Title: Secondary Osteon Variants and Remodelling in Human Bone

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Abstract:

Histomorphometric analysis of human cortical bone has documented the occurrence of secondary osteon variants. These include drifting osteons which form tails as they move erratically through the cortex and type II osteons which show partial resorption and redeposition within the cement line of the osteon. Little is known about the biological significance of these variants. Prior studies suggested correlations with age, biomechanics, diet, and mineral homeostasis. No study has yet tested for osteon variant associations with static measures of bone remodelling. In this study, thin sections (n=112) of the posterior femur representing a late English Medieval adult human osteological collection, subdivided by age, sex, and socio-economic status, were examined to determine whether remodelling indicators reconstructed from osteon parameters (area, diameter, area ratios) and densities differed between categories of presence or absence of type II and drifting osteon variants. Of the 112 sections, 33 presented with type II osteons, and 38 had drifting osteons. Sporadic statistically significant results were identified. Haversian canal:osteon area ratio differed (p = 0.017) differed with type II osteon presence, type II osteons were more prevalent in males than females (p = 0.048), and drifting osteons were associated with smaller osteon (p = 0.049) and Haversian canal area (p = 0.05). These results may be explained through some biological (sex) and social (status) processes such as a period of physiological recovery (e.g. following lactation, malnutrition). However, the general lack of consistent relationships between osteon variants and remodelling indicators suggests they occur as a result of natural variation.

Keywords: histology; cortical bone; drifting osteon; type II osteon

1. INTRODUCTION

Bone remodelling responds to an internal and external environment over the lifespan of an individual (Robling et al., 2006). This occurs through the removal and replacement of bone tissue by the basic multicellular unit (BMU), to optimise bone form and function. Products of bone remodelling, secondary or type I osteons (hereafter 'osteons'), are retained in bone microstructure, and can be studied using histomorphometric methods (Cohen and Harris, 1958; Currey, 2002). Typically, osteons comprise the majority of adult (well remodelled) cortical bone in humans. In cross-section, they appear as circular discs approximately 250µm in diameter, with a central Haversian canal which houses blood, nerve, and lymphatic vessels that maintain bone tissue (Cohen and Harris, 1958; Maggiano et al., 2016). These Haversian systems run longitudinally in bone (along the long bone axis), but can also branch transversely (Volkmann's canals) as they form an interconnected vascular system (Cohen and Harris, 1958; Maggiano et al., 2016).Using histomorphometric techniques, osteon characteristics have been associated with age (Currey, 1964; Martin and Armelagos, 1979; Thompson, 1979), sex (Kelly et al., 2009; Rosenthal et al., 2009), biomechanical loading (Burr et al., 1990; Mulhern and Van Gerven, 1997; van Oers et al., 2008), individual and population-level health (Martin and Armelagos, 1985; Schultz, 2001; Assis et al., 2013), and diet (Richman et al., 1979; Paine and Brenton, 2006), amongst other factors. Osteon densities, geometric properties, and circularity have all been linked to these factors in both observation and experimentation (e.g. Pfeiffer, 1998; Pfeiffer et al., 2006; Britz et al., 2009; Crescimanno and Stout, 2012; Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016; Keenan et al., 2017). However, several secondary osteon variants occur within human cortical bone, including type II and drifting osteons (Stout et al., 2019). These variants have, to date, been studied less frequently than type I osteons and mainly using modern samples (e.g. Watanabe et al., 1998; Robling and Stout, 1999; Raguin and Streeter, 2018; Raguin and Drapeau, 2020). Limited studies have also previously analysed osteon variants qualitatively, proposing associations with health and stress (Epker and Frost, 1965; Pankovich et al., 1974; Ortner, 1975; Richman et al., 1979), or biomechanics (Robling and Stout, 1999; Skedros et al., 2007; Schnitzler and Mesquita, 2013). Whether type II and drifting osteon variants relate to bone remodelling has not yet been tested.

1.1 Type II Osteons

Type II (or embedded) osteons are, in essence, the resorption and reformation of a new, smaller osteon within the cement line boundary of an existing osteon (Cohen and Harris, 1958; Robling and Stout, 1999; Maggiano et al., 2016) (Figure 1). The new resorption space is characterised by a scalloped reversal line which surrounds the central, more recently deposited lamellae and Haversian canal (Raguin and Streeter, 2018). This 'rough' border of the type II reversal line is distinguishable

from the cleanly cut cement line of a typical secondary osteon in polarised light, where the scalloped border can be observed cutting irregularly through existing lamellae (Raguin

and Streeter, 2018) (Figure 2). Studies by Ericksen (1991), Yoshino et al. (1994), and Maggiano et al. (2016) all noted that the frequency of type II osteons increased with age, possibly indicating an association to age-related bone metabolism rates. However, this has not been consistent across studies and populations. Richman et al. (1979), in their study of American and Inuit populations, found no association between type II osteon frequency and age. Rather, Richman et al. (1979) inferred the amount of dietary protein significantly changed the observed number of type II osteons. Populations with high protein intake were found to have significantly more type II osteons than the omnivorous individuals, who in turn were higher in type II osteon frequency than the vegetarian sample (Richman et al., 1979). This was suggested to be linked to protein intake causing metabolic acidosis, associated with bone loss (Lemann et al., 1966; Richman et al., 1979). However, the frequency of type II osteons was not correlated with any changes in the calculated rates of remodelling in this study. The changes in osteonal structure in type II osteons, in both porosity of the resorptive spaces and mineralisation, have been hypothesised to change the mechanical loading tolerances of bone. Limited interconnectivity of the lacunocanalicular network across the boundary of the embedded osteon has been found to cause an increased load-induced fluid velocity than observed in typical osteons (Dallas and Moore, 2020; van Tol et al., 2020). This, in turn, may affect the mechano-sensory network at these sites, though this association is yet to be tested (Dallas and Moore, 2020; van Tol et al., 2020). Alternatively, Skedros et al. (2007) hypothesised that type II osteons may affect mechanical loading responses by local “toughening” to adapt to biomechanical strain. However, experimental modelling in animals did not find any correlations in the type of biomechanical load - tension, shear, or compression – and the cortical region or frequency of type II osteons (Skedros et al., 2007).

This lack of association with biomechanical loading history may be explained by lower mineral density of the embedded osteons (Ortner, 1975). Ortner (1975) hypothesised that the reduced mineralisation of the more recently deposited embedded osteon may function in mineral exchange, quickly releasing minerals in times of need to be restored when resources are more available. However, it should be noted that the type II osteons described in the Ortner (1975) paper were shorter than the 1.3-7.5mm long embedded osteons recorded by others (Nyssen-Behets et al., 1997; Arhatari et al., 2011; Maggiano et al., 2016). This would indicate a larger scale mineral exchange than was suggested in the original paper by Ortner (1975), if multiple osteons of up to 7.5mm were being resorbed and redeposited with less mineralised bone. As such, rather than these osteons being small pockets of mineral exchange, Maggiano et al. (2016) have suggested that these osteons are simply a result of “repathing” of osteons where vascularity is adequate

(Maggiano et al., 2016 p.729). Furthermore, these “repathing” events have been shown to “return” the canal of the original osteon through branching events, where the newly “repathed” embedded osteon diverges out of the surrounding osteon, and the Haversian canal of the existing osteon continues on the original trajectory having been “returned”

(Maggiano et al., 2016 p.729). Maggiano et al. (2016) captured the point at which these osteons form in sequential slices of synchrotron imaging. However, as this study used micro-CT, it can be difficult to observe the finer details of osteon structure, such as collagen orientation, lamellar deposition and the extent of mineralisation that can be determined in thin section or microradiograph examination (Maggiano et al., 2016). The purpose or cause of type II osteons, or possibly lack thereof, is still elusive, and has yet to be assessed experimentally or systematically.

1.2 Drifting Osteons

Drifting osteons, also referred to as “waltzing” (Frost, 1964) or “eccentric” osteons[1] (Sedlin et al., 1963; Epker and Frost, 1965), are identified by their movement in both the longitudinal and transverse planes of bone (Robling and Stout, 1999; Streeter, 2010; Schnitzler and Mesquita, 2013). The resorption of bone on one side of the BMU, and deposition on the other, causes the distinctive hemi-cyclic tail which terminates in a typical secondary osteon structure (Robling and Stout, 1999) (Figure 3). This captures the transverse movement prior to the return to longitudinal movement by the BMU (Robling and Stout, 1999). As shown in the sequential sections of Robling and Stout (1999), this transverse movement does not necessarily continue unilaterally, and in some instances can be erratic, changing directions or doubling back onto its own tail as it moves through the bone. This erratic movement through the cortex may be the cause of inconclusive results of studies that have attempted to identify patterns in drifting osteon direction (Robling and Stout, 1999). As remodelling can respond to biomechanics, specific loading types have been investigated as possibly underlying of the occurrence of drifting osteons. However, the specific load types were not associated with the frequency of drifting osteons (Skedros et al., 1994, 2007), nor was their directionality (Robling and Stout, 1999). Furthermore, Robling and Stout (1999) question whether it is possible for the production, timing, and direction of drifting osteons to coincide with specific loading on bone, to the extent that they would be indicative of biomechanical load type or degree.

There has also been suggestions that the formation of drifting osteons, with their change in canal structure and collagen fibre orientation, may result in alterations to the mechanical properties of the bone (Schnitzler and Mesquita, 2013), as both aspects have been previously linked to the mechanical strength of bone (Martin and Ishida, 1989; Hoc et al., 2006). Additionally, during their deposition, the size of the resorptive canal that forms as a part of a BMU has been reported as up to 30 times larger than that of a typical secondary osteon (Schnitzler and Mesquita, 2013). This may indicate some delay

in deposition by the BMU in the formation of drifting osteons, increasing porosity of the bone in these locations (Schnitzler and Mesquita, 2013), which in turn could also affect the structural integrity of the bone. Yet, no work has yet been conducted on how the structural changes associated with drifting osteons would affect bone strength or stability.As with the interpretation of type II osteons, there have been suggestions that drifting osteons are a way for the body to maintain circulating mineral homeostasis (Coutelier, 1976). The movement through both longitudinal and transverse planes could release more mineral components than typical longitudinal secondary osteons (Coutelier, 1976). However, drifting osteons often change direction, moving back over their tails. Thus, drifting osteons as a mechanism for mineral exchange would be redundant if these osteons continue to resorb the newly deposited, lower mineral density tails as they move erratically (Robling and Stout, 1999). For example, drifting osteons on the pleural cortex of the rib often move towards the younger, less mineralised regions of bone at the medullary cavity (Epker and Frost, 1965).This would be minimally effective in the maintenance of mineral homeostasis (Robling and Stout, 1999).

A number of studies have noted the high frequency of drifting osteons in juveniles, decreasing significantly with increasing age (Sedlin et al., 1963; Burton et al., 1989; Streeter, 2010; Schnitzler and Mesquita, 2013). Burton et al. (1989) note the presence of drifting osteons in the histology of foetal remains, and while touching on their prevalence in juvenile and growing bones, view them as a mechanism of mineral exchange and homeostasis during skeletal growth. In post-natal juveniles, Sedlin et al (1963) found that drifting osteons comprised, on average, 45-55% of observed osteons in those under 25, dropping steeply to a little over 10% between the ages of 25-35. While Schnitzler and Mesquita (2013) have noted that drifting osteons were common in the bone of those under the age of 16, they dropped off significantly at the onset of puberty. This is a far earlier decrease in frequency than the results of Sedlin et al (1963). Regardless, their high frequency in juvenile human bone has resulted in their incorporation into juvenile age estimation techniques, though some discrepancies have been noted when this method is applied to skeletal remains from the archaeological record (Streeter, 2010). As the number of drifting osteons increases from the age of approximately 1 year old, it has been suggested that the predominant occurrence of these osteons is related to muscular forces and the onset of walking at this age (Schnitzler and Mesquita, 2013). However, this link to biomechanical forces would be at odds with the lack of association observed in the studies by Skedros et al. (1994, 2007). While it is well established that these osteons are common in juvenile remains, they continue to form well into adulthood, albeit at a lower rate. There is little understanding of the driving forces of their presence, or function in bone remodelling, if any, in both juvenile and adult bone. Furthermore, the sudden change in their frequency could suggest some intrinsic function, however no research has been able to establish a clear explanation to date.

1.3 Study aims

It is clear that previous research has attempted to associate the presence and morphology of the above outlined osteon variants with various physiological and external factors (e.g. acidosis, mineral homeostasis, biomechanical loading). However, these studies have been inconclusive, so it is necessary to test if the presence of osteon variants associates with nonspecific bone remodelling markers. Here, we used static bone histomorphometry methods to study proxies for cortical bone remodelling by measuring geometric parameters and densities of osteons and Haversian canals in human femur thin sections. We included as series of histological variables. Osteon population density (OPD) and osteon area (On.Ar) measure any change in the number and size of osteons created. Haversian canal area (H.Ar) and diameter (H.Dm) allow to observe changes in the canal size, and thus broader changes in cortical bone porosity (Pfeiffer et al., 2006). The ratio of H.Ar to On.Ar (H.Ar:On.Ar) can indicate if changes in canal area are proportional to osteon area (Skedros et al., 2013). This aids in identifying whether bone quantity alters locally in a uniform (e.g. clusters of similarly sized osteons with roughly equal amounts of lamellar bone present), or heterogeneous (e.g. occurrence of a combination of smaller and larger osteons with relatively larger or smaller canals, or *vice versa*, resulting in localised variations of lamellar content in a sample) fashion (Miszkiewicz & Mahoney, 2019). Quantifying bone remodelling products in this way has previously been linked to a number of factors, including biomechanical loading (e.g. Young et al., 1986; Frost, 1994; Britz et al., 2009; Schlecht et al., 2012; Miszkiewicz, 2015), health and disease (e.g. Martin and Armelagos, 1979, 1985), and even socioeconomic status (SES) (e.g. Miszkiewicz and Mahoney, 2016; Miszkiewicz et al., 2019; Walker et al., 2019), so we know that it is a suitable 'static' reflection of the amount of remodelled bone per region of a thin section to the best of our knowledge, ours is the first study to investigate links between the occurrence of osteon variants and changes in bone remodelling. The study sought to determine if individuals whose bone samples contained type II and drifting osteon variants exhibit different OPD, On.Ar, H.Ar, H.Dm, and H.Ar:On.Ar values. Therefore, this study categorised thin sections into 'present' and 'absent' osteon variants. Our hypothesis was that those with type II and drifting osteon variants will have significantly different histomorphometric measurements reconstructing bone remodelling when compared to individuals without the variants.

2. MATERIALS AND METHODS

2.1 Thin section collection

Histological slides examined in this study had been prepared for previous histomorphometric research assessing socio-economic status (SES), behaviour, and femur cortical robusticity (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016, 2019). The slides had been made from posterior midshaft femur samples representing Medieval (11^{th}-16^{th} centuries) individuals, free from macroscopically diagnosable

pathologies, from Canterbury, England. The larger osteological collection is curated at the University of Kent. This collection represents Medieval individuals who were subject to social stratification, as inferred from burial location and historical records of the St. Gregory's Priory cemetery (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016). Osteological assessment and histological preparation of the sample have been previously described (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016). The assessment and methodologies followed anthropological codes of practice and ethics (American Association of Physical Anthropologists, 2003; British Association for Biological Anthropology and Osteoarchaeology, 2010) and ethical invasive sampling guidelines (Mays et al., 2013). These femur cortical samples were extracted across the *linea aspera* at the posterior midshaft (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016). They were then processed into thin sections following standards methods, including embedding in epoxy resin (Buehler), cutting on a low speed saw (Buehler Isomet 1000), mounting on glass slides, grinding and polishing (using a Buehler Eco-Met 300 Grinder-Polisher), cleaning in an ultrasonic bath, dehydrating in ethanol baths, clearing in Histoclear, and cover-slipping (Bancroft and Gamble 2002; Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016). The resulting thin sections were approximately 100μm thin. There are 450 of these slides curated in the Hard Tissue Histology laboratory at the Australian National University, Canberra. A total of 112 thin section from this collection was included in the present study. Each thin section represented one individual, and was selected at random from the larger collection. They were examined as an entire sample (n = 112), by estimated sex (n = 50 females, n = 62 males), age-at-death (n = 27 aged 20-34 years, n = 83 aged 35-50 years, n = 2 aged >50 years), and SES (n = 28 of 'high' SES, and n = 84 of 'low' SES).

2.2 Identification of Osteon variants

The entire posterior femur section quartile was observed at 100x magnification (total) using an Olympus BX53 microscope. This was to inspect each section for the presence of type II and drifting osteons, which were marked as 'present' or 'absent'. The presence of type II osteons was first identified under simple transmitted light, then confirmed under linearly polarised light, where instances of the cutting cone removing irregular sections of previous lamellae were more noticeable. Drifting osteons were identified under polarised light where the hemi-circular tails were also easily observed. All variants were then captured digitally using an Olympus DP74 camera. We acknowledge that prior research (e.g. Skedros et al. 2007) used a more quantitative criterion for the identification of drifting osteons (i.e. the "tail" of a drifting osteon should be at least twice the length of the osteon's width), but our methodological approach was based on a categorisation of the thin sections into osteon variant absence/presence.

2.3. Histomorphometric Variables and Statistical Analysis

Possible changes in bone remodelling in the presence of osteon variants was measured by the histomorphometric variables of OPD, On.Ar, H.Ar, H.Dm, and the ratio of H.Ar:On.Ar. Data for OPD, On.Ar, H.Ar and H.Dm were collected by Miszkiewicz and Mahoney in previous assessments of this sample (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016). In these previous studies, histomorphometric measurements were made using Olympus CELL Live Biology Imaging software using a maximum of six regions of interest (ROIs) adjacent to the periosteal border of the sections. The analysis of multiple ROIs at magnifications ranging from 100X to 400X (total) allowed for the observation of 60-120 osteons per individual, above the 25-50 osteons per section/individual recommended by Stout and Crowder (2011). Metric variables were collected from each ROI (see figure 3 of Miszkiewicz and Mahoney, 2016 for graphical representation of these measurements), with an average then calculated for each individual for their OPD, On.Ar, H.Ar, and H.Dm across these ROIs. This provided a single value for each variable per individual for comparison across the sample and subgroups. In this study, we calculated the ratio of Haversian canal area to osteon area using the following formula: H.Ar/On.Ar x 100.

All statistical analyses were completed using the software package IBM SPSS (v. 25). In addition to descriptive statistics (mean, standard deviation, minimum, and maximum data) for the entire sample, and broken down into subgroups (sex, age-at-death, SES), inferential comparisons of the histomorphometric traits were undertaken. First, Kolmogorov-Smirnov or Shapiro-Wilk tests, dependent on the sample size, were used to test for normality of the histomorphometric variables for those with and without osteon variants, both as a whole sample and subgroups. To determine whether the presence of osteon variants was linked to sex, age or SES inherently, a chi-square test was completed, which compared the frequency of variants observed within each subgroup to the frequency within the entire sample For those subgroups with normal distributions, a *t*-test was used to compare the histomorphometric variables of those with and without the osteon variants. In instances where the data distributions were not normal, a Mann-Whitney *U* test compared those with and without osteon variants. The use of parametric or non-parametric tests is noted in the results tables (Tables 1 and 2).

3. RESULTS

Of the 112 sections examined in this study, 33 presented with type II osteons (29.5%), and 38 had drifting osteons (33.9%). Based on variant frequencies in the whole sample, there was a statistically significant association (p = 0.048) between osteon variants and sex, with a chi-square test determining that the incidence of type II osteons was more prevalent in males and less prevalent in females. No association between sex and drifting osteons was found. Age-at-death was not found to be significantly linked to the presence of either osteon variant. The age-at-death distribution of this sample was limited to those between the age of 25 and 50 years old. As such, investigating the effects of age, particularly advanced age, in this study is not fully possible. There was no association found between the presentation of either osteon variant and SES.

3.1 Type II osteons

Associations between the presence of type II osteons and markers of bone remodelling, as measured by OPD, On.Ar, H.Ar, H.Dm and H.Ar:On.Ar, were compared across the entire sample. Only H.Ar:On.Ar was found to be statistically significantly different when comparing those with and without type II osteons ($p = 0.017$), with lower H.Ar:On.Ar in those with the variants (7.2) when compared to those without (10.24). All other variables did not differ significantly in the presence of type II osteons (Table 1).

Once the sample was subdivided by sex, in addition to a lower frequency of type two osteons than expected, females with type II osteons were found to have a significantly higher OPD; 18.35/mm^2 in those with type II osteons as opposed to 17.66/mm^2 in those without ($p = 0.003$). No other variable was found to differ significantly in females with type II osteons when compared to those without the variants. While males were found to have more type II osteons than predicted, the only variable that was found to be significantly different was H.Ar:On.Ar ($p = 0.033$). Males with type II osteons had a lower H.Ar:On.Ar, with a mean of 6.77 compared to 9.39 in those without the variants (Table 1).

When the sample was subdivided by SES, those of high SES with type II osteons were found to have significantly lower H.Dm ($p = 0.018$), as those with the variants had a mean H.Dm of 45.06µm, compared to an average H.Dm of 64.38µm in those without. In those of low SES, OPD was significantly different for the presence of type II variants ($p = 0.03$). The when compared low status group without variants had a lower OPD (average of 18.47/mm to those with variants (18.88/mm^2). However, this finding should be treated cautiously given these averages differ by less than one osteon per unit area. The low SES subgroup, also differed in H.Ar:On.AR significantly ($p = 0.044$). Those of low SES with type II osteons had a lower mean H.Ar:On.Ar (6.98) when compared to those without the variants (9.17) (Table 1).

3.2 Drifting Osteons

When comparing those with and without drifting osteons, as a whole sample, those with drifting osteons were found to have a significantly different On.Ar ($p = 0.049$) and H.Ar ($p = 0.05$). Those with drifting osteons had a lower average On.Ar (25,838.45 µm²) than those without the variant (28,983.40 µm²). A lower average H.Ar was also observed at the whole sample in those with drifting osteons (2,400.18 µm²) when compared to those without the variants (2, 591.4 µm²) (Table 2). However, when observed as a ratio of H.Ar:On.Ar, there were no significant differences between those with and without drifting osteons, nor were there differences in OPD or H.Dm at the whole sample level. Females with drifting osteons were found to have a significantly lower OPD than those wthout the variants, with an average OPD of 17.13/mm^2 in those with drifting osteons compared to 18.04/mm^2 in those without ($p = 0.04$) (Table 2). No other variables in the female group, or any variable in the male group, differed significantly in the presence of drifting osteons. Those of high status were not found to differ by any histomorphometric properties assessed in this study in the presence of drifting osteons. Alternatively, those of low status

had a significantly higher OPD (p = 0.047) in those with drifting osteons (18.89/mm^2) when compared to those without the variants (18.44/mm^2). Those of low status with drifting in those osteons were also found to have a significantly lower H.Ar, averaging 1,685.53 µm with drifting osteons compared to 2,427.83 µm^2 (p = 0.045*)* in those without. Osteon population density was not found to change in the presence of drifting osteons in the low status group. Additionally, H.Ar:On.Ar was significantly lower (p = 0.046) in those of low SES with drifting osteons (6.89) when compared to those without drifting osteons (9.33) (Table 2).

4. DISCUSSION

Using a Medieval human sample, posterior femur midshaft OPD, On.Ar, H.Ar, H.Dm and H.Ar:On.Ar were tested for associations with the presence of type II and drifting osteons. A number of type II and drifting osteons was apparent in this sample, however, these osteon variants were inconsistently associated with the histomorphometric measures of bone remodelling. This was the case regardless whether the sample was sub-divided by age-at-death, sex, or SES. While it is possible to hypothesise some biological function for these variants, the results of this study would suggest that there is some extent of randomness in the osteon variant occurrence and should be considered as such until future experimental research can be conducted.

4.1. Type II Osteons

A correlation between age and the presence of type II osteons has been previously suggested, indicating that these variants increase in frequency with age (Ericksen, 1991; Yoshino et al., 1994; Maggiano et al., 2016). However, other studies did not support such an association (Richman et al., 1979). In the present study, no links between age and the presentation of type II osteons was observed either. However, this could be a reflection of the demographic profile in the sample, whereby the number of elderly adults was very small (n = 2). Therefore, further research should examine type II osteons in samples from elderly individuals to validate this question.

Type II osteons occurred more frequently in males than females, significantly outside the expected range given the frequency in the sample as a whole, potentially linking their presence to sex. Neither study by Ortner (1975) nor Richman et al. (1979) found any associations between sex and type II osteons, which makes this finding potentially novel. A possible explanation for their increase frequency, assuming that these are the markers of changes to mineral homeostasis as suggested previously (Ortner, 1975; Richman et al., 1979), is male fragility (Stinson, 1985). This would suggest that while experiencing similar environmental stressors to the females of this population, they are more susceptible and will exhibit more indicators of stress than females (Stinson, 1985). However, this is primarily observed in subadults (King et al., 2005; Stinson, 1985), which is not consistent with the demographic of our study, the majority of whom were middle-aged adults, and these changes would only reflect the previous ten years of life (Clark, 2008). Additionally, this would suggest that those with type II osteons were under systemic stress, which is not reflected in the remodelling traits of males with these variants, nor any of the other subgroups, observed in our study. Indeed, all variables that were found to be significantly different in the whole

sample and subgroups with type II osteons were indicative of increased' bone remodelling, which paints a more complicated picture of what these variants may suggest.

All statistically significant differences in histomorphometric variables observed in our study were found to be indicative of 'increased' rates of remodelling processes (bone gain dominating bone loss) in the presence of type II osteons, with the exception of H.Dm in the high SES subgroup (Table 1). Indeed, OPD significantly increased in females and those of low SES in the presence of type II osteons, and H.Ar:On.Ar significantly decreased with type II osteons in the whole population, as well as in the male and low SES subgroups. This indicates that those with type II osteons show significantly increased remodelling, in terms of the number of osteons created per unit area, as well as a reduced proportion of canal space to lamellar bone in those osteons formed. This misaligns with the previous suggestions that these osteon variants are indicators of critical calcium exchange (Ortner, 1975), or metabolic and dietary distress (Richman et al., 1979), as both of these would be expected to accompany histomorphometric indicators of 'reduced' bone remodelling processes (slower accumulation of larger osteons). Rather, these observations would suggest that type II osteons accompany an uptick in remodelling activities. In females, specifically, a possible cause of this is a period of recovery following lactation, given the demographic observed in this study and the recurring suggestion that these variants are associates with calcium homeostasis (Ortner, 1975). There is substantial evidence in the bone biology literature that females experience bone remodelling variability with lactation status (e.g. Drinkwater & Chestnut, 1991; Miller et al., 1986; Miller & Bowman, 2007; Ruth, 1953). This is supported by the classic study by Ruth (1953) who observed histological changes in lactating rats on a low-calcium diet. They observed that not only did bone volume diminish with lactation and a low calcium diet, but a subsequent period of recovery stimulated the increased formation of both typical and highly variable secondary osteons, which are rarely observed in normal, healthy bone tissue of rats (Ruth, 1953). This significant reduction in bone volume, bone mineral density, and circulating calcium during pregnancy and lactation has been replicated since in mice and rats (e.g. Bowman et al., 2002; Liu et al., 2012), and observed extensively in humans (e.g. Iwaniec, 1997; Kent et al., 1990; Kovacs, 2001, 2005; O'Brien et al., 2006), though little has been done to further investigate the variable osteons reported in Ruth (1953).

Thus, the events of pregnancy or lactation could be stimulating changes in bone remodelling, the recovery of which in turn triggers the formation of type II osteon variants. Although type II osteons did not occur more or less frequently in those of high and low SES, the presence of type II osteons significantly altered a number of histomorphometric variables. Previous assertions on the cause of type II osteons, specifically as manifestations of systemic stress, mineral insufficiency and acidosis (Ortner, 1975; Richman et al., 1979), would predict that the presence of type II osteons would be associated with reduced remodelling, especially in cases of known physiological stress. Indeed, lower SES individuals from this sample were previously inferred to have been under systemic stress (dietary and physiological), but also exposed to more intense physical labour as a result of their disadvantaged status in the Medieval community (Miszkiewicz and Mahoney, 2016; Miszkiewicz et al., 2019; Walker et al., 2019). Medieval

individuals of higher SES from various locations in the United Kingdom are typically associated with diets high in protein, especially if the high SES standing related to the monastery (Rogers and Waldron, 2001). In extreme cases, high protein intake can lead to acidosis, a condition previous research attributed to the increased presence of type II osteons and increased bone remodelling in an Alaskan Inuit population (Richman et al., 1979). From previous observations of these SES subgroups, and the context of this sample more broadly, it was expected that the presence of type II osteons would be accompanied by histomorphometric indicators of reduced remodelling. However, this study found that low SES individuals with type II osteons had significantly higher OPD and decreased H.Ar:On.Ar when compared to those without the variants, indicating that type II osteons accompany an increase in bone remodelling (Table 1). It was previously suggested by Miszkiewicz and Mahoney (2016) that those of low SES were suffering malnutrition, indicated by their lower osteon densities. Thus the increased remodelling in those with type II osteons in this subgroup could, as with the female subgroup, be indicative of a recovery period. Indeed, increased remodelling in the high SES cohort had been attributed to increased access to nutrition in previous analyses of this population (Miszkiewicz and Mahoney, 2016). Thus, the indicators of higher remodelling in conjunction with the presence of type II osteons in those of low SES, could be linked to periods recovery through improved nutritional access.

The significant increase of H.Dm in high SES individuals with type II variants is the only finding at odds with our hypothesis that osteon variants would be linked to increased remodelling processes expecting lower H.Dm (we observed higher H.Dm indicating larger canals) (Table 1). This could be indicative of the association between type II osteons and high protein diet suggested by Richman et al. (1979). Indeed, the high SES individuals in the Medieval Canterbury sample examined in our study were previously shown to have higher nitrogen stable isotope records indicative of protein consumption (Miszkiewicz et al., 2019). However, this increase in H.Dm is not accompanied by an increase in observed H.Ar or H.Ar:On.Ar in those with the osteon variants. As such, this may be more an indicator of changes to Haversian canal circularity than changes in bone porosity or remodelling processes.

While the observed increase in remodelling processes that accompanied the presence of type II osteons does have potential links to recovery, as outlined above, such assertions need to be made with caution. It should also be considered that some of these significances may be artefacts of observations in the larger sample. Previous examinations of bone remodelling in the large sample also noted the high OPD values in females, to the extent that they did not follow patterns of social stratification differences inferred for males (Miszkiewicz and Mahoney, 2016). Thus, the increased OPD in females with type II osteons may be a sampling bias in a historical sample with an ‘unusually’ high OPD. Furthermore, while OPD was found to be significantly higher for those with type II osteons in the female and low SES subgroups, the average number of osteons only increased by approximately one osteon/mm^2. Moreover, the observed decrease in H.Ar:On.Ar, while statistically significant, is only a reduction of approximately 0.03 across the whole sample and subgroups (Table 1). This raises the issue of careful consideration of statistical significance versus biologically meaningful significance. An average difference in osteon

number this small was previously determined to not affect the mechanical strength properties of bone (Skedros et al. 2006). While the findings of this study suggest a possible link to increased remodelling processes, hypothesised here to be caused by a recovery period, this study cannot confidently confirm this correlation due to the inherent limitations of a non experimental sample from an historical context. As such, until experimental (or utilising post-mortem samples) research is conducted on the presence of type II osteons and recovery from physiological stress, such as lactation or malnutrition, their association with physiological or metabolic constraints should be limited to conjecture.

4.2. Drifting Osteons

Links between drifting osteons and age, particularly in juveniles, have been made more convincingly than those suggested for the occurrence of type II osteons (Sedlin et al., 1963; Burton et al., 1989; Streeter, 2010; Schnitzler and Mesquita, 2013). Indeed, drifting osteon prevalence has been included in juvenile age estimation techniques (Streeter, 2010). Drifting osteons appear to decline in frequency as the skeleton grows and reaches maturity (Streeter, 2010), though they continue to be observed in adults, as demonstrated in our study. Our lack of any association with age is likely due to the demographic distribution in the sample, whereby no juvenile or adolescent individuals were included in the analyses.The continued presence of drifting osteons within adult bone suggests that some biological function in both adults and children is possible, and remains to be investigated in experimental work. There was a significant reduction in O.Ar and H.Ar with the presence of drifting osteons in the whole sample (Table 2). This suggests an increase in remodelling processes in the presence of drifting osteons. A reduction in On.Ar and H.Ar has previously been associated with higher biomechanical loading both in analyses of this sample (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016), and in literature (eg. Mulhern and Ven Gervan, 1997; Schlecht et al., 2012). However, here, this broad association between the presence of drifting osteons and increased biomechanical loading is unlikely in light of the work by Skedros et al. (1994, 2007) and Robling and Stout (1999), who were unable to associate the presence or directionality of drifting osteons with biomechanical loading. Despite Keenan et al (2010) suggesting drifting osteons may relate to habitual tension, Henrie et al (2018) and Knight et al (2012) found that collagen fibre orientation in bone histology, instead of OPD or drifting osteons, links better with mechanical loading in human and chimpanzee samples.

Furthermore, research by Pfeiffer et al. (2006) found direct associations of On.Ar and H.Ar with patterns of activity to be more complicated than had previously been assumed, particularly with regards to individual metabolic and developmental processes. Alternatively, our finding may simply be a reflection of the significant histomorphometric differences observed in sub-groups. The low SES group in our sample had a significant increase in OPD, reduction in H.Ar, and lower H.Ar:On.Ar in the presence of drifting osteons (Table 2). These observations are indicative of an increase in bone remodelling, where

more osteons with smaller Haversian canals are produced, possibly as a result of quicker BMU infilling events. Similar results were observed in previous analyses of this subgroup, attributing the increase in remodelling to higher biomechanical loading and overall reduced health (Miszkiewicz, 2015; Miszkiewicz and Mahoney, 2016). However, as outlined earlier, links between drifting osteons and biomechanical loading have been unsuccessful thus far. Alternatively, this increase in remodelling processes that accompanied the presence of drifting osteons could be akin to what has been suggested above for type II osteons. As it was previously established that those of low SES were, as a whole, systemically stressedwith reduced bone remodelling, the increase in remodelling processes associated with the presence of drifting osteons could indicate improved health in these individuals. Again, drifting osteons were previously hypothesised to have a function in mineral homeostasis (Coutelier, 1976). Recovery from dietary insufficiency could be a cause for the increased remodelling with drifting osteons observed in low SES individuals. However, the observance of indicators of increased bone remodelling in conjunction with drifting osteons is not consistent across all subgroups. In this study females had a significant decrease in OPD in the presence of drifting osteons, indicating a reduction in remodelling processes (Table 2), with fewer osteons being formed per unit area, typically interpreted as a reduced mechanical loading or systemic stress (e.g. Evans, 1976; Schaffler and Burr, 1984; Burr et al., 1990; Raab et al., 1991; Mulhern and Van Gerven, 1997; van Oers et al., 2008; Miszkiewicz and Mahoney, 2016; Miszkiewicz et al., 2019; Walker et al., 2019). This is at odds with the aforementioned possible increase in remodelling processes found in the presence of drifting osteons within the whole sample and low SES subgroup.

This finding complicates any association between drifting osteons and changes in remodelling. The presence of drifting osteons within the male and high SES subgroups with no observable changes to the histomorphometric variables assessed in our study also brings any associations between drifting osteons and remodelling into question. As in type II osteons, the differences in remodelling observed in the presence of drifting osteons may be of statistical significance, it is unclear whether this would be of biologically meaningful significance. This is because OPD in those subgroups that differed with statistical significance, yet physically only differed, in both instances, by approximately one osteon per square millimetre. In the low SES subgroup, there was a significant difference in H.Ar, with a similar finding for H.Ar:On.Ar, however the H.Ar:On.A reduction observed is minimal, from 9.33 in those without variants to 6.89 in those with variants (Table 2). For the whole population, both On.Ar and H.Ar saw significant reductions in the presence of drifting osteons. However, this was not reflected in observable changes in OPD and H.Ar:On.Ar. As such, the inconsistency in the occurrence of drifting osteons and the changes in remodelling associated with various other physiological or external aspects of the sample (SES, sex, age, diet, stress) would suggest little biological significance of osteon variants. Thus, there is a need for experimental investigation to further understand the biological function, if any, of this histological phenomenon.

4.3. Limitations

In working with an existing collection of histological slides from a historical population, there are inherent limitations. Primarily, the biological profiles are determined based on observed sexually dimorphic skeletal characteristics (Buikstra and Ubelaker, 1994; Brickley and McKinley, 2004), not known life histories or documented biometric data. As such, age-at death, sex, and social status in our study are estimates only. This is particularly relevant for age and sex, as bone remodelling differences in males and females are known to occur in a 557 number of histological studies, often associated with age and menopause (Martin and Armelagos, 1979; Thompson, 1980; Wronski et al., 1986; Ruff and Hayes, 1988; Britz et al., 2009). However, our use of relatively large sample size hopefully minimises potential issues of misidentification. We reassessed slides that had been prepared from the posterior quartile of the mid-femur, acoss the *linea aspera,* for purposes of another research question (behaviour, femoral robusticity, and SES). As the *linea aspera* is a muscle insertion region, bone microstructure at this location, and at the midshaft femur generally, is sensitive to biomechanical activity (Drapeau and Streeter, 2005; Skedros et al., 2013). Examining a different bone and/or femur quartile for osteon variants and remodelling in the same sample may yield different results. This is also because variances in bone microstructure have been confirmed across mid-femur quartiles (Chan et al., 2007). Finally, this study is limited to an assessment of osteon variants on the basis of absence/presence in the posterior quartile, and does not consider the osteon variants from a three-dimensional perspective. This way, we cannot take into account the variability in variant frequency, or specific size and shape features of these variant osteons.

4.4. Implications

Several studies have posited that the osteon variants, particularly type II and drifting osteons, are associated with systemic stress, acidosis (Richman et al., 1979), mineral exchange and homeostasis (Ortner, 1975), age (Ericksen, 1991; Yoshino et al., 1994; Streeter, 2010; Maggiano et al., 2016), or biomechanical load (Skedros et al., 1994; Robling and Stout, 1999), all of which are linked with changes in cortical bone remodelling, and would be reflected in the bone histomorphometry. Yet, this study did not consistently associate the presence of type II and drifting osteons with changes in measures of remodelling, including OPD, On.Ar, H.Ar, H.Dm, and H.Ar:On.Ar, to statistical and biological significance. The resorption and redeposition within the cement line of Type II osteons, without the complete formation of a new complete osteon, has led previous studies to suggest a pathological or stress-induced causes for their occurrence (Ortner, 1975; Richman et al., 1979). If this were the case and based on the hypothesis of the present study, those with type II osteons would exhibit indicators of significantly lower remodelling, which we did not find.

Furthermore, the association with sex was weak, given that males showed no association with any changes in histomorphologically deduced remodelling. That being said, the changes observed in the remodelling of subgroups may suggest some associations between type II osteons and a period of

increased bone remodelling, perhaps due to recovery. Within the female subgroup, due to their age demographic within this study, it is possible that the recovery observed is linked to pregnancy or lactation. Lactation and pregnancy have been found to cause significant reduction in bone volume, bone mineral density, and circulating calcium, with recovery bringing increased remodelling, OPD, and the presence of osteon variants (Ruth, 1953; Kent et al., 1990; Kovacs, 2001, 2005; Bowman et al., 2002; O'Brien et al., 2006; Liu et al., 2012). For those of low SES, the recovery period could be linked to malnutrition. Low nutrition within those buried in the Cemetery sample had previously been established from lowered remodelling in conjunction with historical data from the population (Miszkiewicz and Mahoney, 2016). Thus, a notable increase in remodelling for those with type II osteons within the low SES cohort may be indicating a period of increased nutrition and recovery. However, while statistically significant, the association of type II osteons and periods of recovery may not be biologically significant. Thus, as this association cannot be experimentally tested or confirmed by this study, it does warrant further investigation. Until such a time that this can be examined further, however, these osteon variants should be considered a part of natural variation observed in bone microstructure.

There is strong evidence that the presence and prevalence of drifting osteons is associated with age in juveniles (Streeter, 2010), though their biological significance at this age is unknown. Their presence in adult bone may be as a result of natural variation in osteon presentation, as remnants of tissue yet to be remodelled from earlier ages, or of an unknown cause or function. Their presence in juvenile tissue, both human (Sedlin et al., 1963; Burton et al., 1989; Streeter, 2010) and non-human (Robling and Stout, 1999; Skedros et al., 2007; Jasinoski and Chinsamy, 2012), is unlikely to be linked to biomechanical forces, as previous investigations into such associations have been unsuccessful (Skedros et al., 1994, 2007). A possible future avenue of investigation, however, may be into their function or presence in ontogenetic growth, and the directional movement of bone during growth and adaptation. For instance, Jasinoski and Chinsamy (Jasinoski and Chinsamy, 2012) note the presence of a drifting osteon in the mandible of a juvenile cynodont, *Tritylodon*, moving laterally, the same direction as the ontogenetic growth of the jaw. However, Jasinoski and Chinsamy (2012) did not comment on any direct association of this. While this is only a single instance, future research should investigate the presentation of these osteons in juvenile tissue in a systematic and quantitative methodology to determine any biological significance, or, as current research suggests, whether they are simply a form of natural variation in bone microstructure.

In regards to the association of drifting osteons to changes in remodelling, the results of this study are inconclusive. The increased remodelling inferred histologically in the whole sample and low status group with drifting osteons is contradicted by the 'depressed' remodelling found in females with drifting osteons. It is possible this is an artefact of the sample studied, however, the clear contradiction in whether the presence of drifting osteons coincides with an increase or decrease in remodelling demonstrates either a more complicated biological cause or function, or that these are random variants that occur infrequently in adulthood.

5. CONCLUSION

This study found no consistent or conclusive association between the presence of the osteon variants, type II osteons and drifting osteons, and changes in bone remodelling. These results do not support interpretations made in previous studies that these osteons variants are as a result of specific factors, such as maintaining mineral homeostasis, acidosis, and types of biomechanical loading. Based on the results of this investigation, and pending any further investigation into their cause or function, we suggest that type II osteons be considered a result of natural variation within the cortical bone microstructure. That being said, this finding should not downplay the potential links between osteon variants and slight increases in remodelling processes; particularly the association of type II osteons and recovery from pregnancy or lactation in females, or malnutrition. The demographic of females within this sample, being predominantly between the ages of 25 and 50 years old, and known low nutritional status of the low SES cohort makes this an attractive possibility. However, it is the nature of ancient populations that it is not possible to tackling this question experimentally. Thus, until further studies are conducted, the results of this study are agreement with the suggestion made by Maggiano et al. (2016), and indicate that type II osteons represent an accidental or coincidental repathing event in regions of adequate vascularisation.

While the prevalence of drifting osteons in juvenile bone is well established, their cause or function both in human and non-human bone is still unknown, as is their enduring, sporadic presence in adult bone. Further experimental research into correlation with remodelling drift during behavioural adaptation and ontogeny could be beneficial to our understanding of these variants. Until more detailed research or experimental modelling can be undertaken to uncover the function and cause of osteon variants in adult tissue, they should be considered a part of the natural variation of cortical bone microstructure.

ACKNOWLEDGMENTS

Cooke is funded by the Australian Government Research Training Program. We thank the University of Kent for facilitating access to the Medieval osteological collection under a Kent GTA PhD studentship 2010-2014 (to Miszkiewicz); David McGregor (ANU) for technical support; ANU College of Arts and Social Sciences and the Australian Research Council (DE190100068) for funding microscopy equipment. We are grateful to the anonymous peer reviewers and Associate Editor Dr. Scott Miller for constructive comments on our manuscript.

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Footnotes

It should be noted that the use of eccentric osteons is not entirely consistent in the literature. For example, Schnitzler and Mesquita (2013) differentiated eccentric and drifting osteons (with eccentric being an intermediary between drifting and typical secondary steons). Papers describing drifting osteons as “tailed”, or specifically noting no differences between drifting, eccentric or waltzing osteons (e.g. Sedlin et al. 1963), were included as comparable to the drifting osteons in our study.

Figure Legends

Figure 1: Type II or embedded secondary osteons observed under transmitted light(individual NGA-88-Sk-725).

Figure 2: Type II or embedded secondary osteons observed under linearly polarised light (B) with the cross cutting of lamellae indicated by the arrow head (individual NGB-89-Sk-56)

Figure 3: Drifting secondary osteons observed under polarised light – left: individual NGB 89-Sk-50, right: NGA-88-Sk-268.

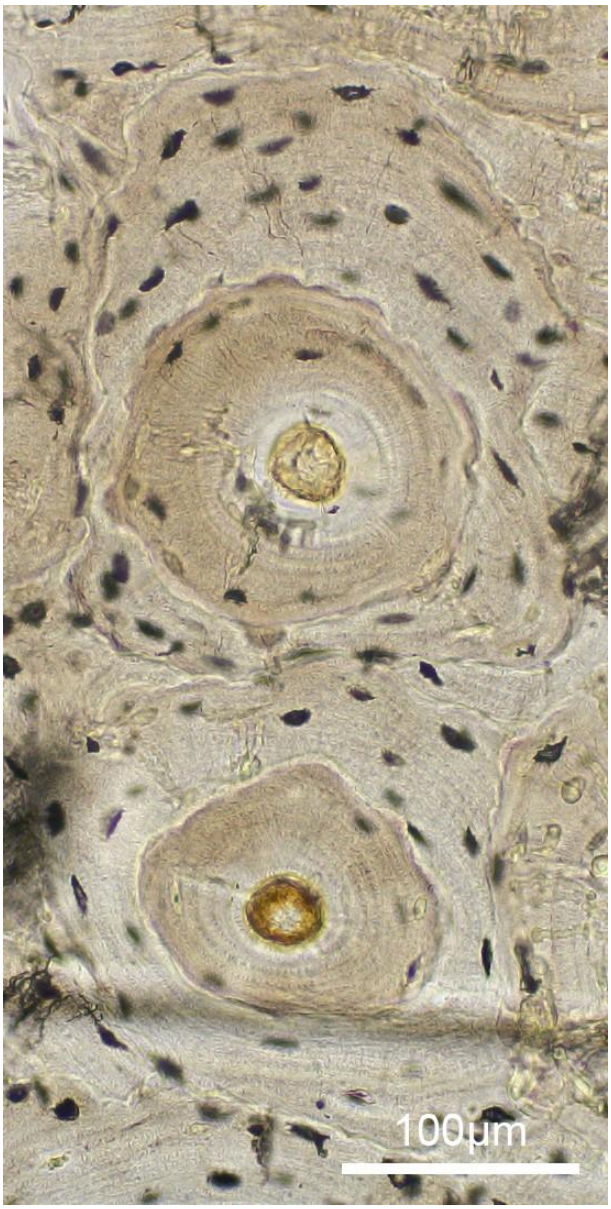

Figure 1: Type II or embedded secondary osteons observed under transmitted light (individual NGA-88-Sk725). Shadow 'line' feature at bottom of image is technical processing artifact.

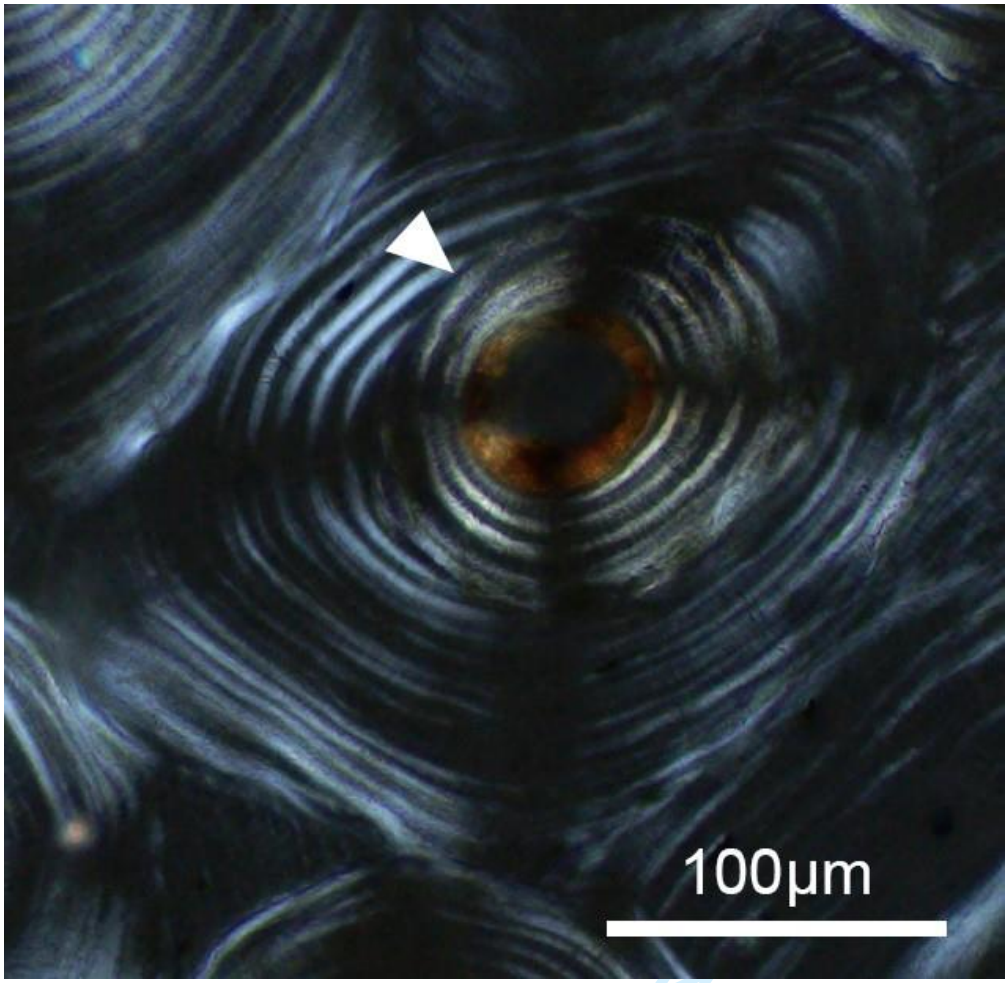

Figure 2: Type II or embedded secondary osteons observed under linearly polarised light (B) with the cross cutting of lamellae indicated by the arrow head (individual NGB-89-Sk-56)

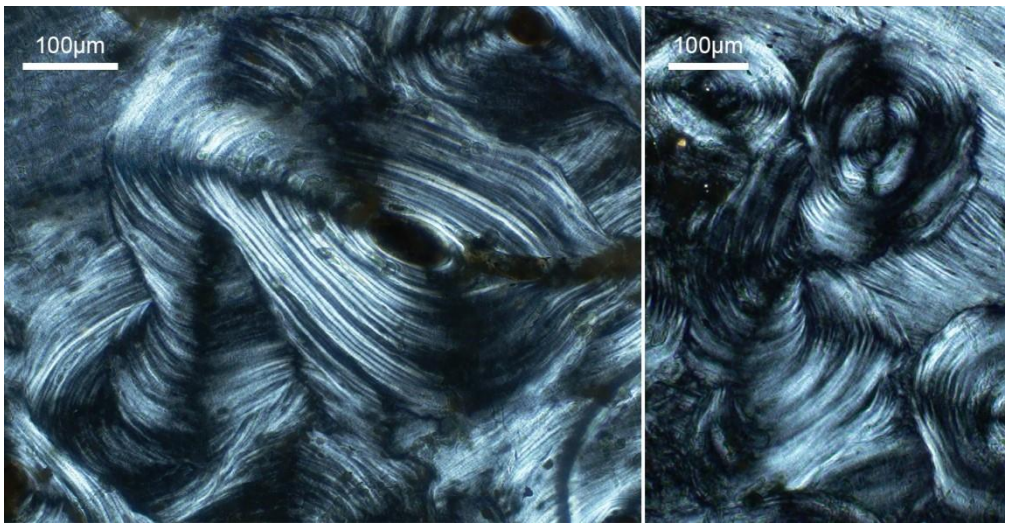

Figure 3: Drifting secondary osteons observed under polarised light – left: individual NGB-89-Sk-50, right: NGA-88-Sk-268.

Table 1. Descriptive data and inferential statistics results for the analysis of type II osteons.(Std.: standard deviation, min.: minimum, max.: maximum). Statistical significance <0.05 is indicated in bold.

		OPD (#/mm^2)		On.Ar (μm^2)		H.Ar (μm^2)		H.Dm (μm)		H.Ar/On.Ar (%)	
		Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT
Whole Sample	*N*	78	32	75	32	79	33	79	32	75	32
	Mean	18.62	19.89	28,297.56	26,954.47	2,704.18	2101.70	49.58	44.23	10.23	7.20
	Std.	3.28	3.36	15,824.18	19,369.86	2,382.89	1821.45	17.40	18.37	7.93	3.19
	Min.	11.77	14.29	3,061.36	7,852.86	558.89	296.45	24.59	21.44	2.69	0.39
	Max.	26.34	28.57	80,120.16	69,051.63	12,894.90	6587.32	121.69	100.71	67	12.40
	p	0.11[a]		0.279[a]		0.61[a]		0.104[a]		**0.017[a]**	
Females	*N*	39	10	38	10	40	10	40	10	38	10
	Mean	17.66	18.35	30,084.76	29,292.88	2,987.41	2,365.50	52.69	47.08	11.04	8.14
	Std.	3.24	1.04	15,319.74	21,498.34	2,371.22	1,773.89	14.81	14.54	10.23	3.06
	Min.	11.77	17.11	3,061.36	7,852.86	700.03	519.93	27.93	27.48	3.97	4.23
	Max.	24.67	20.09	66,272.43	69,051.63	12,000.73	5,058.22	96.06	64.04	67	12.40
	p	**0.003[b]**		0.161[b]		0.451[a]		0.492[b]		0.387[a]	
Males	*N*	39	22	37	22	39	23	39	23	37	22

	Mean	19.59	20.59	26,462.06	25,891.56	2,413.68	1,987.01	46.38	42.98	9.38	6.77
	Std.	3.07	3.81	16,330.11	18,761.39	2,390.29	1,868.97	19.38	19.98	4.49	3.22
	Min.	13.39	14.29	4,671.52	9,448.01	558.89	296.45	24.59	21.44	2.69	0.39
	Max.	26.34	28.57	80,120.16	68,624.33	12,894.9	6,587.32	121.69	100.71	23.09	12.21
	p	0.227[b]		0.594[a]		0.155[a]		0.347[a]		**0.033[a]**	
High Status	*N*	18	10	18	10	18	10	18	10	18	10
	Mean	19.14	22.11	36,588.42	27,139.50	4,133.33	2,394.87	64.38	45.06	13.57	7.68
	Std.	3.70	4.27	19,602.80	23,933.53	3,438.17	2,440.85	20.94	25.73	14.18	9.33
	Min.	14.73	16.74	3061.36	7,852.86	934.69	296.45	39.42	21.44	2.69	2.95
	Max.	26.34	28.57	80,120.16	68,624.33	12,894.90	6,587.32	121.69	100.71	67	11.57
	p	0.356[b]		0.175[a]		0.072[a]		**0.018[a]**		0.133[a]	
Low Status	*N*	60	22	57	22	61	23	61	23	57	22
	Mean	18.47	18.88	25,679.40	26,870.37	2,282.46	1,974.24	45.21	43.86	9.17	6.98
	Std.	3.17	2.32	13,607.60	17,559.57	1,799.65	1,527.21	13.56	14.83	4.15	3.29
	Min.	11.77	14.29	4,671.52	10,773.49	558.89	543.77	24.59	22.69	4.01	0.39
	Max.	24.67	24.22	63,640.89	69,051.63	12,000.73	5,058.22	89.17	70.61	23.09	12.40

	p	**0.03**[b]	1.000[a]	0.29[a]	0.714[a]	**0.044**[a]

[a] Denotes the use of a Mann Whitney *U* test to compare samples

[b] Denotes the use of a *t*-test to compare samples

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Table 2. Descriptive data and Analytical inferential statistics results for the analysis of drifting osteons (Std.: standard deviation, min.: minimum, max.: maximum). Statistical significance <0.05 is indicated in bold.

		OPD (#/mm^2)		On.Ar (μm^2)		H.Ar (μm^2)		H.Dm (μm)		H.Ar/On.Ar (%)	
		Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT	Variants ABSENT	Variants PRESENT
Whole Sample	*N*	73	37	70	37	74	38	74	38	70	37
	Mean	18.86	19.25	28,983.40	25,838.45	2,591.61	2,400.18	49.36	45.35	9.27	9.42
	Std.	3.53	2.96	15,167.51	19,782.31	1,938.03	2,762.71	16.57	19.87	4.33	10.37
	Min.	11.77	14.42	4,671.52	3,061.36	558.89	296.45	24.59	21.44	2.69	0.39
	Max.	28.57	26.79	69,051.63	80,120.16	12,000.73	12,894.9	121.69	100.71	23.09	67
	p	0.147[b]		**0.049[a]**		**0.05[a]**		0.079[a]		0.257[a]	
Females	*N*	36	13	35	13	37	13	37	13	35	13
	Mean	18.04	17.13	30,693.23	27,837.43	2,838.50	2,932.85	52.41	49.15	9.25	13.64
	Std.	3.22	1.85	14,903.40	20,855.00	2,057.84	2,860.12	12.93	19.54	4.13	16.48
	Min.	11.77	1442	10,320.02	3061.36	700.02	519.93	27.93	27.48	3.97	4.23
	Max.	24.67	20.09	69,051.63	66,272.43	12,000.73	9,732.24	89.17	96.06	18.86	67
	p	**0.04[b]**		0.084[b]		0.486[a]		0.193[b]		0.602[a]	
Males	*N*	37	24	35	24	37	25	37	25	35	24

	Mean	19.66	20.39	27,273.56	24,755.66	2,344.73	2,123.19	46.31	43.37	9.29	7.13
	Std.	3.67	2.83	15,451.30	19,548.13	1,804.52	2,728.20	19.25	20.15	4.58	3.35
	Min.	13.39	15.96	4,671.52	9,448.01	558.89	296.45	24.59	21.44	2.69	0.39
	Max.	28.57	26.79	68,624.33	80,120.16	8,626.06	12,894.9	121.69	100.71	23.09	16.09
	p	0.146[b]		0.195[a]		0.106[a]		0.385[a]		0.082[b]	
High Status	*N*	16	12	16	12	16	12	16	12	16	12
	Mean	20.36	19.99	35,250.80	30,497.81	3,185.34	3,948.59	59.23	55.15	9.05	14.69
	Std.	4.35	3.92	15,546.30	27,767.94	2,123.01	4,289.57	21.40	28.33	4.43	17.09
	Min.	14.73	14.88	13,754.56	3,061.36	660.24	296.45	30.14	21.44	2.69	2.95
	Max.	28.57	26.79	68,624.33	80,120.16	8,626.06	12,894.9	121.69	100.71	18.53	67
	p	0.612[b]		0.28[a]		0.599[a]		0.537[a]		0.347[a]	
Low Status	*N*	57	25	54	25	58	26	58	26	54	25
	Mean	18.44	18.89	27,126.39	23,601.95	2,427.82	1,685.53	46.64	40.83	9.33	6.89
	Std.	3.18	2.38	14,687.74	14,749.33	1,870.28	1,232.08	14.01	12.81	4.34	2.66

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	Min.	11.77	14.42	4,671.52	10,773.49	558.89	543.77	24.59	22.69	4.01	0.39
	Max.	24.67	24.22	69,051.63	57,318.64	12,000.73	4,907.18	89.17	70.61	23.09	12.21
	p	**0.047**[b]		0.152[a]		**0.045**[a]		0.087[a]		**0.046**[a]	

2 [a] Denotes the use of a Mann Whitney *U* test to compare samples

3 [b] Denotes the use of a *t*-Test to compare samples