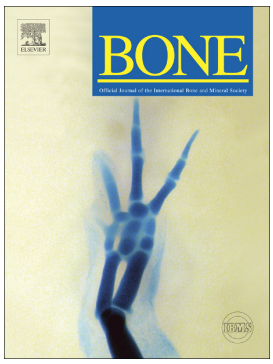

Microscopic markers of an infradian biorhythm in human juvenile ribs

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Microscopic markers of an infradian biorhythm in human juvenile ribs

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Abstract: Recent studies have indicated that there may be an infradian systemic biorhythm that coordinates aspects of human hard tissue growth and influences adult body size. Here we investigate if evidence of this biorhythm retained in human teeth as the periodicity of Retzius lines (RP) corresponds with the microstructural growth of a non-weight bearing bone, the rib, in a sample of 50 human juvenile skeletons. Using static histomorphometric methods, the RP of one permanent tooth from each skeleton was calculated and combined with measures of bone remodeling in a rib from the same individual. Results provide the first evidence that the infradian biorhythm is linked to bone remodeling in children. Retzius periodicity was negatively correlated with relative osteon area ($r = -0.563$, $p = 0.008$) and positively related to Haversian canal area ($r = 0.635$, $p = 0.002$) and diameter ($r = 0.671$, $p = 0.001$) in children between the age of 8 to 12 years. There was also a negative correlation between RP and the relative cortical area of ribs ($r = -0.500$, $p = 0.048$). Relationships between bone remodeling and the biorhythm were much more variable in younger children. Results imply that as the biorhythm speeds up there is increased bone deposition during remodeling of the rib, leading to the larger osteonal lamellar bone areas and smaller Haversian canals in children between 8 and 12 years of age. Our results support the idea that there is an infradian biorhythm that coordinates aspects of human hard tissue growth.

Key words: Histomorphometry; Biorhythm; Osteon; Retzius periodicity

1 Introduction

1.1 Biorhythms

Biorhythms are cyclic changes in an organism's growth or functioning that can be driven by an internal biological clock and synchronized through environmental cues [1]. Evidence of two biorhythms is retained in human tooth enamel in the form of incremental growth lines [2]. The first biorhythm corresponds with a daily, circadian, rhythm that has been linked to the secretory activity of ameloblasts [3,4]. The second biorhythm is a longer period infradian biorhythm that is marked by Retzius lines [5]. The mechanism behind Retzius line formation is currently poorly understood but the chronological time between adjacent lines can be quantified in days as Retzius periodicity (RP) [6]. Retzius periodicity reportedly varies between six to twelve days in modern human permanent teeth, with a modal value of eight days [7–9].

It is hypothesised that RP, as a measure of an underlying biorhythm, has its origin in the hypothalamus and influences pituitary secretions that act upon the endocrine glands and ultimately bone cell proliferation [10]. The proposed biorhythm has been linked to mammalian body size due to the hypothesised link between the biorhythm, RP, and hormone secretion [10]. This hypothesis is consistent with analyses of RP from permanent teeth across mammalian species as smaller bodied mammals tend to have lower RPs than larger bodied mammals [10,11], though exceptions have been noted in some animals such as lemurs [12]. The lower RP, and thus a biorhythm that occurs over a fewer number of days, suggests that smaller bodied species tend to have a faster developmental rate [11]. Life history theory suggests that smaller animals tend to have shorter lifespans and reproduce more frequently and also achieve adult body size quicker when compared to slower maturing animals [13]. It is therefore possible that RP, as a marker of a biorhythm, could provide a link between adult body size, developmental rate, and life history traits when compared between some

mammalian species [10,11].

The role of the infradian biorhythm for human growth and development is poorly understood. Studies of small samples of adult humans indicate that RP is negatively correlated with final attained adult stature [14,15]. This makes sense as the biorhythm is accelerated to achieve greater height within a developmental period that is constrained for humans, relative to inter-specific comparisons of RP [11]. Three previous studies have examined the relationship between RP and bone modeling and remodeling. In the femoral midshaft of one adult human female one bone lamellae formed in the same number of days as the mean human RP, in both the interstitial lamellar bone and in the osteonal lamellar bone [11]. Retzius periodicity was linked to the rate of primary bone deposition in the humeri of seven age-matched children [16]. Femoral osteocyte lacunae density was inconsistently related to RP in a sample of adult humans [15]. Thus, there is currently some evidence that suggests a relationship between RP and stature in adult humans, and between RP and primary bone growth in a small sample of infants. To date, no study has examined the potential relationships of RP and bone remodeling during ontogeny in humans.

1.2 Human skeletal growth

A child's skeletal growth velocity declines from birth until about three years of age and then levels out, followed by a slight increase in velocity around the seventh year, for some but not all children [17–19]. Skeletal growth in height accelerates with the onset of puberty, that typically commences between eight to 11 years of age for girls, and between nine to 13 years of age for boys in modern populations [20–23]. Puberty is often complete between 13-17 years of age for girls, and between 15-18 years of age for boys in modern populations [24]. The timing of puberty is affected by an array of variables including body mass, genetics, nutrition, social status [25], and in particular, poor health which can delay the

onset and completion of puberty [26]. During the recent medieval period in England, which is the period from which the human remains in this study date, puberty commenced at around the same age as contemporary populations but tended to be an extended process that was usually complete between 16-19 years of age [27]. The onset, progress, and completion of puberty is dictated by hormones secreted by the hypothalamus, pituitary glands, adrenal glands, and ovaries or testes. These hormones include leptin, gonadotropin-releasing, and growth hormone [28,29], and also the sex steroids that drive sexual dimorphism [30].

1.3 Bone histomorphometry

Evidence of previously active bone remodeling is retained in human bones and can be accessed using static histomorphometric techniques. As secondary osteons replace primary osteons during skeletal growth secondary osteon density increases during childhood [31–34]. Osteon accumulation continues into adulthood and is closely related with increasing age in younger adults [35]. Secondary osteon population density (On.Dn) can be used as a proxy for regular bone turnover rates, or changes in bone density that are a result of mechanical, pathological, or dietary alterations [36–39].

Geometric properties of osteons can be quantified via variables that include osteon area (On.Ar) and osteon diameter (On.Dm). Previous studies have inferred behavior from On.Ar and On.Dm [40–43] because they can provide an indication of the type of loading on a bone [44–46]. Experimental studies have revealed that osteon size, on average, is inversely related to biomechanical strain [47–49]. Osteon diameter is also generally smaller in regions of the cortex that are exposed to larger loads and resulting strains [44]. Other studies have reported that secondary osteons can become smaller and more circular with advancing age in adults [36,50,51]. However, secondary osteons have been reported to become larger and less circular during adolescence although the cause remains unknown [31]. With age, sex, and

stature controlled for, human femoral osteon size (area and diameter) has also been reported to be inversely related to body mass, a link that may be mechanical or endocrine in nature [36].

The size of the Haversian canal can be quantified as Haversian canal area and diameter (H.Ca.Ar and H.Ca.Dm respectively). Haversian canal size is ultimately related to the infilling of an osteon during BMU activity and can be a reflection of the size of blood vessels residing within bone matrix during life [52]. Consequently, it serves as an insight into bone remodeling activity undertaken during the bone resorption and deposition phases [44]. The proportion of lamellar bone area to Haversian canal area in an osteon can be calculated as a percentage of the total On.Ar, as relative osteon area (Re.On.Ar). This measurement shows the proportion of an osteon that is lamellar bone regardless of its overall size, and is based on a previously described ratio of H.Ca.Ar:On.Ar [53].

1.4 Research Questions and Predictions

The aim in this study is to investigate the relationship between RP in human permanent first molars and past evidence of bone remodeling in ribs from a sample of human juvenile skeletons. The rib was selected because it is less affected by variations in mechanically induced bone remodeling compared to the limbs [54,55]. Standard static histomorphometric techniques are applied.

1. *Is bone remodeling linked to RP?* – Taller adults may attain greater height through an increased developmental rate during ontogeny [10,11,15]. When this hypothesis is applied to juveniles it suggests that those individuals with a lower RP (and thus fewer number of days of enamel growth between each oscillation of the underlying biorhythm) will be on a faster growth trajectory that would lead to greater adult stature [15]. If this is correct, then a faster growth trajectory, indicated by a lower RP,

will be related to higher rates of bone remodeling**.** To account for age-related variation in juvenile bone microstructure [31,34], we test this hypothesis in younger children who died before the onset of puberty and older children who likely would have commenced puberty.

2. *Is osteon size linked to RP?* – In one adult human female one bone lamellae formed in the same number of days as the mean human RP [11]. This finding suggests that larger osteons, with more lamellae, should relate to RP. We test this idea using relative osteon area as a signifier of the amount of osteonal lamellar bone.

3. *Is bone modeling linked to RP?* – One aspect of bone modeling has previously been linked to RP in children [16]. Since periosteal bone apposition during childhood contributes to cortical thickness this sign of bone modeling may also be linked to RP. Given the hypothetical premise that juveniles with lower RPs will be on a faster growth trajectory towards greater stature, we predict that RP will be inversely related to bone size in age-matched children. We use cortical area, total area, and relative rib cortical area as a measure of cross-sectional bone size as a proxy for bone modeling.

2 Materials and Methods

2.1 Sample

Fifty juvenile skeletons, dating to the recent medieval period in England, and without skeletal lesions indicative of pathology, were selected for the present study [56]. No permits were required as these skeletal samples pre-date the time period covered by the Human Tissue Act. All sampling followed appropriate codes of ethics for research conducted on human skeletons from anthropological collections [57].

One permanent tooth was removed from each skeleton. Permanent first molars were preferentially selected (*n*=47). This molar was unavailable in three skeletons so a central incisor (*n*=2) and second molar (*n*=1) were selected. A 5mm ± 2mm rib thick-section was removed from the middle third of an un-sided 3rd to 8th rib from each skeleton (*n*=50). Given that functional adaption is reflected in bone microstructure, the rib was selected for our analyses as it is a non-weight bearing bone and does not vary greatly inter-skeletally when considering respiration induced loading [54,55]. Accordingly, the rib can be considered a skeletal site that reveals systemic bone physiology and remodeling [58].

2.2 Age estimation

The juvenile skeletons were from individuals aged between three and 12 years of age. The age estimations followed standard methods for reconstructing age in juvenile skeletal remains, since the actual chronological age of each skeleton was not known. We collated several age-at-death methods that rely upon the assessment of tooth formation times [59], timing of dental eruption [60], and fusion of the epiphyses [61]. The sample was split into two age groups as follows; younger child (3–7 years, *n* = 29), and older child (8–12 years, *n* = 21). The younger group includes children who had died before puberty commenced. The older child group includes children who died at an age when puberty can commence. All of the children died prior to the mean age that the adult compacta forms in the rib [62].

2.3 Sample preparation

Histological thin-sections of teeth were produced using standard methods [6]. The teeth were embedded in resin (Buehler EpoxiCure®) and sectioned through the tip of the cusp and the dentine horn using a Buehler Isomet 1000 precision saw. The sections were fixed to glass microscope slides (Evo Stick® resin), ground to a final thickness of 50-100μm

with a series of grinding pads (P400, P600, P1200) (Buehler® EcoMet 300), and polished with a 0.3μm aluminium oxide powder (Buehler® Micro-Polish II). Each section was cleaned in an ultrasonic bath, dehydrated in 95-100% ethanol, cleared (Histoclear®), and mounted with a coverslip using a xylene-based mounting medium (DPX®).

Standard histological methods were used to produce bone thin-sections [31,63,64]. Transverse 5mm ± 2mm thick-sections were removed from the middle third of the 3rd-8th ribs using an electronic drill (Dremel Rotary Tool®) [65]. The bone was embedded in resin (Buehler EpoxiCure®), further reduced to 0.3 ±0.1 cm using a Buehler Isomet 1000 precision saw and fixed to glass microscope slides (Evo Stick® resin). Each bone thin section was ground to a final thickness of 50-100 μm, polished, and covered following the same methodology as the tooth thin sections, above.

2.4 Enamel histology

The teeth were examined using a high-powered microscope (Olympus® BX53) with a mounted camera (Olympus® DP72). Images of the teeth were obtained and examined in CELL® Live Biology imaging software. Retzius periodicity (RP) was directly calculated by counting the number of cross striations along an enamel prism between two Retzius lines (figure 1) in the lateral enamel at 20x magnification with a 10x objective. Diagenetic changes, micro-scratches, or blurred lines prevented a direct count being taken for 52% of cases, so RP was calculated from local daily secretion rates (DSR) of enamel [66]. The DSRs were calculated by measuring between six cross striations, which corresponds to five days of enamel formation, and dividing the measurement by five to get a daily mean enamel secretion rate in microns. This was repeated six times within the local enamel so that a grand mean DSR could be calculated [66]. Following this, the distance between four Retzius lines was measured, corresponding to three repeat intervals, divided by three and then divided by the

grand mean DSR to yield an RP value.

2.5 Bone histology

Standard methods were used for imaging undertaken using an Olympus BX51 compound microscope with an Olympus DP25 microscope camera [41,67]. For each rib thin-section the entire cortex was imaged at 20x magnification with a 10x objective and stitched together into a montage using CELL® Live Biology Imaging software (figure 2a). The total subperiosteal area (Tt.Ar, mm^2) and medullary cavity area (Es.Ar, mm^2) were measured and cortical area was calculated (Ct.Ar, mm^2 = Tt.Ar – Es.Ar). Relative cortical area was calculated as a percentage (Re.Ct.Ar, % = (Ct.Ar / Tt.Ar) * 100) to control for allometry [68]. Higher percentages relate to higher proportions of cortical bone in the rib cross sectional area. The number of secondary osteons and secondary osteon fragments were counted across the entire cross section. Secondary osteons were identified by the presence of a complete cement line and intact Haversian canals, and secondary osteon fragments were identified as partial secondary osteons [69–71] (figure 2b). These osteon counts formed the osteon population density (On.Dn), which was calculated by dividing the number of osteons and fragments by the area of the rib cortex. Histomorphometric studies quantify bone turnover as osteon population density by adding the number of intact and fragmentary osteons and dividing the result by the area of the ROI to produce a density score [71]. The intact osteons are the most recently formed and they intercut the preceding generations of osteons, leaving fragments of the older osteons visible.

Secondary osteon structure was quantified by measuring the osteon area (On.Ar, μm^2) and diameter (On.Dm, μm), and the Haversian canal area (H.Ca.Ar, μm^2), and diameter (H.Ca.Dm, μm) (figure 2c). Incomplete Haversian systems, identified by scalloped Haversian canal margins, and drifting osteons were excluded. Drifting osteons have been reported to

account for 70% of cortical porosity in children [72]. Osteon lamellar area (On.Lm.Ar, μm^2) was determined (On.Lm.Ar = On.Ar – H.Ca.Ar) to calculate relative osteon area as a percentage (Re.On.Ar, % = (On.Lm.Ar / On.Ar) * 100) to provide a measure of the proportion of lamellar bone in an osteon within the cement line irrespective of overall secondary osteon size. Higher percentages relate to higher proportions of lamellar bone area to total osteon area.

2.6 Statistical 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. The normality of each variable was assessed with Kolmogorov-Smirnov tests. Subsequently, count variables (On.Dn) were square root transformed and measurement variables (On.Ar, Re.On.Ar, On.Dm, H.Ca.Ar, H.Ca.Dm) were log transformed. Independent samples *t*-tests were used to check for differences in microstructure between younger and older children. Linear regression was used to seek relationships between the variables.

3 Results

3.1 Is bone remodeling linked to RP?

Descriptive statistics and comparisons of the rib microstructure between older and younger children are in Table 1, whereas descriptive statistics of the transformed data appear in Supplement Table 1. Osteon density was higher in the older children ($p < 0.001$) and the osteon area and relative osteon area were also larger in the older children ($p = 0.046$ and $p = 0.049$ respectively). However, the dimensional measurements just met the threshold of significance. Haversian canal dimensions remained consistent between the two age groups.

Regression statistics for RP and rib microstructure amongst the older children, and younger children, are in Table 2. Amongst the older children, RP was significantly and negatively related to relative osteon area, (r = -0.563, p = 0.008), but positively related to Haversian canal area (r = 0.635, p = 0.002), and Haversian canal diameter (r = 0.671, p = 0.001) (figure 3a, b, and c).

3.2 Is bone modeling linked to RP?

Descriptive statistics and comparisons of the rib cortical area, and relative cortical area are in Table 1, whereas descriptive statistics of the transformed data appear in Supplement Table 1. Total rib cross-sectional area and rib cortical area were significantly larger in the older children than in the younger children (both $p < 0.001$), although relative rib cortical area remained consistent between the age groups.

The regression statistics for RP and rib cortical area and relative rib cortical area are in Table 2. Within the older child group there was a statistically significant negative correlation between RP and rib relative cortical area (r = -0.500, p = 0.048) (figure 3d).

4 Discussion

This study investigated the relationship between RP in human permanent first molars – as a measure of an underlying biorhythm – and past evidence of bone remodeling in ribs from human juvenile skeletons from a single population. We found evidence that the biorhythm was linked to the relative area of osteons, the size of Haversian canals within osteons, and relative cortical area in the ribs of children aged eight to twelve years. There was no evidence that the biorhythm was linked to the frequency of osteons in this sample of children, or to any histomorphometric variable amongst younger children aged 3 to 7 years of age.

4.1 Is bone remodeling linked to RP?

Our results show that osteons with proportionally larger lamellar areas and smaller Haversian canal areas relate negatively to RP amongst the older children. We did not observe a relationship between osteon area and RP in either age group. This implies that a faster biorhythm predicts increased bone deposition during remodeling in the rib, leading to larger osteonal lamellar areas relative to their Haversian canal size. However, it does not predict overall osteon size. Prior research in one adult human observed that one lamellae, from both interstitial and osteonal bone, formed in the same number of days as the species specific mean RP in one adult human female [11]. Data presented here could be consistent with that observation if the difference in lamellar bone quantity was attributable to a greater number of lamellae. The finding that relative osteon area is related to RP, but not osteon area, could indicate that the biorhythm is related to the number of lamellae in each osteon rather than to the overall osteon size. A faster biorhythm indicated by a lower RP could form more lamellae in the same time period as an individual with a slower biorhythm, indicated by a higher RP. Alternatively, the number of lamellae may not have changed but they may have become thicker which would also account for the link to RP.

Retzius periodicity and Haversian canal size were positively correlated in the ribs of the older children. This implies that a slower biorhythm predicts larger Haversian canals in the rib. Haversian canal size is related to the size of the blood vessels that were contained within the canal during life [52] suggesting that children with higher RPs could have had larger capilaries within their Haversian canals. Alternatively, the observed link between RP and Haversian canal size could simply be a consequence of the observed relationship between RP and relative osteon area. Since the overall osteon area is consistent between different RPs, the overall amount of bone removed by the BMU during remodeling will also be constant.

But given that the proportion of lamellar bone in the osteon varies according to RP, the amount of bone deposited by the BMU during remodeling will also vary with RP and the resultant Haversian canal area may be a by-product of the increased bone deposition. Thus the relationship between RP and Haversian canal size observed here may be an incidental relationship to the relationship between RP and osteon infilling by BMUs. A previous study examined Haversian canal to osteon area scaling in multiple mammal species and suggested that the proportion of lamellar bone area to Haversian canal area in osteons may scale with either body size or longevity, but this was not a focus of the study [73].

Another consideration is the rapid remodeling rate of cortical bone in children that causes increased cortical porosity when compared to adults [74]. The link between RP and Haversian canal size could potentially be explained by more rapid bone turnover and increased cortical porosity in children with higher RPs, and *vice versa*, rather than by the amount of osteonal bone deposition. Despite attempting to only include completed Haversian canal in our study, it is possible that some active osteons could have been included. Although, mean Haversian canal area in both the younger children and the older children of this study (1616.90μm^2 and 1606.94μm^2 respectively) are similar to the mean Haversian canal area reported for inactive concentric Haversian canals (1544μm^2) and inactive eccentric Haversian canals (1984μm) from external iliac cortices of 0-25 year olds [72]. This suggests that the canals measured in this study were fully formed.

Retzius periodicity was only related to bone microstructure in the older children. Our microstructural data are consistent with expectations for this age group. Both mean On.Dn and mean On.Ar are greater in the older children compared to the younger children (Table 1). This is similar to previous findings from the juvenile humerus where On.Dn was significantly higher in older children (eight to 12 year olds) than in younger children and infants [31]. Hence, the bone microstructural data are not unusual or indicative of pathological change, but

it does follow the anticipated age-related pattern. The increased osteon density in the older children is also consistent with the increased growth rates that might be expected as puberty commences.

It is hypothesized that a centrally coordinated infradian biorhythm will be accelerated in children that are on a faster growth trajectory [10,11,15]. It is thought that this biorhythm stems from the hypothalamus that stimulates pituitary secretions that are linked to puberty, metabolism and body mass [10]. Our findings show that RP is related to the proportion of lamellar area to overall osteon size in juveniles who were at an age consistent with the onset of puberty, when compared to younger children. If the hypothesized biorhythm is applied to this finding it suggests that amongst the older children, those with a faster biorhythm have more rib osteons with more lamellar bone and smaller Haversian canals, even though overall osteon size remains constant. Thus, RP appears to relate to the infilling of osteons rather than the amount of bone removed by osteoclast during remodeling and the overall osteon size. Since the loading environment of the rib is relatively consistent between individuals [54,55] the rib is considered to reveal systemic influences on bone microstructure and a centrally coordinated biorhythm could be one such systemic influence.

4.2 Is bone modeling linked to RP?

Our results support the hypothesis that RP will be inversely related to bone size in children. Retzius periodicity was inversely related to relative cortical area in the older but not the younger children, although the residual was high and the relationship was just $p = 0.048$. There was no relationship between RP and rib cortical area. Relative cortical area was used to standardise the bone area relative to the medullary area and account for variation in overall bone size to assess whether RP could relate to cortical bone area independently of total bone size. A larger relative cortical area means that, as a proportion of overall size, more

interstitial lamellar bone was deposited during the process of bone modeling. Our findings indicate that an older child with a lower RP, and faster biorhythm, will have a larger cortical bone area proportionally to the total cross-sectional area and vice versa. This relationship between RP and relative cortical area indicates that RP is related to bone modeling.

Potentially, this finding that RP relates to Re.Ct.Ar may support the prediction that individuals possessing a faster biorhythm will attain a greater stature or larger body size. However, we did not test the relationship between Re.Ct.Ar and stature or body size directly. Relative cortical area may not relate to body size, since relative cortical area varies across the skeleton in response to biomechanical demands [75]. Our data do not address whether these children were on a trajectory towards a large body size, but it does provide a new foundation for future studies to investigate this potential link.

4.3 Limitations

Since the samples in this study were archaeological, we could not evaluate formation periods directly with vital labelling and we were unable to count lamellae directly, nor measure lamellar thickness due to taphonomy. These measures would help to explain how RP relates to osteonal lamellar area proportion by showing if there was a change in lamellar number or lamellar thickness. Another consideration when using archaeological samples is that fewer factors can be controlled. We were unable to account for variation in dietary, sex-specific, or genetic factors potentially contributing to the expression of rib microstructure that did not manifest macroscopically. A sample of fully documented skeletal samples would allow for a more controlled investigation of our research questions. Nevertheless, the clear links between dental and bone microstructure here certainly invite further research into biorhythms of the juvenile skeleton.

5 Conclusion

We explored the relationship between RP, as a sign of an underlying infradian biorhythm, and bone microstructure in a sample of 50 human juvenile skeletons from a single population. We found evidence that a fewer number of days between adjacent Retzius lines – indicating a faster biorhythm - was positively related to Haversian canal size, but negatively related to relative osteon area and relative cortical area in the ribs of children aged eight to 12 years old. Our results support the idea that an infradian biorhythm coordinates aspects of human hard tissue growth. This biorhythm may be one of the many factors that affect bone growth and remodeling.

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Table 1 Descriptive (non-transformed) statistics for the rib microstructure for the younger children (3-7 yrs) and the older children (8-12 yrs), and the results (*p* value) of a comparison between these groups using an independent samples *t*-test.

Age group	Variable	*N*	Min.	Max.	Mean	SD	*p*
Younger	Tt.Ar	24	15.61	38.14	26.84	7.05	< 0.001**
child	Ct.Ar	24	8.23	23.42	14.81	3.92	< 0.001**
	Re.Ct.Ar	24	37.00	80.00	53.00	9.62	0.612
	On.Dn	29	0.00	9.33	3.38	2.58	< 0.001**
	On.Ar	26	22401.10	53443.69	35073.37	7736.00	0.046*
	Re.On.Ar	26	92.54	97.07	95.31	1.39	0.049*
	On.Dm	26	140.13	257.45	178.54	24.10	0.120
	H.Ca.Ar	26	709.47	2872.17	1616.90	487.13	0.802
	H.Ca.Dm	26	24.62	48.23	36.09	5.67	0.837
Older	Tt.Ar	18	26.37	48.71	37.53	6.31	
child	Ct.Ar	18	11.51	25.10	18.87	3.51	
	Re.Ct.Ar	18	39.00	66.00	51.44	8.66	
	On.Dn	21	1.55	8.79	6.08	1.82	
	On.Ar	21	23383.73	67798.76	40774.59	10638.78	
	Re.On.Ar	21	93.68	97.61	96.06	1.08	
	On.Dm	21	131.49	265.84	190.58	27.63	
	H.Ca.Ar	21	710.38	4078.07	1606.94	677.73	
	H.Ca.Dm	21	25.04	52.41	35.77	6.03	

[a] Statistical significance value: *P<0.05, **P<0.01.

Table 2 Linear regression analyses of log-RP against log-bone size and log-rib microstructure.

	N	Intercept	Slope	*r*	R^2	*p*	Residual
RP vs Ct.Ar							
younger child	24	0.788	0.090	0.145	0.021	0.498	98%
older child	16	1.112	-0.154	-0.166	0.028	0.511	97%
RP vs Re.Ct.Ar							
younger child	24	-0.136	-0.164	-0.160	0.026	0.456	98%
older child	16	0.205	-0.544	-0.500	0.250	0.048*	75%
RP vs On.Dn							
younger child	29	1.054	0.280	0.174	0.030	0.366	97%
older child	21	7.152	-0.131	-0.108	0.012	0.640	99%
RP vs On.Ar							
younger child	26	4.354	0.196	0.161	0.026	0.433	97%
older child	21	4.189	0.449	0.322	0.104	0.154	90%
RP vs Re.On.Ar							
younger child	26	-0.045	0.026	0.323	0.104	0.107	97%
older child	21	0.014	-0.034	-0.563	0.317	0.008**	68%
RP vs On.Dm							
younger child	26	2.135	0.122	0.171	0.029	0.403	96%
older child	21	1.980	0.327	0.413	0.171	0.062	83%
RP vs H.Ca.Ar							
younger child	26	3.486	-0.322	-0.183	0.033	0.371	97%
older child	21	2.067	1.226	0.635	0.403	0.002**	60%

RP vs H.Ca.Dm							
younger child	26	1.724	-0.187	-0.210	0.044	0.302	96%
older child	21	0.994	0.611	0.671	0.450	0.001**	55%

[a] Statistical significance: *P<0.05, **P<0.01.

Figure 1 Histological measurements. A – Average enamel thickness is calculated at 2x magnification. Blue line encompasses the enamel area. AET = total enamel area (mm2) / total enamel-dentine junction length (mm). B – Retzius periodicity is measured at 20x-40x (polarised). Large white arrows show Retzius lines. C – Small white arrows show the daily cross striations between two Retzius lines. RP is the number of days between Retzius lines. White dotted line shows the direction of the prism path.

Figure 2 A – Rib cross section. B – Counting secondary osteons and fragments (10×). White arrows point to Haversian canals. Area bounded in blue indicates an intact osteon and area highlighted in blue indicates a fragmentary secondary osteon (N.On, N.On.Fg, and On.Dn). C – Measurements of osteon structure (20×). Circles indicate the secondary osteons (blue) and Haversian canals (white) measured (On.Ar, H.Ca.Ar, and H.Ca.Dm). Dashed lines indicate the diameters of secondary osteons (blue) and Haversian canals (white) (On.Dm, H.Dm).

Figure 3 Plots of log-RP against a) log-relative osteon area, b) log-osteon canal area, c) log-osteon canal diameter, and d) log-relative rib cortical area for the older child group.

Highlights

- A faster multidien biorhythm relates to increased osteon infilling in children
- A faster biorhythm relates to increased cortical bone modeling in children
- The biorhythm is one of many factors affecting bone growth and remodeling

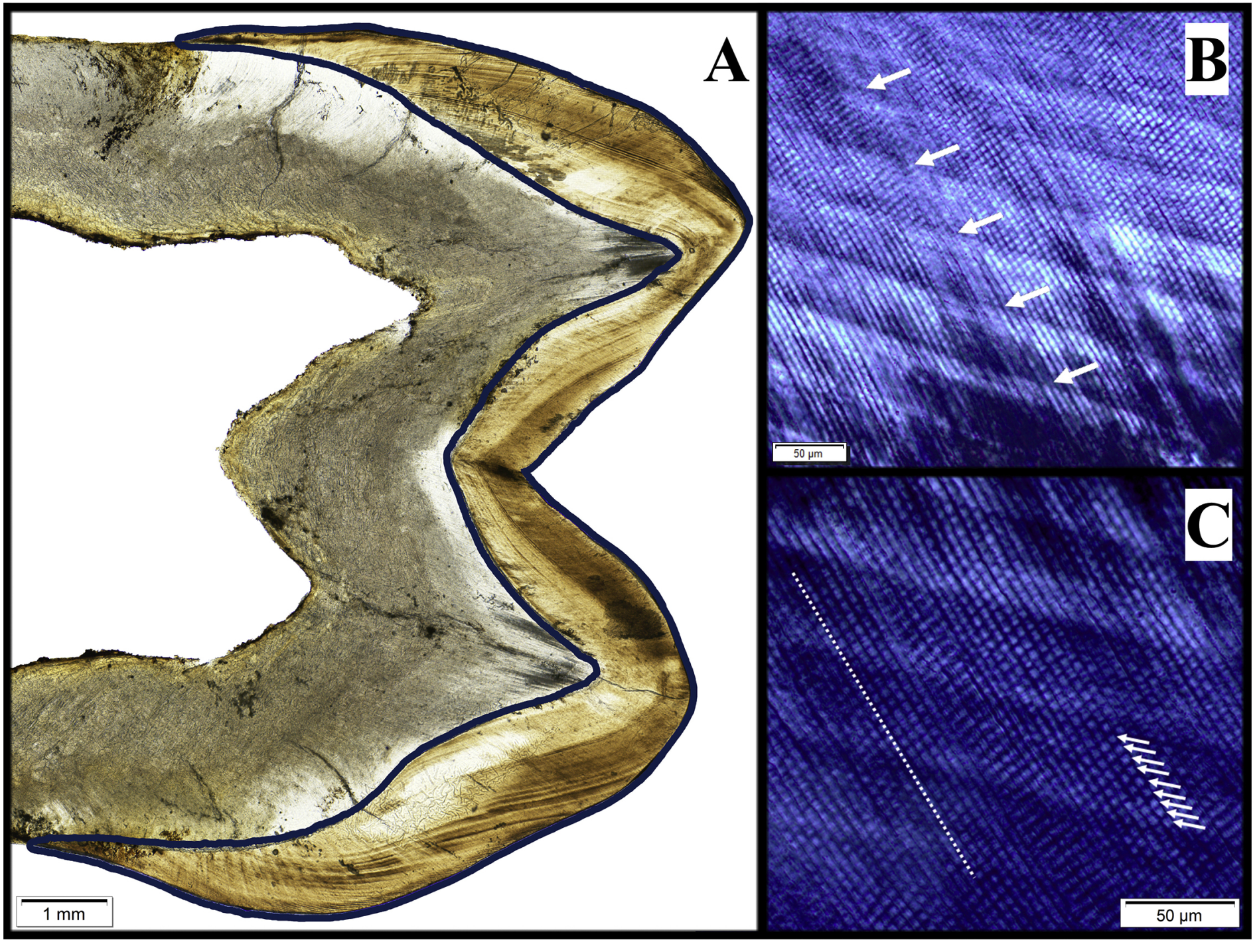

Figure 1

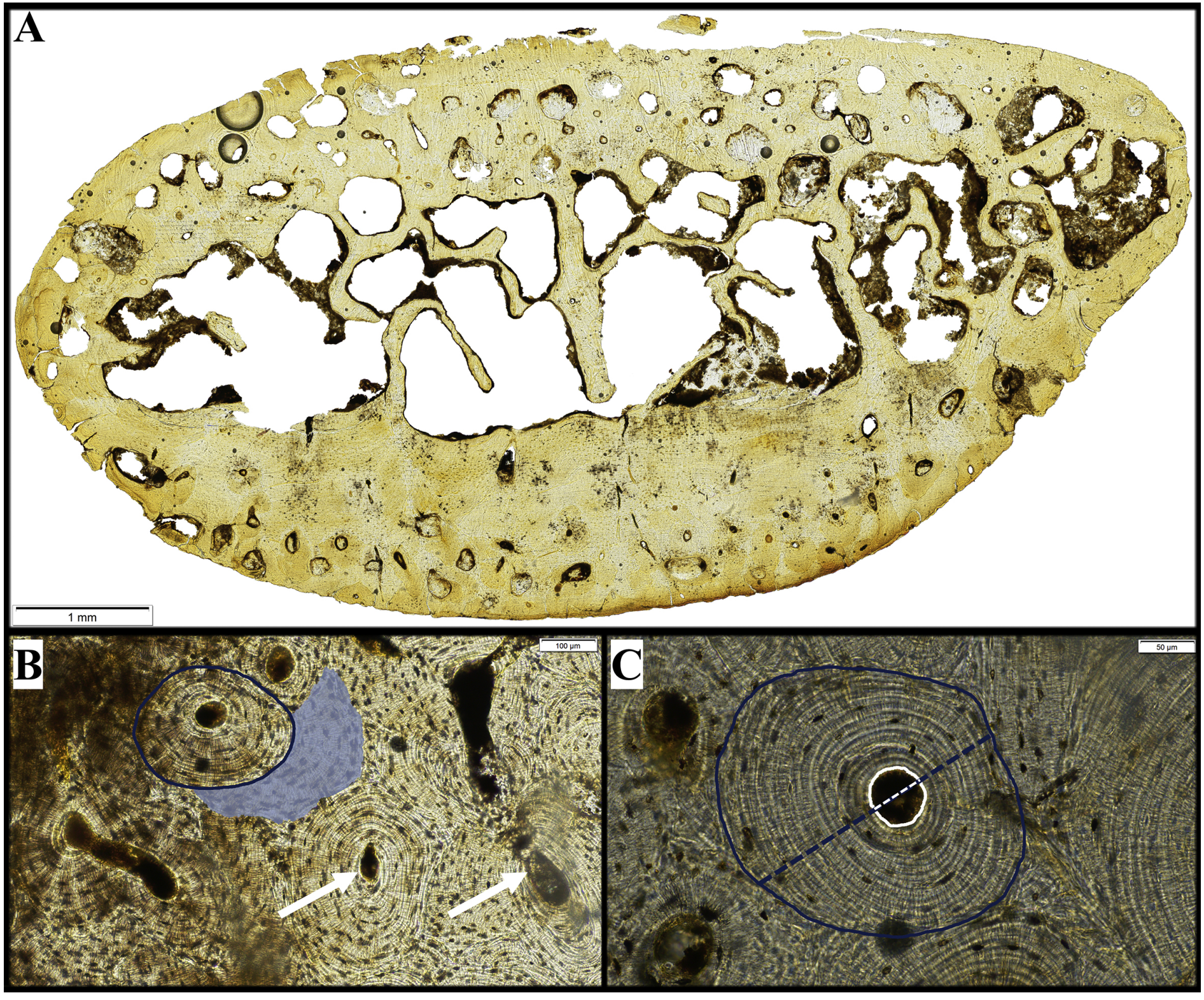

Figure 2

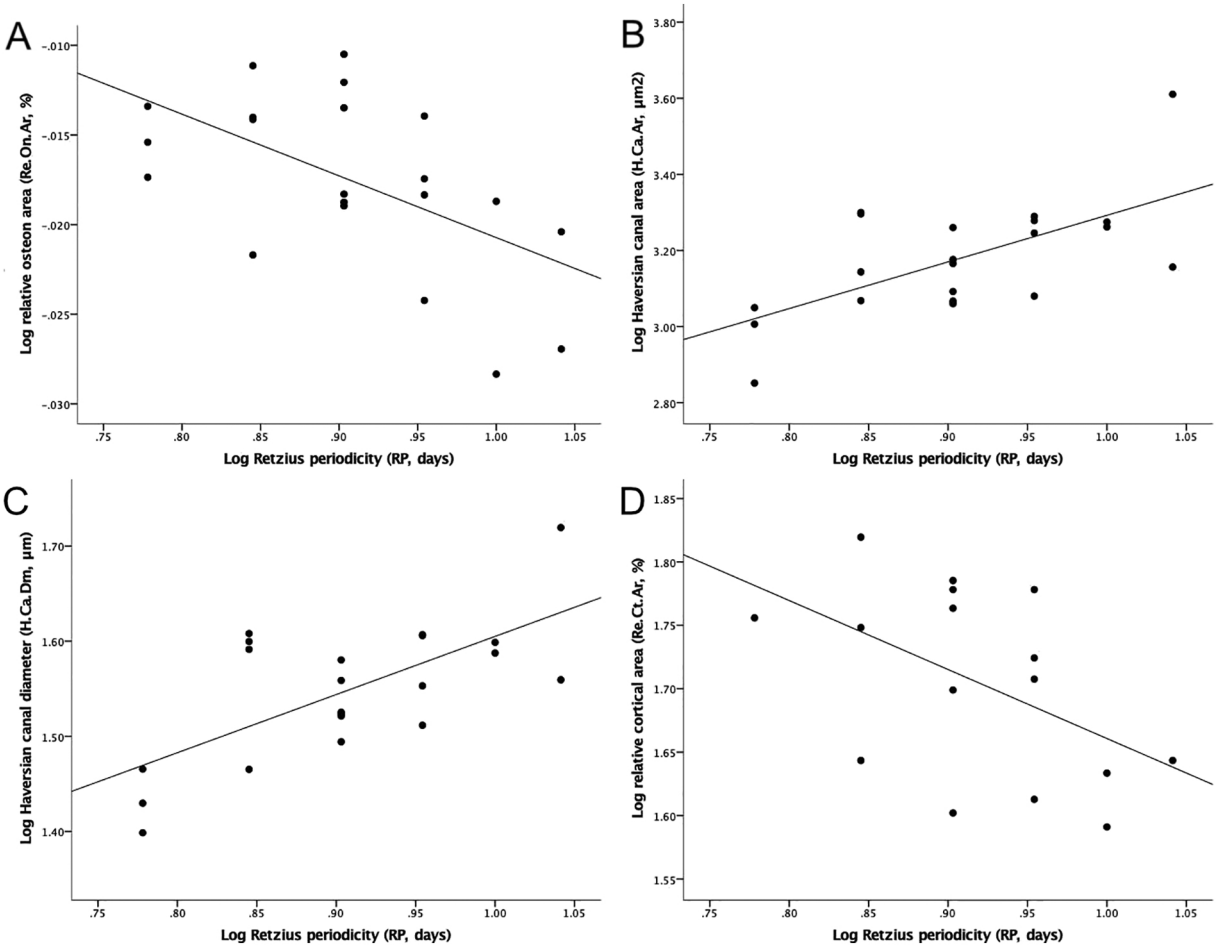
A
Log relative osteon area (Re.On.Ar, %)
-.010
-.015
-.020
-.025
-.030
.75
.80
.85
.90
.95
1.00
1.05
Log Retzius periodicity (RP, days)
B
Log Haversian canal area (H.Ca.Ar, µm2)
3.80
3.60
3.40
3.20
3.00
2.80
Log Retzius periodicity (RP, days)
C
Log Haversian canal diameter (H.Ca.Dm, µm)
1.70
1.60
1.50
1.40
Log Retzius periodicity (RP, days)
D
Log relative cortical area (Re.Ct.Ar, %)
1.85
1.80
1.75
1.70
1.65
1.60
1.55
Log Retzius periodicity (RP, days)

Figure 3