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TITLE: Bone histomorphometric measures of physical activity in children from Medieval England

RUNNING TITLE: Bone histomorphometry of medieval children

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ABSTRACT:

Objectives: Histomorphometric studies show consistent links between physical activity patterns and the microstructure underlying the size and shape of bone. Here we adopt a combined bone approach to explore variation in microstructure of ribs and humeri related to physical activity and historical records of manual labor in skeletal samples of children (*n*=175) from medieval England. The humerus reflects greater biomechanically induced microstructural variation than the rib which is used here as a control. Variation in microstructure is sought between regions in England (Canterbury, York, Newcastle), and between high- and low-status children from Canterbury.

Materials and Methods: Thin-sections were prepared from the humerus or rib and features of bone remodeling were recorded using high-resolution microscopy and image analysis software.

Results: The density and size of secondary osteons in the humerus differed significantly in children from Canterbury when compared to those from York and Newcastle. Amongst the older children, secondary osteon circularity and diameter differed significantly between higher and lower status children.

Discussion: By applying bone remodeling principles to the histomorphometric data we infer that medieval children in Canterbury engaged in less physically demanding activities than children from

York or Newcastle. Within Canterbury, high-status and low-status children experienced similar biomechanical loading until around seven years of age. After this age low-status children performed activities that resulted in more habitual loading on their arm bones than the high-status children. This inferred change in physical activity is consistent with historical textual evidence that describes children entering the work force at this age.

KEY WORDS: Histomorphometry; Social status; Osteon; Juvenile; Behavior

1 | INTRODUCTION

Studies of bioarcheology in recent years have inferred aspects of childhood behavior and physical activity from skeletal remains (Lewis, 2016; Mays, Gowland, Halcrow, & Murphy, 2017; Shapland, Lewis, & Watts, 2015). However, juvenile skeletons are often poorly preserved and fragmented when recovered from the archaeological record. Previous histomorphometric studies have inferred behavior and patterns of physical activity from fragments of human skeletons (Miszkiewicz, 2016; Miszkiewicz & Mahoney, 2016), within the context of evidence that has linked physical activity to bone microstructure (Hedgecock et al., 2007; Skedros, Hunt, Hughes, & Winet, 2003; Skedros, Keenan, Williams, & Kiser, 2013; Skedros, Sybrowsky, Parry, & Bloebaum, 2003). Several studies have described age-related changes in the juvenile rib or humerus (C. M. Maggiano, Maggiano, Tiesler, Chi-Keb, & Stout, 2016; Pitfield, Miszkiewicz, & Mahoney, 2017; Streeter, 2010) or used a 'combined bone' approach that incorporated the rib, humerus, and femur, to investigate the effect of biomechanics and metabolism on bone microstructure during ontogeny (Eleazer & Jankauskas, 2016). Here we calculate histomorphometric measures of bone microstructure for the humerus and rib from skeletal samples of children that date to the English medieval period. Variation in their microstructure is related to textual evidence that describes behavior and manual labour for children from this period.

1.1 | Childhood lifestyles in Medieval England

Several aspects of medieval lifestyles could potentially influence the bone microstructure of growing children, including social status and environment, diet, and behavior (Eleazer & Jankauskas, 2016; Miszkiewicz & Mahoney, 2016; Specker & Vukovich, 2007). Medieval England had a hierarchical socio-economic structure that dictated medieval lifestyles, with a particular influence over diet, occupation and physical activity, and disease risk (Dyer, 2000). Poverty can have a more significant impact on childhood growth than any other environmental factor (Tanner & Eveleth, 1976). Poverty could affect both rural and urban populations in medieval England. Canterbury and York were diverse urban centers that attracted migration from the surrounding areas (Lyle, 2002; Schofield & Vince, 2003), but the population of the Northgate area of Canterbury and the Fishergate area of York were predominantly poor. However, there was a minority group of wealthier individuals living in Northgate, Canterbury who were of a higher socioeconomic status. The modern city of Newcastle did not exist in the early medieval period when the Black Gate cemetery was in use, so the origin of these skeletons is uncertain but it is likely that they were from a rural population (Nolan, 2010; Swales, 2012).

Childhood growth profiles were similar in rural and urban medieval places, as were incidences of skeletal manifestations of stress, such as hypoplastic enamel defects, maxillary sinusitis, cribra orbitalia, and non-specific indicators of stress (Lewis, 2002). However, there is evidence that maternal stress was more common in urban areas than in rural areas (Lewis, 2002). The prevalence of non-specific infection and specific infection, including treponemal disease, leprosy, and tuberculosis were similar in urban and rural populations of older children and adolescents (Lewis, 2016). However, spinal and joint disease was more common for urban adolescents, which could indicate that these people engaged in more strenuous physical activity than their rural counterparts (Lewis, 2016).

In addition to regional differences, social status could influence childhood growth and disease (Bennike, Lewis, Schutkowski, & Valentin, 2005). There is dental evidence that non-specific stress during childhood and adolescence could vary according to social status. High-status people in medieval Canterbury had significantly fewer enamel indicators of non-specific stress than low-status people (Miszkiewicz, 2015). This indicates that, while children of both high- and low-status experienced physiological stress during growth, the higher social status of some children provided a buffer against some of these stressors. The high-status children were more likely to have better health than low-status children due to better living conditions and diets.

Everyday life and diet were broadly similar for younger children from different regions of England, but it could vary greatly with socioeconomic status for older children. Historical records report that weaning began between 7-9 months of age (Fildes, 1986) and isotopic studies have shown that medieval children were entirely weaned by 2 years old (Burt, 2013; Haydock, Clarke, Craig-Atkins, Howcroft, & Buckberry, 2013; Mays, Richards, & Fuller, 2002). Dental macroscopic and microscopic wear analyses suggest that the physical properties of diet for children up to approximately 8-10 years old was similar when compared between different geographic locations and between children of different social status (Dawson & Brown, 2013; Mahoney et al., 2016). Despite this similarity in one aspect of childhood diet, social status determined the quality, variety, and type of adult diet (Woolgar, Serjeantson, & Waldron, 2006). Various studies using different methodologies have indicated a change in diet occurred at approximately 7-10 years old, after which time a more adult-like diet was consumed (Macpherson, 2005; Mahoney et al., 2016). The historical and archaeological evidence shows that after weaning at 2 years old, there was a transitional period of several years before children were consuming an adult diet at approximately 7-10 years of age. At this time, children began to be treated like adults and consume an adult diet that was dependent on their social status.

Medieval children spent more time with their parents until the age of 4 and were given greater independence between the ages of 8-12 years (Hanawalt, 1977). In rural areas, boys of this age from lower-status families commonly worked as shepherds, mill hands, reapers or servants, and girls worked gathering wood, child-minding, or in the fields at harvest time (Hanawalt, 1977). This was also the age at which the children of crafting, mercantile, or landed families would begin to learn the necessary skills to carry on the family trade (Fleming, 2001). Some adolescents would migrate to nearby towns and cities for employment, particularly after the Black Death (Goldberg, 1986). In urban areas, some high-status boys would attend school (Hanawalt, 1977), or go into apprenticeships as grocers, mercers, or merchant tailors (Ben-Amos, 1994). Lower-status children could be apprenticed to craftsmen as weavers, carpenters, builders, farriers, and smiths (Ben-Amos, 1994). More prestigious apprenticeships were longer lasting and more expensive (Fleming, 2001). Many low-status adolescents, whose families could not afford an apprenticeship, would gain work and lodgings as a household servant on yearly contracts (Fleming, 2001). Female household servants were usually employed as unskilled domestic servants rather than in a craft or trade (Goldberg, 1992). The occupations of low-status people usually involved more physically demanding manual labor than the occupations of high-status people.

Few historical records of childhood physical activity exist, and it is unknown if physical activity varied between children from different geographic locations. However, historical records show that adult occupation varied across regions of England. The area surrounding Newcastle was a politically unstable border area between Scotland and England in the 10th and 11th centuries (Rollason, 2003). The population were subjected to ongoing scorched earth and siege campaigns, whilst also undertaking intensive farming to provide for themselves and the occupying armies (Lomas, 1996). York and Canterbury were both major medieval cities, but each had a different character. The Fishergate area of York was a center of industry and manufacturing in the medieval period, with a particular emphasis on textiles (Nicholas, 2014; Palliser, 2014). The Northgate area of Canterbury was a relatively poor area of the city, but the local people could be employed by the wealthy religious houses in domestic jobs or construction (Lyle, 2002). A medieval English person's experience of manual labor could vary according to age, social status, and geographic area. If the regional differences in occupation caused sufficiently different patterns of loading on the skeleton, it is possible that this may be reflected in bone microstructure, during later childhood after the children had taken on their more adult-like roles.

1.2 | The archaeological sites

St Gregory's Priory (1084-1537 AD). Canterbury Archaeological Trust excavated the site of St Gregory's Priory, Canterbury, between 1988 and 1991 (Figure 1) (Hicks & Hicks, 2001). During this time, 1342 articulated skeletons were retrieved (Anderson & Andrews, 2001). St Gregory's Priory was located just outside the city walls of Canterbury, Kent and it was in use as a burial ground between 1084AD and 1537AD. High status lay people could pay for burial within the Priory, alongside the members of the clergy. Juvenile skeletons excavated from graves within the foundations of the Priory were likely members of high-status families and juvenile skeletons buried in the surrounding cemetery were members of the lower-status lay community. Given that this archaeological site has two socioeconomic groups, it is an ideal population to study whether bone microstructure varies according to social status in children.

Fishergate House (1399-1539 AD) and All Saint's Church (1091-1539 AD). Fishergate House, York, was excavated between 2000 and 2002 (Figure 1) (Spall & Toop, 2005). During this time, 244 articulated skeletons were retrieved. It has been suggested based on documentary evidence that the cemetery would have belonged to St Helen's Church. St Helen's Church and Hospital was founded in Fishergate in 1399AD, but its exact location is unknown (Holst, 2005). While St Helen's may be the source of the burials, it remains uncertain, and there has been no archaeological evidence of a church found at the Fishergate House site (Holst, 2005).

All Saints' Church, York (Figure 1), more commonly known as Fishergate Barbican, was excavated between 2007 and 2008 (McIntyre & Bruce, 2010). During this time, 667 articulated skeletons were retrieved. The first documentary evidence for a church at the site dated to 1091 and 1095 AD. The church did not survive long after 1539 AD, and its location was lost as the parish was merged with the parish of St. Lawrence in 1586 A.D. The shroud burials and lack of grave goods indicate that the population of Fishergate were low-status.

Black Gate cemetery (c. 800-1168 AD). The Black Gate cemetery, Newcastle, was excavated between 1973 and 1992 (Figure 1). There were 663 articulated skeletons excavated from 660 burials between 1977 and 1992, representing the largest group of Christian Anglo-Saxon burials excavated in Northeast England (Nolan, 2010; Swales, 2012). Radiocarbon dating of burials indicates that the cemetery was established around 800 AD (Nolan, 2010; Swales, 2012). However, there was no documented settlement at the site at that time, but some dateable archaeological finds, such as coins, indicate that people were in the area in the eighth and ninth centuries AD. It is possible that the cemetery was linked to an unrecorded seventh-century monastic settlement (Nolan, 2010). By the tenth century, the cemetery was well established and was most likely serving a local lay population. Analysis of oxygen isotopes suggests some degree of migration in this population (Macpherson, 2005). All of the burials are most likely Christian, based on the east-west burial orientation, and the lack of grave goods (Nolan, 2010).

1.3 | Bone biology

Ontogeny of Humerus. The humeri begin to develop *in utero*. Primary ossification centers appear for the diaphyses between the 8th-9th week of gestation (Noback & Robertson, 1951). Three secondary ossification centers appear at the proximal end and coalesce between 5-7 years, and four secondary ossification centers appear at the distal end and coalesce between 10-12 years (Cunningham, Scheuer, & Black, 2016). Bone modeling thickens the cortices and modeling drift moves the humerus diaphysis postero-medially during childhood (Maggiano, Maggiano, Tiesler, Chi-Keb, & Stout, 2015). The distal epiphyses typically fuse around 11-18 years with the medial epicondyle fusing separately at approximately 13-18 years, followed by the proximal epiphyses at around 14-21 years (Cunningham et al., 2016).

Ontogeny of Ribs. Ribs begin to develop *in utero* through chondrofication followed by ossification. Primary ossification centers appear at the posterior angle between the 8th-12th week of gestation (Noback & Robertson, 1951). Bone modeling thickens the cortices and modeling drift ensures the endosteal area remains in the center of the diaphysis while the ribs move ventrally during childhood as the thorax expands (Streeter, 2010). Rib growth is complete between 15-24 years of age (Cunningham et al., 2016).

Microstructure. Primary vascular canals are formed when blood vessels are incorporated into the periosteal cortex during bone modeling (Parfitt, 1983). They contain few or no lamellae and do not have a boundary cement line (Currey, 2002). Remodeling differs to modeling because existing bone is removed prior to the deposition of new bone only in remodeling. During remodeling, osteoblasts and osteoclasts are coupled in a basic multicellular unit (BMU) that produces a basic structural unit of bone, also known as a secondary osteon (R. B. Martin, Burr, Sharkey, & Fyhrie, 1998). Each BMU follows a defined sequence: Activation – Resorption – Formation. The activation phase is prompted by the disruption of an inhibitory signal from local osteocytes (Burger & Klein-Nulend, 1999). Osteoclasts within a cutting cone remove a core of existing bone during the resorption phase (Vaananen, Zhao, Mulari, & Halleen, 2000). This results in a resorption cavity that is bounded by a cement line separating the osteon from the surrounding bone. The action of the osteoclasts determines the diameter of the cutting cone and of the future secondary osteon, which is usually between 150μm and 350μm (van Oers, Ruimerman, Tanck, Hilbers, & Huiskes, 2008). The resorption cavity will subsequently by infilled with concentric rings of lamellae during the formation phase. Osteoblasts, lining a closing cone, deposit osteoid and calcium phosphate crystals at the cement line and deposition proceeds towards the central Haversian canal (R. B. Martin et al., 1998).

As remodeling occurs, existing osteons are intercut by new osteons and fragments of the osteons accumulate in the bone and cause an age-related increase in OPD (Stout & Paine, 1992). Targeted remodeling replaces areas of strain induced micro-cracks and cause higher OPDs in those regions (Burr, 2002). The number of intact and fragmentary osteons can be combined as osteon population density (OPD) to give an indication of bone turnover rate since secondary osteons are the product of bone remodeling. OPD is linked to age (Goliath, Stewart, & Stout, 2016; Pfeiffer, Heinrich, Beresheim, & Alblas, 2016; Stout & Paine, 1992), and biomechanics (Britz, Thomas, Clement, & Cooper, 2009; Young, Niklowitz, Brown, & Jee, 1986), as well as diet (Richman, Ortner, & Schulter-Ellis, 1979), and health (Martin & Armelagos, 1979). Osteon density and osteon morphology are closely linked, and high OPDs are often associated with smaller osteons (Miszkiewicz, 2016). Osteon size and shape are quantified as osteon area, diameter, and circularity (On.Ar, On.Dm, On.Cr). Generally, On.Ar, On.Dm, Haversian canal area (H.Ca.Ar), and Haversian canal diameter (H.Ca.Dm) are inversely related to biomechanical strain magnitude (van Oers, Ruimerman, van Rietbergen, Hilbers, & Huiskes, 2008). Osteon morphology can provide an indication of the type of loading on a bone which may be used to infer aspects of behavior (Miszkiewicz & Mahoney, 2016).

Cortical bone microstructure is influenced by a) age, b) metabolism, and c) biomechanics (Eleazer & Jankauskas, 2016; Goldman, McFarlin, Cooper, Thomas, & Clement, 2009; Specker & Vukovich, 2007). Here we use a ‘combined bone’ approach to infer population level differences in factors affecting systemic remodeling. and biomechanical loading induced local remodeling (Eleazer & Jankauskas, 2016). When age is accounted for, the biomechanical forces that affect the ribs do not vary substantially between individuals because the ribs are non-weight bearing and the biomechanical effect of breathing is consistent between people (Tommerup, Raab, Crenshaw, & Smith, 1993). Accordingly, due to the limited inter-personal variation in biomechanical forces affecting the ribs, they reflect systemic bone remodeling (Agnew & Stout, 2012). Whereas the biomechanical forces that affect the humerus can vary between people to a greater extent, since physical activity and behavior can vary between people. The humerus reflects both systemic bone remodeling and greater biomechanically induced microstructural variation (Eleazer & Jankauskas, 2016). Here, we study the cortical bone microstructure of the humerus and rib within the context of behavior and manual labour practices described in textual evidence.

1.4 | Research questions and predictions

1. *Is there a difference in remodeling in the humerus compared to the rib?* – Since the ribs are reported to have a higher remodeling rate than the long bones (Frost, 1969) we expect the rib to show evidence of greater remodeling as an increased OPD, intact osteon density (N.On), and osteon fragment density (N.On.Fg).

2. *Are there age-related differences in remodeling of the humerus, or the rib?* – Osteons accumulate with advancing age (Goliath et al., 2016; Pfeiffer et al., 2016; Stout & Paine, 1992). Given this, we expect to see this in an increase in OPD, N.On, and N.On.Fg in older children compared to younger children. Osteon size decreases with advancing age (Britz et al., 2009; Dominguez & Agnew, 2016) and we expect to see this in a reduction in On.Ar, On.Dm, H.Ca.Ar, and H.Ca.Dm in older children compared to younger children. We expect to see the same age-related pattern in both bones since age has a systemic effect on the skeleton.

3. *Does the histomorphometry of the humeri and rib vary between children of different social status in Medieval England?* – Historical and archaeological evidence indicates that children could transition to a different and sometimes more active lifestyle at around 7-8 years of age, when they engaged in occupations determined by social status. Low-status children could sometimes undertake more physically strenuous work than high-status children (Ben-Amos, 1994). We hypothesize that older children and adolescents from the low-status group will show more strain induced remodeling in their humeri, as smaller and more dense osteons, than the older children and adolescents from the high-status group. We do not expect to see differences in rib microstructure when compared between groups of different social status. However, there could be differences in the rib microstructure if the health and nutritional status of the low-status children was poor enough that the energetic demands of normal bone remodeling could not be met.

4. *Does the histomorphometry of the humeri and rib vary between children from different locations in Medieval England?* – It is possible that there will be a change in the microstructure of the humerus when compared between the samples of older children, if there are regional differences in the type of occupation and physical activity undertaken. There is historical and archaeological evidence for regional variation in adult occupation in medieval England (Lomas, 1996; Lyle, 2002; Nicholas, 2014; Palliser, 2014). If children from different regions follow a similar trend, then those that are more physically active might have higher habitual loading on the humerus and show more strain induced remodeling than juveniles from a less active region. This would manifest as smaller and more dense secondary osteons. We do not expect to see any significant differences in the rib microstructure between regional groups, unless the health and nutritional status of a population was poor enough that the energetic demands of normal bone remodeling could not be met.

2 | MATERIALS AND METHODS

2.1 | Study Sample

This study included 175 medieval juvenile skeletons excavated from three locations in England (Figure 1). St Gregory's Priory and Cemetery, Canterbury *n* = 125 (high-status *n* = 19 and low-status *n* = 116), York *n* = 33 (Fishergate House *n*= 20 and All Saint's Church *n* = 13), and The Black Gate Cemetery, Newcastle *n* = 17. No permits are required for the present study as these skeletal samples pre-date the Human Tissue Act, and all of the sampling followed the appropriate codes of ethics for research conducted on human skeletons (Mays, Elders, Humphrey, White, & Marshall, 2013).

2.2 | Age-at-death Estimation

Age estimations were based on multiple standard methods for estimating age in sub-adult skeletal remains. These methods were the assessment of tooth formation times (Moorrees, Fanning, & Hunt, 1963), the timing of dental eruption (Al Qahtani, Hector, & Liversidge, 2010), and epiphyseal union timing (Scheuer, Black, & Christie, 2000). Each skeleton was assigned to one of the following age groups; younger child (3–7 years, *n* = 71), older child (8–12 years, *n* = 52), or adolescent (13–18 years, *n* = 52).

2.3 | Histomorphometric methods

The rib was selected as it is less influenced by physical activity patterns, it is a non-weight bearing bone and as such has limited inter-skeletal variation in respiration induced loading (Tommerup et al., 1993). Accordingly, ribs reflect systemic bone remodeling (Agnew & Stout, 2012), whereas the humerus reflects both systemic bone remodeling and greater biomechanically induced microstructural variation (Eleazer & Jankauskas, 2016). Here we use the rib as a control for inferring physical activity related changes in the cortical microstructure of the humerus. A single bone section was removed from each skeleton, from either the humeri (*n*=102) or the rib (*n*=73). The right humerus was preferentially chosen (*n*=70), but the left humerus was sampled when the right was damaged, pathological, or absent (*n*=32). The rib samples were taken from the middle third of un-sided 3rd-8th ribs.

Transverse sections of a 90° orientation to the long axis of the shaft were removed from either the anterior mid-shaft region of the humerus or complete sections of the mid-shaft rib using a Dremel Rotary Tool®. The location of the humerus sections were standardized by finding the mid-shaft at 50% of the maximum length – or diaphyseal length where epiphyses were not united with the shaft – of the complete humerus (Pitfield et al., 2017). When the humerus was fragmented, the midshaft was located by comparing it to the complete antimere. The location of the rib sections were standardized by finding the mid-shaft at 50% of the length between the tubercle and the sternal end (Agnew & Stout, 2012). Each section was approximately 0.7±0.2cm thick. Thick-sections were embedded in epoxy resin (Buehler EpoxiCure®), reduced to 0.3 ±0.1cm thickness using a Buehler Isomet 1000 precision saw and fixed to glass microscope slides (Evo Stick® resin). Each section was ground to a final thickness of 50-100µm (Buehler EcoMet 300), polished with a 0.3µm Al_2O_3 powder (Buehler® Micro-Polish II), cleaned in an ultrasonic bath, dehydrated in 95% and 100% ethanol, cleared (Histoclear®), and mounted with a coverslip using a xylene-based mounting medium (DPX®).

2.4 | Microscopy

An Olympus BX51 microscope and an Olympus DP25 camera were used to collect images from five regions of interest (ROIs) from each humerus thin-section. The histological variables, with their abbreviations and definitions, appear in Table 1. The anterior humerus cortical width (Ct.Wi, mm) was measured from the endosteum to the periosteum at the most anterior part of the humeri. The rib thin-sections had much smaller cortical areas, so the entire cortex was imaged and stitched together into a montage. The total rib subperiosteal area (Tt.Ar, mm^2) and endosteal area (Es.Ar, mm^2) were measured, and the cortical area was calculated (Ct.Ar, mm^2 = Tt.Ar – Es.Ar). Each ROI within the humerus was positioned sub-periostealy in the cortex to exclude the endosteal and periosteal surfaces. The rib thin-sections were divided into two ROIs, one for the pleural cortex and one for the cutaneous cortex. Histomorphometry was performed using CELL® Live Biology Imaging software.

The number of primary vascular canals, secondary osteons, and secondary osteon fragments were counted in each ROI. Primary vascular canals were identified by the presence of a vascular canal with no associated cement line (Currey, 2002). Primary vascular canal density (Pr.Ca.Dn) was calculated by dividing the number of primary vascular canals by the area of the ROI (Humerus = $2.24mm^2$. Rib = pleural cortical area or cutaneous cortical area). Secondary osteons were identified by the presence of a complete cement line and intact Haversian canals, and fragments were identified as partial secondary osteons (Currey, 2002). Osteons were included if they were within or touching the ROI boundary (Britz et al., 2009). These osteon counts formed the osteon population density (OPD), which was calculated by dividing the number of osteons and fragments by the area of the ROI (Humerus = 2.24mm^2. Rib = pleural cortical area or cutaneous cortical area).

In both the humerus and rib, secondary osteon structure was quantified in each ROI (Figure 2) by measuring the osteon area (On.Ar, μm^2), diameter (On.Dm, μm), and perimeter (On.Pm, μm), and the Haversian canal area (H.Ca.Ar, μm^2), and diameter (H.Ca.Dm, μm). Osteon circularity (On.Cr) was calculated from the area and perimeter measurements using the equation $4 * \pi * (On.Ar)/(On.Pm^2)$ (Dominguez & Crowder, 2012). Values closer to 1 indicate more circular osteons, and values closer to 0 indicate more elliptical osteons.

2.5 | Analyses

All statistical analyses were performed in IBM SPSS® 24 with the Type 1 error alpha value set at $p < 0.05$ for all tests. Differences between the left and right humeri were tested with Mann-Whitney *U* tests. The normality of each variable was assessed with Shapiro-Wilks tests, and any not normally distributed variables were transformed by square root for the count variables (Pr.Ca.Dn, OPD, N.On, N.On.Fg) or log10 for the measurement variables (Ct.Wi, Ct.Ar, On.Ar, On.Cr, On.Dm, H.Ca.Ar, H.Ca.Dm). Mann-Whitney *U* tests were used to test for differences in microstructure between the rib and humerus and between high-status and low-status children because of the unequal sample sizes. Non-parametric Kruskal Wallis tests, combined with Dunn-Bonferroni post-hoc analyses, were used to test for interpopulation differences in the microstructure of each bone. Finally, a discriminant function analysis (DFA) was performed to classify the juveniles to a population (either Canterbury, York, or Newcastle) based on their bone microstructural properties.

3 | Results

There were no significant differences between right and left humeri, so the sample of humeri were pooled for all further analyses. Shapiro-Wilks tests indicated that the transformed variables met the normality assumption.

3.1 | Variation in microstructure between the rib and humerus

Descriptive statistics for the histomorphometric variables of each bone are shown in Table 1. The Mann-Whitney *U* results are in Table 2. Each variable, except Pr.Ca.Dn, On.Ar and On.Cr, differed significantly between the rib and the humerus, when all ages were pooled. Osteon density was higher in the ribs than in the humeri (OPD, N.On, and N.On.Fg) and osteons were larger in the ribs than in the humeri (On.Ar and On.Dm). However, Haversian canals (H.Ca.Ar) were smaller in the ribs compared to the humeri.

3.2 | Age-related variation in microstructure

Descriptive statistics for the histomorphometric variables are split by age category and bone type and are shown in Table 2. Kruskal Wallis tests (Table 3) revealed that there are significant differences in the microstructure of the humerus, when compared between the age groups. The Dunn-Bonferroni post-hoc tests show significant changes between the age groups. The variables Ct.Wi (all $p<0.05$) and OPD (all $p<0.01$) increased from the younger children aged 3 to 7 years to the older children aged 8 to 12 years, and to the adolescents aged 13 to 18 years, and also from the older children to the adolescents. Whilst the variable Pr.Ca.Dn (all $p<0.01$), decreased between each age group (from young children to older children, from young children compared to adolescent, and from older child to adolescent). The variables N.On (both $p<0.01$), and N.On.Fg (both $p<0.01$) also increased from the younger children to the older children aged 8 to 12 years, and to the adolescents aged 13 to 18 years. The On.Ar of the humerus was significantly larger in the adolescents compared to the younger children ($p<0.05$).

Kruskal Wallis (Table 3) showed additional significant differences in the rib microstructure when compared between the age groups. The Dunn-Bonferroni post-hoc tests show significant increases between every age group (from young children to older children, from young children compared to adolescent, and from older child to adolescent) in Ct.Ar (all $p<0.05$) and OPD (all $p<0.05$), and decreases between every age group in Pr.Ca.Dn (all $p<0.01$). The variable N.On (both $p<0.01$) increased from the younger children to the older children, and from the younger children to the adolescents. The variable N.On.Fg also increased between the younger children and the adolescents and between the older children and the adolescents (both $p<0.05$).

3.3 | Social Status and microstructure of the humerus and rib

Descriptive statistics are shown for the humerus microstructure of a high-status group and a low-status group from medieval Canterbury in Table 4 alongside Mann-Whitney *U* results. Individuals from the adolescent group were not tested because of the low sample number in the high-status group. None of the histomorphometric variables from the humeri differed significantly between the high-status and low-status children when the young children and older children were pooled, or when just the young children were considered. Osteons were significantly more circular ($p<0.05$) with larger diameters ($p<0.05$) in the humeri of high-status compared to low-status older children between the ages of 8-12 years, though sample sizes were very small.

There were no significant differences in histomorphometric variables from the ribs when sub-divided by age-group and compared between high-status and low-status children in Canterbury.

3.4 | Bone microstructure compared between the regions

Descriptive statistics are shown for the microstructure of the humerus of each population in Table 5. Kruskal Wallis tests (Table 5) revealed significant differences in the microstructure of the humerus, but not the ribs, when compared between the regions (Humeri: OPD $p<0.01$; On.Dm, $p<0.01$; H.Ca.Dm $p<0.01$). Dunn-Bonferroni post-hoc tests show that OPD of the humerus is significantly lower in juveniles from Canterbury than from York ($p<0.01$) or Newcastle ($p<0.01$). The On.Dm of the humerus is significantly lower in juveniles from Canterbury than from York ($p<0.01$) or Newcastle ($p<0.01$). The H.Ca.Dm of the humerus was significantly lower in juveniles from Canterbury than from York ($p<0.01$) or Newcastle ($p<0.01$). This result was consistent within each age group, except there was no significant difference in OPD among the adolescents from different regions.

A DFA was performed with the regional groups as the dependent variable and OPD, On.Dm, and H.Ca.Dm as predictor variables. The analysis produced two discriminant functions, but the first function, which was composed of the variable H.Ca.Dm, was the most successful at discriminating between the dependent variables (Table 6). The DFA assigned 83.7% of cases to the correct regional group. The Eigen value of 3.11 and the *U* (canonical correlation) value of 0.87, together with the visual representation for the result in Figure 3A, confirms the good separation between the groups. When the analysis was repeated, and the sample was split by age group, the DFA successfully classified 82.4% of younger children (Figure 3B), 87.5% of older children (Figure 3C), and 88.5% of adolescents (Figure 3D).

A second DFA was performed with the regional groups as the dependent variable, but with Canterbury split according to social status, and OPD, On.Dm, and H.Ca.Dm as the predictor variables. The analysis produced three discriminant functions. The DFA classified 72.8% of cases to the correct regional group (Table 7). The Eigen value of 3.21 and the *U* (canonical correlation) value of 0.87, together with the visual representation for the result in Figure 4A, confirms the good separation between the groups. When the sample was split by age group, the discriminant function successfully classified 79.4% of younger children (Figure 4B), 75.0% of older children (Figure 4C), and 76.9% of adolescents (Figure 4D).

4 | Discussion

4.1 | Variation in microstructure between the humerus and rib

For all age groups combined, and when each age group was considered separately, osteons were larger in the ribs than in the humeri (On.Ar and On.Dm). but the Haversian canals were smaller (H.Ca.Ar) though they had larger diameters. This means that the osteons of the rib were larger, and more bone was deposited during infilling resulting in smaller canals, than the osteons in the humerus. The small Haversian canal area but large Haversian canal diameters could suggest that the canals in the rib are less circular than the canals in the humerus. This morphological variation could be due to a difference in the levels of strain that affected the ribs compared to the humerus. Smaller osteons are associated with larger strains (van Oers, Ruimerman, van Rietbergen, et al., 2008). When applied to our findings, this suggests that the humerus experienced larger habitual strain than the ribs even though the ribs are in constant motion during respiration throughout life. Secondary osteon appearance can also relate to targeted remodeling that repairs microdamage and maintains bone structural integrity (Burr, 2002). Thus, osteon size may vary intra-skeletally according to differences in the habitual loading environment of each bone. Studying the microstructure of the humerus and rib together may therefore provide a way of accessing information about physical activity. The humerus is relatively more liable to be affected by physical activity patterns than the rib.

4.2 | Age-related variation in microstructure

Primary vascular canal density decreased between the younger children and older children and then decreased again between the older children and the adolescents in both the humerus and the ribs of the children from all populations pooled together. Secondary osteons and secondary osteon fragments accumulated with advancing age in the humerus and the ribs of the children from Canterbury, York and Newcastle, when pooled together. All of the osteon count variables (OPD, N.On, N.On.Fg) in both bones increased significantly from younger children to the older children and again to the adolescents. This age-related feature of bone remodeling has been well described previously for adults (Cho, Stout, Madsen, & Streeter, 2002; Pfeiffer et al., 2016; Stout & Paine, 1992) and children (Goldman et al., 2009; Pitfield et al., 2017; Streeter, 2010). Osteons accumulate in bones because of targeted remodeling to repair microdamage and nontargeted remodeling for mineral homeostasis (Burr, 2002) and the primary vascular canals are removed by this remodeling (Pitfield et al., 2017). The mean age for the birth of adult compacta in the rib is 12.5 years, which could explain some of the differences observed in the rib histology between the children and the adolescents (Frost & Wu, 1967).

Osteon area was significantly larger in the adolescent humeri than in the humeri of the younger children. Yet, in adults, osteon area is negatively correlated with age (Britz et al., 2009; Dominguez & Agnew, 2016). The opposite relationship seen in the juveniles of this study could be accounted for by changes to bone size. The anterior humerus cortical width also increased significantly between the younger children and the adolescents, and the larger osteons of adolescents were associated with these thicker cortices. A similar scaling pattern has been noted in the adult tibia (Goldman, Hampson, Guth, Lin, & Jepsen, 2014) and adult rib (Dominguez & Agnew, 2016). There may be a site-specific balance between limiting the spread of microdamage and maintaining a low porosity for optimum bone stiffness (Goldman et al., 2014) and minimal skeletal weight (R. B. Martin, 2003), and a thicker cortex may support the formation of larger osteons (Dominguez & Agnew, 2016). The complicated relationship between bone dimensional structure and bone functional adaptation needs further exploration, especially in relation to the interplay between modeling and remodeling during ontogeny.

4.3 | Social Status and microstructure

Humerus microstructure was not related to social status amongst the younger children from Canterbury. Amongst the older children age eight to twelve years, osteon circularity and diameter differed significantly between those of higher and lower status from Canterbury. The low-status older children had smaller (On.Ar, On.Dm) and less circular osteons (On.Cr) than the high-status older children. Smaller osteons are linked to higher strains (van Oers, Ruimerman, van Rietbergen, et al., 2008), indicating a response to greater habitual loading. These results indicate that high-status and low-status children did activities that led to broadly similar levels of strain in their upper limbs in early childhood, but physical activity patterns differed after seven years of age. Historical records report that from around the age of seven onward children became more independent and could begin work, or apprenticeships outside of their family homes (Hanawalt, 1977). The bone microstructural data support this change in habitual physical activity for older children. The microstructural data also indicate that this was around the age that differences in social status began to have a noticeable influence on the lives and activities of mediaeval children in Canterbury. There were higher levels of biomechanical loading for the low-status children, which is compatible with the idea that they had started to become involved in more physical forms of manual labor from around the age of seven or eight years of age onwards. The low-status people in medieval Canterbury were probably employed in relatively more strenuous manual labor including domestic jobs, construction, trading, or farming (Lyle, 2002). Whilst the high-status children were likely to be involved in relatively less strenuous occupations (Ben-Amos, 1994), which in medieval Canterbury would have meant learning the skills needed to become members of the clergy, land holders, or business owners (Lyle, 2002).

4.4 | Regional variation in bone microstructure

The humerus cortical bone microstructure of the children varied between Canterbury, York, and Newcastle. Children from Canterbury had lower OPDs and smaller osteon and Haversian canal diameters in their humeri than children from York or Newcastle. However, rib cortical bone microstructure did not vary between these regions. This pattern was consistent for the whole sample, and when each of the three age groups when tested separately to account for age-related variation.

Our results imply that there was regional variation in the factors that affect localized bone remodeling in the humerus. Differences in the microstructure of the humeri only, and none in the rib, supports the idea that the children of all ages from Canterbury engaged in less strenuous behavior or physical activity than children from York or Newcastle. Differences in the intensity or type of physical activity result in differences in habitual loading, which in turn cause differences in the habitual strain magnitude and mode that affects the humerus. Increased OPD is linked to increased strain magnitudes (van Oers, Ruimerman, Tanck, et al., 2008; van Oers, Ruimerman, van Rietbergen, et al., 2008) and larger osteon diameters are linked to strain mode. On this basis, we can infer that humeri of children from medieval Canterbury experienced lower habitual strain magnitudes than the humeri of children from medieval York or Newcastle. This suggests that children from Canterbury engaged in activities that were less physically demanding on their upper arms, compared to children from medieval York or Newcastle.

Microstructural variation increases with age when compared between regions. This indicates that the habitual loading and the behavioral differences causing the loading became larger, or else changed, as the children became older. If this can be attributed to the start of manual or skilled labor in the older children and adolescents (Hanawalt, 1977), it would imply that people in York and Newcastle undertook more physically demanding occupations than people in Canterbury. This idea is supported by historical records that show a general north-south divide in lifestyles in medieval England (Jewell, 1991, 1994). The population of the region surrounding modern day Newcastle were living in a politically unstable area and had to undertake physically demanding intensive farming to provide for themselves and the occupying armies (Lomas, 1996). The population of Fishergate York would have been involved in heavy industry and manufacturing, with a particular emphasis on textiles (Nicholas, 2014; Palliser, 2014). These occupations would have involved relatively more physically demanding manual labor than the common occupations for the people of Northgate Canterbury, which included religious orders, land holding, or business owning for the high-status people, and domestic jobs, construction, trading, or low-intensity farming for the low-status people (Lyle, 2002).

While OPD was found to increase with age, this study found no significant relationship between age and On.Dm or H.Ca.Dm, indicating that our findings are not attributable to aging. Additionally, when the social status of the children from Canterbury was taken into account, it showed that the difference between high-status and low-status children from Canterbury was less than the difference between children from Canterbury (with their status pooled) when compared to the children from York and Newcastle. This indicates that while low-status children in Canterbury were more physically active and undertook more intense manual labor tasks than their high-status contemporaries, all children from Canterbury, regardless of status, were less physically active than the children from York or Newcastle, who, according to historical records, may have undertaken more intense manual labor (Lomas, 1996; Lyle, 2002; Nicholas, 2014; Palliser, 2014). This implies that social status had less influence on bone microstructure than where a child lived in medieval England.

Recent studies have related bone microstructural properties to bone gross morphology (Goldman et al., 2014; Miszkiewicz & Mahoney, 2018). Robusticity and cortical width have been linked to larger secondary osteons in the adult human tibia (Goldman et al., 2014) and rib (Dominguez & Agnew, 2016) but to smaller secondary osteons and Haversian canals in the human femur (Miszkiewicz & Mahoney, 2018). Scaling analysis has shown negative allometry between humerus On.Ar and adult body size and humerus H.Ca.Ar and adult body size (Felder et al., 2017). These relationships between microstructure and macrostructure cannot explain the difference found in the current study because the children from York and Newcastle did not have thicker cortices than the children from Canterbury. The population-level difference in microstructure seen here was not attributable to a difference in the dimensional bone adaptation, but it can be attributed to bone functional adaptation.

5 | Conclusions

This study applied histomorphometric methods to bone thin-sections from medieval English children. Results were interpreted though principles of bone remodeling within the context of historical records that describe behavior and manual labor. Population specific variation in microstructure was detected in the humeri of the children, but not their ribs. Children from Canterbury had a lower osteon population density and smaller osteon diameters than children from York and Newcastle. It was inferred that these differences in microstructure related to different loading environments caused by different physical activity levels and manual labor practices during life. Both high-status and low-status children from Canterbury had a lower level of habitual loading and were likely to have been less physically active with their upper limbs than children from York and Newcastle. The historical evidence supports the histological findings, since children from Canterbury could have undertaken less strenuous forms of manual labor than the children from York or Newcastle. High-status and low-status children from Canterbury experienced similar biomechanical loading until around seven years of age. After this age low-status children performed activities that resulted in more habitual loading on their bones than the high-status children. The historical evidence describes children taking on more adult-like occupations after eight years of age. Taken together, our findings imply that social status and habitual physical activity from manual labor influenced the bone microstructure of children who lived and worked in medieval England.

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FIGURE LEGENDS

FIGURE 1 Map displaying the locations of the archaeological sites in England. St Gregory's Priory and Cemetery, Canterbury is south-east. The Black Gate Cemetery, Newcastle is north. Fishergate House and All Saint's Church, York are central

FIGURE 1 Bone histomorphometry in one ROI (2.24mm^2, 10×). The area bounded in dark blue indicates an intact osteon, and the area highlighted in blue indicates a fragmentary secondary osteon (N.On, N.On.Fg, and OPD). The area bounded in light blue indicates a Haversian canal, and the dark structure to the left of the canal is a Volkmann's canal. Measurements of osteon structure (20×) – the circles indicate the secondary osteons (dark blue) and Haversian canals (light blue) measured (On.Ar, On.Pm, and H.Ca.Ar). The dashed lines indicate the diameters of secondary osteons (dark blue) and Haversian canals (light blue) (On.Dm, H.Ca.Dm)

FIGURE 3 Discriminant function analysis demonstrated how well the calculated functions discriminated (separation is shown by the group centroids) between the populations (Canterbury, York, and Newcastle). A) all ages, E (Eigen value) = 3.11, U (canonical correlation) = 0.87, B) 3-7 year olds, E = 1.61, U = 0.79, C) 8-12 year olds, E = 6.00, U = 0.93, D) 13-18 year olds, E = 23.53, U = 0.98

FIGURE 4 Discriminant function analysis demonstrated how well the calculated functions discriminated (separation is shown by the group centroids) between the populations (Canterbury high-status, Canterbury low-status, York, and Newcastle). A) all ages, E = 3.21, U = 0.87, B) 3-7 year olds, E = 1.63, U = 0.79, C) 8-12 year olds, E = 6.67, U = 0.93, D) 13-18 year olds, E = 24.07, U = 0.98