present in baseline images but are not present in follow-up indicating bone formation has infiltrated the cortical pore (Metzger et al., 2021; Swallow et al., 2022). Static pores are pores which are classified in the same location at both image timepoints. Developed pores are newly formed pores present in follow-up images but not in baseline images. Contracted pores are those that are present at both timepoint but have reduced in area between baseline and follow-up. Finally, expanded pores are those which gain area between baseline and follow-up imaging timepoints.

Cortical geometry parameters were measured in three consecutive slices at both the tibial and radial sites using CTAn software (Bruker, Billerica, MA, USA). Following registration, the endocortical and periosteal surfaces of the bone were isolated in the baseline and 1 year follow-up images and total area (TLa), cortical area (TLa), marrow area (Ma.A), cortical area fraction (BA/TA), and cortical thickness (Ct. Th) were calculated.

All data were visualized, and statistical analysis was performed in Prism (GraphPad) with the exception of intraclass correlation coefficient analysis which was performed in SPSS (v.28, Chicago, IL, USA). For all analyses, a priori α-levels were set at 0.05 to determine statistical significance. All data was assessed for normality, if a parameter failed a Shapiro-Wilk normality test, then the appropriate non-parametric test was performed.

To determine inter-rater reliability of the pore dynamic analysis presented in the manuscript, intraclass correlation coefficients (ICC, two way random, average of eight measures, absolute agreement) and 95% confidence intervals were calculated for the following outcomes: total porosity, total pore number, filled pore area, area lost to contraction, area remaining static, area gained from expansion, and developed pore area. The ICC values were graded using the scoring method described by Fleiss et al.: 0.75–1.00 = excellent reliability, 0.40–0.75 = fair to good reliability, 0–0.4 = poor reliability (Fleiss, 1986). To determine the effects of time on cortical porosity, cortical pore area, and cortical pore number in the tibia, paired t-tests were performed. In the radius, pore...
number was assessed by a paired t-test, whereas cortical porosity and pore area were assessed via a paired non-parametric t-test (due to lack of normality). To assess whether pores gained or lost area (filled/contracted vs. expanded/developed) in the tibia or radius, a Mann Whitney unpaired non-parametric t-test was performed. A one-way ANOVA was used to determine the impacts of pore relationships (filled, contracting, static, expanding, or developed) on pore number in the tibia and was followed by a Tukey’s multiple comparison test when significance was reached. Similarly, tibial pore area, radii pore number and radii pore area were assessed via a Kruskal-Wallis non-parametric ANOVA.

Fig. 1. Methodological notes. A) Pores are classified as open (pore perimeter is partially opened on the endosteal or periosteal surface) or closed (pore perimeter is fully contained within the cortex). Panel ‘A’ depicts a representative example of a binarized cross-section of the tibia with open pores displayed as green and closed pores displayed as pink. B) Schematic of dynamic pore action classifications (blue pixels representing porosity). C) Binarized cross-sectional tibia with pore dynamics output displayed: filled = dark blue, contracting = light blue, expanding = peach, developed = burnt orange.

Fig. 2. A) Signal noise ratio (SNR) assessment for each volunteer (black dots with lines connecting the timepoints) at both tibia and radius sites. Lower SNR was seen in the tibia of one individual (highlighted red), therefore additional despeckling was performed. There was one subject which had a 1-year radial scan which had higher SNR. Upon further inspection we did not feel this image required further processing thus no additional operations were utilized. B) The individual with the lower SNR was theorized to be associated with the tibia being locationally positioned above the isocenter of the bone due to the size/mass of the volunteer’s extremities. The other image is a representative example of how the lower extremities typically sit within the holder.
Multiple comparisons were corrected using a Dunn’s test. Descriptive statistics were used to observe the patterns between pore dynamic relationships and their pore status (open vs. closed). Descriptive statistics were utilized because of a limited number of pores available to measure in each tibial or radial sample. Finally, changes in cortical geometry parameters from baseline to follow-up HR-pQCT acquisitions were assessed by paired t-tests.

3. Results

3.1. Participant characteristics

Seven female volunteers were included in the study. Mean time between baseline and follow-up visits were 11.71 ± 1.75 months. Representative baseline and 1 year HR-pQCT images at the tibial and radial sites can be found in Supplementary Fig. 1. Participants were 65.9 ± 3.1 years old, 1.63 ± 0.7 m tall, and weighed 68.8 ± 16.5 kg. Participants had an average BMI of 26.0 ± 3.1 kg/m² and an average appendicular lean mass (sum of lean tissue in arms and legs) of 7.15 ± 1.40 kg/m². The average total hip aBMD t-score was −1.11 ± 1.27 and the average spine aBMD t-score was −0.62 ± 0.97. All individuals identified as white, non-Hispanic or non-Latino.

3.2. Signal efficiency was maintained over the course of the study

There were no significant differences in SNR between baseline and 1-year acquisitions in the tibia (paired t-test p = 0.623) or radius (p = 0.316) (Fig. 2A). The average SNR at the tibia (combined baseline and 1-year) and radius were 26.86 (min = 17.35, max = 33.01) and 39.49 (range = 33.44–52.78), respectively. SNR results from one individual for the baseline (18.97) and follow-up (17.35) tibial diaphysis image deviated substantially from all other volunteers (−29 % and −35 % below the mean SNR for baseline and 1-year, respectively) likely due to the size/mass of the patient’s extremities which placed the bone out of the isocenter of the bore (Fig. 2B). As a result, baseline and 1-year HR-pQCT images from this individual were subject to a median filter (3 × 3 neighborhood) utilizing the ‘despeckle’ function in ImageJ allowing the dynamic cortical pore tracking program to remain conservative about what was defined as a cortical pore. This process improved the SNR by +7 % and +10 % for the baseline (20.28) and follow-up (19.14) images, respectively.

3.3. Intraclass correlation coefficients exhibited good to excellent inter-rater reliability

The inter-rater reliability expressed by the ICCs are reported in Table 1. Inter-rater ICC values showed good-to-excellent agreement for six of the seven dynamic pore measurements we assessed. Only ‘area remaining static’ demonstrated poor agreement.

3.4. Over 1-year, there were significant increases in overall cortical porosity in the tibia which was driven by increases in pore area

Static microstructure analysis of the tibia showed significantly higher percent total cortical porosity after one year (2.29 ± 1.56) compared to baseline (1.93 ± 1.51; p = 0.0257, Fig. 3). Tibial pore area also increased after one year (p = 0.0385, baseline 4.49 ± 3.17 vs. one year 5.4 ± 3.36) however, pore number did not differ between timepoints (p = 0.3940, baseline 32.71 ± 18.09 vs. one year 34.14 ± 18.71). In the radius, neither cortical porosity (baseline 3.65 ± 5.71 vs. one year 4.09 ± 5.97), cortical area (baseline 2.88 ± 4.66 vs. one year 3.23 ± 4.89), nor pore number (baseline 6.00 ± 4.32 vs. one year 6.29 ± 2.75) were significantly different between baseline and 1-year timepoints (p = 0.1094, p = 0.1094, and p = 0.7261, respectively. Fig. 3).

3.5. Over a 1-year period, pore area and number drove changes that favored bone loss

In alignment with higher cortical porosity, individual pore tracking analysis of the tibia showed that both expanding and developed pores had a significantly greater changes in pore area compared to filled and contracting (p = 0.0034, filled/contracting 0.26 ± 0.18 vs. expanding/developed 0.73 ± 0.54, Fig. 4). In the radius, a similar pattern occurred with expanding and developed pores showing greater pore area change after one year compared to filled and contracting pores (p = 0.0474, baseline 0.13 ± 0.25 vs. one year 0.30 ± 0.28, Fig. 4).

Individual pore tracking analysis in the tibia demonstrated that pore area change over a year differed depending on pore action. Specifically, expanding and developed pores drove the gain in porosity since they had significantly higher pore number (p = 0.0015 and 0.0140, respectively) and pore area (p = 0.0012 and p = 0.0152, respectively) compared to unchanged pores (static) (Fig. 5). There were no other differences between pore action groups for either pore number or pore area change. There were significantly more developing pores with significantly greater pore area compared to static pores in the radius (Fig. 5). The remaining pore action comparisons did not differ between groups for either pore number or pore area change.

3.6. Closed expanding pores accounted for the greatest changes over a 1-year period

Observing whether pore actions differ based on pore characteristics (open vs. closed at baseline), exploration of the tibia found that closed pores were more frequently identified (Fig. 6). Additionally, closed pores, or pores found fully contained within the cortex, that were expanding accounted for the greatest number of pores that changed. In the radius, there were no differences between the frequency of open and closed pores with respect to individual pore action (Fig. 6).

3.7. Cortical geometry was largely unchanged over 1-year

Detailed cortical geometry outcomes in the tibia and radius, including p-values, can be found in Table 2. Only total area significantly increased in the tibia (1336.29 ± 126.42 vs. 1341.09 ± 127.08, p = 0.010) after one year. No other cortical outcome was significantly different from baseline to follow-up scans.
4. Discussion

This study demonstrates an approach to longitudinally track individual cortical pore activity in female tibial and radial sites. HR-pQCT images taken one year apart revealed cortical pores were actively developing, contracting, expanding, and infilling. In this cohort of females aged 60+ years, we showed that tibial pores were more likely to get bigger in size (expand/develop) while the radius was more likely to...
develop new pores over one year. Closed pores in the tibia, those that were not connected to the endosteal or periosteal surfaces, were the most dynamic of any pore type (open/closed) at either site. For closed tibial pores, all actions (infilling, contracting, expanding, and developing) occurred at a higher number than open pores, but the number of expanding/developing drove the overall increase in pore area. These data expand conventional parameters for assessing cortical porosity and could help understand intracortical bone physiology.

The negative effect of cortical porosity on bone mechanical properties is well-established (Dong and Guo, 2004; Schaffler and Burr, 1988; Turner, 2002). These data have focused on global porosity – that is overall levels of porosity on various mechanical outcomes. Experimental and computational modeling have indicated that differences in pore radius are more representative than changes in pore number when examining bones with different overall porosities in cross-sectional design studies (Lerebours et al., 2015; Thomas et al., 2006). If or how differences in pore size/number affect mechanics was not addressed. Although always possible to assess pore number and size using histological approaches – such work was limited to cadaveric material and cross-sectional study design. HR-pQCT technology has now made it easier to make these measures in those settings and, as we show, has opened the potential to assess individual pores and their changes over time. This opens the potential to ask exciting and new questions which have not been possible to date.

Bone histomorphometry from transiliac biopsies remains the gold standard for both static and dynamic quantification of bone micro-architecture. Although traditionally focused on trabecular bone, ample data exists regarding cortical bone dynamics of the iliac crest (Vedi et al., 2011). However, iliac crest biopsy studies are often cross-sectional because of its invasive nature of biopsy and high variability makes comparing to control populations challenging. As a result, new approaches to evaluate longitudinal changes over time are needed in the field. Recently, a surrogate cortical porosity measure termed the ‘porosity index’ has been described utilizing non-radiating magnetic resonance imaging (MRI) (Rajapakse et al., 2015). Porosity index correlates significantly with CT-based cortical porosity boasting a safer alternative to invasive biopsies (Rajapakse et al., 2015), yet MRI is based on the hydrogen signal (i.e. water) in bone which limits the resolution capacity. So, although it is without radiation and allows repeat scanning MRI cannot directly resolve cortical microstructure at the level of HR-
HR-pQCT, like most high-resolution in vivo imaging techniques, is susceptible to motion artifact which can cause blurring of the image impacting the ability to precisely resolve cortical pores at the micron level. To limit the impact of motion blurring in the current study, HR-pQCT image quality was visually graded at the time of acquisition and subjects were re-scanned when artifact was indicated. It is entirely plausible that minor impacts of motion still impacted analyses highlighting the value of motion compensation algorithms to be developed similar to cone-beam CT algorithms (Sisniega et al., 2019) to assure accuracy of microarchitecture data. Our attempt to minimize small artifacts was to set a threshold (5 pixels) under which we ignored pores. It is both plausible and likely that by performing this step, we removed some true pores in addition to noise. Even so, we believe the addition of this step increased the rigor of our assessment and made our estimation of pore (number, area etc.) more conservative.

Our workflow utilized a 3D rigid registration scheme to align baseline and 1-year follow-up images from aged individuals. We assessed inter-rater reliability via ICC which demonstrated good-to-excellent agreement in six of the seven dynamic pore outcomes assessed. The good-to-excellent agreement between raters suggests that analysis could be carried out by a singular rater in future studies when using the workflow presented. It is plausible that when the registration scheme is applied to scans from individuals with bone and joint disease, the rigid scheme will underperform in cases that have severe cortical thinning and/or endocortical surfaces with severe trabecularization. To mitigate these effects, we employed a scheme which allows for manual alignment. We found this beneficial for cases from two individuals which failed using fully automated registrations and required manual alignment by the trained user. The addition of matched angle analysis or boundary transformations to the 3D registration scheme may improve these outcomes in cases of severe bone disease where there is a desire to align images over time (Hosseinibatabaei et al., 2022). When considering the time cost of analysis, another potential drawback was the use of semi-automatic, rather than fully automated, segmentation of the bone images. However, in unpublished pilot work, we determined that fully automated segmentation routines had challenges, particularly at the endocortical surfaces in subjects who had trabecularization occurring. In these cases, the porosity was either inflated or underreported depending on the resultant segmentation approach. As a result, we chose to utilize a semi-automated methodology which allows correction by a trained user to improve accuracy of our pore measures.

While the detrimental mechanical impact of increasing cortical pores is known, little is understood about individual pores’ dynamic activity, including how and what drives the rate in which they develop, expand, contract, and infill. In our aged female cohort, we observed that individual cortical pores were actively changing over one year including expanding, contracting, infilling, and developing. Furthermore, the net increase in pore area in the tibia was driven by expanding and developing pores, mainly closed pores, while in the radius, new pores were forming pores, mainly open pores. To mitigate these effects, we employed a scheme which allows for manual alignment. We found this beneficial for cases from two individuals which failed using fully automated registrations and required manual alignment by the trained user. The addition of matched angle analysis or boundary transformations to the 3D registration scheme may improve these outcomes in cases of severe bone disease where there is a desire to align images over time (Hosseinibatabaei et al., 2022). When considering the time cost of analysis, another potential drawback was the use of semi-automatic, rather than fully automated, segmentation of the bone images. However, in unpublished pilot work, we determined that fully automated segmentation routines had challenges, particularly at the endocortical surfaces in subjects who had trabecularization occurring. In these cases, the porosity was either inflated or underreported depending on the resultant segmentation approach. As a result, we chose to utilize a semi-automated methodology which allows correction by a trained user to improve accuracy of our pore measures.

**Table 2**

|                  | Baseline | 1-Year | Paired T-test (p-values) |
|------------------|----------|--------|-------------------------|
| **TIBIA**        |          |        |                         |
| Total area (mm²) | 1336.29  | 1264.22| 1341.09 ± 127.08 0.010  |
| Cortical area (mm²) | 990.84  | 1632.73| 993.58 ± 162.63 0.286  |
| Marrow area (mm²) | 71.58   | 21.93  | 71.80 ± 21.53 0.326   |
| Cortical area fraction (%) | 73.96 | 7.88   | 73.90 ± 7.74 0.699   |
| Cortical thickness (mm) | 4.40  | 0.83   | 4.40 ± 0.83 0.610   |
| Radius            |          |        |                         |
| Total area (mm²) | 368.85  | 39.45  | 368.80 ± 39.84 0.966  |
| Cortical area (mm²) | 296.47  | 36.20  | 296.83 ± 39.45 0.861  |
| Marrow area (mm²) | 14.95   | 5.26   | 14.87 ± 5.28 0.756   |
| Cortical area fraction (%) | 80.47 | 6.82   | 80.50 ± 7.10 0.942   |
| Cortical thickness (mm) | 2.77 | 0.47   | 2.78 ± 0.51 0.736   |

Data presented as mean ± standard deviation. P-values from paired t-tests.

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**Disclosures**

All authors declare that they have no conflicts of interest.

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Declarations of competing interest

All authors declare that they have no competing interest related to the present study.

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