



Research paper

Evaluating differential nuclear DNA yield rates and osteocyte numbers among human bone tissue types: A synchrotron radiation micro-CT approach



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ARTICLE INFO

Article history:

Received 24 October 2016

Received in revised form 24 January 2017

Accepted 2 March 2017

Available online 6 March 2017

Keywords:

Nuclear DNA

3D imaging

Osteocyte lacunae

Cortical and cancellous bone

ABSTRACT

Molecular human identification has conventionally focused on DNA sampling from dense, weight-bearing cortical bone tissue, typically from femora or tibiae. A comparison of skeletal elements from three contemporary individuals demonstrated that elements with high quantities of cancellous bone yielded nuclear DNA at the highest rates, suggesting that preferentially sampling cortical bone may be suboptimal (Mundorff & Davoren, 2014). Despite these findings, the reason for the differential DNA yields between cortical and cancellous bone tissues remains unknown. The primary goal of this work is to ascertain whether differences in bone microstructure can be used to explain differential nuclear DNA yield among bone tissue types observed by Mundorff and Davoren (2014), with a focus on osteocytes and the three-dimensional (3D) quantification of their associated lacunae. Osteocytes and other bone cells are recognized to house DNA in bone tissue, thus examining the density of their lacunae may explain why nuclear DNA yield rates differ among bone tissue types. Lacunae were visualized and quantified using synchrotron radiation-based micro-Computed Tomographic imaging (SR micro-CT). Volumes of interest (VOIs) from cortical and cancellous bone tissues ($n = 129$) were comparatively analyzed from the three skeletons sampled for Mundorff and Davoren's (2014) study. Analyses tested the primary hypothesis that the abundance and density of osteocytes (inferred from their lacunar spaces) vary between cortical and cancellous bone tissue types. Results demonstrated that osteocyte lacunar abundance and density vary between cortical and cancellous bone tissue types, with cortical bone VOIs containing a higher lacunar abundance and density. We found that the osteocyte lacunar density values are independent of nuclear DNA yield, suggesting an alternative explanation for the higher nuclear DNA yields from bones with greater quantities of cancellous bone tissue. The use of SR micro-CT allowed for a scale of analysis that revealed a high range of variation in lacunar abundance in both tissue types. Moreover, high-resolution SR micro-CT imaging revealed potential soft tissue remnants within marrow spaces not visible macroscopically. It is hypothesized that soft tissue remnants observed among the trabeculae of skeletal elements with high quantities of cancellous bone tissue are responsible for the high nuclear DNA yields. These findings have significant implications for bone-sample selection for nuclear DNA analysis in a forensic context when skeletal remains are recovered from the ground surface.

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1. Introduction

Mundorff and Davoren [1] recently presented a comparison of nuclear DNA quantity and quality from modern skeletal elements

from three individuals who naturally decomposed on the ground surface. A unique feature of their work is that they tested representatives of all element types within each skeleton. This was the first empirical study to evaluate the differential preservation of DNA by skeletal element under controlled conditions. The researchers compared 55 skeletal elements from three recently deceased individuals ($n = 165$). Results revealed that bones traditionally considered unsuitable for DNA testing,

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such as phalanges, tarsals, and patellae yielded both higher quantity and better quality nuclear DNA than cortical bone tissue from weight-bearing long bones. Results further demonstrated that, in general, elements with high quantities of cancellous bone may preserve and yield DNA at higher rates than cortical bone. While these findings conflict with certain previous studies that report superior data from weight-bearing cortical bone [2–4], similar results were found during a comprehensive retrospective study conducted by the International Commission on Missing Persons during efforts to identify individuals recovered throughout the Western Balkans [5]. Their results revealed that tarsal bones, small elements predominantly comprised of cancellous bone tissue, yielded more complete nuclear Short Tandem Repeat (STR) DNA profiles than all other bones, and were thus identified at a higher rate. In fact, vertebrae produced the same success rates as femora. The authors further reported that other small primarily cancellous bones, such as the patella, also “exhibited good results”. The sample size, however, was considered too small to be very informative [5]. Other researchers have reported similar findings in case studies, determining that phalanges served well as a replacement for sampling femora, based on their high success rate and ease of sampling [6,7]. As such, these findings have significant implications for the extraction of DNA, since cancellous bone is typically dismissed as a potential DNA source in favor of cortical bone.

While recent studies [1,5–7] clearly indicate that skeletal elements with a greater proportion of cancellous bone produced higher DNA yields, the reason(s) remain unknown. As DNA is becoming the preferred human identification method, understanding why these results diverge from expectations requires further scientific investigation. Evidence from bone microarchitecture may help explain this variation and further enrich our understanding of the density and morphology of bone microstructural features.

This study sought to ascertain whether differences in bone microstructure may be used to explain differential nuclear DNA yield among bone tissue types, with a focus on osteocytes and the three-dimensional (3D) quantification of their associated lacunae. The use of Synchrotron Radiation-based micro-Computed Tomography (SR micro-CT) permitted for a scale of analysis that allows two central research questions to be addressed, without destruction of the specimen: 1) How does osteocyte lacunar density and the amount of bone matrix surrounding osteocytes compare between cortical and cancellous bone tissue? 2) Can osteocyte lacunar density in cortical and cancellous bone be used to explain differential nuclear DNA yield in the Mundorff and Davoren [1] sample?

2. Materials and methods

2.1. Samples and experimental design

2.1.1. Skeletal selection

Specimens for this work were sourced from the William M. Bass Donated Skeletal Collection, housed in the Department of Anthropology at the University of Tennessee, Knoxville. Prior to bone sampling, ethical approval was obtained from the University of Tennessee (UTK IRB-15-02071-XM) and the University of Saskatchewan (Bio # 15–47). The study sample comprised cortical and cancellous bone tissues from the three skeletons used in the Mundorff and Davoren [1] study. A subset of 43 elements from the original 55 sampled for nuclear DNA were selected per skeleton for SR micro-CT imaging. Representatives from each skeletal element type were chosen (Table 1) and bones from the left side only were selected to maintain consistency ($n = 129$).

Table 1

Sample list of the forty-three skeletal elements, apportioned by body region.

Body Region(s)	Skeletal Element
Skull	Frontal
	Parietal
	Temporal
	Occipital
	Maxilla
	Mandible
Thorax and Abdomen	Cervical Vertebra
	Thoracic Vertebra
	Lumbar Vertebra
	First Rib
	Middle Rib
	Twelfth Rib
	Scapula
	Clavicle
	Ilium
	Ischium
	Pubis
	Sacrum
Lower Limb	Femur
	Tibia
	Fibula
	Patella
Foot	Metatarsal 1
	Metatarsal 2
	Metatarsal 3
	Metatarsal 4
	Cuneiform 1
	Cuneiform 2
	Cuneiform 3
	Navicular
	Cuboid
	Talus
Upper Limb	Calcaneus
	First Proximal Phalanx
	First Distal Phalanx
	Humerus
	Ulna
Hand	Radius
	Capitate
	Metacarpal 2
	Metacarpal 3
	Metacarpals 4
	First Proximal Hand Phalanx
	First Distal Hand Phalanx

2.2. Sample preparation

2.2.1. Specimen sampling methodology for 3D imaging

Rectilinear bone blocks with dimensions of approximately $2 \times 2 \times 10$ mm were removed from each skeletal element directly adjacent to the DNA sampling site [1]. This sampling location was selected in order to directly compare the osteocyte lacunar parameters with the DNA yield from the same general location on each bone. Each bone specimen was procured from the same region of each element for all three individuals to eliminate inter-individual variation in lacunar properties.

Bones were secured in a gripping device for stability during sampling. All samples were removed using a Dremel 8200 drill at 15 RPM with a 7/8-inch diamond wheel attachment. Specimens were further refined using a Buehler IsoMet precision diamond-wafer saw.

2.3. SR micro-CT

SR micro-CT scanning was conducted at the Canadian Light Source (CLS) national synchrotron facility on the BioMedical and Imaging Therapy (BMIT) Insertion Device (ID) beamline [8]. A custom built stage and custom Bruker (Kontich, Belgium) micro-CT set-up was used for this study. Each fixed bone specimen was

mounted on a brass pin holder on the stage. To ensure that the sample did not shift during scan rotation, which would increase the possibility of motion artifacts, dental wax and an adhesive were applied to the brass pin. The adhesive was dissolved following scanning.

Images were obtained using monochromatic X-rays with a photon energy of 31 keV and an effective pixel size of 0.9 μm . An exposure time ranging from 360 to 1250 milliseconds and four-frame averaging was employed. As the storage ring current decreases from a peak of 250 mA over time following injection, the exposure time was tuned to maintain 20% saturation on the detector. Prior to each scan, ten flat-field and ten dark-field projections were collected to correct for noise in the detector and X-ray beam. A rotation step of 0.25 and 2×2 binning were used to obtain 720 projections spanning 180° of rotation. Applying 2×2 binning results in 1.8 μm pixels/voxels for analysis. This protocol resulted in a scan time of approximately 75 min per sample. The projection images were reconstructed to create a 3D dataset of the 720 slices, representing a height of 1.296 mm, for each specimen.

2.3.1. SR micro-CT: 3D quantitative morphometry

The SR micro-CT projection images were reconstructed using NRecon 1.6.10.2 (Bruker, Kontich, Belgium), a commercial GPU accelerated filtered back projection based reconstruction software package. Image stacks were cropped and analyzed using CT Analyser 1.15.4.0 (Bruker, Kontich, Belgium), following protocols described by Carter and colleagues [9]. Cylindrical Volumes of Interest (VOIs) were identified within each bone sample with a diameter of 0.7 mm, a height of 0.7 mm, and a volume of 0.27 mm^3 for both cortical and cancellous bone regions (Figs. 1 and 2). This produced matching VOIs from which all measurements were acquired.

Osteocyte lacunae were separated from the high-density bone using global thresholding. The same threshold values were applied to each VOI (-0.512661 , 0.353718). Despeckling (denoising) was conducted to remove noise (structures less than $10 \mu\text{m}^3$). Elements above $2000 \mu\text{m}^3$ were assumed to be canals and remaining structures were designated as lacunae (Fig. 3). The above volume

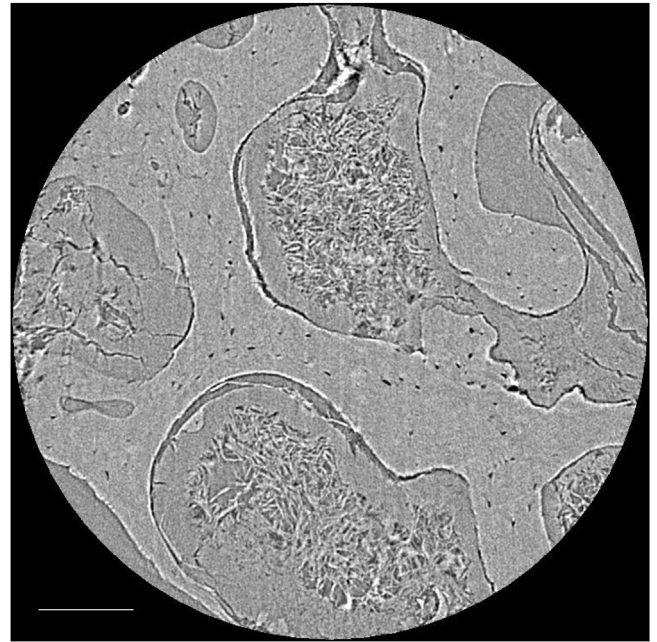


Fig. 2. SR micro-CT single slice of a cancellous bone cylindrical VOI. Scale = 100 μm .

limits are based on previous confocal microscopy measurements, which determined human osteocyte volumes range from 28 to $1713 \mu\text{m}^3$ [9,10]. Subsequently, 3D renders of bone

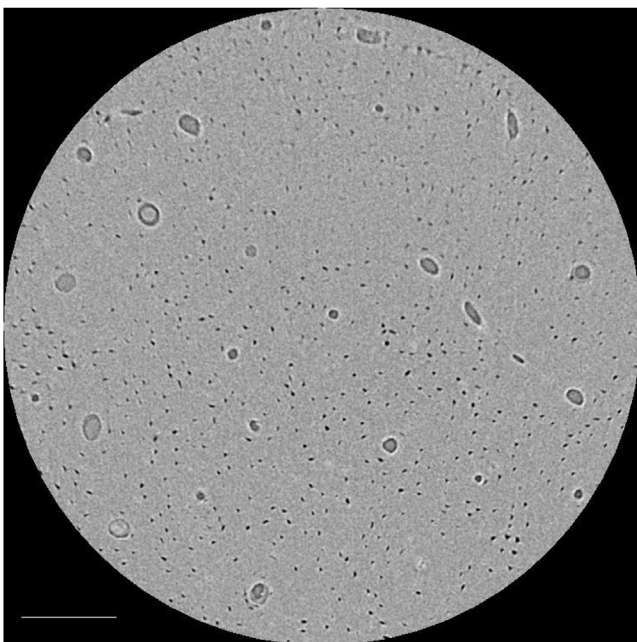


Fig. 1. SR micro-CT single slice of a cortical bone cylindrical VOI. Scale = 100 μm .

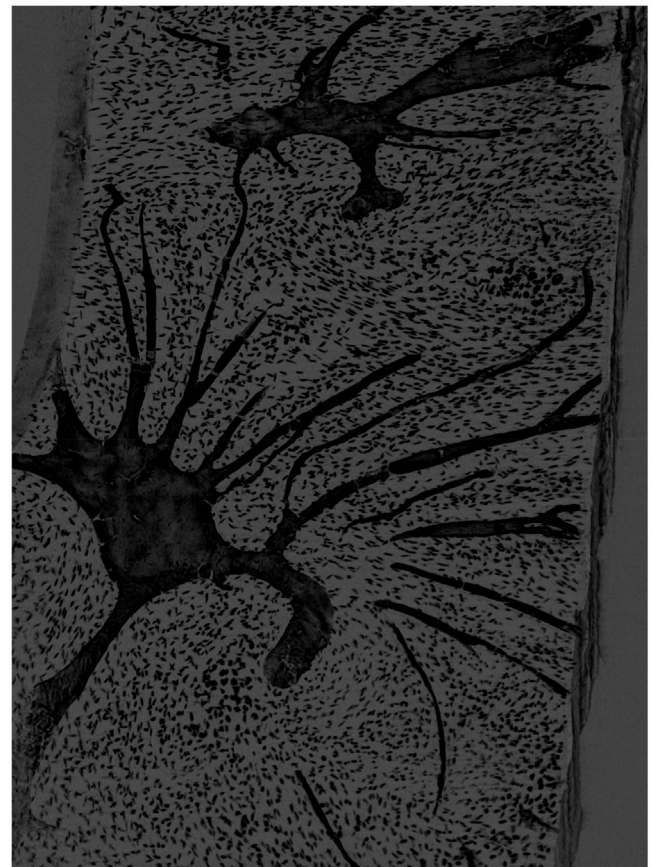


Fig. 3. SR micro-CT minimum projection re-slice of a cortical bone VOI to visualize lacunar spaces and canals (black) from higher-density bone.



Fig. 4. SR micro-CT 3D render of cortical bone showing vascular porosity (red) and osteocyte lacunae (grey). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

microarchitecture were created using AMIRA 5.4.1 (Visage Imaging, Berlin, Germany) imaging software (Figs. 4 and 5).

Standard nomenclatures for canal and lacunar indices were applied for the analysis of 3D lacunar parameters within the VOIs [10–12]. The variables measured included: (1) total VOI volume (TV); (2) total canal volume within VOI (Ca.V); (3) average canal diameter (Ca.Dm); (4) total number of lacunae (N.Lc); and (5) average lacunar volume (Lc.V). To determine lacunar density per mm^3 (N.Lc/BV), bone volume (BV) was calculated as total volume minus canal volume (TV–Ca.V). As cancellous bone generally lacks Haversian systems, and thus central vascular channels, BV for cancellous bone VOIs was calculated as total volume minus the volume of the marrow spaces.

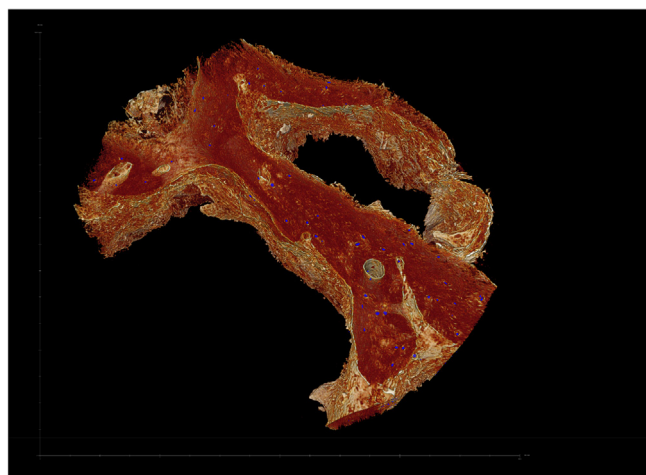


Fig. 5. SR micro-CT 3D render of cancellous bone showing microarchitecture and osteocyte lacunae (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Analytical methods

Statistical analyses were accomplished using SPSS 23.0 statistical software (Chicago, IL, USA). The statistical approach was modeled after Carter and colleagues [9]. In accordance with their methodology, individual outliers were kept in the study since previous analyses have demonstrated that variation is normal and restricted to one or two variables [9,13,14]. All statistical tests for the lacunar parameters were conducted on the entire sample rather than by skeletal element due to the limited sample size.

N.Lc, N.Lc/BV, Lc.V, Ca.Dm, and Ca.V for cortical and cancellous bone VOIs were calculated using Individual Object Analysis. Repeated Measures Analysis of Variance (RM-ANOVA) tests were performed to compare the N.Lc and N.Lc/V variables from cortical and cancellous bone VOIs within each individual and among individuals. If a value was found to be significant at $\alpha \leq 0.05$, a post-hoc Bonferroni adjustment was applied to identify factors contributing to significance.

Spearman's Rank-Order Correlations were computed to examine the relationship between N.Lc and N.Lc/BV values in cortical and cancellous bone VOIs and the nuclear DNA yield results per gram of sample (ng/g).

3. Results

3.1. Total number of lacunae (N.Lc)

Due to the minute specimen size required for SR micro-CT imaging, certain bone blocks with dense cortical regions (femur, tibia, fibula, humerus, radius, ulna, temporal, mandible, occipital) did not contain sufficient cancellous bone for the evaluation of a separate VOI. For the noted skeletal elements, only cortical bone VOIs were quantitatively analyzed.

Results for all osteocyte lacunar and canal parameters within each cortical bone and cancellous bone VOI are summarized in Table 2. Results of the within-subjects test (Table 3) indicated that mean N.Lc in cortical and cancellous bone were significantly different overall ($F_{(1,99)} = 171.928$, $p = 0.000$). There was no significant interaction observed between overall N.Lc in cortical and cancellous bone and individual ($F_{(1,99)} = 2.561$, $p = 0.082$). The test of between-subjects effects (Table 4) demonstrated significant differences in N.Lc between cortical and cancellous bone among the three individuals ($F_{(1,99)} = 47.969$, $p = 0.000$). Lower N.Lc values are evident for individual #3 in both cortical and cancellous bone tissues. Alternatively, individual #2 exhibited higher lacunar counts than #3 and #1 in both cortical and cancellous bone tissues. A Bonferroni *post-hoc* test revealed significant differences between individuals at $\alpha \leq 0.05$. Differences were noted in N.Lc between individuals #3, #2 ($p = 0.000$), and #1 ($p = 0.000$). Significant differences were also observed between individual #2 and #3 ($p = 0.000$), and individual #1 and #3 ($p = 0.000$).

3.2. Lacunar density per mm^3 (N.Lc/BV)

Results of the within-subjects test (Table 3) indicated that mean N.Lc/BV in cortical and cancellous bone are significantly different overall ($F_{(1,99)} = 196.189$, $p = 0.000$). There is no significant

Table 2

Individual Object Analysis results for all osteocyte lacunar and canal parameters for cortical bone and cancellous bone VOIs.

Bone Tissue Type	n	Average number of lacunae	Average lacunar density ($\text{mm}^{-3} \pm \text{sd}$)	Average lacunar volume ($\mu\text{m}^3 \pm \text{sd}$)	Average canal diameter ($\mu\text{m} \pm \text{sd}$)	Average canal volume ($\text{mm}^3 \pm \text{sd}$)
Cortical	129	4725 $\pm$ 1170.34	22723.32 $\pm$ 7441.88	274 $\pm$ 28.66	52.49 $\pm$ 8.6	0.085 $\pm$ 0.033
Cancellous	99	1931.6 $\pm$ 811.37	10542.87 $\pm$ 3440.09	164 $\pm$ 40.44	N/A	N/A

Table 3

RM-ANOVA Results for N.Lc and N.Lc/BV in Cortical and Cancellous Bone: Within-Subjects Effects.

Source	df	Mean Square	F	p-value
N.Lc	1	233129658	171.928	0.000 [*]
N.Lc*Individual	2	3473326.43	2.561	0.082
N.Lc/BV	1	6.484E+9	196.189	0.000 [*]
N.Lc/BV*Individual	2	13413143.5	0.406	0.668

^{*} Significance at $p < 0.05$.**Table 4**

RM-ANOVA Results for N.Lc and N.Lc/BV in Cortical and Cancellous Bone: Between-Subjects Effects.

Source	df	Mean Square	F	p-value
N.Lc/Individual	2	65004213.5	47.969	0.000 [*]
N.Lc/BV/Individual	2	459448156	14.205	0.000 [*]

^{*} Significance at $p < 0.05$.

interaction observed between overall N.Lc/BV in cortical and cancellous bone and individual ($F_{(1,99)} = 0.406$, $p = 0.668$). The test of between-subjects effects (Table 4) demonstrated significant differences in N.Lc/BV between cortical and cancellous bone among the three individuals ($F_{(1,99)} = 14.205$, $p = 0.000$). N.Lc/BV in cortical and cancellous bone was significantly different between individuals, with lower lacunar density overall in cancellous bone tissue.

Lower N.Lc/BV values are evident for individual #3 in both cortical and cancellous bone tissues. Alternatively, higher lacunar counts are evident in both cortical and cancellous bone tissues for individual #2 compared to individuals #3 and #1. These differences are more substantial in cancellous bone than cortical bone.

A Bonferroni post-hoc test revealed significant differences between individuals at $\alpha \leq 0.05$. Differences were noted in N.Lc/BV between individuals #3, #2 ($p = 0.000$), and #1 ($p = 0.001$). Significant differences were also noted between individuals #2 and #3 ($p = 0.000$), and individuals #1 and #3 ($p = 0.001$).

3.3. Lacunar parameters and nuclear DNA yield

Correlation coefficients indicated which variables shared significant relationships at $\alpha \leq 0.05$ (Table 5). Significant negative correlations were observed between cortical bone N.Lc and nuclear DNA yield ng/g ($r = -0.410$), and cortical bone N.Lc/BV and nuclear DNA yield ng/g ($r = -0.408$). Positive correlations exist between cancellous bone N.Lc and nuclear DNA yield ng/g ($r = 0.150$), and cancellous bone N.Lc/BV and nuclear DNA yield ng/g ($r = 0.107$), though they are not statistically significant. Despite the low correlation, a slight positive trend was observed between cancellous bone N.Lc and nuclear DNA yield ng/g ($r = 0.150$) (Fig. 6), though a positive trend was not evident for cortical bone N.Lc/BV and nuclear DNA yield ng/g (Fig. 7).

Table 5

Spearman's Rank-Order Correlation Coefficients for Osteocyte Lacunar Parameters and Nuclear DNA Yield.

	Nuclear DNA Yield (ng/g)	Cort. Bone N.Lc	Canc. Bone N.Lc	Cort. Bone N.Lc/BV (mm ³)	Canc. Bone N.Lc/BV (mm ³)
Nuclear DNA Yield (ng/g)	1.00	−0.410 [*]	0.150	−0.408 [*]	0.107
Cort. Bone N.Lc	−0.410 [*]	1.00	0.073	0.939 [*]	−0.018
Canc. Bone N.Lc	0.150	0.073	1.00	0.043	0.925 [*]
Cort. Bone N.Lc/BV	−0.408 [*]	0.939 [*]	0.043	1.00	0.001
Canc. Bone N.Lc/BV	0.107	−0.018	0.925 [*]	0.001	1.00

^{*} Significance at $p < 0.05$.

4. Discussion

To our knowledge this work represents the first examination of inter-element variation in osteocyte lacunar properties from cortical and cancellous bone tissues in various modern human skeletal elements. As this is the first study to use this approach, the results have implications that improve current understandings of normal variation of osteocyte lacunar parameters in adult males and the relationship between nuclear DNA yield and osteocyte lacunar abundance. Results presented here also have broader applications as they offer promise for the development of a refined method for identifying the bone tissue type most likely to yield nuclear DNA from skeletal material recovered from the ground surface.

Significant differences in osteocyte lacunar abundance and density were found between cortical and cancellous bone tissues, with greater lacunar counts found in cortical bone. Significant negative correlations observed between cortical bone N.Lc and nuclear DNA yield ng/g, and cortical bone N.Lc/BV and nuclear DNA yield ng/g suggest that although osteocyte lacunar counts are higher in cortical bone tissue, these specimens do not share a positive relationship with nuclear DNA yield per mass of sample, indicating an alternative factor at play.

Ancillary to the study's focus on the 3D quantification of osteocyte lacunae, potential remnants of soft tissue between trabeculae were observed using SR micro-CT, offering a plausible explanation for the relationship between cancellous bone and greater DNA yield. Though soft tissue was not present on the surface of the bones, 3D scans consistently revealed soft tissue otherwise non-visible to the naked eye, within the marrow spaces of skeletal elements with high cancellous content (Fig. 8). It is hypothesized that these residual soft tissues, which likely include periosteum, bone marrow cells, and bone lining cells contributed to the higher nuclear DNA yields from cancellous bone.

Cancellous bone tissue has an open, porous structure characterized by interconnected trabecular struts. In growing bone, spaces around and within cancellous tissue are sites for hematopoietic marrow. With age, the majority of hematopoietic marrow tissue is modified to yellow bone marrow and found within medullary cavities [15,16]. The internal surfaces of cancellous bone are also covered by endosteum. This soft tissue layer consists of connective tissue housing bone cells, and physically separates the bone surface from the marrow. Due to this composition, it is hypothesized that the surface to volume ratio of cancellous bone and adhering soft tissues removed during DNA sampling is likely much higher compared to cortical bone samples.

The three skeletons under study are of modern origin and decomposed naturally on the ground surface. Forensic laboratory procedures prior to nuclear DNA testing include cleaning the outer surface of the bone to eliminate contaminating DNA resulting from handling. Since the interior of intact bone is not accessible, cleaning is typically limited to the bones' surface. If remnant soft tissue was present between the trabeculae of cancellous bone, it

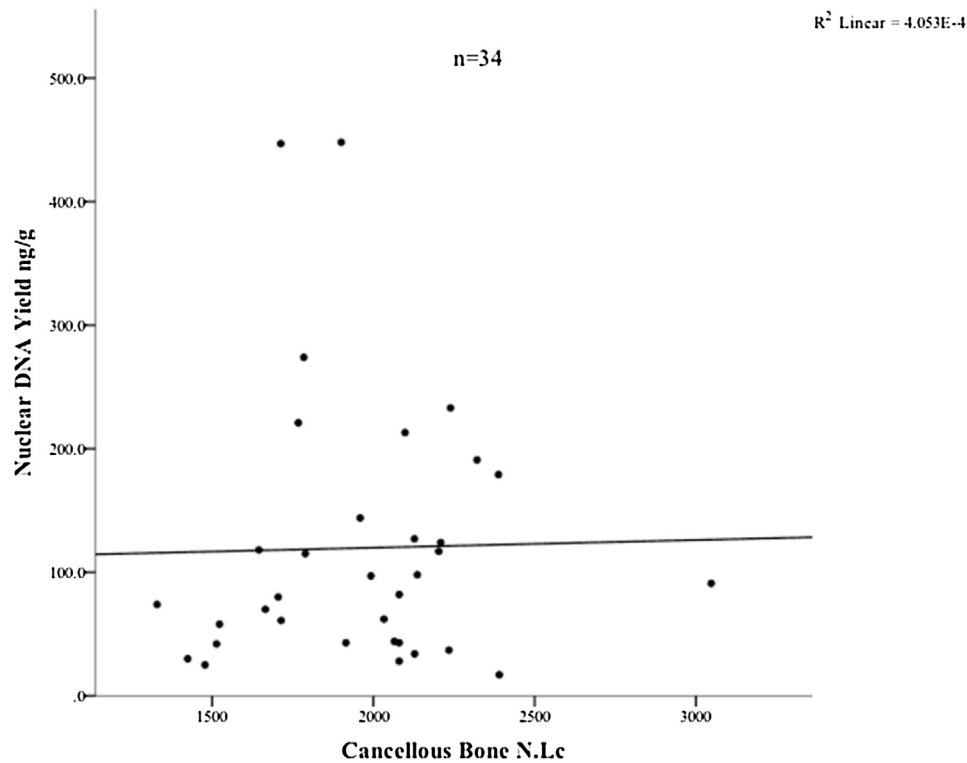


Fig. 6. Scatterplot Depicting Average Cancellous Bone N.Lc and Nuclear DNA Yield (ng/g).

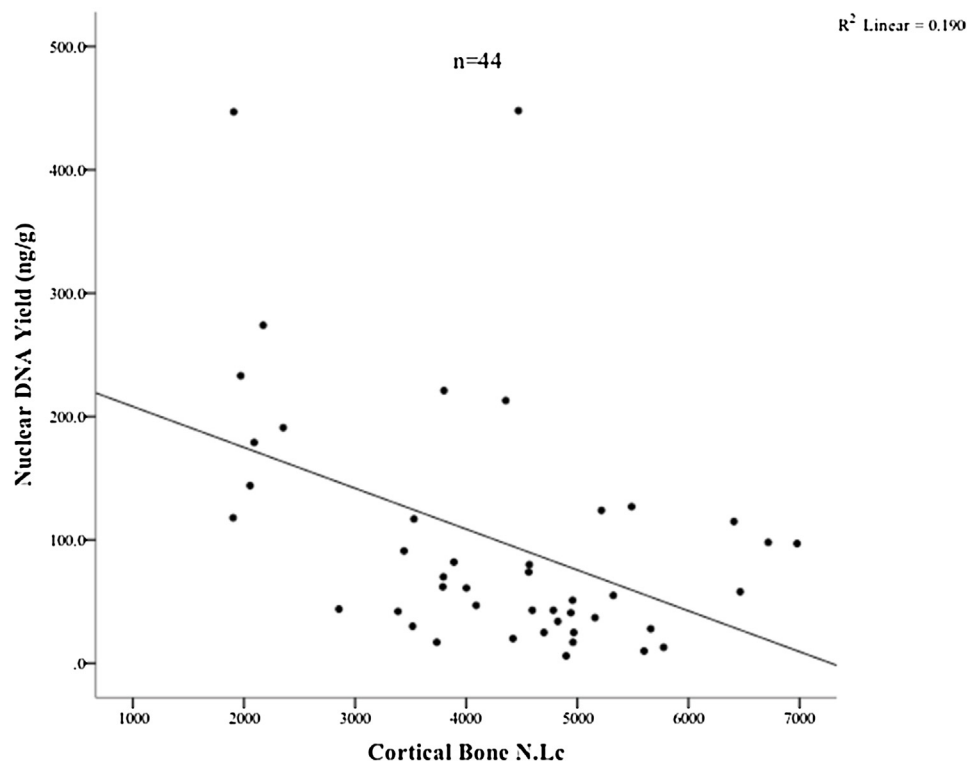


Fig. 7. Scatterplot Depicting Average Cortical Bone N.Lc/BV and Nuclear DNA Yield (ng/g).

would not be removed by surface cleaning. Comparatively, organ donation procurement follows a bone washing protocol to remove blood and bone marrow components from the medullary cavities [17], and such measures were not employed for this study. During

sample preparation for SR micro-CT, no soft tissue was evident on the bones' surface or visible within the sampling site cavity.

Though the presence of soft tissue remnants between trabeculae in cancellous bone was consistent for all three

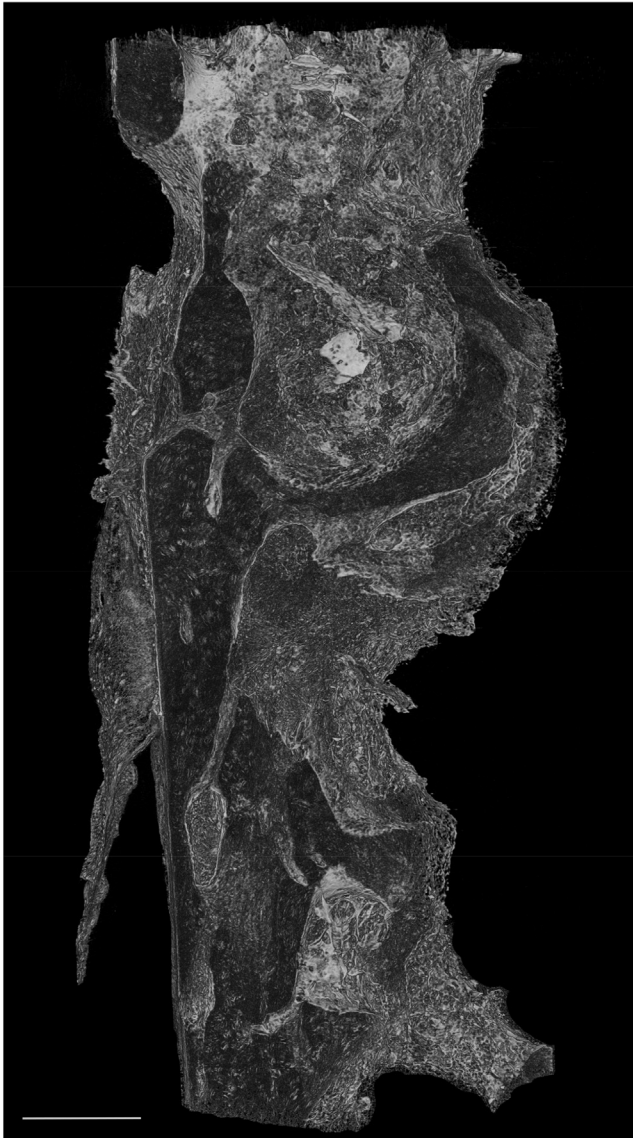


Fig. 8. SR micro-CT cancellous bone 3D render. Soft tissue remnants (light grey) are evident between the trabecular struts (dark grey). Scale bar = 300 μ m.

individuals in this study, skeletal elements from individuals of increased postmortem intervals (PMIs) were not assessed for the presence of similar amounts of soft tissue. As such, further investigation is warranted to assess if these results will hold true for skeletons from increased PMIs and archaeological specimens.

In a follow-up to the primary study (Phase 2), Mundorff and Davoren [1] examined a subset of skeletal elements ($n = 120$) from twelve additional skeletons of increasing PMIs through 21 years following death. This second phase was designed to determine if the same rank order of skeletal elements by DNA yield would maintain over increased PMIs, and to give an indication of how nuclear DNA degradation occurs over time. Results revealed that as PMI increased, DNA yield generally decreased, however, the skeletal elements largely maintained a comparable rank order as the first phase. Consistent with phase one of Mundorff and Davoren's [1] study, skeletal elements predominantly comprised of cortical bone were generally lower yielding than elements with greater cancellous bone content.

To further investigate whether soft tissue between trabeculae of cancellous bone tissue is responsible for higher nuclear DNA yields

in the Mundorff and Davoren [1] sample, all skeletal elements from phase two will be evaluated using 3D imaging modalities including clinical CT and SR micro-CT. If remaining soft tissue is revealed within the marrow spaces of bones with increased PMIs, this could aid in explaining why the DNA yields observed remained generally consistent over time, and strengthen the argument that soft tissue remnants may be accountable for this trend. Additional SR micro-CT research confirming these results with increased PMIs may lead to proposed DNA sampling guidelines that involve the procurement of skeletal elements with higher quantities of cancellous bone tissue.

The proposed "soft tissue hypothesis", as an alternative explanation for why cancellous bone tissue yielded higher rates of nuclear DNA compared to cortical bone, has forensic implications for bone sampling protocols, specifically in Disaster Victim Identification. In particular, for skeletonized or partially skeletonized remains recovered from the ground surface that are likely to be re-associated by piece-to-piece DNA matching [18]. For a discussion on DNA sampling protocols specific to buried commingled remains, which often require re-association by pair matching and element articulations, see Hines and colleagues 2014 [5].

5. Conclusions

By employing SR micro-CT, this study explored osteocyte lacunar density in representatives of each skeletal element type within a single individual and among multiple individuals for the first time. Though a higher density of osteocyte lacunae in cancellous bone tissue was not revealed as hypothesized, the residual soft tissue among the trabeculae likely contributed to the higher DNA yields found by Mundorff and Davoren [1]. As such, procuring elements with high cancellous content for DNA testing, potentially housing DNA rich soft tissues, offers promise for selecting elements that will yield higher quantities of nuclear DNA.

Acknowledgements

Research described in this paper was performed at the BMIT facility at the Canadian Light Source, which is supported by the Canada Foundation for Innovation, Natural Sciences and Engineering Research Council of Canada, the University of Saskatchewan, the Government of Saskatchewan, Western Economic Diversification Canada, the National Research Council Canada, and the Canadian Institutes of Health Research. We are grateful to the BMIT beamline user support staff, Drs. George Belev, Adam Webb, Ning Zhu, and Tomasz Wysokinski. The authors would also like to thank the Forensic Anthropology Center at the University of Tennessee, Knoxville for their assistance coordinating the selection of the skeletons used for this project. Janna M. Andronowski and Isaac V. Pratt are fellows in the Canadian Institutes of Health Research Training program in Health Research Using Synchrotron Techniques (CIHR-THRUST).

References

- [1] A.Z. Mundorff, J.M. Davoren, Examination of DNA yield rates for different skeletal elements at increasing post mortem intervals, *Forensic Sci. Int: Genet.* 8 (2014) 55–63.
- [2] S. Edson, J. Ross, M. Coble, T.J. Parsons, S. Barritt, Naming the dead: confronting the realities of rapid identification of degraded skeletal remains, *Forensic Sci. Rev.* 16 (1) (2004) 63–90.
- [3] S. Anđelinović, D. Sutlović, I.E. Ivkošić, V. Škaro, A. Ivkošić, F. Paić, B. Režić, M. Definis-Gojanović, D. Primorac, Twelve-year experience in identification of skeletal remains from mass graves, *Croat. Med. J.* 46 (4) (2005) 530–539.
- [4] A. Milos, A. Selmanovic, L. Smajlovic, R. Huel, C. Katzmarzyk, A. Rizvic, T. Parsons, Success rates of nuclear short tandem repeat typing from different skeletal elements, *Croat. Med. J.* 48 (2007) 486–493.

- [5] D. Hines, M. Vennemeyer, S. Amory, R. Huel, I. Hanson, C. Katzmarzyk, T. Parsons, Prioritizing sampling of bone and teeth for DNA analysis in commingled cases, in: B.J. Adams, J.E. Byrd (Eds.), *Commingled Human Remains: Methods in Recovery, Analysis, and Identification*, Elsevier Science, Oxford, 2014, pp. 275–305.
- [6] S.T.G. Ferreira, H.H. Kuser, R.G. Garrido, A. Trindade-Filho, K.A. Paula, M.F. Galvão, A.V. Moraes, Floods and mudslides in the State of Rio de Janeiro and a plane crash in the Brazilian Amazon rainforest: a study of two different experiences in disaster victim identification (DVI), *Forensic Sci. Int: Genet. Suppl.* 3 (2011) e516–e517.
- [7] S.T.G. Ferreira, R.G. Garrido, K.A. Paula, R.C. Nogueira, E.S. Oliveira, A.V. Moraes, Cartilage and phalanges from hallux: alternative sources of samples for DNA typing in disaster victim identification (DVI). A comparative study, *Forensic Sci. Int: Genet. Suppl.* 4 (2013) e366–e367.
- [8] T.W. Wysokinski, D. Chapman, G. Adams, M. Renier, P. Suortti, W. Thomlinson, Beamlines of the biomedical imaging and therapy facility at the Canadian Light Source-Part 3, *Nucl. Instrum. Methods Phys. Res. A* 775 (1) (2015) 1–4.
- [9] Y. Carter, C.D.L. Thomas, J.G. Clement, A.G. Peele, K. Hannah, D.M.L. Cooper, Variation in osteocyte lacunar morphology and density in the human femur – a synchrotron radiation micro-CT study, *Bone* 52 (2013) 126–132.
- [10] B.R. McCreadie, S.J. Hollister, M.B. Schaffler, S.A. Goldstein, Osteocyte lacuna size and shape in women with and without osteoporotic fracture, *J. Biomech.* 37 (2004) 563–572.
- [11] D.M.L. Cooper, A.L. Turinsky, C.W. Sensen, B. Hallgrímsson, Quantitative 3D analysis of the canal network in cortical bone by micro-computed tomography, *Anat. Rec. B* 274 (2003) 169–179.
- [12] P. Schneider, M. Stauber, R. Voide, M. Stampanoni, L.R. Donahue, R. Muller, Ultrastructural properties in cortical bone vary greatly in two inbred strains of mice as assessed by synchrotron light based micro- and nano-CT, *J. Bone Miner. Res.* 22 (10) (2007) 1557–1570.
- [13] Y. Carter, C.D.L. Thomas, J.G. Clement, D.M.L. Cooper, Femoral osteocyte lacunar density: volume and morphology in women across the lifespan, *J. Struct. Biol.* 183 (3) (2013) 519–526.
- [14] Y. Carter, J.L. Suchorab, C.D.L. Thomas, J.G. Clement, D.M.L. Cooper, Normal variation in cortical osteocyte lacunar parameters in healthy young males, *J. Anat.* 225 (2014) 328–336.
- [15] J.D. Currey, *Bones: Structure and Mechanics The Structure of Bone Tissue*, Princeton University Press, 2002, pp. 3–25.
- [16] D. Carter, G. Beaupré, *Skeletal Function and Form Mechanobiology of Skeletal Development, Aging, and Generation*, Cambridge University Press, NY, 2001.
- [17] M.J. Eagle, J. Man, P. Rooney, P. Hogg, J.N. Kearney, Assessment of an improved bone washing protocol for deceased donor human bone, *Cell Tissue Bank.* 16 (2015) 83–90.
- [18] C.H. Brenner, B.S. Weir, Issues and strategies in the DNA identification of World Trade Center victims, *Theor. Popul. Biol.* 63 (3) (2003) 173–178.