Stressed Volume around Vascular Canals Explains Compressive Fatigue Life Variation of Secondary Osteonal Bone but not Plexiform Bone

Loundagin, L.L., Edwards, W.B.

Human Performance Laboratory, Faculty of Kinesiology, University of Calgary

McCaig Institute for Bone and Joint Health, Cumming School of Medicine, University of Calgary

Abstract

The fatigue life of bone illustrates a large degree of scatter that is likely related to underlying differences in composition and microarchitecture. Vascular canals act as stress concentrations, the magnitude and volume of which may depend on the size and spatial distribution of canals. The purpose of this study was to establish the relationship between vascular canal microarchitecture, stressed volume and the fatigue life of both secondary osteonal and plexiform bovine bone. Twenty-one cortical bone samples were prepared from bovine femora and tibiae and imaged using micro-computed tomography (μ CT) to quantify canal diameter, canal separation and canal number. Samples were cyclically loaded in zero-compression and fatigue life was defined as the number of cycles until fracture. Finite element models were created from μ CT images and used to quantify the stressed volume, i.e., the volume of bone stressed higher than yield. Fatigue life ranged from 162-633,437 cycles with the fatigue life of plexiform bone ($n=15$) being more than 4.5 times longer than secondary bone ($n=6$). The fatigue life of secondary bone was negatively correlated with canal diameter ($r^2=0.73$) and canal separation ($r^2=0.56$), while the fatigue life of plexiform bone was negatively correlated with canal separation ($r^2=0.41$), but positively correlated with canal number ($r^2=0.36$). Stressed volume was related to canal microarchitecture in secondary bone only, where canal diameters and canal separation were larger than $50\mu\text{m}$ and $200\mu\text{m}$, respectively. Consequently, stressed volume explained 89% of the fatigue life variance in secondary bone but was not related to the fatigue life of plexiform bone. These findings suggest that the volume of the stress concentration surrounding vascular canals is dictated by canal diameter and may play an important role in the fatigue failure of bone. The detrimental impact of canal diameter was only evident above $50\mu\text{m}$, potentially indicating a critical canal size. We suspect that a larger stressed volume is more likely to encounter and facilitate the propagation of pre-existing microcracks, thereby leading to a reduction in fatigue life.

Highlights

- Increased canal diameter, rather than canal number, determined the fatigue life of secondary bone.
- The longer fatigue life in plexiform bone was associated with more abundant canals.
- In secondary bone, stressed volume increased linearly with increasing canal diameter.
- Stressed volume explained 89% of the fatigue life variance in secondary bone.
- Stressed volume was not related to the fatigue life of plexiform bone.

1. Introduction

Cortical bone exhibits a considerable degree of scatter in fatigue life measurements [1], which is likely related to underlying differences in bone composition and microarchitecture. Previous research investigating the mechanical fatigue of cortical bone has primarily focused on the role that material properties and loading conditions may have on fatigue life measurements [2–8]; however, very few studies have investigated the influence of intracortical microarchitecture beyond simple measures of porosity [9,10]. It is known that increased cortical porosity is associated with a shorter fatigue life, and may explain some 25% of the variance in fatigue strength [4,6]. Cortical porosity is largely attributed to the void space created by vascular canals (i.e., Haversian and Volkmann's canals) and is therefore a function of canal number and size. Given the wide range of canal sizes and spatial distributions observed in cortical bone [11–13], it is possible that the observed scatter in fatigue life measures is better explained by differences in canal morphology. Furthermore, measures of intracortical microarchitecture that are more descriptive than porosity may provide mechanistic insights as to why increased porosity is detrimental to the fatigue life of bone.

In general, porosity may affect the mechanical competence of bone in two ways; by reducing its effective load-carrying capacity and by the introduction of stress concentrations. Vascular canals were first described as stress concentrators by Currey [14], who approximated canals as circular holes in the bone matrix and reported the stress concentration factors associated with both Haversian and Volkmann's canals. It was argued that because Haversian canals are typically parallel to the applied load and Volkmann's canals are relatively small, the stress concentrations induced by either type of canal would not have an appreciable effect on the mechanical behavior of bone. However, the stress concentration factor is only indicative of the peak stress magnitude associated with a particular feature (e.g., a vascular canal) and does not consider other important aspects of the stress distribution. The Theory of Critical Distances (TCD), for example, describes a failure criterion that accounts for both the peak stress and stress gradient surrounding a stress concentration [15]. According to TCD, a stress concentration will only cause failure when the stress at a critical distance, or average stress over a critical volume, reaches some material specific threshold. In this regard, larger canals would be associated with a larger stressed volume, which according to TCD, would result in a decrease in fatigue resistance [16].

In addition to canal size, the spatial distribution of canals may affect the magnitude and volume of stress concentrations. The interaction of two or more stress concentrations can be complex and depends on the relative size and distance between stress concentrators, as well as their orientation with respect to each other and the loading axis [17]. When the alignment of two pores is perpendicular to the applied load their stress concentrations may be additive, resulting in a higher average stress but less stressed volume, as their elevated stress fields potentially overlap. Conversely, when their alignment is parallel with the applied load, the interaction of two pores may dampen the stress concentration [17]. Fatigue testing of bone cement would suggest that closely packed pores are detrimental to fatigue life as cracking occurs between pores, resulting in a larger, irregularly shaped, pore cluster that initiates fatigue failure [18,19]. On the other hand, a high osteonal density has been related to increased yield strength, toughness, and fatigue life [5,20,21], suggesting that cortical bone with more canals in close proximity are more capable of

withstanding repetitive loading and microdamage accumulation. However, much of this research was based on 2D analysis of microarchitecture and only reports the area density of osteons, not the spatial distribution of vascular canals. It is well accepted that osteons provide effective toughening mechanisms [22], but more abundant canals associated with a higher osteon density may increase the overall stressed volume and counteract the beneficial toughening contribution of osteons.

The microarchitecture of bovine plexiform bone exhibits numerous small canals closely packed together in a lattice pattern, whereas bovine secondary osteonal bone contains significantly larger, but fewer, canals that are less uniformly distributed throughout the bone matrix. These distinct differences in bovine bone microarchitecture may provide a unique model to study the effects of canal morphology on the fatigue life of cortical bone and investigate the role of canals as stress concentrators in the bone matrix. The purpose of this study was to quantify the influence of intracortical microarchitecture on stressed volume and fatigue life measurements of both secondary osteonal and plexiform bovine bone. We hypothesized that the fatigue life of bone would be more strongly correlated with canal size rather than the number of canals, and therefore considerably lower in secondary bone. Cylindrical bone samples were extracted from the cortex of bovine femora and tibiae, imaged using micro-computed tomography (μ CT), and cyclically loaded in zero-compression until failure. Relationships between fatigue life measurements and the canal size, spacing, and number, as well as the stressed volume measured using μ CT-based finite element (FE) models, were quantified.

2. Methods

2.1 Sample preparation

Twenty-one cortical bone samples were created from fresh-frozen skeletally mature bovine tibiae (n=4) and femora (n=4) obtained from a local butcher. Cylindrical cores, approximately 35mm in length and 7mm in diameter, were extracted from the cortex of the mid-diaphysis. The exact dimensions and weight of the bone cores were recorded to calculate apparent density. A mini-lathe was used to turn down the cylindrical cores into a “dog-bone” geometry, with a central gauge region 7mm in length, 5.25mm in diameter and a transition radius of 5.55mm. Samples were wrapped in saline-soaked gauze, sealed in plastic wrap and stored at -30°C.

2.2 Quantifying microarchitecture

A 5mm section in the center of the gauge length was imaged using a Scanco μ CT 100 scanner (Scanco Medical AG, Bassersdorf, Switzerland) with an isotropic voxel resolution of 5 μ m. Scans were acquired with a 0.5mm aluminum filter, an x-ray tube voltage and current of 70 KVp and 85 μ A, respectively, and an integration time of 550ms. Due to computational costs, only the central 2.5mm was evaluated in the microstructural and FE analyses. The vascular microarchitecture was segmented and quantified using the Fiji software package (v1.51, NIH, USA) [23]. First the images were filtered and binarized using Fiji’s built in ‘despeckle’ and ‘make binary’ commands, respectively. The despeckle operation applied a 3x3 pixel median filter to remove noise from the image and improve edge detection. The binarization algorithm uses an iterative process that first assumes a tests threshold and segments the image into groups of pore space or bone and then increments the test threshold value until it is above the composite average of these two groups.

Porosity was calculated as the fraction of void volume to total volume. The thickness and separation options included in the BoneJ plugin [24] were used to determine canal diameter (Ca.Dm) and canal separation (Ca.Sp), respectively. The images were then skeletonized and canal number (Ca.N) was calculated as one plus the number of junctions in each skeleton. Two blinded examiners independently grouped the samples by bone type, labelling a sample as secondary osteonal bone if more than 50% of the bone illustrated evidence of osteonal remodeling (i.e., presence of Haversian systems).

2.3 Mechanical testing

Samples were fitted with brass end caps and secured with wedge-face grips in an Instron Electropuls E3000 test frame (Instron, Norwood, MA, USA). The initial elastic modulus was quantified using an extensometer (632.29F-30, MTS, Eden Prairie, MN, USA) secured to the sample within the gauge length. A compressive pre-load was applied followed by a compressive ramp load from 1000-1220N (46-56MPa). The elastic modulus was calculated as the slope of the stress-strain curve over the entirety of the ramp load. In preparation for fatigue testing, the extensometer was removed and samples were wrapped in saline-soaked gauze. Samples were fatigue tested in zero-compression under load-control at a frequency of 4.35Hz. The applied load was scaled according to the sample's cross-sectional area such that the stress range of the cyclic waveform was 95MPa with a stress ratio $R=0$. Fatigue life was quantified as the number of loading cycles to failure, which was defined as complete fracture. All mechanical testing was performed at room temperature and samples were continuously hydrated throughout the fatigue tests.

2.4 Ash Fraction

A portion of each broken sample was reserved for ash fraction measurements. Samples were placed in ceramic crucibles and dried in a muffle furnace (FB1415M, Fisher Thermo Scientific) at 100°C for one hour. The weight of the samples at this stage was recorded as the dry weight. Samples were then ashed in the furnace at 600°C for 12 hours and weighed again to obtain the ash weight. Ash fraction was calculated as the ratio of ash weight to dry weight [25,26]. Preliminary testing demonstrated that one hour at 100°C was sufficient to achieve a stable measurement of dry weight, as an additional 5 hours in the furnace changed dry weight measurements by less than a 3%. Similarly, the ash weight did not change when measured after 12, 24 or 36 hours at 600°C.

2.5 Finite element modeling

Finite element (FE) models were generated and solved using FAIM software (v8.0, Numerics88 Solutions Ltd, Canada). MicroCT images were coarsened to a resolution of 10 μ m and the central 2.5mm was converted into an FE model with linear hexahedral elements. Elements were assigned homogenous, linear-elastic material properties with a modulus of 18 GPa and Poisson's ratio of 0.3 [27]. The boundary conditions of the models were consistent with experimental testing. A uniform compressive load was applied to the top surface, equating to an apparent stress of 95MPa distributed over the entire cross-section, and all nodes on the bottom surface were fixed in the vertical direction. Stressed volume was calculated as the volume of elements experiencing a von Mises stress greater than the yield stress of cortical bone, which was assumed to be 108MPa [28–30]. For an elastic modulus of 18GPa, this yield stress corresponds to a strain magnitude of 6,000 μ ε.

2.6 Statistical analysis

The Shapiro-Wilk test of normality was used to determine the distribution of all experimental variables. The apparent density, modulus, Ca.Sp, and Ca.N were normally distributed ($p \geq 0.066$), while ash fraction, porosity, Ca.Dm, and fatigue life were not ($p \leq 0.001$). Due to the unequal sample size in each group, and small sample size overall, non-parametric Mann-Whitney U tests were used to examine statistical differences between secondary and plexiform bone for all the experimental variables. The relationships between material properties, microarchitectural parameters, stressed volume and the logarithmic fatigue life, $\ln(N_f)$, were examined using Pearson product-moment correlations. As expected, nearly all the experimental variables were intercorrelated. Therefore, a stepwise linear regression analysis was used to evaluate the relative influence of the primary microarchitectural variables of interest (i.e., Ca.Dm, Ca.Sp, and Ca.N) on fatigue life measures within each bone type. The effect of each additional explanatory variable was assessed by the significance of its standardized coefficient upon entering the model. All statistical analyses were performed in SPSS (SPSS Inc., Chicago, IL, USA) with $\alpha=0.05$.

3. Results

Evidence of secondary osteonal remodeling was easily identifiable upon examination of the μ CT images as indicated by 100% agreement between blinded examiners when independently separating samples into groups of plexiform ($n=15$) or secondary osteonal bone ($n=6$; Fig. 1a). Of the 21 samples, 10 samples were obtained from the tibia and 11 samples from the femur. Only one of the femoral samples was classified as secondary bone; however, there was no significant difference in material properties or microarchitectural parameters between the tibia and femur. Therefore, the samples were pooled within the plexiform and secondary groups and bone location was not accounted for in the remaining analyses.

The ash fraction was not significantly different between the plexiform and secondary bone ($p=0.622$), however, the elastic modulus and apparent density were higher in plexiform bone compared to secondary osteonal bone ($p \leq 0.05$; Table 1). The number of canals was also significantly higher in plexiform bone ($p=0.001$), while increased vascular porosity and a larger canal diameter was observed in secondary osteonal bone ($p \leq 0.001$; Table 1). Despite being loaded to the same apparent stress, the number of cycles to failure varied more than three orders of magnitude. The fatigue life of secondary osteonal bone ranged from 162 cycles to 9,611 cycles and plexiform samples exhibited fatigue failure from 5,604 cycles to 633,437 cycles.

When the samples were pooled together ($n=21$), nearly all the material properties and microarchitectural parameters were intercorrelated and were strongly related to fatigue life measurements (Table 2). However, when the two bone types were considered independently, the influence of density and modulus was diminished and fatigue life was most strongly related to microarchitectural parameters. Ca.Dm ($r^2=0.73$, $p=0.03$) was the strongest predictor of fatigue life in secondary osteonal bone, whereas Ca.Sp ($r^2=0.41$, $p=0.011$) or Ca.N ($r^2=0.36$, $p=0.018$) explained more of the fatigue life variance in plexiform bone (Fig. 2). A stepwise linear regression of canal diameter, canal separation and canal number, indicated that only canal diameter was

1 significantly related to the fatigue life of secondary osteonal bone ($R_{adj}^2 = 0.66$, $p=0.03$), whereas
2 canal separation was the best predictor of fatigue life in plexiform bone ($R_{adj}^2 = 0.36$, $p=0.03$).

3
4 The stressed volume, as determined by FE modeling based on μ CT images, was on average more
5 than three times higher in secondary bone than plexiform bone ($p \leq 0.001$). Furthermore, stressed
6 volume was strongly correlated with the Ca.Dm ($r^2=0.91$, $p=0.003$) and Ca.Sp ($r^2=0.83$, $p=0.01$)
7 of secondary bone, but was not related to measures of canal microarchitecture in plexiform bone
8 ($r^2 \leq 0.23$, $p \geq 0.07$; Fig. 3). Consequently, the fatigue life of secondary bone ($r^2=0.89$, $p=0.005$), but
9 not plexiform bone ($r^2=0.01$, $p=0.381$), was associated with stressed volume (Fig. 4).

11 4. Discussion

12 It is well established that the fatigue life of bone decreases with porosity, yet the specific details
13 of the canal microarchitecture that determine porosity and its relation to fatigue life are unknown.
14 The purpose of this research was to establish the relationship between measures of canal
15 microarchitecture, stressed volume, and the fatigue life of cortical bone to better understand the
16 role of canals as stress concentrations in the bone matrix. In secondary osteonal bone, a larger
17 canal diameter was strongly associated with an increase in stressed volume and a reduced fatigue
18 life. However, the fatigue life of plexiform bone was correlated with canal separation and the
19 number of canals, neither of which were related to stressed volume. The findings from this study
20 suggest that the detrimental effects of increased porosity on the fatigue life of bone can be primarily
21 attributed to an increase in canal diameter.

22
23 Intracortical porosity is primarily dictated by the size and abundance of vascular canals. The canal
24 microarchitecture of bovine bone exhibits a wide spectrum of these parameters with secondary
25 bone containing larger, but fewer, canals compared to the smaller and more numerous canals of
26 plexiform bone. Carter et al. [9] investigated the effects of bovine microstructure on the fatigue
27 life of bovine cortical bone and demonstrated that, even after accounting for differences in apparent
28 density, the fatigue life of secondary osteonal bone was more than five times shorter than plexiform
29 bone. Although no microstructural measures were reported, they attributed this reduction in fatigue
30 life to the inherently weaker and more porous structure of secondary osteonal bone. The findings
31 from the current study support their hypothesis, as the more porous secondary osteonal bone
32 illustrated a shorter fatigue life than the less porous plexiform bone. The negative relationship
33 between the fatigue life of secondary bone and porosity is also consistent with previous
34 observations in human cortical bone [4]. However, the correlation between porosity and fatigue
35 life measures of human bone was not as strong as the present study ($r^2=0.25$ versus $r^2=0.79$,
36 respectively), and may be attributed to differences in 2D histological or 3D measures of porosity.
37 The porosity of secondary bone was positively associated with canal diameter, but was not
38 significantly related to canal number, suggesting that increased canal diameter, rather than canal
39 number, determined fatigue life variation in this bone type.

40 Understanding the relative importance of cortical bone microarchitecture in the fatigue failure
41 process may have important implications to the pathophysiology of stress fracture. It has been
42 hypothesized that the development of stress fracture is a biological process mediated by bone
43 remodeling. Martin et al. [31] developed a mathematical model describing this process as a

positive feedback loop in which continuous mechanical loading causes the accumulation and subsequent repair of microdamage, resulting in a temporary increase in intracortical porosity and bone strain. The model predicted a critical porosity threshold, above which continued loading would make the system unstable; the strain would increase rapidly, and the bone would become saturated with microdamage and quickly progresses to failure. The increased porosity of the model was attributed to the presence of large resorption canals that are typically 150-300 μ m in diameter [32,33]. For the bone volume analyzed in the currently study, the addition of a 250 μ m canal would increase the porosity by 1%, which would cause more than a 70% reduction in fatigue life (Fig. 2a), whereas a 50 μ m canal would increase porosity by only 0.04% and reduce fatigue life by less than 5%. To achieve a similar 1% increase in porosity, it would take 25 canals with a diameter of 50 μ m; however, the data from this study suggest these additional small canals would have a negligible effect on fatigue life measures.

Evidence of a critical canal size is emphasized by the sharp transition in the fatigue life relationship between secondary and plexiform bone (Fig. 2b). A median canal diameter of approximately 50 μ m, or smaller, did not seem to greatly influence fatigue life measurements while those greater than 50 μ m exhibited a rapid reduction in fatigue life. Consistent with the effects of feature size predicted by the TCD [16], the reduced fatigue life associated with larger canals is likely related to the strong relationship between stressed volume and canal diameter. In secondary osteonal bone, the stressed volume increased linearly with increasing canal diameter (Fig. 3b). When normalized to bone volume, a median canal diameter of 50 μ m corresponded to roughly 2% of the total bone volume being stressed beyond yield (108MPa) and a fatigue life on the order of 10^5 cycles. A larger median canal diameter of 250 μ m, akin to a resorption canal, may increase the stressed volume more than 25% and decrease the fatigue life by three orders of magnitude. Interestingly, the porosity and canal diameter of plexiform bone was not related to fatigue life (Fig. 2a) but was associated with an increase in stressed volume (Fig. 3a). This confirms that the measure of stressed volume derived from the linear-elastic FE model is driven by the model's porosity; however, the small amount of stressed volume caused by low levels of porosity and small canals of plexiform bone was not enough to influence the fatigue failure observed experimentally.

In addition to more of the bone volume yielding and developing new microcracks, a larger stressed volume is also more likely to encounter and facilitate the propagation of pre-existing microcracks. It has been suggested that the majority of microdamage occurs in highly mineralized interstitial bone and rarely at high stress concentrations, i.e. the canal edge [34,35]. A larger stressed volume may exceed the osteon boundaries, subject the interstitial bone tissue to elevated stresses and promote the nucleation of microcracks. While the nucleation of microcracks is governed by material properties, the propagation of microcracks is determined by the intracortical microarchitecture. The modeling work of Ural and Vashishth [36] suggested that a 4% increase in porosity is associated with only a 6% decrease in crack initiation toughness but a 62% decrease in crack growth toughness. Moreover, using a two-point correlation function, Turnbull et al. [37] determined that while the majority of fatigue-induced microdamage was located within highly mineralized regions such as interstitial bone, the microcracks that initiated fracture were located near elevated porosity. This suggests that fracture may occur due to a single microcrack located within proximity of a canal. Large canals would increase the probability of a microcrack being

1 located within a highly stressed volume and enable the propagation of the microcrack to failure. It
2 is well accepted that bone readily develops and tolerates microdamage accumulation but must
3 resist the propagation of microcracks to avoid fracture [38]. Therefore, the role of stressed volume
4 in fatigue failure may be more related to the interaction between the stressed volume and pre-
5 existing microcracks and less dependent on its contribution to crack nucleation.

6 The number of canals and canal separation did not influence the stressed volume of plexiform bone
7 but was associated with fatigue life measurements. The effects of canal separation and canal
8 number on the fatigue life are likely related to the toughening mechanisms that are not captured in
9 the FE models and, consequently, indicated no relation with stressed volume. Increased osteon
10 density has been related to an increase in the fracture toughness of bone by providing more barriers
11 to deflect or arrest propagating cracks [21,39]. Furthermore, O'Brien et al. [40] demonstrated that
12 microcracks shorter than 100 μ m were regularly arrested by osteons but microcracks longer than
13 300 μ m had enough energy to break through cement lines and propagate to failure. Although
14 plexiform bone lacks osteonal barriers, the fatigue life was improved by having more canals in
15 close proximity, suggesting that the canals of plexiform bone could sufficiently arrest microcracks.
16 In fact, the average canal separation of plexiform bone was 150 μ m, similar to the previous estimate
17 of critical crack length of 100 μ m, so it is unlikely that a microcrack in plexiform bone had the
18 opportunity to grow to 300 μ m without being arrested or deflected by a canal. Unlike plexiform
19 bone, the canal separation of secondary osteonal bone was related to both fatigue life and stressed
20 volume. Because the remodeling of plexiform bone into secondary osteonal bone results in fewer
21 and larger canals that are inherently spaced further apart, the positive relationship between stressed
22 volume and canal separation may be a reflection of the strong correlation between canal diameter
23 and canal separation. Alternatively, the stressed volume may be lower when canals are closer
24 together because the affected volumes from each stress concentration are more likely to overlap
25 and share elements of high stress. In this way, a dense arrangement of canals may mitigate their
26 negative impact as stress concentrations by minimizing the amount of material subjected to
27 elevated stresses.

28 Although the relative importance of individual material properties differs between studies,
29 apparent bone density, mineral content and elastic modulus have been shown to account for a
30 portion of the scatter in fatigue life measures [2,4,6,41]. In general, more stiff and denser bone
31 exhibits a longer fatigue life. Our results established similar relationships when both secondary
32 osteonal and plexiform bone were considered; however, none of the material properties could
33 explain as much of the variance in fatigue life as microarchitectural parameters and their effects
34 were weak when secondary osteonal and plexiform bone were evaluated separately. This study
35 was limited by the low number of secondary osteonal bone samples and a larger sample size is
36 needed to confirm that the present results are generalizable to all osteonal bone. Nonetheless, the
37 material properties of secondary and plexiform bone were consistent with previous reports
38 [9,41,42], with secondary osteonal bone being less dense and having a lower ash fraction and
39 elastic modulus compared to plexiform bone. The elastic modulus of bone has contributions from
40 both tissue-level material properties as well as intracortical microarchitecture. In this study, we
41 chose not to normalize loading magnitude by the elastic modulus such that microstructural
42 contributions were preserved and fatigue-life variation was maximized. It is interesting to note that

1 the majority of scatter in the fatigue life of plexiform bone was unexplained by any of the
2 microarchitectural or material properties investigated in this study. While the influence of density
3 approached significance, ash fraction had no effect on the fatigue behavior. Heterogeneity
4 throughout the bone matrix is an important determinant of bone toughness and fatigue resistance
5 [4,43] and measures more specific than ash fraction may be needed to assess the importance of
6 mineralization.

7 This study was focused almost exclusively on the importance of microarchitecture, and for that
8 reason we chose to assign a uniform tissue modulus to each FE model. An alternative approach
9 would have been to adjust the assigned tissue modulus such that the apparent modulus of the model
10 and the experiment were consistent. However, in addition to potentially introducing experimental
11 error into FE model results, it is important to note that our primary outcome measure of stressed
12 volume is independent from the chosen tissue modulus. In other words, assigning sample-specific
13 moduli would have no influence on our findings. Given that bone failure is ultimately strain
14 controlled [39], it may be interesting in future work to explore additional strain-based criteria (e.g.,
15 strained volume above a given strain threshold). Owing to the small canal diameters of plexiform
16 bone, it is possible that coarsening the image data to 10 μ m may have biased the observed
17 relationship between stressed volume and microarchitecture for this type of bone. Unfortunately,
18 it was not computationally feasible for us to solve the FE models at the full resolution of 5 μ m.
19 However, there was a strong linear correlation between all microarchitectural parameters measured
20 at a resolution of 5 μ m and 10 μ m for both bone types ($r^2=0.82-0.99$), suggesting that binning the
21 image data affected all measures and samples comparably. We can only speculate that stressed
22 volume correlations would be similar. The models were also limited to a sample height of 2.5mm
23 for computational efficiency; however, this was likely a sufficient representative sample volume.
24 Decreasing FE models to a smaller sample height of 1mm provided nearly identical measures of
25 relative stressed volume when compared to the 2.5mm models (e.g., 1.5% and 1.47% for 1mm and
26 2.5mm models, respectively, from a representative sample). Finally, we recognize that the FE
27 model only simulated the first loading cycle and did not capture the plastic deformation or
28 redistribution of stresses that would occur with continued cyclic loading. Despite this fact, stressed
29 volume accounted for 89% of the variance in the fatigue life of secondary bone, demonstrating the
30 efficacy of using linear analysis to predict fatigue failure.

31 To summarize, this was the first study to quantify the influence of intracortical microarchitecture
32 on stressed volume and the fatigue failure of cortical bone. The findings from this study
33 demonstrate that vascular canals act as stress concentrations in the bone matrix that increase the
34 stress magnitude and stressed volume from an applied load. The stressed volume increased with
35 the size of the canals resulting in a shorter fatigue life; however, the detrimental impact of canal
36 diameter was only evident when canal diameter was larger than 50 μ m, potentially indicating a
37 critical canal size. The smaller canals and lower porosity of plexiform bone produced minimal
38 stressed volume, as defined herein, and had no effect on fatigue life measurements. Stressed
39 volume is thought to cause fatigue failure by propagating pre-existing microcracks, but the exact
40 mechanisms cannot be determined from the current study. Further studies are needed to establish
41 the relationship among fatigue life measures and the magnitude and volume of stress
42 concentrations associated with specific canal morphologies.

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Tables

Table 1: Median and range (*min-max*) of the material properties, microarchitectural measures, stressed volume and fatigue life for secondary and plexiform bone. *significantly different than plexiform bone ($p \leq 0.05$).

	Secondary (n=6)	Plexiform (n=15)
Density (g/cm³)	1.95* (1.93-2.02)	2.04 (1.96-2.08)
Ash Fraction (g/g)	0.71 (0.67-0.74)	0.72 (0.67-0.82)
Elastic Modulus (GPa)	17.04* (12.15-22.06)	21.02 (16.34-25.81)
Fatigue Life (cycles)	6,161* (162-9,611)	28,540 (5,604-633,437)
Porosity (%)	4.65* (4.25-7.43)	3.09 (2.58-3.67)
Ca.Dm (µm)	92.50* (44.00-164.00)	22.00 (20.00-30.00)
Ca.Sp (µm)	224.50* (211.00-284.00)	145.00 (115.00-227.00)
Ca.N (#)	17,424* (7,682-24,378)	48,805 (15,554-77,639)
Stressed Volume (mm³)	2.53* (0.97-8.84)	0.76 (0.64-1.10)

1 **Table 2:** Correlation between material properties, microarchitectural measurements, stressed volume and the fatigue life. The top
2 row is the Pearson's correlation coefficient (r) and is bolded if significant ($p \leq 0.05$).

	Density	Ash Fraction	Elastic Modulus	Porosity	Median Ca.Dm	Median Ca.Sp	Ca.N	Stressed Volume
Density	1							
Ash Fraction	0.399 (0.073)	1						
Elastic Modulus	0.402 (0.071)	0.434 (0.049)	1					
Porosity	-0.457 (0.037)	-0.235 (0.305)	-0.537 (0.012)	1				
Median Ca.Dm	-0.472 (0.031)	-0.384 (0.086)	-0.644 (0.022)	0.927 (0.000)	1			
Median Ca.Sp	-0.757 (0.000)	-0.367 (0.102)	-0.612 (0.003)	0.727 (0.000)	0.810 (0.000)	1		
Ca.N	0.662 (0.001)	0.365 (0.104)	0.585 (0.005)	-0.500 (0.021)	-0.589 (0.005)	-0.831 (0.000)	1	
Stressed Volume	-0.337 (0.135)	-0.331 (0.142)	-0.578 (0.006)	0.933 (0.000)	0.944 (0.000)	0.719 (0.000)	- 0.484 (0.026)	1
ln(N _f) Pooled (N=21)	0.516 (0.017)	0.206 (0.371)	0.560 (0.008)	-0.744 (0.000)	-0.786 (0.000)	-0.825 (0.000)	0.704 (0.000)	-0.778 (0.000)
ln(N _f) Secondary (n=6)	-0.469 (0.348)	0.290 (0.578)	0.408 (0.421)	-0.889 (0.018)	-0.856 (0.030)	-0.750 (0.086)	0.229 (0.663)	-0.943 (0.005)
ln(N _f) Plexiform (n=15)	0.471 (0.076)	-0.066 (0.816)	0.345 (0.207)	0.079 (0.779)	-0.338 (0.218)	-0.638 (0.011)	0.602 (0.018)	-0.244 (0.381)

1 Figures

Figure 1: Representative samples of a secondary (top row) and plexiform (bottom row) bone sample. From left to right, the panels illustrate a 2D cross-sectional view (a), 3D rendering of the vascular canals (b), semi-transparent FE model (c), and the stressed volume i.e., the volume stressed above the yield, 108 MPa (d).

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2

3 **Figure 2:** The relationship between measures of microarchitecture and the fatigue life of secondary (closed circles) and plexiform
 4 bone (open circles).

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Figure 3: The relationship between measures of microarchitecture and the stressed volume in secondary (closed circles) and plexiform bone (open circles).

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2

3 **Figure 4:** The relationship between the stressed volume and fatigue life of secondary (closed circles) and plexiform bone (open
4 circles).

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