Intracortical Bone Mechanics Are Related to Pore Morphology and Remodeling in Human Bone

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Relating Intracortical Bone Mechanics to Pore Morphology and Remodeling Characteristics in the Human Fibula†

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Abstract

During aging and in osteoporosis, cortical bone becomes more porous, making it more fragile and susceptible to fractures. The aim of this study was to investigate the intracortical compression-induced strain energy distribution, and determine whether intracortical pores associated with high strain energy density (SED) in the surrounding bone matrix have a different morphology, distribution and remodeling characteristics than matrix with normal SED. Fibular diaphyseal specimens from 20 patients undergoing a jaw reconstruction (age range 41–75 years, 14 men and 6 women) were studied. Bone specimens were μCT-scanned, plastic embedded, and sectioned for histology. Three-dimensional micro-finite element models of each specimen were tested in compression, and the SED of the bone immediately surrounding the intracortical pores was calculated within a plane of interest corresponding to the histological sections. The SED of a pore, relative to the distribution of the SED of all pores in each specimen, was used to classify pores as either a high or normal SED pore. Pores with high SED were larger, less circular, and were located closer to the endosteal surface of the cortex than normal SED pores ($p < 0.001$). Histological analysis of the remodeling events generating the pores revealed that the high SED pores compared to normal SED pores had a 13.3-fold higher odds of being an erosive (70%) or formative (7%) pore versus a quiescent pore ($p < 0.001$); a 5.9-fold higher odds of resulting from remodeling upon existing pores (type 2 pore) versus remodeling generating new pores (type 1 pore) ($p < 0.001$); and a 3.2-fold higher odds of being a coalescing type 2 pore versus a non-coalescing type 2 pore ($p < 0.001$). Overall, the study demonstrates a strong relationship between cortical bone mechanics and pore morphology, distribution, and remodeling characteristics in human fibular bone. This article is protected by copyright. All rights reserved

Keywords: cortical bone, microstructure, finite element, porosity, strength
Introduction

Cortical bone is the outer compact layer of bone that is structurally significant for non-vertebral bones,(1,2) and accounts for a significant proportion of fractures in the elderly.(3–5) The pores in cortical bone are part of a complex intracortical network of vascularized canals, which allow for nutrient and cell transport within the cortex.(6,7) This canal network is generated and modulated by intracortical bone remodeling events.(8–10) In adult bone, the initiation of intracortical bone remodeling events are believed to be largely driven by mechanical stimuli(11–13) likely detected by mechanosensing osteocytes,(14,15) and results in renewal of the cortical microarchitecture and bone matrix. The microarchitecture of the intracortical canal network and the surrounding bone matrix material properties affect the local strain distribution within cortical bone, which in turn can affect intracortical remodeling events, thereby providing a mechanism for continual renewal of the cortex. However, during aging cortical bone becomes increasingly porous and thin, suggesting a disruption in this process,(16–18) ultimately leading to reduced mechanical strength of the cortices and, as a consequence, increased fracture risk.(2,19–21)

Classically, intracortical bone remodeling events were illustrated by a cutting cone with bone-resorbing osteoclasts excavating a new canal that is subsequently refilled by a closing cone of bone-forming osteoblasts until only a narrow canal remains.(9,22) However, intracortical remodeling events have been shown not only to generate new canals, defined as type 1 remodeling, but also to remodel existing canals as well, defined as type 2 remodeling.(10,23–25) The age-induced increase in cortical porosity is the result of a cumulative abundance of dysfunctional intracortical remodeling events that manifests as pores with enlarged diameters.(16,18,26) A recent study on the ilium of women has shown that these enlarged pores are preferentially eroded pores generated by type 2 remodeling events.(27) Here, the accumulation of eroded type 2 pores likely reflects a delayed or absent initiation of bone formation, as reported to be the case in cancellous bone of osteoporosis patients.(10,28–30)

The local mechanical strain environment influences pore remodeling events and therefore cortical microstructure. Local cortical surface strain measurements have shown that both osteocyte lacunae and intracortical pores result in strain concentrations around pore surfaces, and that the surrounding bone matrix has a heterogeneous strain distribution at the osteon level.(31–33) This heterogeneity in strain may be, in part, due to local differences in bone matrix material properties and constituents. However, the strain variation observed in cortical bone could only partly be explained by variations in bone mineral content.(31) Finite-element based evaluations of cortical
bone have shown variations in microstrain measurements when material properties were held constant. Microstructural variation is therefore an important determinant of local strain distributions and has been suggested to magnify the inhomogeneous strains predicted in cortical bone. Mechanically, increased cortical porosity has been shown to be critical for bone strength, and previous studies have focused on cortical bone pore structure, macro-scale strength of cortical bone, or the effects of gender or age. However, the relationship between microstructural variation and local strain distributions, as well as the intracortical remodeling events that lead to specific pore morphologies, remains to be investigated. From a mechanical perspective, increases in pore size and irregularity are likely factors that increase the local strain energy in the surrounding bone; however, the degree to which these factors can discriminate increased strain energy and therefore serve as potential indicators of fracture risk is not clear.

The objective of the present study was to investigate the relationship between calculated bone mechanical measurements (strain energy density, SED) in the bone matrix surrounding cortical pores and both their morphology and the type of intracortical remodeling events generating the pores using a newly established classification. The specific aims were (1) to investigate whether pores of different SED in the surrounding bone matrix are characterized by their size, shape, and location within the cortex, and (2) to determine whether the SED is associated with pores of a specific remodeling type – based on their position relative to existing osteons – and remodeling stage. Understanding the relationship between the remodeling events, their pores and the resulting mechanics is important for identifying determinants of bone fragility during aging.

Materials and Methods

Bone samples
Fibula diaphyseal bone specimens were acquired from 20 patients (6 women, aged 41–73 years and 14 men, aged 43–75 years) undergoing mandibular reconstructive surgery at the Department of Plastic Surgery, Aarhus University Hospital (Figure 1A). The bone specimens were approximately 12mm thick discs obtained from the fibular diaphysis 7–12 cm proximal from either the medial or lateral malleolus. The lateral half of this discs was fixed in 70 percent ethanol, μCT imaged, methylmethacrylate embedded and sectioned, as described below. The study was approved by the Danish National Committee on Biomedical Research Ethics (Project-ID: S-2012-0193).
Micro-computed tomography imaging

Prior to embedment, the bone samples were scanned in a desktop micro-computed tomography (μCT) scanner (μCT35, Scanco Medical AG, Brüttisellen) in high-resolution mode (1000 projections per 180 degrees) with an isotropic voxel size of 6 μm (voltage = 70 kVp, current = 114 μA, integration time = 3200 ms). The data sets were low-pass filtered with a Gaussian filter (σ = 0.8, support = 1) and segmented with a fixed threshold (508.4 mg HA/cm³). The threshold was based on the mid-point between histogram peaks identifying bone and pore using the scanner software (IPL). The resulting binary image data was overlayed with an image mask delineating the cortical bone and exported as 8-bit TIFF images. The images were processed to remove bone fragments not connected to the main cortical structure (Matlab R2015-Academic, Mathworks, Natick, MA, USA).

Histological Analysis

After μCT, the undecalcified bone samples were embedded in methylmetacrylate, and cut into 7-μm-thick sections on a hard tissue microtome (Reichert Jung GmbH, Heidelberg, Germany). The tissue block was mounted so the cutting plane matched the xy-plane in the μCT scanner as closely as possible. A central histological section was obtained from each bone specimen and Masson-Goldner trichrome stained. Next, the histological section was registered to the μCT image stack using Amira (version 5.6., FEI Visualization Science Group, Merignac, France). Approximately 50 pores within both the central histological section and the complementary μCT image of each specimen was given an identification number and marked on an image of the cortex (Figure 1C). Note that the cortex investigated histologically reflected a sub-part of the lateral half of the fibular bone.

The pores were classified according to our recently established classification (Figure 2).(10) Specifically, pores with a resorption area showing no overlap with the pore of an existing osteon were defined as type 1 pores, and pores with a resorption area physically overlapping with the estimated position of an existing osteon were defined as type 2 pores. Note that in formative or quiescent pores, the resorption area was defined by the cement line. Type 2 pores were further divided based on the position of their resorptive area with respect to their existing parent osteon (Figure 2). Type 2 pores with a resorptive area that remained within the boundary of an existing osteon were classified as intra-osteonal type 2 pores (type 2\textsubscript{IN} pores), type 2 pores with a resorptive area breaking the boundary of an existing osteon were classified as cement-line breaking type 2
pores (type 2BK pores), and type 2 pores with a resorption area that merged with the pores of neighboring osteons were classified as osteon coalescing type 2 pores (type 2CO pores). Finally, the pores were classified according to their surface characteristics as: newly eroded surfaces (type E), pores with mixed eroded and osteoid (formative) surfaces (type EF), formative pores with osteoid surfaces only (type F), or pores with quiescent surfaces (type Q).

2D structural analysis of intracortical porosity
For each bone sample, the location within the stack of binarized μCT images corresponding to the histological section was identified and used for 2D structural analyses (Matlab R2015-Academic, Mathworks, Natick, MA, USA) (Figure 1C). Within this slice approximately 50 cortical pores were identified and the size, shape, and location with respect to the periosteal or endosteal boundary was quantified and linked to its identification number (Figure 1C). The pore diameter was defined as the diameter of the largest circle that could be inscribed in the pore.(37) The shape of each pore was defined as the degree of circularity (ratio of the area of the largest circle that can be inscribed in the pore to the actual area of the pore). Pore location was calculated by first identifying the endosteal and periosteal boundaries as defined by Andreasen et al.(10) Next, the linear distance from the centroid of each pore to the respective edge of the bone was calculated taking into account the varying cortical thickness. The median pore size, circularity, and location was calculated per bone sample.

Micro finite element models and classification of pore mechanics
High-resolution three-dimensional finite element models were generated using 100 consecutive binarized μCT images centered around the same plane of interest used in the 2D structural analyses. The images were used to create a mesh of quadratic tetrahedral volume elements (ScanIP, Simpleware, Exeter, UK). To facilitate the finite element mesh generation, very small pores (regions less than 20 connected voxels) were removed by filling their space as bone. Cortical bone was modeled with homogeneous, isotropic, elastic properties (Young’s Modulus, E = 17.5 GPa and Poisson’s ratio, ν = 0.3).(38,39)

Linear implicit calculations of SED within the cortical bone under compression were performed using a commercial finite-element solver (Abaqus v. 6.13 and v. 6.14; Dassault Systèmes Simulia Corp., Pawtucket, RI, USA). Specifically, a unit load in uniaxial compression was applied uniformly to the top surface of the model (Figure 1D, left). Nodes on the opposing surface were
vertically fixed, and three nodes on the same face were constrained in all three dimensions. The central third of the model centered around the same section analyzed for structural and histological measurements was post-processed. Within this central region, the SED of elements closest to each pore boundary was calculated and averaged for each pore (Figure 1D, center). Specifically, the location of each voxel along the pore border was identified within the micro-CT data. These locations were then cross-referenced with the centroids of all elements within the model and used to identify the element closest to the pore border. Similar to the 2D structural analyses, the median SED of all pores per bone sample was calculated.

Statistical Analysis
The interquartile range (IQR) for each sample (Matlab, Mathworks, Natick, MA USA) was used to identify pores with high strain energy density. “High” SED pores were those with mean SED values 1.5 times the IQR away from the 75th percentile (Figure 1D, right), “low” SED pores were 1.5 times below the IQR from the 25th percentile, and the remaining pores were classified as “normal” (Figure 1D). In our analysis, there were no pores that fell within the low category and hence all pores were either categorized as having high or normal SED. Following categorization, the mean SED of all pores within each category was calculated for each specimen. Kolmogorov-Smirnoff test revealed that multiple parameters were not normally distributed, and therefore Wilcoxon Rank-Sum tests were used for comparison between different groups of variables including SED (high, normal, and low), pore size, pore circularity, and pore location (GraphPad Prism 6, GraphPad software Inc., La Jolla, CA, USA). For comparison between SED and remodeling type, a clustered logistic regression analysis was used taking into consideration that pores were clusters from different individuals (Stata/IC 10.1, Statacorp, College Station, TX, USA). Data is reported as median ± median absolute deviation. P-values less than 0.05 were considered statistically significant.
RESULTS

Across the twenty cortical bone specimens, a total of 1064 pores were 2D analyzed in the μCT images (Figure 3). The majority of pores were small: 52% of pores were less than 50 μm in diameter and 32% between 50 μm and 100 μm in diameter while only 16% had a diameter above 100 μm. Pores tended to be circular with 45% of the pores having a circularity greater than 50%. Cortical porosity ranged from 2% to 22% with a median of 6 ± 4.6% and the bone specimens had a median cortical thickness of 2.23 ± 0.62 mm.

Morphology and location of pores with increased strain energy density

Within the section of each specimen, a subset of pores was identified as having a high SED according to the quartile stratification, and the remaining pores were classified as normal SED pores \( (p < 0.001, \text{Figure 4A}) \). The diameter of pores with high SED was three times larger than those with normal SED \( (p < 0.001, \text{Figure 4B}) \) and were significantly less circular than normal SED pores \( (p < 0.001, \text{Figure 4C}) \). Similar results were found for pore area, and there was no correlation between pore area and circularity \( (r^2 = 0.08) \). Across the specimens, high SED pores were more often located closer to the endosteal than the periosteal surface \( (p < 0.001, \text{Figure 4D}) \).

Remodeling characteristics of pores with increased strain energy density

Ninety-one percent of high SED pores reflected intracortical remodeling upon an existing pore (type 2), while 63% of the normal SED pores reflected generation of a new pore (type 1) (Figure 5A). High versus normal SED pores had 5.9-fold higher odds of being a type 2 pore versus a type 1 pore \( (p < 0.001, \text{Figure 5A}) \).

Of the type 2 pores, 41% of the high SED pores, and only 9% of the normal SED pores, reflected the coalescence of pores (see type 2\textsubscript{CO}, Figure 5B). On the other hand, 17% of the high SED pores versus 54% of the normal SED pores were classified as intra-osteonal type 2 pores (Figure 5B). High versus normal SED pores had a 3.2-fold higher odds of being an osteon coalescing type 2 pore versus an intra-osteonal or cement-line breaking type 2 pore \( (p < 0.001, \text{Figure 5B}) \).

Eighty-one percent of the high SED pores, compared to only 27% of the normal SED pores, were non-quiescent. High versus normal SED pores had a 13.3-fold higher odds of being a non-quiescent pore (type E, EF, or F pore) versus a quiescent pore (type Q pore) \( (p < 0.001, \text{Figure 5C}) \). Here, the majority of the non-quiescent pores were eroded pores (type E pores).
DISCUSSION
With aging, the intracortical bone remodeling process becomes dysfunctional causing increased cortical porosity.(40,41) Such bone loss results in structural damage, reduced mechanical strength, and increased fracture risk.(19–21)(3,42) Currently, there are no clinical assessments of fragility risk, which incorporates cortical porosity directly. Our results indicate that cortical pores with increased strain energy density are characterized by specific micro-architectural features and remodeling characteristics. Specifically, high SED pores compared to normal SED pores are: larger, less circular, positioned closer to the endosteal surface of the cortex, and more frequently associated with non-quiescent remodeling events upon existing canals causing a coalescence of pores.

Association between cortical pores SED and their size, shape, and position
While increased cortical porosity is associated with decreased bone strength,(21,43) total porosity and pore density may not be the best indicators of fragility. Even though the majority of cortical pores are relatively small, the less frequent larger pores account for a significant fraction of the porosity in cortical bone.(44,45) The present study shows that high SED pores are larger than normal SED pores, suggesting that pore size matters from a mechanical perspective. The occurrence of larger pores has been shown to increase with age,(10,46,47) and is more prominent in the anterior region of the femoral neck of postmenopausal women with osteoporotic fractures compared to controls.(45) Future studies are needed to address whether the assessment of fracture risk may be improved by taking the presence of larger pores into consideration.

Pore size alone does not determine whether the surrounding bone will have high or normal SED as seen by the overlap in pore sizes that were found in both categories. We evaluated pore circularity independent of pore size, and found that high SED pores were less circular than normal SED pores. The pore size and circularity correlated poorly, suggesting that they may be independent contributors to the SED in the surrounding bone. While in general, the cortex surrounding less circular pores had an increased SED, there were some circular pores that also had increased SED. Small irregularly shaped pores may reflect the irregular geometry of branching-points in the canal network.(25) One may speculate that the larger irregular pores are those found in the transition zone of the cortex at the endosteal surface.(48) This highlights the need to characterize porosity in terms of the three-dimensional canal structure rather than as two-dimensional pores, which is part of
current research efforts by our groups.

The high SED pores were located closer to the endosteal surface than the normal SED pores. This endosteal portion of the cortex has been reported to have an increased porosity,(49–52) larger pores,(52) and increased remodeling activity(53) than the periosteal part of the cortex. We found similar characteristics in the high SED cortex surrounding pores in the present study.

**Association between cortical pores surrounding SED and their remodeling characteristics**

Using our recently developed histological classification of pores,(10) and their associated remodeling characteristics that combines many of the criteria previously reported in the literature,(23–25,46,54–56) we investigated the remodeling characteristics of high versus normal SED pores. The high SED pores were shown to reflect remodeling upon existing canals (type 2 pores), while normal SED pores primarily reflected penetrative remodeling events generating new canals (type 1 pores). Type 2 pores were recently shown to accumulate with age, are present in bone with increased porosity, and have a larger pore diameter than type 1 pores.(10) In the present study, we also found that the type 2 pores associated with increased SED were larger. Moreover, the age-induced accumulation of type 2 pores supports the hypothesis that age induces the accumulation of high SED pores thereby contributing to increased fracture risk. Future studies are warranted to investigate this notion.

Within type 2 pores, high SED pores more frequently reflected osteon-coalescing pores compared to normal SED pores. These osteon-coalescing type 2 pores correspond, in part, to so-called composite osteons, which result from the coalescence of two or more pores of existing osteons and are more abundant in the femoral neck of osteoporotic fracture patients than controls.(56) These pores have been proposed to primarily originate from clusters of remodeling events that merge together.(10,56,57) In addition, the osteon-coalescing type 2 pores as well as the composite osteons were reported to have an irregular shape and reflect the largest pores,(10,56) as was the case for the high SED pores in the current study. On the other hand, high SED pores classified as type 2 pores were less frequently classified as intra-osteonal type 2 pores compared to normal SED pores. These intra-osteonal type 2 pores have previously been described as new osteons in old osteons,(23,24) double-zoned osteons,(55) and type II osteons.(25,54) Intra-osteonal pores have been shown to be smaller pores,(10) as were the normal SED pores in the present study.

Finally, high SED pores were primarily non-quiescent pores, while normal SED pores were mainly classified as quiescent pores with a terminated remodeling. These non-quiescent high SED
pores were primarily classified as eroded pores, reflecting remodeling events in the reversal-resorption phase. (10, 58) This phase was recently shown to be a temporal link between initial resorption and bone formation, and was suggested to play a critical role in osteoclast and osteoblast coupling. (58) Particularly enlarged eroded type 2 pores have been shown to accumulate and coalesce with age, demonstrating that a prolonged reversal-resorption phase is a major contributor to the age-induced cortical porosity. (10)

These same characteristics were found in the high SED pores of this study supporting the notion that high SED pores also accumulate with age, making cortical bone more fragile. The data presented here may support the hypothesis that the fate of these specific types of pores is influenced by the high SED sensed by the matrix-embedded osteocytes in the surrounding bone. If increased strain energy is the result of irregular or large pores, the osteocytes on these irregular surfaces may undergo increased mechanical strain compared to the strain they may experience when on a more regular surface. However, the nature of the causal relationship between bone strain energy and remodeling characteristics remains to be elucidated. This relationship may in fact be bidirectional such that the resulting mechanics induces certain remodeling characteristics and vice versa.

Limitations

There are several limitations to this study. First, the fibula likely endures multi-axial loading rather than the uniaxial compression boundary condition imposed in the present study. Bending or other loading conditions may change our results, but to the best of our knowledge the physiological loading conditions of the fibula due to muscle and joint forces is not currently known. However, in using a unit compressive load we maintained consistent boundary conditions between the samples and our analysis focused on differences between pores within the same specimen. Second, the finite element models were also limited by the thickness of bone evaluated. This study focuses on the SED within an internal sub-section and considered only the bone immediately around each pore (less than 20 μm away from the pore edge) and may be influenced by proximity to the global boundary conditions. Analyses over a larger spatial range were limited by computational resources. The models used in the study had an average of 5.9 million elements. The images were also limited by the resolution of the μCT scanner wherein a voxel size of 6 μm means that the theoretical smallest pores that could be resolved was 12 μm. However, our previous work in iliac bone revealed that 5 percent of pores identified in histological sections had diameters below 12 μm. (10) Our analysis was also limited to older adults and does not allow for a comparison to young bone.
Finally, the study assumed homogeneous material properties for all models and therefore did not account for variation in these properties. However, given that the bone surrounding quiescent pores that have terminated remodeling is likely more mineralized than the bone surrounding non-quiescent pores, the study has likely overestimated the cortical stiffness and underestimated the strain energy density. Further studies are required to investigate this assumption.

Conclusion

This interdisciplinary study provides a novel insight into the relationship between cortical bone mechanics, pore morphology, and cellular-driven remodeling characteristics. We have demonstrated a link between cortical porosity, increased strain energy, and the intracortical remodeling characteristics across the fibula specimens. The study shows that the intracortical pores with a high SED in the surrounding bone actually have a similar morphology and remodeling characteristics as the pores reported to accumulate with age.(10,48,52) The investigation of alternative mechanical parameters more directly linking to fragility, such as stress intensity factor, may also provide new insights into the role of porosity. Importantly, this should be done using three-dimensional techniques to better account for the cortical canal network. These findings warrant future studies addressing the link between bone mechanics and remodeling, which likely plays a role in aging- and osteoporosis-associated cortical porosity, and fragility.

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Author roles: The study was designed by MEK, TLA, and JST. The bone specimens were obtained by EMH and BJK. The analysis was conducted by LPB, CMA, and JST, whom also take responsibility for the integrity of the data analysis. The data was analyzed by LPB, MEK, and TLA and interpreted by all authors. The manuscript was drafted by MEK and TLA, and revised by all authors, whom also approved the final version.
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Figure Legends

Figure 1. Measurements of structure and remodeling activity using (A) µCT data of fibular bone specimens. (B) Histological sections from the bone specimens were used to characterize remodeling activity. Structural 2D measurements of apparent pore diameter and circularity (C) were quantified for each pore within a µCT image corresponding to the same histological section. Pore location with respect to the endosteal or periosteal edge. Finite-element models (D) were created using the data above and below the histological slice to calculate the average SED around each pore. Pores with an average SED greater than 1.5 times the interquartile range (IQR) were classified as “high” SED pores, and those within ±1.5 times the IQR were classified as “normal” SED pores (right image). There were no pores that fell below 1.5 times interquartile range (IQR).

Figure 2. Pore type and remodeling pathways identified using histological sections. Four phases of remodeling were identified based on surface characteristics of each pore: erosion, erosion and formation, formation, and quiescence. Within these phases, the pores were further classified according to the pore location with respect to existing osteons. Independent pores were identified as type 1, while pores, overlapping with a pore of an existing osteon, were called type 2. Type 2 pores were further identified as intraosteonal (IN), breaking (BK), or coalescing (CO) depending on their position relative to the existing osteon.

Figure 3. Distribution of pores with respect to their size and circularity across all samples in the FE and histology analysis (n=1064 pores across 20 biopsies). In general, the pores tended to be small and circular.

Figure 4. Comparisons of pore morphology and location in pores with high and normal strain energy density (SED). (A) Each specimen had a group of pores that had SED values higher than the remaining pores (>1.5 IQR) and were therefore classified as high SED pores. High SED pores were larger (B), less circular (C), and tended to be located towards the endosteal edge (D). *** indicates p < 0.001
Figure 5. Remodeling characteristics associated with high and normal SED pores. (A) Compared with normal SED pores, a greater proportion of high SED pores were type 2 pores. (B) Within the type 2 pores, more of the high SED pores were the result of coalescence of pores. (C) Amongst all pores, the high SED pores were more often in their eroded phase compared to the normal SED pores, which tended to be quiescent. Clustered logistic regression analysis was used for the calculation of odds ratios (A–C).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5