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Cortical histomorphometry of the human humerus during ontogeny

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Corresponding Author:	Rosie Pitfield, M.Sc University of Kent Canterbury, Kent UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Kent
Corresponding Author's Secondary Institution:	
First Author:	Rosie Pitfield, M.Sc
First Author Secondary Information:	
Order of Authors:	Rosie Pitfield, M.Sc Justyna J Miskiewicz, PhD Patrick Mahoney, PhD
Order of Authors Secondary Information:	
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Cortical histomorphometry of the human humerus during ontogeny

Rosie Pitfield^a, Justyna J. Miszkiewicz^b, Patrick Mahoney^a

^aHuman Osteology Lab, Skeletal Biology Research Centre, School of Anthropology and Conservation, University of Kent, Canterbury, United Kingdom.

^bSkeletal Biology and Forensic Anthropology Research Group, School of Archaeology and Anthropology, The Australian National University, Canberra, Australia.

Correspondence: Rosie Pitfield

Human Osteology Lab, Skeletal Biology Research Centre,

School of Anthropology and Conservation,

University of Kent, Canterbury. UK. CT2 7NR.

E: rjp41@kent.ac.uk

T: +44 1227 82 3982

Abstract

Modeling and remodeling are two key determinants of human skeletal growth though little is known about the histomorphometry of cortical bone during ontogeny. In this study we examined the density and geometric properties of primary and secondary osteons (osteon area and diameter, vascular canal area and diameter) in sub-periosteal cortical bone from the human humerus ($n=84$) between birth and age 18 years. Sections were removed from the anterior midshaft aspect of humeri from skeletons. Age-at-death was reconstructed using standard osteological techniques. Analyses revealed significant correlation between the histomorphometric variables and age. Higher densities of primary osteons occurred between infancy and seven years of age but were almost completely replaced by secondary osteons after 14 years of age. The geometry of primary osteons was less clearly related to age. Secondary osteons were visible after two years of age, and reached their greatest densities in the oldest individuals. Osteon size was positively but weakly influenced by age. Our data implies that modeling and remodeling are age dependent processes that vary markedly from birth to adulthood in the human humerus.

Key Words

Primary osteon; secondary osteon; modeling; remodeling; humerus, cortex

Introduction

1
2 Current understanding of human cortical bone histomorphometry during ontogeny is limited.
3
4 Histomorphometric studies have explored cortical drift, the gross geometry of the human femur
5
6 [1, 2] and humerus [3-5], and bone remodeling with age in the iliac bone [6]. Recently,
7
8 relationships between secondary osteon area and mechanical loading have been reported for the
9
10 femur, humerus and rib [7]. Following earlier observations that osteon frequency increases
11
12 with age [8], qualitative analyses of rib bone histology have been incorporated into methods for
13
14 reconstructing juvenile age-at-death [9]. Collectively, these studies have begun to characterize
15
16 ontogenetic change in human bone microstructure, and how this variation relates to
17
18 macroscopic indicators of bone growth.
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24 Describing age-related changes in cortical bone tissue may be useful to better understand
25
26 juvenile fracture risk. Fracture risk is partially dependent on bone strength, which in turn is
27
28 dependent on bone composition [10, 11]. Primary bone has different mechanical properties
29
30 compared to secondary bone, which is related in part to differences in microstructure. For
31
32 example, the presence of primary or secondary osteons can affect bone density, and modulus
33
34 of elasticity just prior to failure, which has been linked to different types of childhood fractures
35
36 [10, 11]. Unfortunately, studies describing age-related variation in the histomorphometry of
37
38 primary and secondary osteons in human cortical bone are still limited [6, 12]. Thus we decided
39
40 to explore the density and geometric properties of osteons in human humeral cortical bone
41
42 during ontogeny. For that purpose we used a skeletal collection available in Canterbury, UK.
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47 Our data can provide a new insight into one factor that contributes to juvenile fracture risk.
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Growth of the human humerus

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2 The human humerus attains its adult shape and size through an ontogenetic growth phase that
3
4 encompasses two physiological processes - modeling and remodeling [12]. Modeling largely
5
6 involves the deposition of primary bone tissue onto a cartilaginous model of bone. Primary
7
8 osteons can form when existing blood vessels become entrapped in the bone matrix at the bone
9
10 surfaces during appositional growth [3, 13] and during lamellar compaction [4], when
11
12 trabecular spaces are 'in-filled' to form cortical bone [12]. Unlike modeling, remodeling
13
14 primarily replaces existing bone with new bone through the linked action of osteoclasts and
15
16 osteoblasts (Bone Multicellular Units - BMUs) to produce secondary osteons [14]. Together,
17
18 these processes lead to the attainment of a mostly genetically determined adult bone size and
19
20 morphology [3], though mechanical load, diet, and hormones, amongst other factors, influence
21
22 humeral growth during ontogeny [7]. The spatial distribution of bone tissues changes through
23
24 ontogeny with an overall trend for immature woven bone to be replaced first by primary
25
26 lamellar bone, and then secondary bone in the form of secondary osteons that are surrounded
27
28 by cement lines. Thus we expect primary osteon density to be negatively correlated with age,
29
30 whilst secondary osteon population density should be positively correlated with age.
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39 Previous studies investigating bone modeling in relation to the micro-structure of primary
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41 osteons have been based on experiments in non-human vertebrates such as birds [15], guinea
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43 pigs and monitor lizards [16], and sheep [17]. It has been shown that centripetal osteogenesis
44
45 occurs during primary osteon formation in modeling in bird taxa [18], decreasing the size of
46
47 vascular osteonal canals due to new lamellar bone apposition. It is currently unknown whether
48
49 the few concentric lamellae that can be observed in primary osteons in human juveniles are
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51 formed by centripetal osteogenesis. If this process does occur in humans we would expect to
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53 see a reduction in primary vascular canal size with increasing age.
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1 Bone morphology is partially influenced by mechanical loading. Modeling strengthens
2 bone by increasing cross-sectional area, reducing compressive stress, and by sub-periosteal
3 apposition to increase resistance to bending and twisting [19]. As a result, children who
4 undertake intensive physical activity often develop more robust bones than those who are more
5 sedentary [14]. Over time, stress may cause microscopic cracks (micro-damage) to appear and
6 accumulate in the bone. Targeted remodeling (an estimated 30% of remodeling activity [20])
7 replaces these micro-cracks to maintain mechanical strength [14]. The cement lines of the
8 secondary osteons prevent the spread of micro-cracks. In previous studies of adult bone smaller
9 osteons are correlated with larger strains [21] and advancing age [22]. It is not known when this
10 trend begins and it is possible that secondary osteon size will decrease throughout the juvenile
11 period. Stochastic remodeling is not site dependent and is associated with mineral homeostasis
12 [20]. Both targeted and stochastic remodeling are active through the entire human lifespan and
13 so secondary osteons accrue with age [23].

34 **Histomorphometric measurements of bone growth**

35 Here, we calculate histomorphometric parameters of cortical bone growth for the human
36 humerus, and assess these against age. Osteon population density is a measure of complete and
37 fragmentary secondary osteons per section area, which together represent past remodeling
38 events [24]. Primary osteon density, a feature of bone modeling, particularly in periosteal region
39 of long bones [5, 13], can increase with age [e.g. 25]. The size and shape of primary canals can
40 be an indicator of primary bone deposition during modeling [e.g. 16]. The size and shape of
41 secondary osteons and their vascular canals has also been linked to age [e.g. 24], as well as
42 mechanical stress [e.g. 22], diet [e.g. 26], and health [e.g. 27]. Based upon prior research we
43 predict primary osteon density will decrease with age [5], while secondary osteon population
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1 density will increase [3]. Primary osteon canal size will decrease with age [18], while secondary
2 osteons will become smaller and more circular [22].
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6 **Samples and methods**

7 **Sample**

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10 The study sample comprised eighty-four human juvenile skeletons that did not retain skeletal
11 evidence from pathology, or a healed fracture. Many of these skeletons have accompanying
12 radiographs. Radiographs were produced at Kent and Canterbury Hospital (Radiology
13 Department) for any skeleton with suspected trauma or skeletal pathology. The skeletons had
14 previously been recovered from one cemetery in Canterbury, England, which dated to the 16th
15 century. Historical texts state that the burials were from a single socio-economic group that
16 lived and worked in Canterbury [28]. The skeletons are curated in the Skeletal Biology
17 Research Centre, University of Kent, UK. No permits were required for the present study as
18 these skeletal samples pre-date the Human Tissue Act. All sampling followed appropriate codes
19 of ethics for research conducted on human skeletons [29]. These skeletons have previously been
20 incorporated into a study that examined histomorphometric variables in adult femoral cortical
21 bone [30].
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44 **Age**

45 We used multiple standard osteological methods to reconstruct age-at-death for each skeleton,
46 as the actual biological age of each skeleton was not known. Age-at-death estimations for
47 juvenile skeletons are more accurate than those for adults. We collated several age-at-death
48 estimates using established methods that rely upon the assessment of tooth formation times
49 [31], timing of dental eruption [32], and fusion of cervical vertebrae [33]. Histomorphometric
50 descriptive statistics were subdivided into four age groups, which were: Infant (0-1.9 years,
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n=6), Young Child (2-7.9 years, *n*=42), Older Child, (8-12.9 years, *n*=22) and Adolescent (13-18 years, *n*=14). These age groups roughly correspond to different developmental phases of childhood growth, i.e. the accelerated growth rate in early childhood and adolescence. We were unable to account for any hormonal changes associated with puberty that may affect bone growth during adolescence in this sample as biological sex estimation based upon gross anatomical measurements from sub-adult skeletal remains is not possible.

Sample selection and preparation

One humerus was sampled from each skeleton. The right humerus (*n*=57) was selected based on availability of the midshaft for sectioning, meaning that the left humerus (*n*=27) was only chosen as a substitute when the right side was not preserved. The humerus was selected because the cortical area is large enough to study histologically in perinatal remains. Furthermore, the histomorphometry of the human humerus is less well described in the literature, relative to the femora and ribs [1, 7, 9, 25].

Standard histological methods were used [e.g. 34, 35]. Thin sections were removed from the anterior midshaft region (located by dividing the maximum length - or diaphyseal length where epiphyses were not united with the shaft - of the complete humerus by two). When the humerus was fragmented, the midshaft was located by comparing it to the complete antimeres. Removing anterior sections is less destructive than removing entire cross sections and preserves the bone for future study. All sections were removed using an electronic drill (Dremel Rotary Tool®). Each section removed was approximately 0.7±0.2cm thick. Sections were embedded in resin (Buehler EpoxiCure®), further reduced to 0.3±0.1cm using a Buehler Isomet 4000 precision saw, and fixed to glass microscope slides (Evo Stick® resin). Each section was ground (Buehler EcoMet 300), polished with a 0.3µm aluminum oxide powder (Buehler® Micro-

1 Polish II), cleaned in an ultrasonic bath, dehydrated in 95-100% ethanol, cleared (Histoclear®),
2 and mounted with a coverslip using a xylene-based medium (DPX®).
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6 **Microscopy**

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9 Imaging and histomorphometric procedures followed standard methods [e.g. 30, 35]. Imaging
10 was undertaken using an Olympus BX51 compound microscope with an Olympus DP25
11 microscope camera. Figure 1a illustrates that images were obtained from five regions of interest
12 (**ROIs**) using CELL® Live Biology Imaging software. Each section was divided into locations:
13 medial (**1**), antero-medial (**2**), anterior (**3**), antero-lateral (**4**), and lateral (**5**).
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22 Each ROI within each of these locations was positioned sub-periosteally in the cortex to
23 exclude the endosteal and periosteal surfaces that have non-remodeled interstitial lamellae in
24 juveniles [1]. In addition, given the increased bone resorption in the endosteum and increased
25 bone formation in the periosteum during modeling, bone sites in the subperiosteal region should
26 reflect osteons that can be, at least partly, associated with age related growth of the cortex.
27
28 Because our study examines samples from a series of bones representing individuals of different
29 ages, we acknowledge that the overall size of bone differs inter-individually. However,
30 selecting ROIs in a relatively consistent way allows us to undertake comparisons between data
31 sampled from the same region of the bone. Sometimes the ROI would have to be moved
32 fractionally to avoid diagenesis and taphonomy. However, the ROI would still be within the
33 medial, anteromedial, medial, anterolateral, or lateral compartment of the section (ie., medial
34 would still be medial).
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51 The number of primary osteons, secondary osteons, and secondary osteon fragments were
52 counted in each ROI at a magnification of 10x. Primary osteons (**Fig. 1b**) were identified by a
53 Haversian circular canal that is surrounded by few, if any, circumferential lamellae. Secondary
54 osteons (**Fig. 1c**) were identified by cement lines [13], and fragments were identified as partial
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1 secondary osteons with more than 10 percent of the Haversian canal remodeled by subsequent
2 secondary osteons [25]. The osteon counts formed two density variables, which were calculated
3
4 by dividing the number of osteons by the area of the ROI (2.24mm²):
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- 7 1. Primary Osteon Density: $Pr.On = \#Pr.On./2.24$. In the absence of a cement line, we used
8 the presence of one Haversian primary canal as a proxy for one primary osteon.
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- 10 2. Osteon population density: $OPD = (N.On.+N.On.Fg.)/2.24$. Only osteons that had
11 identifiable cement lines that were over 90% intact were counted as complete secondary
12 osteons. Osteon fragments were identified as partial osteons with more than 10% of the
13 Haversian canal showing evidence of remodeling [25].
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21 Each ROI was subdivided into quarters resulting in four sub-ROIs for the purpose of increasing
22 measurement accuracy. Osteonal geometric properties (**Fig. 1d**) were measured in each
23 subdivision at a magnification of 20x. At this level of magnification the following features were
24 measured in μm^2 :
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- 31 3. Primary Osteons: canal area (Pr.On.Ar), minimum diameter (Pr.On.Dm_{min}), maximum
32 diameter (Pr.On.Dm_{max})
33
- 34 4. Secondary Intact Osteons: area (On.Ar), minimum diameter (On.Dm_{min}), maximum
35 diameter (On.Dm_{max}). Haversian canal area (H.Ar), minimum canal diameter (H.Dm_{min}),
36 maximum canal diameter (H.Dm_{max}). The average value was calculated for each variable,
37 from all ROI's combined, for each individual.
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48 **Statistical Analyses**

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50 Data were analyzed in IBM SPSS® 22 (2014). A one-sample Kolmogorov-Smirnov test
51 indicated that distribution of the data for each variable was normal. Data from right and left
52 humeri were pooled into a single sample. The strength of the relationship between the age of
53 each individual, and each histomorphometric variable, was assessed using correlation and linear
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1 regression statistics. In linear regressions, r values indicate the proportion of variance in the
2 dependant variable that is explained by the independent variable. A residual value is the error
3 not explained by the regression equation. Further comparisons between histomorphometric
4 variables when sub-divided by age groups were undertaken using a non-parametric Kruskal-
5 Wallis test combined with a Dunn-Bonferroni post hoc test.
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11 **Results**

12 **Osteon density and age**

13 Descriptive statistics subdivided by age group are in Table 1. Comparisons between the age
14 groups are in Table 2 and Table 3. Some individuals did not have *both* primary and secondary
15 osteons visible within the ROIs resulting in different sample sizes in Table 2. The density of
16 primary osteons differed significantly between age groups. Post-hoc tests revealed that
17 adolescents had a significantly reduced density of primary osteons compared to all younger
18 childhood age-groups. Older children also had a reduced Pr.On compared to infants. Regression
19 statistics are in Table 4. The density of primary osteons was significantly and negatively
20 correlated with age, decreasing from infants to adolescents (**Fig. 2a**). Primary osteons were
21 almost absent after age 14 years.
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43 Secondary osteon population density differed significantly between the groups.
44 Adolescents had a significantly greater secondary osteon population density compared to
45 younger childhood age-groups. Older children also had a greater OPD compared to the younger
46 childhood age group and infants. In contrast to primary osteon density, the number of secondary
47 osteons was significantly and positively correlated with age (**Fig. 2b**). Secondary osteons were
48 absent until after two years of age, and the greatest density occurred amongst adolescents.
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Osteon size and age

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2 Comparisons between the age groups revealed that the minimum diameter of primary osteon
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4 vascular canals amongst the adolescents was significantly larger, and the maximum diameter
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6 was significantly smaller, compared to all of the younger age groups. Regression analyses
7
8 revealed that primary osteon vascular canal area did not correspond with the age of the juveniles
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12 **(Fig. 3a)**.

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14 Secondary osteons of adolescents had a significantly greater area, and their maximum
15
16 diameter was significantly larger, relative to the younger childhood age group. Haversian canals
17
18 of adolescents also had a significantly smaller minimum diameter, and larger maximum
19
20 diameter, compared to the other younger childhood age groups. Secondary osteon area increased
21
22 significantly with age **(Fig. 3b)**, which was associated with an increase in maximum diameters.
23
24 Regression analyses revealed that the area of secondary osteons was also significantly and
25
26 positively correlated with the density of secondary osteons ($r=0.63$; $p=0.00$). The minimum and
27
28 maximum diameters of secondary osteon Haversian canal area increased with age, though the
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30 residual associated with these correlations was high.
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Discussion

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43 Here, we examined the density and geometric properties of both primary and secondary osteons
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45 in cortical bone from the anterior mid-shaft region of the human humerus. We have expanded
46
47 upon recent research into the histology of the humerus [3, 5, 7], by assessing parameters of
48
49 cortical bone modeling and remodeling between birth and 18 years of age. Our study shows
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51 that the density of both osteon types is linked to age, but the relationship between age and
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53 osteon geometry is more complicated.
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Age related change in primary osteons

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2 As primary osteons form around vascular canals by becoming entrapped by lamellar bone tissue
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4 during growth, our data suggest that modeling at the periosteal envelope producing primary
5
6 osteons may peak by seven years of age. In our sample, there were hardly any primary osteons
7
8 remaining in the sub-periosteal region of the humeri of the eldest children. Neither did the size
9
10 of the primary vascular canals change greatly with age. At present, our understanding of
11
12 primary osteon formation in humans is still limited, but based on previous experimental
13
14 research on animals it is thought that primary vascular canals are ‘in-filled’ by bone deposition
15
16 [16]. Therefore, it might be expected that primary osteon canal area would decrease with
17
18 advancing age if they form in the same way in human cortical bone. Our data do not support
19
20 this idea, though there are several reasons why the association between canal area and age may
21
22 not be present in our sample. Relatively decreased canal size might become apparent when
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24 compared within one age group, rather than when compared between children of different age.
25
26 Prior research on adult cortical bone also reports inconsistent association with age [22, 36].
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Age related change in secondary osteons

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37 Primary osteons are gradually replaced by secondary osteons through remodeling [34],
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39 resulting in an increase in the number and density of secondary osteons as bone ages. The
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41 correlation between an increasing density of secondary osteons and advancing age is well
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43 documented in adult bone [8, 24], and has previously been confirmed in the juvenile humerus
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45 [4]. However, our data reveal that secondary osteons were present in early childhood from two
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47 years of age, and after 14 years of age the subperiosteal region of the anterior mid-shaft humerus
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49 is composed of mainly secondary osteonal bone. This finding is similar to results reported for
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51 the femur [25], where secondary osteons were also present in children between the age of 6 to
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53 10 years. Cambra-Moo and colleagues [3] report that Haversian bone accounted for 47.37%
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1 and 78.67% respectively of the entire cortical area of the humeri of two juveniles aged between
2 10 and 20 years.
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4 Approximately three quarters of childhood fractures occur in the upper limbs [37].
5 Fracture risk varies with age but seems to peak during early adolescence [37]. The change from
6 primary and secondary bone, to predominantly secondary osteonal bone at around 14 years of
7 age in our data (Fig 4a-b), could be one factor that contributes to the increased fracture risk of
8 adolescents relative to younger children. For example, cement lines of secondary osteons have
9 been shown to be sites of weakness [38] and remodeled bone is more brittle than primary
10 lamellar bone [11]. The presence of mainly secondary osteonal bone from 14 years of age also
11 coincides with peak bone mineral accrual rates (females=13yrs, males=14ys) reported in a
12 study of Canadian children and young adults [39].
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26 Our results for the geometric properties of secondary osteons in the humerus are similar to
27 those reported for the ilium [6]. Generally, the size of secondary osteons and their vascular
28 canals did not change greatly with age, relative to the relationship between the density of
29 secondary osteons and age. When the size of osteons changed, there was substantial variation
30 between individuals, which has been reported previously [36]. However, some trends can still
31 be discerned. Secondary osteons become larger with age ($r=0.40$) with a clear increase in their
32 maximum diameter ($r=0.52$). When compared between the age groups, mean secondary osteon
33 area of $40239.09\mu\text{m}^2$ and maximum diameter of $364.09\mu\text{m}$ for adolescents was significantly
34 greater compared to the younger childhood age group (area= $30844.68\mu\text{m}^2$, max
35 diameter= $265.91\mu\text{m}$). These values are within the range of mean osteon areas ($20184\mu\text{m}^2$ to
36 $64391\mu\text{m}^2$) reported for iliac cortices of juveniles, aged between birth and 25 years, [40].
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38 However, in a sample of juvenile transiliac sections there was no significant correlation
39 between osteon diameter and age [6]. This difference between studies may represent underlying
40 age-related differences in remodeling activity between the humerus and ilium, or population
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1 differences. Intra-skeletal variation in osteonal bone has been recently observed in a juvenile
2 skeletal sample [7]. Alternatively, it might reflect the different measurements employed in the
3
4 two studies. Rauch and colleagues [6] measured one diameter dimension, but our data showed
5
6 a correlation between age and maximum diameter.
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9 We observed a significant trend in the dimensions of secondary Haversian canals, when
10 compared between age groups. Vascular canals of secondary osteons become more irregular
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12 with increasing age. This is because canal minimum diameters became smaller, ranging from
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14 26.82 μm in young children to 19.19 μm in adolescents, while maximum diameter becomes
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16 larger, ranging from 72.63 μm in young children to 99.47 μm in adolescents. There was no
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18 associated change in canal area with age. Contrary to the results presented here, Rauch and
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20 colleagues [6] found no significant correlation between secondary vascular canal diameter and
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22 age. Although, as with secondary osteon diameters, only one measure of diameter was taken
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24 rather than maximum and minimum dimensions.
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31 Secondary osteon size is determined by the amount of bone that is removed by osteoclasts
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33 [25]. In principle, the wider the resorption diameter the larger the resulting osteon will be.
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35 Osteoblasts subsequently deposit bone within the osteon from the cement line inwards towards
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37 the center, determining the size of the Haversian canal [41]. Thus, the area and diameter of
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39 osteon and Haversian canal transverse surfaces viewed in thin sections should be an indicator
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41 of BMU activity. Larger osteons of older children may indicate a slower rate of BMU activity
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43 because it takes longer to remove a large area of bone and deposit more lamellae [42], or, it
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45 may reflect an increase in osteoclast resorption resulting in ‘tunneling’ larger osteons composed
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47 of larger vascular canals. These age-related changes in the morphology of secondary osteons in
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49 juveniles exhibit a different pattern compared to those of adults.
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55 As discussed previously, in adult bone, the change in the size of secondary osteons in
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57 relation to age is still not fully understood [22], especially as variation in osteon geometric
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1 properties may relate to the type of load that bone is exposed to. Smaller and more circular
2 osteons have been linked to compressive forces, and larger and less circular osteons have been
3 shown to correspond to tensile forces [43]. If these findings are applied to our data, then perhaps
4 bone remodeling in the subperiosteal region of the anterior humerus was influenced by tensile
5 load during adolescence in response to upper arm physical activity. We were unable to
6 investigate the effect of mechanical loading on bone histology in our skeletal sample, which
7 could have contributed to the variation in secondary osteonal structure with age.
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17 Alternatively, the apparent increase in maximum diameter of secondary osteons may be
18 the result of an accumulation of mature drifting osteons. Drifting osteons are a type of
19 secondary osteon with continuous resorption and continuous formation at opposite sides of the
20 osteon, resulting in a transverse elongation in the horizontal plane [44]. Early histological
21 studies found that drifting osteons occur most commonly in sub-adult bone [45]. Indeed,
22 drifting osteons have been observed in the juvenile humerus [4], rib [9], and femur [1].
23 However, the biomechanical implications of drifting osteons in juvenile cortex remain poorly
24 understood [44].
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36 When the structure and density of secondary osteons are considered together, it suggests
37 that adolescents have a greater number of these bone functional units, which are slightly larger
38 with more irregular vascular canals, relative to younger children and infants. Although some
39 characteristics of primary and secondary osteons are age-related, other factors will also exert
40 an influence. Differences in secondary osteon structure and density between studies imply that
41 there is substantial variation between human populations, and so in order to expand our
42 understanding of human humeral bone growth further research is needed to gain more
43 representative sampling from different human populations to establish the range of bone
44 microstructural variability.
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There are several limitations within our study. The sample was undocumented so age-at-death was estimated using multiple standard osteological methods. Even though age-at-death estimations for juvenile skeletons are more accurate than those for adults, these estimates may have introduced variation into our analyses of histomorphometry and age. The uneven sample sizes in each age group in this study reflect the pattern of infant mortality in medieval Canterbury. Additionally it is possible that aspects of cortical bone growth have changed since the medieval period. Future studies on known age and sex populations can expand upon our study.

Conclusion

This study has shown that the density of primary and secondary osteons is markedly age related in the human humerus, but the relationship between the geometric properties of bone modeling and remodeling units and age is more complicated. Future studies might further explore how other factors affect the age-related change in juvenile bone microstructure by examining sex, puberty, biomechanics, or diet to reveal broader perspectives into skeletal growth.

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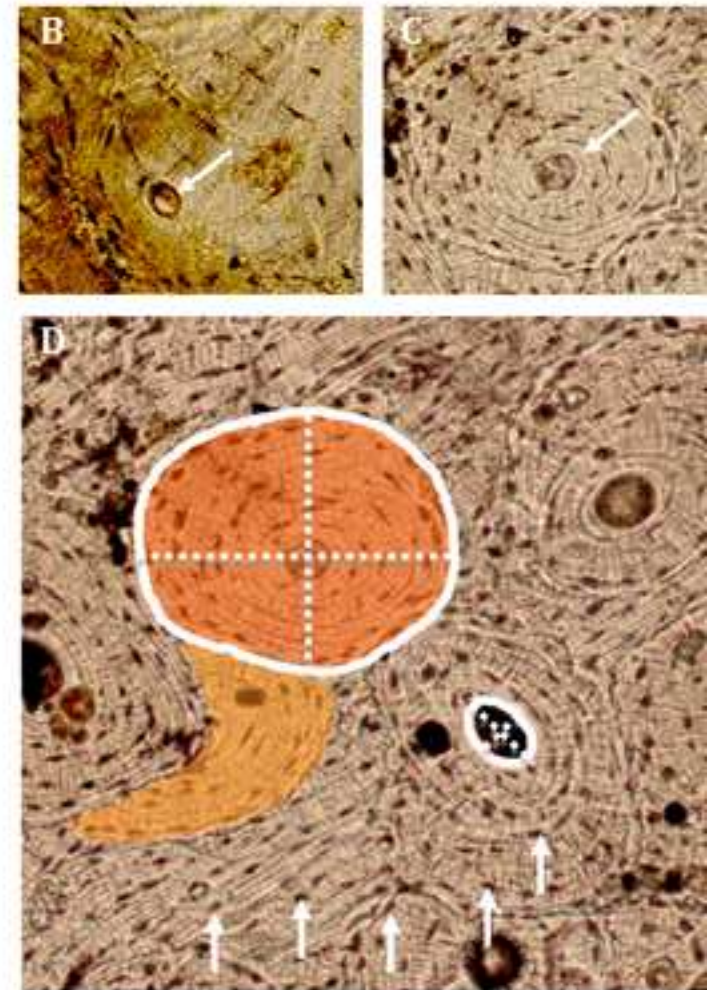
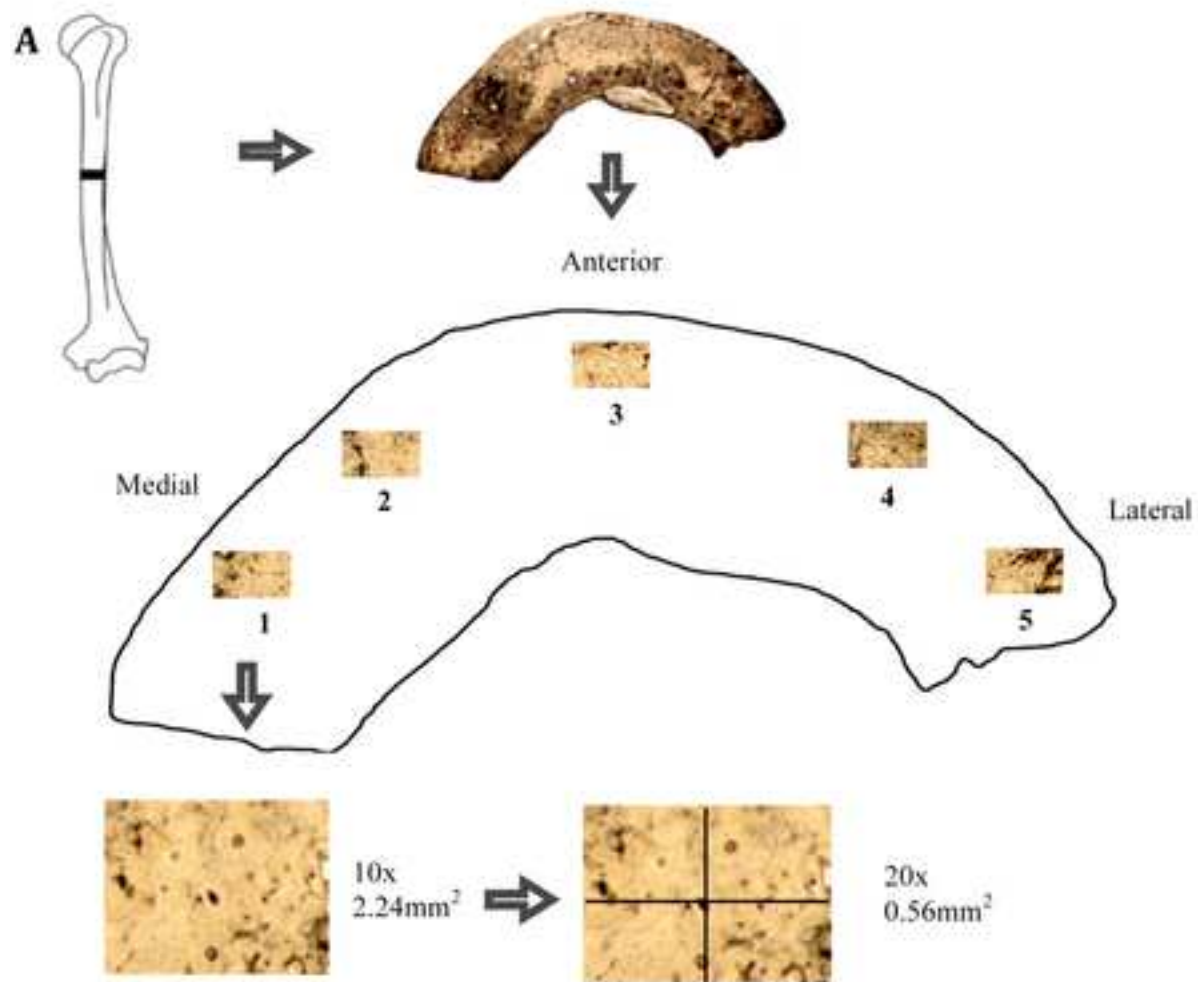
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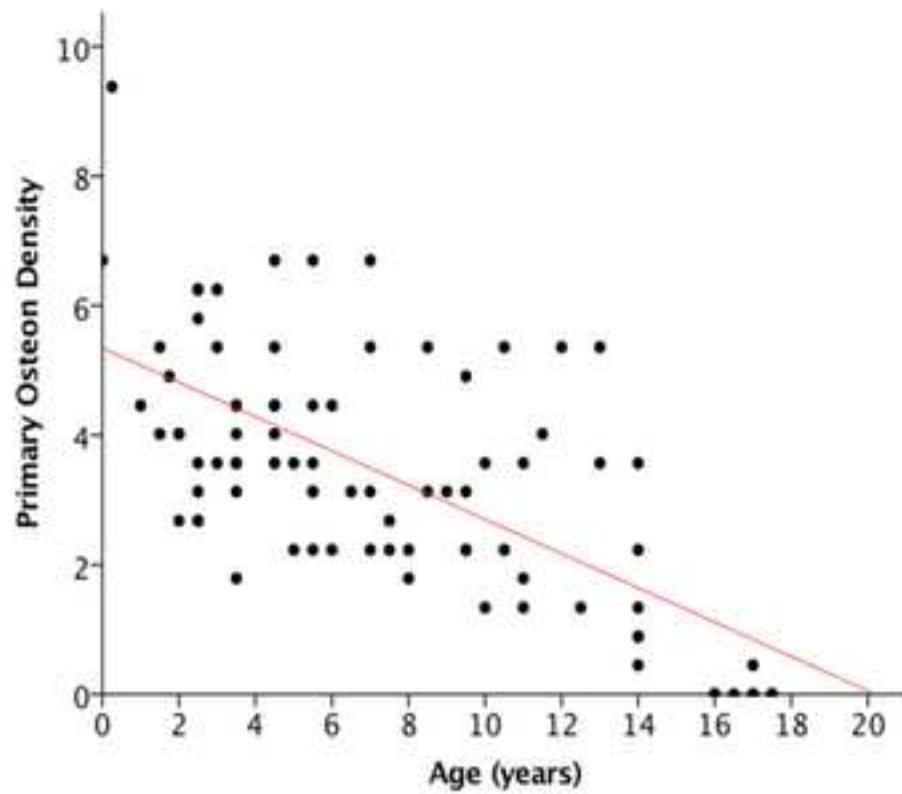
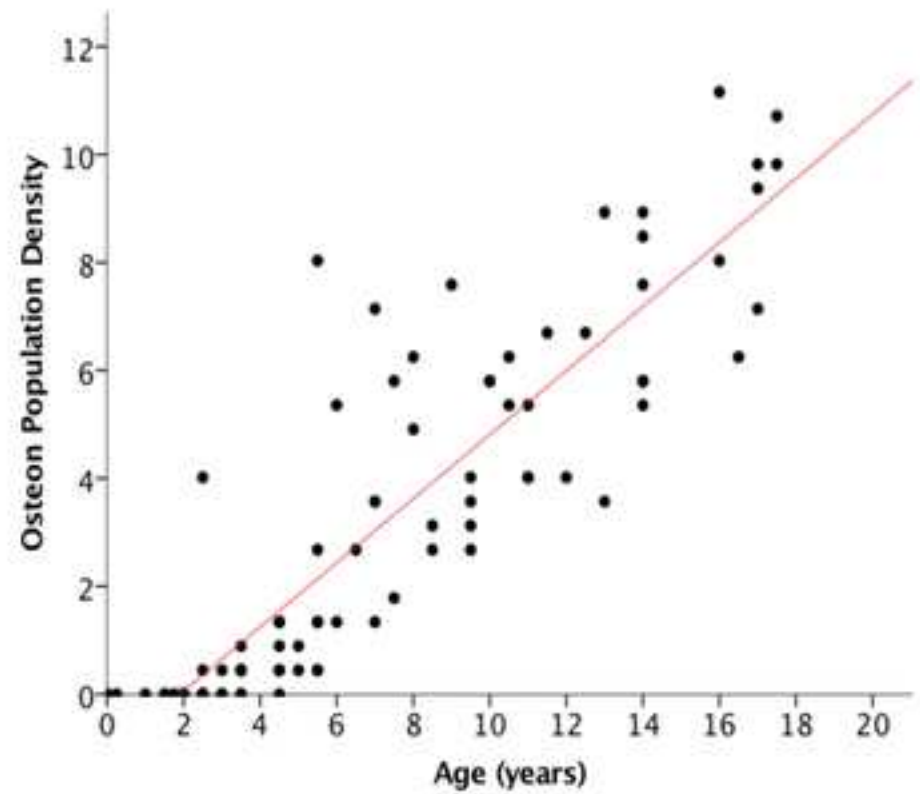
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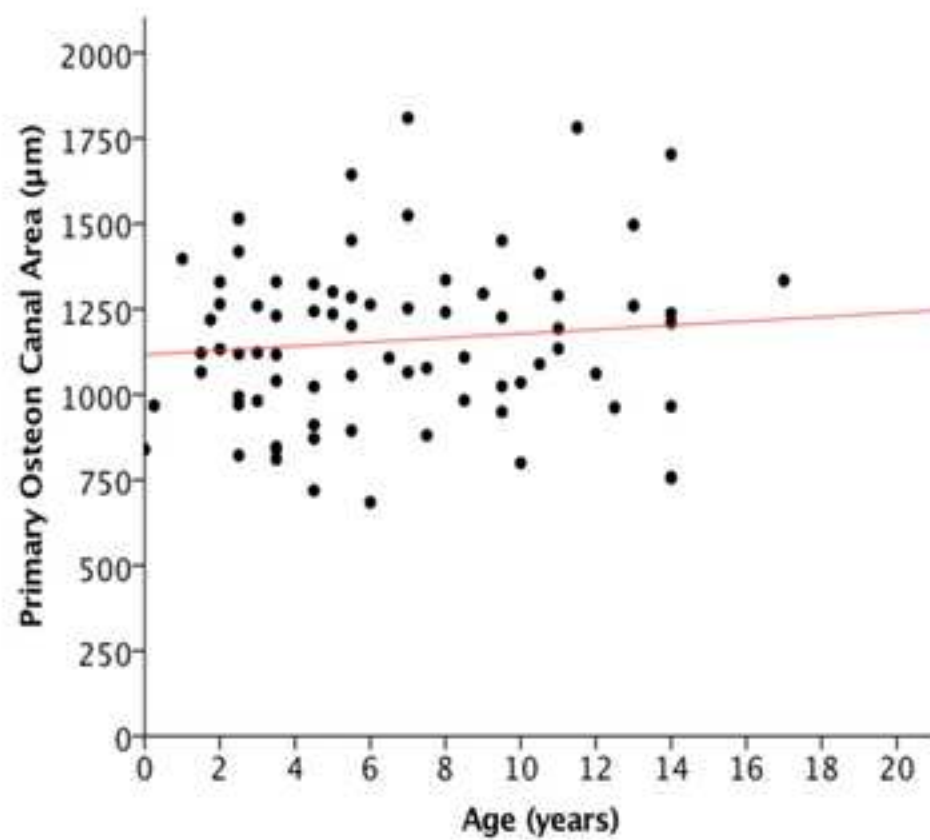
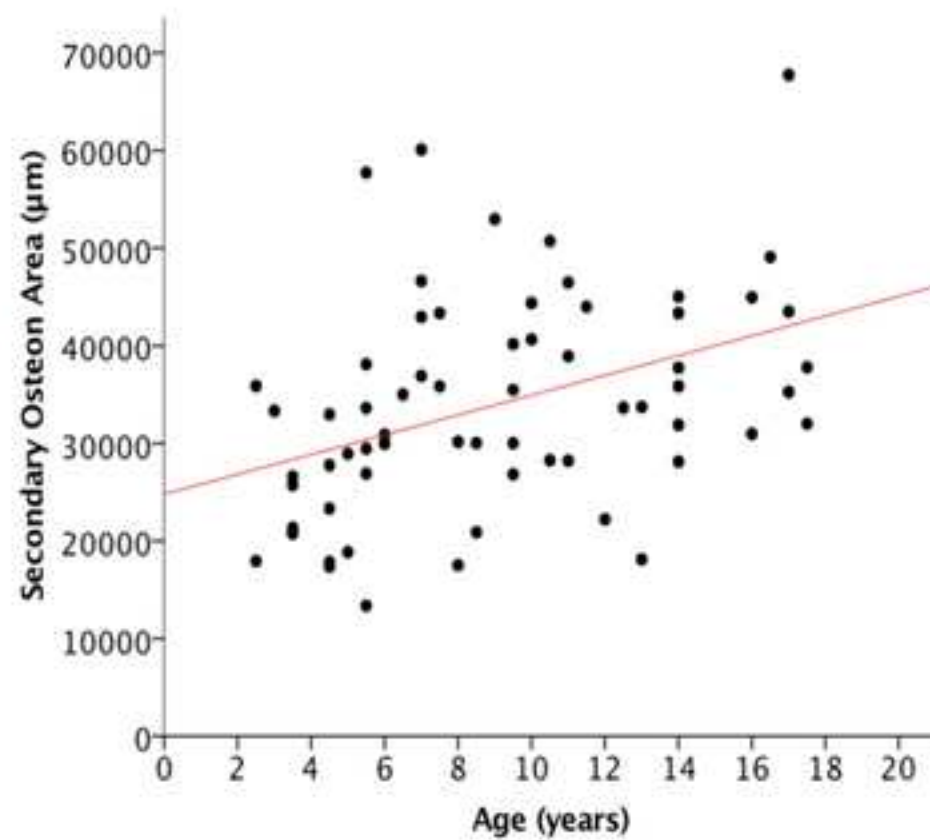
1 **Fig. 1 (a)** Anterior mid-shaft humerus region with five regions of interest (1-5), used for
2 counting primary and secondary osteons (10x), and measuring osteonal structure (20x). Primary
3 osteon **(b)** and secondary osteon **(c)** micrographs (20x magnification). White arrows point to
4 vascular canals. **(d)** Micrograph (20x magnification) illustrating the different histology
5 variables examined in the present study. Areas highlighted in orange indicate an intact (top)
6 and a fragmentary (bottom) secondary osteon (N·On, N·On·Fg, and OPD). White circles
7 indicate the secondary osteon and canal areas measured (On·Ar, and H·Ar). Dashed lines
8 indicate minimum and maximum diameters of secondary osteon (top) and canal (bottom)
9 (On·Dm_{max}, On·Dm_{min}, H·Dm_{max}, H·Dm_{min}). White arrows indicate osteocyte lacunae (Ot·Dn)
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14 **Fig. 2 (a)** Plot of primary osteon density, and **(b)** secondary osteon population density, against
15 the age of the children. Linear regression lines are fitted to the data. Regression equations for
16 cortical bone are in Table 1
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20 **Fig. 3 (a)** Plot of primary osteon canal area, and **(b)** secondary osteon area, against the age of
21 the children. Linear regression lines are fitted to the data. Regression equations for cortical bone
22 are in Table 1
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(a) Primary osteons**(b) Secondary osteons**

(a) Primary osteons**(b) Secondary osteons**

1 **Table 1** Histomorphometric descriptive statistics subdivided into age groups

		Infant	Young child	Older child	Adolescent
		birth to 1.9	2 to 7.9	8 to 12.9	13 to 18
Histomorphometric variable		<i>n</i> = 6	<i>n</i> = 42	<i>n</i> = 22	<i>n</i> = 14
Number of Primary Osteons	Mean	13.00	9.00	7.05	1.57
	SD	4.43	3.08	3.15	2.38
Number of Secondary Osteons	Mean	0.00	2.76	9.36	14.79
	SD	0.00	3.72	3.40	3.66
Number of Secondary Osteon Fragments	Mean	0.00	0.36	1.45	3.50
	SD	0.00	0.98	1.10	1.91
Primary Osteon Density	Mean	5.47	4.01	3.14	0.70
	SD	1.31	1.37	1.40	1.06
Secondary Osteon Population Density	Mean	0.00	1.39	4.82	8.16
	SD	0.00	2.02	1.79	1.91
Primary Canal Area	Mean	1101.75	1157.88	1179.45	1137.66
	SD	194.89	252.97	226.38	339.37
Primary Canal Minimum Diameter	Mean	17.86	18.90	18.78	23.540
	SD	4.08	2.97	4.07	4.83
Primary Canal Maximum Diameter	Mean	69.72	67.61	66.04	49.06
	SD	14.10	14.66	13.25	6.42
Secondary Osteon Area	Mean		30844.68	34059.09	40239.09
	SD		11450.67	10029.61	10102.21
Secondary Osteon Minimum Diameter	Mean		129.78	124.00	121.84
	SD		23.96	27.81	18.07
Secondary Osteon Maximum Diameter	Mean		265.91	317.63	364.09
	SD		80.67	72.70	59.58
Secondary Canal Area	Mean		1876.84	1827.63	1842.21
	SD		772.85	415.09	539.36
Secondary Canal Minimum Diameter	Mean		26.82	23.69	19.19
	SD		9.22	4.25	2.33
Secondary Canal Maximum Diameter	Mean		72.63	80.97	99.47
	SD		21.88	14.74	20.63

2 **Table 2** Histomorphometric variables compared between the age groups¹

Variable	n	X²	df	p
<i>Density</i>				
Pr.On	84	5.34	3	0.000*
OPD	84	54.931	3	0.000*
<i>Size</i>				
Pr.On.Ar	77	0.579	3	0.901
Pr.On.Dm _{min}	76	10.628	3	0.014*
Pr.On.Dm _{max}	76	12.559	3	0.006*
On.ar	64	8.158	2	0.017*
On.Dm _{min}	64	1.430	2	0.489
On.Dm _{max}	64	13.890	2	0.001*
H.ar	64	0.393	2	0.822
H.Dm _{min}	64	12.096	2	0.002*
H.Dm _{max}	64	13.388	2	0.001*

3 1=Age groups and descriptive statistics are in Table 1

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16 **Table 3** Post-hoc tests¹ of the significant histomorphometric variables in Table 2

	Statistic	<i>p</i>	Statistic	<i>p</i>
DENSITY	Pr.On		OPD	
Adolescent v Older child	28.013	0.004*	-16.146	0.050*
Adolescent v Younger child	40.405	0.000*	-44.405	0.000*
Adolescent v Infant	59.452	0.000*	-63.464	0.000*
Older child v Younger child	12.392	0.316	-22.259	0.000*
Older child v Infant	31.439	0.030*	-47.318	0.000*
Young child v Infant	19.048	0.435	19.060	0.427
GEOMETRIC	Pr.On.Dm_{min}		Pr.On.Dm_{max}	
Adolescent v Older child	-31.901	0.001*	28.810	0.002*
Adolescent v Younger child	-25.238	0.005*	30.355	0.001*
Adolescent v Infant	-26.310	0.032*	34.595	0.004*
Older child v Younger child	5.952	1.000	1.546	0.792
Older child v Infant	4.881	1.000	5.786	0.566
Young child v Infant	-1.071	1.000	4.240	0.656
	On.Ar		On.Dm_{max}	
Adolescent v Older child	-10.123	0.117	-11.162	0.080
Adolescent v Younger child	-17.456	0.005*	-22.250	0.002*
Older child v Younger child	-7.332	0.170	-11.088	0.037*
	H.Dm_{min}		H.Dm_{max}	
Adolescent v Older child	17.256	0.006*	-14.234	0.025*
Adolescent v Younger child	20.679	0.001*	-22.286	0.000*
Older child v Younger child	3.153	0.552	-8.052	0.129

17 1=Dunn-Bonferroni post hoc test. *P value <0.05 significant

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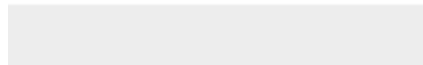
22 **Table 4** Linear regression analyses of the relationship between histomorphometric variables
 23 and age

Variable¹	<i>N</i>	Intercept	Slope	<i>R</i>	<i>P</i>	Residual
Pr.On	84	5.34	-0.26	-0.67	0.00*	51%
OPD	84	-1.12	0.59	0.88	0.00*	22%
Pr.On.Ar	77	1172.20	6.08	0.10	0.48	97%
Pr.On.Dm _{min}	77	17.56	0.24	0.27	0.03*	92%
Pr.On.Dm _{max}	77	69.99	-0.66	-0.19	0.11	96%
On.Ar	64	25973.00	837.02	0.40	0.00*	84%
On.Dm _{min}	64	130.91	-0.53	-0.09	0.45	99%
On.Dm _{max}	64	215.97	9.78	0.52	0.00*	72%
H.Ar	65	1702.90	8.17	0.08	0.58	96%
H.Dm _{min}	65	27.64	-0.48	-0.38	0.02*	81%
H.Dm _{max}	65	61.00	2.23	0.45	0.00*	80%

24 1 = See methods section for definitions. *P value <0.05 significant.



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