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Histomorphometric analysis of the variability of the human skeleton: forensic implications

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Abstract

In the last decades, the histomorphometric analysis of bone tissue has been utilized to develop equations for species discrimination of fragmentary bone. Although this technique showed promising results, its main limitation concerns the lack of knowledge on the histomorphometric variability which may exist between different bones of the skeleton. In a previous study, we demonstrated a significant histomorphological variability in different bones of the same individual and even in different sections of the same bone. The present study aimed at investigating the extent of intra-individual variability in bone histomorphometry throughout the human adult skeleton and areas of a single bone.

Samples were taken along an entire medieval male adult human skeleton (aged between 26-45 years), including long, flat, irregular and sesamoid bones for a total of 49 cross-sections.

The histomorphometric analysis revealed that the size of both Haversian systems and Haversian canals were statistically significantly larger in long and irregular bones compared to flat bones. Moreover, osteons were generally bigger in the diaphysis compared to the proximal and distal metaphyses, whereas Haversian canals showed a higher uniformity in the different portions of each bone.

The present study has highlighted the importance of conducting similar studies on both human and nonhuman skeletons at different stages of skeletal maturity in order to shed light on the extent of variability in the size of osteons and Haversian canals. This, in fact, represents an important prerequisite to develop reliable histological methods for species discrimination of fragmented bone.

Keywords: BONE HISTOLOGY; HISTOMORPHOMETRIC VARIABILITY; HAVERSIAN SYSTEM; HAVERSIAN CANAL; HUMAN SKELETON; FORENSIC ANTHROPOLOGY

1. Introduction

In recent decades, the histological analysis of bone tissue has been undertaken both in forensic and archaeological contexts to address a number of questions, such as species (human vs non-human) discrimination (1) and age-at-death estimation (2). These application come due to its low cost and its applicability even in case of human skeletal remains which have been affected by taphonomic alterations (e.g. fire, water) (3).

This technique is particularly useful in case of highly fragmented remains for which a macroscopic approach may not be sufficient. Indeed, in the event of mass fatalities as a result of natural disasters, fatal fires and transportation accidents as well as in clandestine burials, human skeletal remains can be highly fragmented and they can become commingled with the remains of pets, wildlife animals or other animals used for meat consumption (4-5).

In such cases, forensic anthropologists are often asked to assist law enforcement in identifying the human or nonhuman origin of the remains and assess their biological profile.

Several authors have investigated bone microarchitecture in human and nonhuman species at different stages of skeletal maturity and provided equations for species discrimination (6-12) based on osteon and Haversian canal parameters (e.g. diameter, area, perimeter). Although these investigations have shown promising results, their main limitation is that they have focused exclusively on specific bones (e.g. femur, rib), without considering the extent of histomorphometric variability which may exist in the different bones of the skeleton. In forensic and archaeological contexts, it is often difficult, if not impossible, to identify with certainty the precise anatomical origin of tiny bone fragments. Accordingly, when trying to determine if the material is human or nonhuman, the analyst has to take into consideration the possibility that the fragment might belong to any part of the skeleton.

In a previous study (13), we demonstrated a significant intra-individual histomorphological variability in different bones of the skeleton and even in different sections of the same bone. Long bones showed a higher variability, especially in the pattern of osteon organization, compared to flat and irregular bones. In particular, the sites of muscular insertion, experience a higher rate of remodeling and therefore, a higher osteon density. Flat and irregular bones, which are not directly involved in locomotion and are subjected to bidirectional loading are mainly characterized by lamellar tissue with few scattered secondary osteons.

The present study aimed at investigating the extent of intra-individual variability in bone histomorphometry throughout a human adult skeleton since it an important prerequisite in order to develop reliable histological methods for species discrimination of fragmented bone.

2. Materials and methods

Bone samples were taken from a medieval adult human skeleton with no evident sign of pathological conditions. A morphological analysis was performed to estimate sex and the age at death of the individual following a number of techniques (14-18), and it revealed that the skeleton belonged to a European male individual aged between 26 and 45 years. The skeleton was well preserved with only minor signs of post-mortem erosion on long bone epiphyses.

In order to test the intra-individual histomorphometric variability samples were taken along the entire skeleton, including long, flat, irregular and sesamoid bones, for a total of forty-nine samples (Table 1). The choice of the bones to sample was based on their availability (e.g. carpal and tarsal bones were not present) and their state of preservation. Different portions of long bones were sampled (e.g. diaphysis, and proximal and distal metaphysis) since different mechanical loads to which the different parts of the bones are subjected to may result in regional variation of bone microarchitecture (19-22). Since cervical vertebrae and ilia primarily consist of spongy bone, they were sectioned in both longitudinal and transversal planes so as to verify the presence of Haversian systems.

The method used to produce bone thin sections was based on the procedure commonly utilized at LABANOF (Laboratory of Forensic Anthropology and Odontology) for histological investigations of bone tissue (3, 6, 23).

Complete cross-sections approximately 5 mm thick were obtained from each bone by making two parallel cuts, perpendicular to the long axis of the bone using a hack-saw. Each bone sample was ground and then polished using a Struer DAP-7 grinding wheel for geologist equipped with different Buehler® abrasive papers up to 4000 grit. The smoothed face of the bone samples was glued to the slides using Pertex® mounting medium (HistoLab, Göteborg, Sweden). Once the mounting medium dried, the other face of the bones was ground down to approximately 70-100 μm. The slides were then polished with 2400 and 4000 grit silicon carbide abrasive papers in order to remove surface scratches and finally coverslipped.

The histological analysis was performed using an Axio Scope.A1® polarized light microscope connected to a Tucsen's TrueChrome II HD® camera. Measurements were taken using the tools "line" and "polygon" of IScapture® software.

The list of measurements for the histomorphometric analysis was made following previous investigations on species discrimination (6-9). For each osteon the following measurements were taken: maximum and minimum diameter ($On.Dm_{max}$ and $On.Dm_{min}$), area (On.Ar) and perimeter (On.Pm). Similarly, for each Haversian canal, maximum and minimum diameter ($H.Ca.Dm_{max}$ and $H.Ca.Dm_{min}$), area (H.Ca.Ar) and perimeter (H.Ca.Pm) were measured.

The choice of the osteons to measure was in accordance with the following criteria commonly used in histomorhometric studies (24-26): a) mature osteon (the Haversian canal area must be smaller than ¼ of the osteon area); b) not in resorption phase; c) with a well-defined and complete cement line; d) absence of Volkmann's canals crossing the osteon; e) the ratio between the Haversian canal maximum and minimum diameter must be lower than 2:1. Criterion "e" was chosen in order to minimize the bias that may be introduced when measuring osteons which are not transversely sectioned. Therefore, when the ratio between the maximum and minimum diameter of the Haversian canal was higher than 2:1, the secondary osteon was excluded from the analysis. For each cross-section, the entire cortex was analyzed and all the osteons that fit the above criteria were measured. Adobe Photoshop CS® "photomerge" command was used to create whole slide images of each sample, allowing to map the osteons that were measured and ensuring that osteons were not measured twice.

Statistical analysis of the results was computed using SPSS 22 software (SPSS Inc., Chicago, IL, USA). The descriptive statistics of the mean value, the standard deviation of the mean, the minimum value and the maximum value were obtained for each of the parameter measured. One-way ANOVA combined with Tukey post-hoc test was used to compare the size of osteon and Haversian canal in different bone types and in different part of the same bone (e.g. proximal metaphysis vs diaphysis). Cohen's *d* effect size was calculated to determine the standardized differences between the means. In addition, Intraclass Correlation Coefficient (ICC) was calculated for repeating measurements of thirty secondary osteons by the main operator and an additional trained operator after 24, 48 and 72 hours in order to test the inter-rater and intra-rater reliability.

3. Results

The Intraclass Correlation Coefficient (ICC), indicated an excellent agreement between the observations of the main observer as well as those of the two observers (main and additional observer) (Table 2). The minimum correlation coefficient regarded the measurement of the Haversian canal minimum diameter, even though the agreement remains excellent (27-28).

The histomorphometric analysis of the human adult individual involved the measurement of 1317 secondary osteons and Haversian canals. The descriptive statistics of the mean value, standard deviation, minimum and maximum value for osteon and Haversian canal parameters in long, flat and irregular bones are shown respectively in Table 3 and Table 4 (see supplemental material for the descriptive statistics of each bone). No data were provided for the cross-sections that showed no secondary osteons (or no osteons fit the criteria): the patella, the base of the metacarpal, the cervical vertebra, the lateral end of the clavicle, the mandibular condyle and the zygomatic process of the frontal bone.

Osteons were generally bigger in irregular bones compared to long and flat bones, with a mean area of 31701.29(±15850.60) μm^2, 29385.27(±13268.86) μm^2 and 21812.48(±11004.53) μm^2 respectively.

Similarly, the largest mean value for the Haversian canal area was observed in irregular bones (1813.60±942.07 μm^2), followed by long bones (1626.35±784.76 μm^2) and flat bones (1422.76±744.93 μm^2). Concerning the minimum Haversian canal size, flat bones showed the lowest value (219.14 μm^2) followed by long bones (231.56 μm^2) and irregular bones (346.24 μm^2).

ANOVA test (Table 5) revealed that both the sizes of osteon and Haversian canal were statistically significantly different between the three groups, except for the Haversian canal maximum diameter. Tukey post-hoc test indicated that the size of the osteon was statistically significantly larger in long bones (p=0.000, Cohen's d=0.589, 95% CI for Cohen's d: 0.449 – 0.729) and irregular bones (p=0.000, Cohen's d=0.841, 95% CI for Cohen's d: 0.496 – 1.185) compared to flat bones. There was no statistically significant difference between long and irregular bones.

Similarly, the size of the Haversian canal was statistically significantly larger in long bones (p=0.001, Cohen's d=0.262, 95% CI for Cohen's d: 0.123 – 0.401) and irregular bones (p=0.011, Cohen's d=0.505, 95% CI for Cohen's d: 0.165 – 0.845) compared to flat bones. There was no statistically significant difference between long and irregular bones.

With regard to the differences between different portions of the same bone, the descriptive statistics are presented in Table 6. As previously stated some cross-sections showed no secondary osteons and for those bones a comparison between the different parts was not possible. However, statistically significant differences ($p<0.05$) were found in the size of osteons in the different parts of the radius, ulna, femur, tibia and fibula (Table 7). Osteons were generally bigger in the diaphysis compared to the proximal and distal metaphyses, except in the tibia in which osteons were bigger in the proximal metaphysis. On the contrary, humerus, clavicle, metacarpal, metatarsal and rib showed no statistically significant differences in the size of osteons along their length ($p>0.05$).

Haversian canals showed a higher uniformity in the different portions of each bone ($p<0.05$), except for the humerus, the radius and the fibula. In the humerus both the proximal (1613.96±666.31 μm^2) and distal metaphyses (1601.17±779.32 μm^2) showed statistically significantly smaller Haversian canals compared to diaphysis (2063.91±958.44 μm^2), whereas in the fibula, Haversian canal were larger at the distal metaphyses (1819.70±857.46 μm^2) compared to the proximal metaphysis (1223.59±802.30 μm^2). With regard to the radius, Haversian canals were statistically significantly larger in the distal metaphysis (2042.44±742.52 μm^2) compared to the diaphysis (1088.72±436.73 μm^2) and the proximal metaphysis (1262.43±588.42 μm^2).

4. Discussion

The histomorphometric analysis on the human adult individual revealed that the size of both Haversian systems and Haversian canals were statistically significantly larger in long and irregular bones compared to flat bones. Patella (sesamoid bone) showed no secondary osteons. Although literature does not provide an explanation for the variation in the size of osteons and Haversian canals in long and flat bones, this may be due to the smaller cross-sectional areas of the latter, as well as differences in the habitual loading (29-30). The smaller cross-sectional diameter in flat bones may yield a packing effect for osteons which could lead to smaller observed osteons, especially in older individuals who are near or at OPD asymptote. Indeed, Dominguez and Agnew (31) demonstrated a correlation in ribs between the size of osteons, the age of the specimen and the cortical area. With increasing age, the cortical area decreases and this may limit the size of forming osteons.

The differences observed in different portions of each bone were consistent with the hypothesis that cortical thickness determines the size of osteons. Indeed, in the proximal metaphysis, which in most of long bones is characterized by a thinner cortex compared to that of the diaphysis and distal metaphysis, osteons were statistically significantly smaller. The only exception regarded the tibia, in which the proximal metaphysis, although characterized by a thinner cortex, showed bigger

osteons compared to those of the diaphysis. Along with the cortical thickness, there may be other factors playing a role in determining the size of secondary osteons such as habitual loading and locomotion. Moreover, the diaphysis of long bones showed systematically higher standard deviations probably due to a larger area of cortical bone compared to that of the metaphyses, allowing a higher variability in terms of the size of osteons. On the contrary, in ribs, as well as in metatarsals and metacarpals, the cross-sectional area is rather uniform along the length of the bone, and this could explain why in these bones there is a lower variability in terms of the size of osteons.
The variation observed in different portions of long bones should be taken into account for the implications that it may have on the reliability of the equations developed in previous studies on species discrimination by histological analysis (6-9), which are based on measurements taken on few skeletal elements (generally femur, tibia and rib). Applying those formulas with fragments belonging to other parts of the skeleton may led to wrong conclusions. By comparing the results of our study with the current literature on mammalian bone histomorphometry, the human bones whose values may overlap with those of non-human bones are the proximal metaphysis of the fibula and the metacarpal. The mean value obtained for the fibula (On.Ar=12433.08±4482.49 μm^2) overlaps with those of cow, sheep, pig and goat metacarpals (32), whereas the mean value of the human metacarpal (mean On.Ar=24505.17±9628.85 μm^2) overlaps with those of the sheep, domestic pig and wild pig femora (7,32) as well as those of the horse metacarpal (32) and domestic pig humerus (33).

Further studies should investigate the intra-individual and intra-species histomorphometric variability in both human and nonhuman skeletons at different stages of skeletal maturity since it is well known that, with increasing age, there is a decrease in the size of secondary osteons and an increase in the size of the Haversian canals (34-39). This would help to shed light on the extent of variability in the size of osteons and Haversian canals and it would represent a starting point in order to develop reliable methods for species discrimination and age-at-death estimation.

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Conflict of interest

The authors declare that they have no conflict of interest

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Long bones	Humerus (PM, D, DM)
	Ulna (PM, D, DM)
	Radius (PM, D, DM)
	Clavicle (medial end, D, lateral end)
	Femur (neck, PM, D, DM)
	Tibia (PM, D, DM)
	Fibula (PM, D, DM)
	Metacarpal (base, shaft, head)
	Metatarsal (base, shaft, head)
Flat bones	Glabella (frontal bone)
	Zygomatic process of frontal bone
	Parietal (middle portion)
	Occipital
	Scapula superior border
	Scapula acromion
	Sternum
	Rib (head, body)
	Iliac crest (longitudinal, transversal)
	Ischiopubic ramus
	Iliopubic ramus
Sesamoid	Patella (sagittally)
Irregular bones	Petrous (temporal bone)
	Gonion (mandible)
	Mental protuberance (mandible)
	Mandibular condyle (mandible)
	Cervical vertebra (longitudinal, transversal, spinous process)

Table 1 – Study sample (PM=proximal metaphysis; D=diaphysis; DM=distal metaphysis)

	Intraclass Correlation Coefficient (ICC) Intra-rater reliability	Intraclass Correlation Coefficient (ICC) Inter-rater reliability
$On.Dm_{max}$	0.957	0.874
$On.Dm_{min}$	0.979	0.925
On.Ar	0.995	0.997
On.Pm	0.988	0.990
$H.Ca.Dm_{max}$	0.874	0.973
$H.Ca.Dm_{min}$	0.782	0.889
H.Ca.Ar	0.832	0.969
H.Ca.Pm	0.886	0.983

Table 2 – Histomorphometric analysis of osteons and Haversian canals: Intraclass correlation coefficient (ICC) for intra-rater and inter-rater reliability

OSTEON					
Bone type		**On.Dm$_{max}$ (µm)**	**On.Dm$_{min}$ (µm)**	**On.Ar (µm^2)**	**On.Pm (µm)**
Long bones (n=1028)	Mean	216.79	166.77	29385.27	623.80
	SD	54.36	40.08	13268.86	144.65
	Min	100.77	73.98	7122.36	310.65
	Max	413.23	321.01	86173.82	1137.03
Flat bones (n=250)	Mean	192.38	139.58	21812.48	536.99
	SD	54.11	37.67	11004.53	139.29
	Min	84.54	64.26	4472.39	249.95
	Max	351.42	248.99	60628.03	924.58
Irregular bones (n=39)	Mean	235.67	162.39	31701.29	654.85
	SD	68.68	41.77	15850.60	176.40
	Min	124.09	80.58	9017.82	361.57
	Max	384.96	260.74	68078.28	1004.43
Total (n=1317)	Mean	212.61	161.41	27998.45	607.97
	SD	55.82	41.11	13305.33	148.98
	Min	84.54	64.26	4472.39	249.95
	Max	413.23	321.01	86173.82	1137.03

Table 3 - Descriptive statistics of osteon parameters

HAVERSIAN CANAL					
Bone type		**H.Ca.Dm$_{max}$ (µm)**	**H.Ca.Dm$_{min}$ (µm)**	**H.Ca.Ar (µm^2)**	**H.Ca.Pm (µm)**
Long bones (n=1028)	Mean	50.18	38.40	1626.35	146.25
	SD	13.53	10.60	784.76	36.29
	Min	17.01	13.83	231.56	56.43
	Max	101.42	71.72	3592.42	245.09
Flat bones (n=250)	Mean	48.67	34.44	1422.76	138.08
	SD	14.98	9.48	744.93	37.70
	Min	17.50	14.39	219.14	55.08
	Max	91.30	61.61	3930.87	230.57
Irregular bones (n=39)	Mean	54.04	39.25	1813.60	156.11
	SD	15.34	10.63	942.07	41.44
	Min	25.37	17.24	346.24	71.60
	Max	86.37	60.16	3890.45	236.55
Total (n=1317)	Mean	49.99	37.66	1592.26	144.93
	SD	13.91	10.52	787.31	36.94
	Min	17.01	13.83	219.14	55.08
	Max	101.42	71.72	3930.87	245.09

Table 4 - Descriptive statistics of Haversian canal parameters

HUMAN ADULT – LONG VS FLAT VS IRREGULAR BONES								
	On.Dm (max)	**On.Dm (min)**	**On.Ar**	**On.Pm**	**H.Ca.Dm (max)**	**H.Ca.Dm (min)**	**H.Ca.Ar**	**H.Ca.Pm**
				Sig.				
Between groups	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.001
Long vs flat	0.000	0.000	0.000	0.000	0.273	0.000	0.001	0.005
Long vs irregular	0.088	0.777	0.517	0.387	0.203	0.873	0.307	0.227
Flat vs irregular	0.000	0.003	0.000	0.000	0.064	0.020	0.011	0.012

Table 5 - Results of ANOVA and Tukey post-hoc test on osteon and Haversian canal parameters

Bone			**On.Ar (μm^2)**	**H.Ca.Ar (μm^2)**	**Bone**			**On.Ar (μm^2)**	**H.Ca.Ar (μm^2)**
HUMERUS	PM	Mean	37057.52	1613.96	FIBULA	PM	Mean	12433.08	1223.59
	(n=50)	St.dev	12530.40	666.31		(n=32)	St.dev	4482.49	802.30
	D	Mean	31538.02	2063.91		D	Mean	35354.56	1679.99
	(n=36)	St.dev	13877.72	958.44		(n=42)	St.dev	15416.09	810.42
	DM	Mean	31488.50	1601.17		DM	Mean	25095.26	1819.70
	(n=50)	St.dev	11970.50	779.32		(n=33)	St.dev	7509.45	857.46
RADIUS	PM	Mean	28406.51	1262.43	CLAVICLE	D	Mean	32666.70	1686.44
	(n=37)	St.dev	11610.90	588.42		(n=44)	St.dev	17008.90	664.41
	D	Mean	36387.90	1088.72		M	Mean	28303.78	1711.91
	(n=50)	St.dev	13310.31	436.73		(n=26)	St.dev	11890.90	715.79
	DM	Mean	29198.18	2042.44	METACARPAL	Head	Mean	25827.43	1685.93
	(n=43)	St.dev	9798.13	742.52		(n=44)	St.dev	9880.35	818.65
ULNA	PM	Mean	35655.79	1583.04		D	Mean	23152.17	1716.71
	(n=49)	St.dev	14025.38	760.63		(n=43)	St.dev	9284.02	815.62
	D	Mean	34389.81	1966.50	METATARSAL	Head	Mean	23951.92	1374.73
	(n=37)	St.dev	18876.18	933.71		(n=4)	St.dev	7950.02	477.96
	DM	Mean	24417.89	1613.47		D	Mean	27165.60	1816.20
	(n=41)	St.dev	10373.38	791.87		(n=39)	St.dev	14272.01	894.24
FEMUR	Neck	Mean	25600.70	1496.92		Base	Mean	20382.33	1288.32
	(n=45)	St.dev	9425.44	699.12		(n=9)	St.dev	6201.17	560.39
	PM	Mean	24683.90	1305.07	RIB	Head	Mean	14216.88	1042.23
	(n=50)	St.dev	8367.31	578.70		(n=5)	St.dev	4606.21	508.49
	D	Mean	25521.31	1640.01		Body	Mean	20279.75	1063.24
	(n=44)	St.dev	10883.85	749.58		(n=48)	St.dev	11030.51	583.51
	DM	Mean	30872.51	1529.30	SCAPULA	SB	Mean	12712.92	782.90
	(n=44)	St.dev	12324.90	850.74		(n=6)	St.dev	5202.65	162.82
TIBIA	PM	Mean	38318.25	1604.51		A	Mean	25362.88	1426.22
	(n=46)	St.dev	11601.89	791.10		(n=15)	St.dev	12711.58	465.14
	D	Mean	29574.32	1731.87	MANDIBLE	MP	Mean	28811.88	1857.77
	(n=43)	St.dev	15616.75	824.90		(n=24)	St.dev	12763.71	913.43
	DM	Mean	26974.41	1844.89		Gonion	Mean	42204.44	1955.65
	(n=49)	St.dev	10482.21	693.51		(n=11)	St.dev	19530.72	1056.88

Table 6 - Descriptive statistics of osteon and Haversian canal parameters in different portions of the same bone. PM=proximal metaphysis; D=diaphysis; DM=distal metaphysis; M=medial; SB=superior border; A=acromion; MP=mental protuberance.

	Between groups (On.Ar)	**PM vs D**	**PM vs DM**	**D Vs DM**	**Between groups (H.Ca.Ar)**	**PM Vs D**	**PM Vs DM**	**D Vs DM**
	Sig.							
Humerus	0.052	0.119	0.076	1.000	0.014	0.028	0.996	0.023
Radius	0.001	0.006	0.952	0.011	0.000	0.372	0.000	0.000
Ulna	0.002	0.917	0.001	0.009	0.075	0.087	0.983	0.146
Femur *	0.019	0.980	0.021	0.076	0.157	0.119	0.437	0.891
Tibia	0.000	0.004	0.000	0.588	0.317	0.715	0.284	0.762
Fibula	0.000	0.000	0.000	0.000	0.006	0.052	0.012	0.746

Table 7 - Results of ANOVA and Tukey post-hoc test on osteon and Haversian canal area in the different portions of each bone. PM=proximal metaphysis; D=diaphysis; DM=distal metaphysis. *the comparison between the neck and the other portions of the femur showed no statistically significant differences

Supplemental material

Descriptive statistics of osteon and Haversian canal parameters of each bone

Bone		On.Dm$_{max}$ (μm)	On.Dm$_{min}$ (μm)	On.Ar (μm^2)	On.Pm (μm)	H.Ca.Dm$_{max}$ (μm)	H.Ca.Dm$_{min}$ (μm)	H.Ca.Ar (μm^2)	H.Ca.Pm (μm)
Humerus (n=136)	Mean	239.58	173.69	33549.04	677.03	53.84	38.45	1728.36	152.7
	St.dev	54.81	34.11	12892.33	137.29	14.55	10.74	812.78	36.98
	Min	111.2	79.75	7122.36	310.65	20.94	16.98	290.24	66.2
	Max	383.91	260.5	68174.21	980.36	101.42	64.36	3474.24	245.09
Radius (n=130)	Mean	228.7	176.03	31738.13	650.18	48.64	36.05	1453.62	139.13
	St.dev	50.52	39.35	12242.89	132.59	12.64	10.05	725.42	34.22
	Min	132.16	87.2	8705.67	353.69	20.47	14.43	245.44	58.45
	Max	350.25	266.23	66297.41	960.5	78.21	63.38	3558.33	217.45
Ulna (n=126)	Mean	222.41	173.04	31480.39	641.19	51.05	39.07	1690.49	147.99
	St.dev	57.75	44.55	15302.72	161.05	13.9	11.3	823	37.29
	Min	116.01	88.79	8462.18	340.74	23.04	16.01	388.61	76.02
	Max	413.23	321.01	84195.76	1137.03	96.86	63.01	3581.22	219.71
Femur (n=182)	Mean	208.18	158.81	26604.58	599.59	48.42	36.24	1485.85	140.57
	St.dev	47.24	33.46	10492.91	123.2	13.34	9.85	725.92	34.9
	Min	122.06	79.06	8427.61	349.13	19.98	15.15	305.33	66.96
	Max	367.6	244.27	61261.59	960.65	83.39	63.77	3566.13	224.67
Tibia (n=138)	Mean	222.84	174.35	31565.81	645.61	51.14	40.35	1729.55	150.98
	St.dev	51.54	40.85	13474.83	140.84	12.45	10.15	769.86	34.66
	Min	114.55	89.06	8879.52	357.6	21.19	15.74	298.52	64.02
	Max	348.94	302.79	75311.94	1009.65	78.53	61.86	3491.29	219.07
Fibula (n=107)	Mean	189.48	155.98	25335.45	566.25	46.31	38.1	1586.58	141.4
	St.dev	55.93	46.9	14313.32	162.34	14.18	11.5	851.11	40.96
	Min	100.77	83.33	7253.73	317.75	17.01	13.83	231.56	56.43
	Max	315.54	278.45	65870.96	925.91	84.99	60.96	3401.04	222.4
Metacarpal (n=87)	Mean	206.63	148.39	24505.17	579.77	50.91	40.71	1716.71	149.67
	St.dev	48.69	32.17	9628.85	120.58	13.49	11.23	815.62	36.56
	Min	125.65	85.4	9376.72	361.31	26.05	19.3	425.62	79.22
	Max	329.03	223.08	49300.97	878.08	79.49	71.72	3592.42	228.99

Bone		**On.Dm$_{max}$ (µm)**	**On.Dm$_{min}$ (µm)**	**On.Ar (µm^2)**	**On.Pm (µm)**	**H.Ca.Dm$_{max}$ (µm)**	**H.Ca.Dm$_{min}$ (µm)**	**H.Ca.Ar (µm^2)**	**H.Ca.Pm (µm)**
Metatarsal (n=52)	Mean	198.05	159.83	25744.36	580.03	49.93	39.78	1690.88	147.36
	St.dev	47.01	37.22	12976.49	135.98	13.49	10.89	840.82	37.1
	Min	109.04	73.98	7456.58	321.55	23.73	20.48	431.46	75.72
	Max	332.25	283.11	86173.82	1088.39	74	63.67	3586.09	218.93
Clavicle (n=70)	Mean	219.03	175.02	31046.18	635.27	52.23	39.85	1695.9	151.64
	St.dev	60.02	43.35	15363.21	155.28	12.18	8.92	678.89	31.38
	Min	110.21	90.64	7637.73	320.1	26.53	19.98	450.94	81.65
	Max	393.78	286.13	74180.58	1025.29	85.42	58.63	3421.49	220.94
Frontal (n=14)	Mean	215.07	156.66	26420.05	595.39	59.21	43.03	2041.26	166.98
	St.dev	47.55	24.42	9033.55	112.25	8.33	8.6	647.75	24.14
	Min	126.98	114.6	10981.8	379.25	50.11	27.55	1053.82	127.57
	Max	280.28	193.09	41031.63	758.7	81.75	61.61	3930.87	230.57
Parietal (n=25)	Mean	224.96	143.66	25155.1	594.06	55.54	35.98	1705.35	153.76
	St.dev	57.81	37.69	11385.37	138.31	15.41	10.27	775.74	37.14
	Min	120.82	89.64	8323.88	340.42	29.20	16.29	434.87	81.54
	Max	333.77	227.47	53714.43	859.01	87.11	54.74	2987.6	222.21
Occipital (n=31)	Mean	214.17	145.54	25539.27	588.13	57.18	37.05	1755.99	158.06
	St.dev	47.46	33.88	10663.17	123.63	14.47	9.27	764.17	34.89
	Min	146.23	90.39	10550.82	391.19	32.14	19.66	630.15	96.45
	Max	308.54	215.42	48281.24	822.77	86.99	55.52	3347.47	222.33
Petrous (n=4)	Mean	193.41	134.09	20154.11	525.75	47.7	27.83	1157.91	127.82
	St.dev	17.18	14.91	3582.49	44.9	15.68	9.77	677.35	41.44
	Min	172.13	120.03	16431.55	476.08	25.37	17.24	346.24	71.6
	Max	213.61	147.76	23727.91	567.28	60.37	40.78	1980.53	169.24
Rib (n=53)	Mean	177.07	135.68	19707.78	506.08	39.89	31.12	1061.26	117.42
	St.dev	50.28	40.96	10714.75	136.19	10.25	9.22	572.43	30.42
	Min	94.92	68.3	6661.35	298.84	17.5	14.39	219.14	55.08
	Max	328.22	248.99	48832.5	853.92	69.73	50.9	2922.83	199.99

Bone		**On.Dm$_{max}$ (µm)**	**On.Dm$_{min}$ (µm)**	**On.Ar (µm^{2})**	**On.Pm (µm)**	**H.Ca.Dm$_{max}$ (µm)**	**H.Ca.Dm$_{min}$ (µm)**	**H.Ca.Ar (µm^{2})**	**H.Ca.Pm (µm)**
Scapula (n=21)	Mean	192.02	138.38	21748.61	535.95	45.83	33.14	1242.41	130.67
	St.dev	56.87	39.04	12416.37	148.37	8.84	6.6	469.75	25.61
	Min	111.21	79.04	6813.77	311.58	31.61	19.75	544.44	88.94
	Max	351.42	232.35	60628.03	924.58	61.99	46.55	2345.28	181.7
Sternum (n=32)	Mean	161.88	123.29	15822.17	458.63	40.88	30.05	1029.39	117.08
	St.dev	38.18	27.33	6387.68	99.59	13.56	7.64	545.08	32.45
	Min	84.54	66.2	4654.52	249.95	18.89	15.45	235.7	57.45
	Max	242.08	163.03	29449.39	646.71	73.12	45.88	2218.66	187.09
Iliac crest (n=19)	Mean	158.27	119.43	14860.02	450.93	47.22	34.13	1338.9	134.57
	St.dev	40.71	26.62	6267.96	107.06	15.17	8.95	650.6	35.31
	Min	99.94	72.85	6150.99	286.37	22.72	21.22	418.8	75.06
	Max	229.55	162.4	25602.28	617.16	79.79	52.69	2683.24	200.92
Iliopubic ramus (n=45)	Mean	203.33	152.45	25161.87	573.82	53.19	37.26	1685.55	151.02
	St.dev	53.12	43.41	12205.78	147.63	16.47	9.91	854.13	41.25
	Min	85.33	71.96	4472.39	252.11	26.85	17.87	396.4	75.78
	Max	311.9	235.92	53745.11	858.41	91.3	59.73	3693.71	228.35
Ischiopubic ramus (n=10)	Mean	206.64	142.78	24047.66	568.66	50.27	32.89	1347.56	137.06
	St.dev	63.53	33.75	11189.93	150.11	14.99	7.59	551.44	32.71
	Min	101.82	64.26	5839.82	286.45	24.67	17.76	444.12	78.67
	Max	304.43	185.44	43896.02	802.58	81.21	43.83	2108.12	193.35
Mandible (n=35)	Mean	240.5	165.62	33020.97	669.6	54.77	40.55	1888.53	159.34
	St.dev	70.79	42.73	16192.2	180.04	15.36	10.04	946.08	40.77
	Min	124.09	80.58	9017.82	361.57	31.21	22.5	572.68	90.52
	Max	384.96	260.74	68078.28	1004.43	86.37	60.16	3890.45	236.55