OPEN ACCESS

Scholars International Journal of Anatomy and Physiology

Abbreviated Key Title: Sch Int J Anat Physiol
ISSN 2616-8618 (Print) |ISSN 2617-345X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Comparative Analysis of Long and Short Bones from Skeletal Collections of the Humerus and Carpal Bones

Clinton David Orupabo[1]*, Cherish Chigonum Dickson[1]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University

DOI: https://doi.org/10.36348/sijap.2026.v09i06.001 | **Received:** 27.09.2026 | **Accepted:** 20.11.2026 | **Published:** 27.11.2026

***Corresponding author:** Clinton David Orupabo
Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University

Abstract

Histomorphometric assessment of skeletal specimens has been widely applied in human identification and provides a reliable outcome when combined with macroscopic findings. The aim of this study, however, is to compare the histomorphometric features of long and short bones. Skeletal collections of the humerus and carpal bones from the Department of Human Anatomy of the Rivers State University were used for this study. Each bone provided 10 specimens with cross sections, collected using a hacksaw and cut to sizes of less than 1cm to facilitate grinding and bone preparation. The modified Frost manual method of bone preparation was adopted. Quantitative microscopic parameters, including osteon diameter, Haversian canal diameter, and osteon fragment count, were evaluated to identify structural variations between bone types with differing biomechanical and functional demands. The results showed comparative analysis of histomorphometric parameters between humerus (long bones) and carpals (short bones) using Student's t-test. Significant differences were observed in the number of secondary osteons ($p < .05$) and primary osteons ($p < .05$), with higher values in the humerus. Maximum osteon diameter also differed significantly between the two bone types ($p = .05$). In contrast, no statistically significant differences were found for osteon fragments. The findings demonstrated that humeri possess significantly higher secondary and primary osteon counts and larger maximum osteon diameters than carpals, consistent with greater remodeling associated with long bones.

Keywords: Carpals, Histomorphometry, Humerus, Long bones, Osteons, Short bones.

Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Quantitative bone histology, or histomorphometry, examines the microscopic architecture of cortical bone including secondary osteon density, Haversian canal properties, and osteon fragmentation. This has become an indispensable tool in forensic and bioarchaeological investigations where macroscopic indicators are absent or skeletal material is fragmented. Histomorphometric methods are widely applied to estimate age-at-death, assess remodeling rates, and infer biomechanical history because intracortical features accumulate and remodel predictably over a person's lifespan and respond to mechanical loading and pathology (Oghenemavwe *et al*., 2022, Orupabo *et al*., 2020, Andronowski *et al*., 2020, Dominguez *et al*., 2019).

Despite this broad utility, significant practical and theoretical problems remain when applying histomorphometry across the skeleton: different bones remodel at different rates, mechanical loading produces intra-skeletal variability, and many established equations perform poorly when transferred between populations or when applied to short (e.g., metacarpals, ribs) versus long bones (e.g., femur, humerus). These issues complicate forensic casework where only partial or non-standard elements are recovered, and they limit the reliability and generalizability of histology-based age and identity assessments (Da Silva *et al*., 2023, Karydi *et al*., 2022).

Recent literature has advanced both methodology and our understanding of these limitations. Domínguez's *et al*., 2019 overview synthesized established histomorphometric age-estimation approaches and emphasized method selection, sampling location, and population effects. Andronowski *et al*. (2017) reviewed emerging imaging and quantitative frameworks, highlighting how new metrics and imaging

modalities can improve measurement repeatability but also underscoring remaining inter-skeletal and inter-population variability. Karydi *et al*. (2022) validated cortical histomorphometry protocols on femora and ribs from a British sample and demonstrated that rib and femur parameters can differ markedly in their age signal and method performance, calling attention to element-specific calibration. More recently, experimental and comparative work has explicitly linked mechanical loading regimes to differences in intracortical remodeling among highly loaded long bones versus minimally loaded elements such as ribs, reinforcing that functional loading is a major source of intra-skeletal histomorphometric variance (Da Silva *et al*., 2023, Crowder 2013, Stout *et al*., 1996).

These studies however, show relevance but also reveal a clear research gap: while many investigations focus on single elements or validate methods for specific populations, comparatively few studies systematically quantify and contrast histomorphometric parameters between short and long bones using standardized sampling and up-to-date quantitative measures across modern reference collections (Orupabo *et al*., 2025, Chang *et al*., 2021). This gap matters for forensic practice because recovery scenarios frequently present an incomplete skeletal inventory, and element-specific differences may bias age estimates or obscure biomechanical inferences if unaccounted for.

The present study aims to address that gap by performing a comparative histomorphometric assessment of human skeletal remains, directly contrasting long and short bones using standardized midshaft (or analogous) sampling, a suite of established and novel quantitative metrics (e.g., secondary osteon population density, osteon area and fragmentation indices, Haversian canal metrics), and statistical models that evaluate element-specific performance for age estimation and biomechanical interpretation. By explicitly quantifying intra-skeletal variability and testing whether element-type-specific calibration improves accuracy and robustness, this research seeks to produce practical recommendations and reference data that strengthen the forensic and bioarchaeological application of bone histomorphometry.

MATERIALS AND METHODS

Study Design

A comparative analytical, cross-sectional study design was employed to assess histomorphometric characteristics of selected short and long bones obtained from adult human skeletal remains. Quantitative microscopic parameters, including osteon diameter, Haversian canal diameter, and osteon fragment density, were evaluated to identify structural variations between bone types with differing biomechanical and functional demands.

Study Setting

The study was conducted in the Human Anatomy Laboratory, Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt, Nigeria. Bone processing, slide preparation, and microscopic evaluation were performed in the department's histology and imaging facility, which is equipped with digital light microscopy and morphometric analysis systems.

Sample Collection

Human skeletal materials were obtained from the Anatomy Department's approved skeletal collection, consisting of preserved adult human remains used for academic and research purposes. A total of 20 bone samples were analyzed, including 10 carpals representing short bones and 10 humeri long bones. Inclusion criteria comprised adult skeletal specimens with intact cortical bone and no visible deformities, fractures, or pathological lesions. Bones exhibiting severe post-mortem damage, pathological alterations, or belonging to infants or juveniles were excluded.

Ethical Approval

Ethical clearance for the study was obtained from the Faculty Research and Ethics Committee, College of Medical Sciences, Rivers State University (RSU/RBMS/REC/25/311). Authorization to access and utilize the skeletal materials was granted by the Head of the Department of Anatomy. All procedures conformed to institutional guidelines and internationally accepted ethical standards for research involving human skeletal remains.

Preparation of Undecalcified Bone Sections

Undecalcified ground bone sections were prepared using Frost's manual grinding technique (George *et al*., 2001, Frost 1958). Initially, bone samples were sectioned into transverse slices approximately 2–3 mm thick using a hacksaw or electric band saw, with precautions taken to minimize excessive force and heat generation. Fragile specimens were cut at a thickness of 4–5 mm to reduce the risk of damage during processing. Waterproof abrasive paper was affixed to a glass slab using petroleum jelly to prevent slippage and water infiltration. Bone slices were ground manually on the abrasive surface using light and even pressure until sufficient translucency and thickness suitable for microscopic examination were achieved. Throughout the grinding process, specimens were periodically rinsed with water to remove bone debris and abrasive particles.

Following grinding, bone sections were thoroughly washed with liquid soap and repeatedly rinsed in clean tap water. The specimens were briefly dehydrated in absolute ethanol and subsequently cleared in xylene to enhance optical clarity. Each bone section was mounted on a clean glass slide using DPX mounting medium and covered with an appropriately sized

coverslip. Prepared slides were allowed to dry for 24–48 hours before microscopic examination under transmitted and polarized light to visualize cortical bone microstructure.

Histomorphometric Analysis

Histological examination was performed using a digital light microscope, and images were captured for quantitative analysis. Histomorphometric parameters measured included osteon diameter, Haversian canal diameter, and osteon fragment density. Measurements were obtained from standardized cortical regions to ensure consistency across specimens.

Primary osteons are identified as osteons within a compact bone possessing a small central canal. They are usually surrounded by a few or three rings of concentric lamellae.

Secondary osteons are identified as matured remodeled bone possessing a Haversian canal which contains blood vessels. They are surrounded by lots of concentric lamellae.

Osteon fragments are remnants of old secondary osteons and are seen sometimes to surround the matured osteons. They are secondary osteons with partially visible Haversian canals that have been obscured or breached by a neighbouring osteon.

Osteon diameter is measured as the maximum distance from one point of the osteon to the other across its circle. Haversian canal diameter is measured as the maximum diameter across the haversian canal.

Statistical Analysis

Data were analyzed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Results were expressed as mean ± standard deviation. Independent-samples *t* tests were used to compare mean histomorphometric values between short and long bones, while one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to assess differences among multiple bone groups. Pearson's correlation analysis was used to evaluate relationships among histomorphometric parameters. Statistical significance was set at $p < .05$.

RESULTS

Table 1: Descriptive statistics of the Histomorphometric parameters of the humerus

Parameters	N	Mean (µ)	SEM	Min	Max
OS	10	11.5	1.49	6	19
OP	10	5.4	1.12	2	11
OF	10	15.6	1.34	8	22
HCD	10	14.01	0.63	9.92	16.61
Max OD	10	44.06	1.59	38.3	55.58

N-Number of subjects, OS-Secondary osteons, OP-Primary osteons, OF-Osteon fragments, HCD-Haversian canal diameter, Max OD-Maximum Osteon diameter, Min-Minimum, Max-Maximum, SEM-Standard error of mean

Table 1 presents the descriptive statistics of histomorphometric parameters measured in humerus bones. The mean number of secondary osteons was 11.5 ± 1.49, while primary osteons averaged 5.4 ± 1.12, indicating substantial intracortical remodeling activity. Osteon fragments showed a mean value of 15.6 ± 1.34, reflecting advanced remodeling and osteonal turnover. The mean Haversian canal diameter was 14.01 ± 0.63 µm, with values ranging from 9.92 to 16.61 µm. The maximum osteon diameter averaged 44.06 ± 1.59 µm, demonstrating relatively large osteonal structures in the humerus.

Table 2: Descriptive statistics of the Histomorphometric parameters of Carpal bones

Parameters	N	Mean (µ)	SEM	Min	Max
OS	10	4.4	1.05	0	11
OP	10	1.3	0.54	0	4
OF	10	15.9	1.46	11	25
HCD	10	12.62	0.77	9.39	16.59
Max OD	10	39.62	1.96	27.54	46.28

N-Number of subjects, OS-Secondary osteons, OP-Primary osteons, OF-Osteon fragments, HCD-Haversian canal diameter, Max OD-Maximum Osteon diameter, Min-Minimum, Max-Maximum, SEM-Standard error of mean

Table 2 summarizes the descriptive statistics of histomorphometric parameters in carpal bones. Secondary osteons had a lower mean value of 4.4 ± 1.05 compared with long bones, while primary osteons were sparsely distributed, with a mean of 1.3 ± 0.54. Osteon fragments exhibited a mean value of 15.9 ± 1.46,

comparable to that observed in the humerus. The mean Haversian canal diameter was 12.62 ± 0.77 µm, with a range of 9.39 to 16.59 µm. The maximum osteon diameter had a mean value of 39.62 ± 1.96 µm, indicating smaller osteonal dimensions relative to the humerus.

Table 3: Comparative analysis of histomorphometric properties of long bones and short bones using Student T test

Bone type	Parameter	t stat	t critical	P value
Humerus Carpals	Secondary osteon	3.90	1.75	0.00*
Humerus Carpals	Primary osteon	3.31	1.77	0.00*
Humerus Carpals	Osteon Fragments	-0.15	1.73	0.44
Humerus Carpals	HCD	1.42	1.73	0.09
Humerus Carpals	Max OD	1.75	1.73	0.05*

HCD- Haversian canal diameter, Max OD-Maximum Osteon diameter, P ≤ 0.05 is significant (*)

Table 3 shows the comparative analysis of histomorphometric parameters between humerus (long bones) and carpals (short bones) using Student's t test. Significant differences were observed in the number of secondary osteons ($p < .05$) and primary osteons ($p < .05$), with higher values in the humerus. Maximum osteon diameter also differed significantly between the two bone types ($p = .05$). In contrast, no statistically significant differences were found for osteon fragments ($p = .44$) or Haversian canal diameter ($p = .09$).

DISCUSSION

The present study compared cortical histomorphometric features of humeri (long bones) and carpals (short bones) to evaluate intra-skeletal variation and its implications for interpretation and forensic application. Descriptively, humeri exhibited higher mean counts of secondary and primary osteons compared with carpals (Table 1 & 2), whereas osteon fragment counts were similar between the two bone types. The comparative *t* tests confirmed that secondary osteon and primary osteon number were significantly greater ($p<0.05$) in the humerus than in carpals (Table 3), while osteon fragment count and Haversian canal diameter did not differ significantly. The maximum osteon diameter was larger in the humerus than the carpals, and this variation was statistically significant. These patterns indicate element-specific remodeling signatures consistent with differential mechanical loading and bone size effects reported in recent histological studies (Orupabo *et al*., 2026, Karydi *et al*., 2022). In previous studies, Orupabo *et al*., 2026 reported similar findings in a study involving short and flat bones, where greater counts of osteons were noted in short bones compared to flat bones. Likewise in a similar vein, Orupabo *et al*., 2025 documented that the tibia (long) bones had greater secondary and primary osteons than the talus (short bones).

Higher counts of secondary and primary osteons in the humerus are concordant with the expectation that large, weight-bearing or highly loaded long bones undergo more extensive intracortical remodeling than small, less-loaded short bones. Long bones typically experience greater and more variable mechanical strains during locomotion and manipulation, stimulating remodeling that increases osteon population density and the occurrence of primary and secondary osteons (Pedersen *et al*., 2024; Karydi *et al*., 2022). The results of this study therefore support the view that osteon counts reflect functional loading histories as well as age-related remodeling, and they emphasize that bone type must be considered when using osteon metrics for comparative or forensic purposes.

The lack of a significant difference in osteon fragment count between humeri and carpals (Table 3) suggests that osteon fragmentation may be less sensitive to gross differences in bone size or loading regime than osteon counts. Osteon fragments can arise from ongoing remodeling, osteon drift, or resorption events and thus may accumulate in both bone types irrespective of overall osteon production rate. This implies that fragment count alone may be an unreliable discriminator of long- versus short-bone remodeling intensity and should be interpreted alongside other metrics (Cooke *et al*., 2021).

Haversian canal diameter showed a numerical difference in mean values between humeri and carpals but the variation was not statistically significant. This finding aligns with recent syntheses that document considerable inter- and intra-skeletal variability in canal dimensions and note that Haversian canal size is influenced by local vascularization patterns, age, and remodeling dynamics rather than bone type alone (Orupabo *et al*., 2025, Stan *et al*., 2024, Chang & Liu, 2021). Consequently, Haversian canal diameter should be used cautiously as an isolated diagnostic metric; when combined with osteon counts and osteon size, it may nevertheless contribute useful information about remodeling and microstructural organization. This was well demonstrated in the study by Orupabo and associates, 2026 on calcaneus and sternum.

Maximum osteon diameter was larger on average in humeri than carpals and the variation was statistically significant ($p\leq0.05$). Larger osteon size within long bones may reflect differences in the geometry of remodeling units needed to repair mechanically demanding regions or differences in the scale of vascular channels supporting cortical turnover.

This observation supports recent work showing that osteon area and perimeter vary with bone robusticity and functional loading and that these size metrics can confound age-estimation models if not adjusted for bone size or robusticity (Pedersen *et al*., 2024). Thus, the present study reinforces the need to consider bone-types during forensic investigations.

From an applied perspective, the bone-specific differences documented in the present study, have two practical implications. First, age-at-death estimation equations and histology-based identification protocols developed on long bones may overestimate remodeling intensity if applied directly to short bones without adjustment; significant differences in osteon counts and maximum osteon diameter highlight this risk. Second, the similarity in osteon fragment count and Haversian canal diameter between bone types suggests that some microstructural features are broadly comparable across the skeleton and may still be informative when only short bones are available, provided their limitations are acknowledged. It is therefore imperative that for any accurate and reliable results to be obtained, bone-type and population-specific data must be generated, and more so, if such data is to be applied to forensic research.

CONCLUSION

The present comparative analysis demonstrated that humeri possess significantly higher secondary and primary osteon counts and larger maximum osteon diameters than carpals, consistent with greater remodeling associated with long-bones. Haversian canal diameter and osteon fragment count were not markedly different between bone types. These findings reinforce the need for bone-specific calibration of histological metrics in forensic and bioarchaeological applications and suggest areas of priority for future research, including larger multi-population samples, and the incorporation of age/sex/robusticity covariates.

REFERENCES

- Andronowski, J. M., & Cole, M. E. (2020). Current and emerging histomorphometric and imaging techniques for assessing age-at-death and cortical bone quality. Wiley Interdisciplinary Reviews Forensic Science, 3(2). https://doi.org/10.1002/wfs2.1399
- Andronowski, J. M., Cole, M. E., Davis, R. A., & Crowder, C. M. (2017). 3D quantitative analysis of human clavicle cortical microstructure using micro-CT. American Journal of Physical Anthropology, 164(4), 670–680. https://doi.org/10.1002/ajpa.23307
- Chang, B., & Liu, X. (2021). Osteon: structure, turnover, and regeneration. Tissue Engineering Part B Reviews, 28(2), 261–278. https://doi.org/10.1089/ten.teb.2020.0322
- Cooke, K. M., Mahoney, P., & Miszkiewicz, J. J. (2021). Secondary osteon variants and remodeling in human bone. The Anatomical Record, 305(6), 1299–1315. https://doi.org/10.1002/ar.24646
- Crowder, C. (2013). Estimation of Age at Death Using Cortical Bone Histomorphometry. Submitted to the U.S. Department of Justice Office of Justice Programs, National Institute of Justice, Award no.240692 2010-DN-BX- K035.
- Da Silva, R. G. C., Sun, T. C., Mishra, A. P., Boyde, A., Doube, M., & Riggs, C. M. (2023). Intracortical remodelling increases in highly loaded bone after exercise cessation. Journal of Anatomy, 244(3), 424–437. https://doi.org/10.1111/joa.13969
- Dominguez, V. M., & Mavroudas, S. (2019). Bone histology for skeletal age-at-death estimation. In Elsevier eBooks (pp. 145–159). https://doi.org/10.1016/b978-0-12-814491-6.00010-8
- Frost, H.M. (1958). Preparation of thin undecalcified bone sections by rapid manual method. Stain Technology, 33: 271–276.
- George, J.R.M, Robert, P.M.V & MJ (JOB), A. (2001). Manual preparation of ground sections for the microscopy of natural bone tissue: Update and Modification of Frost's'Rapid Manual Method'. International Journal of Osteoarchaeology. 11: 366–374.
- Karydi, C., García-Donas, J. G., Tsiminikaki, K., Bonicelli, A., Moraitis, K., & Kranioti, E. F. (2022). Estimation of Age-at-Death using Cortical bone histomorphometry of the rib and femur: A validation study on a British population. Biology, 11(11), 1615. https://doi.org/10.3390/biology11111615
- Oghenemavwe, L.E, Orupabo, C.D. (2022). Sex Estimation from Histomorphometric Analysis of Cortical Bone: A Hospital-based Study. Arab Journal of Forensic Sciences and Forensic Medicine. Vol.4 Issue (1): 1-12
- Orupabo, C.D, Oghenemavwe, L.E, Diamond, T.E. (2020). Histomorphometric estimation of age from bone samples of Nigerians. International Journal of Medicine and Medical Research. 6(2): 67-76.
- Orupabo C.D, Echomgbe J.O, Bokoko H. (2025). "Histomorphometric Assessment of Human Skeletal Remains: Comparative Analysis of Long and Short Bones". Archives of Current Research International 25 (12):533–542. https://doi.org/10.9734/acri/2025/v25i121691
- Orupabo, C. D. & `Elenwo, S. K. (2026). Comparative Histomorphometric Analysis of Short and Flat Bones in Human Skeletal Remains. Asian Journal of Advanced Research and Reports, 20(4), 21–29
- Pedersen, L. T., Miszkiewicz, J., Cheah, L. C., Willis, A., & Domett, K. M. (2024). Age-dependent change and intraskeletal variability in secondary osteons of elderly Australians. Journal of Anatomy, 244(6), 1078–1092. https://doi.org/10.1111/joa.14010

- Stan, E., Muresan, C., Daescu, E., Dumache, R., Ciocan, V., Ungureanu, S., Costachescu, D., & Enache, A. (2024). A Review of Histological Techniques for Differentiating Human Bone from Animal Bone. Methods and Protocols, 7(4), 51. https://doi.org/10.3390/mps7040051
- Stout, S. D., Lueck, R., & Holvig, L. (1996). Histomorphometric analysis of the human clavicle: Implications for age estimation. American Journal of Physical Anthropology, Supplement 22, 220.