A structural biorhythm related to human sexual dimorphism

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Periodicity of incremental marking in human enamel

Periodicity relates to dimorphism in enamel thickness

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ABSTRACT

Life on earth is regulated by biological rhythms, some of which oscillate with a circadian, monthly or lunar cycle. Recent research suggests that there is a near weekly biorhythm that may exert an influence on human skeletal growth. Evidence for the timing of this biorhythm is retained in tooth enamel as the periodicity of Retzius lines. Studies report that Retzius periodicity (RP) relates to adult human stature and enamel thickness. Adult human stature is sexually dimorphic, and so is enamel thickness of maxillary third molars (M3) but not mandibular M3. Yet, previous studies report sex differences in RP are apparent in some populations but not others, and it is unknown if dimorphism in enamel thickness relates to RP. To further our understanding of this biorhythm we analysed sex-related variation in RP and its relationship with enamel thickness in a sample of M3's ($n=94$) from adults in Northern Britain. Results reveal RP was significantly higher in our sample of female molars compared to those of males, which is consistent with the previously reported correlation between the biorhythm and adult stature. The RP of maxillary M3 related to sex differences in enamel thickness, but this relationship was not present in mandibular M3. Our results support previous findings suggesting that this biorhythm is sexually dimorphic and provide the first evidence that RP may be one factor influencing sex differences in enamel thickness. Our study also shows that correlations between RP and enamel thickness appear to be most readily detected for tooth types with sufficiently wide ranges of enamel thickness variation, as is the case for maxillary but not mandibular M3. Achieving a sufficient sample size was critical for detecting a sex difference in periodicity.

Key Words: Histology; enamel microstructure; growth and development.

1. Introduction

Human teeth retain a record of their growth in the form of incremental markings (**Fig. 1**). Retzius lines are one type of marking that are created by ameloblasts during the secretory phase of enamel growth. Ameloblasts periodically alter the way they secrete new matrix, which changes the density, size and shape of rods and inter-rod regions (Weber et al., 1974; Risnes, 1990, 1998; Li and Risnes, 2004). The altered rod morphology becomes visible as Retzius lines when a tooth is prepared as a thin section and viewed under a microscope using transmitted or polarised light. The number of days of enamel secretion between two adjacent Retzius lines is termed Retzius periodicity (RP), or a long period repeat interval.

Fig. 1. Enamel incremental growth markings preserved as Retzius lines. (a) Illustration of a mandibular permanent third molar and the approximate location of the lateral enamel region on a crown. (b) Thin section of lateral enamel (to the right of the image) imaged at a total (including ocular) magnification of 40x. Black arrows point in the direction of Retzius lines that emerge on the outer lateral enamel surface as perikymata. (c) Retzius lines imaged at 100x magnification and (d) 200x magnification. The 'layers' that are visible in (c) and (d) are an optical phenomenon created by Retzius lines.

Within individuals, RPs typically range from 6 to 12 days with population averages centering on seven, eight, or in some cases, nine days (e.g., Gysi, 1939; Fukuhara, 1959; FitzGerald, 1998; Reid et al., 1998; Risnes, 1998; Schwartz et al., 2001; Reid and Dean, 2006; Reid and Ferrell, 2006; Smith et al., 2007; Mahoney, 2008; Smith, 2008; McFarlane et al., 2014; Nava et al., 2019). A growing body of evidence suggests RP reflects an underlying and newly discovered infradian biorhythm that relates to human skeletal growth. Human RP relates to bone modelling and remodelling (Bromage et al., 2009; Mahoney et al., 2016; Pitfield et al., 2019), attained adult stature (Bromage et al., 2016a; Mahoney et al., 2018), and molar enamel thickness (Mahoney et al., 2016, 2018). The role of this biorhythm for human skeletal sexual dimorphism is poorly understood, and it is unknown if dimorphism in enamel thickness relates to RP.

The source of the infradian biorhythm for humans has been investigated but has not been conclusively identified (Gysi, 1939; Newman and Poole, 1993; Appenzeller et al., 2005; Bromage et al., 2009, 2016b; also see Kierdorf et al., 2019). It has been suggested that RP may result from an underlying centrally controlled (possibly hypothalamic) metabolic oscillation (Bromage et al., 2009) or the autonomic nervous system that controls cardiac activity (Appenzeller et al., 2005). The underlying process is likely to be complex as there is now a growing body of evidence that points towards a sequence of repeat intervals within individuals rather than a single value (Mahoney et al., 2018). Retzius periodicity changed between deciduous and permanent molars of four children (Mahoney et al., 2017), and recent preliminary analyses revealed RP changed when compared along the human permanent tooth row when molars were compared to incisors (McFarlane et al., 2020). The range of RPs in human deciduous teeth also extends below those of human permanent teeth (Mahoney et al., 2016). In these cases, where RP changes within an individual, in different tooth types that have overlapping formation times, and when this change is unrelated to pathology (Mahoney et al., 2017),

it seems unlikely that a single underlying oscillation can explain the expression of this biorhythm in enamel. It seems increasingly likely that there are more local - even tooth specific - genetic or hormonal regulatory controls underpinning, or having a role, in the timing of these repeat intervals, similar to recent findings for the circadian rhythm in mammals (Ray et al., 2020). Whether or how local control may relate to a broader systemic influence on the infradian biorhythm is unknown. Regardless of the debate about the source, the modal timing is not a unique biorhythm for humans as a near seven-day oscillation has been identified in blood pressure during pregnancy, adult heart rate, and adult core body temperature (Halberg et al., 1964; de la Pena et al., 1989; Otsuka et al., 1994; Ayala and Hermida, 1995; Cornélissen et al., 1996, 2001).

Taller adults tend to have lower RPs (Bromage et al., 2016a; Mahoney et al., 2018), meaning their Retzius lines repeat over intervals of fewer days than do those of shorter adults. When considered as a biorhythm, a lower RP has fewer days between Retzius lines representing a faster underlying oscillation. A faster biorhythm has been suggested to result in greater adult height (Bromage et al., 2009). When this hypothetical relationship between RP and height is applied to human sexual dimorphism there is a basis to expect sex differences in the biorhythm. Within any one human population the average adult stature of males will usually be greater compared to that of adult females (e.g., Eveleth, 1975; Hauspie et al., 1985). Thus, following this logic, RP should, on average, be lower in males than it is in females, if RP reflects a faster underlying biorhythm(s) that impacts rates of skeletal growth.

Retzius periodicity has been shown in some populations to exhibit the expected sex differences in central tendencies. Permanent molars from South Africans, and canines from England and South Africa, revealed females had higher RPs on average than males (Schwartz et al., 2001; Smith et al., 2007). Other studies of sex differences in RP for samples from North America (Smith et al., 2007) and China (Tan et al., 2017) reported

no differences. The sample size of Chinese adults may not however have been sufficiently large enough to detect a difference. Amongst $n=34$ Chinese adults, the lowest RPs of 6 days were present in males but not females, even though there was no sex difference in their median RP (Tan et al., 2017: their Fig 2).

Retzius periodicity has an association with the average enamel thickness (AET) of deciduous second, and permanent first and second human molars (Mahoney et al., 2016, 2018). We do *not* suggest RP has a deterministic effect on enamel thickness. Enamel thickness is determined by the number of active ameloblasts, their secretory life span, and the total period over which these cells are active (Grine and Martin, 1988; Dean, 2000). We have previously proposed that RP can relate to some enamel formation processes and enamel thickness in a predictable way for human molars (Mahoney et al., 2017). We develop this proposal here (**Fig. 2**). Deciduous second (Mahoney et al., 2017) and permanent first molars (Mahoney et al., 2018) with higher repeat intervals can have a greater distance between adjacent Retzius lines producing thicker enamel layers relative to layers from equivalent regions of molars with lower repeat intervals. Layers become wider because ameloblasts deposit enamel for additional 24-hour periods in crowns with higher repeat intervals without greatly changing the rate at which they secrete new matrix (Mahoney et al., 2017, 2018). Wider layers with higher RP's should result in a molar with a thicker enamel cap but only if the period of time over which the layers accumulate is extended. There is reason to suspect that this can be the case. Retzius periodicity relates to formation time (Mahoney et al., 2016), and formation time relates to enamel thickness (Dean et al., 2001; Mahoney, 2011; Pitfield et al., 2018). Thus, there are clear links between repeat intervals, formation time, and enamel thickness. Taken together these links suggest that when daily secretion rates (DSRs) are held constant higher repeat intervals will relate to thicker enamel caps if crown formation periods or ameloblast lifespans are extended (Mahoney et al., 2017).

Fig. 2. Model of human molar enamel formation processes incorporating Retzius periodicity.

When daily enamel secretion rates are constrained, higher repeat intervals can lead to thicker enamel if formation periods are extended (see Text for details). The model is partially constrained by the total number of Retzius lines within a tooth. If both crowns in Figure 2 had 50 Retzius lines, and DSRs were the same in each tooth, then the crown with an RP of 9 would lead to a thicker enamel cap and a greater quantity of enamel compared to the crown with an RP of 6. However, if the number of lines *increased above 70* in the crown with an RP 6, it would have more enamel than the crown with an RP of 9 and 50 Retzius lines. Perikymata packing of Retzius lines in the cervical region of enamel would also be a factor in the relationship between the number of these lines related to RP and enamel thickness. Our model of molar enamel formation processes and RP does *not* transfer along the tooth row to other tooth types (see discussion in Mahoney et al., 2017).

Sexual dimorphism in molar dental tissues has been reported. The AET of first and second mandibular molars can be greater in females but it is not always the case that it is (Smith et al, 2006; Feeney, 2009; Daubert et al., 2016). Thicker enamel has also been related to a smaller dentin core (and shorter EDJ length) for some female molar types, which can produce a greater AET compared to males (Smith et al., 2006) depending upon the quantity of crown enamel.

When just permanent third molars (M3s) are considered, analyses of thin sectioned mesial cusps and 3D volumetric reconstructions reveal sex differences in dental tissues but there is a different pattern for the upper (M^3) and lower molar (M_3). Female M^3 s can have a greater AET (Smith et al, 2006: their Appendix B). In this case, dimorphism in AET relates to the quantity of crown enamel, not a reduction to the underlying dental components as neither EDJ length nor the quantity or proportion of dentin in M^3 differs greatly between the sexes (Smith et al., 2006; see Feeney, 2009: her Table 3.2, 3.3, 3.4). The lower third molar differs to its isomere in that there are usually no sex differences in AET (Stroud et al., 1994; Schwartz and Dean, 2005; Smith et al., 2006; Feeney, 2009). Furthermore, the range of enamel thickness values is reduced in M_3 compared to the upper molar (Schwartz and Dean, 2005; Smith et al. 2006: their Appendix A). The M_3 of females also have proportionally less dentin compared to male M_3 's, which occurs in first and second mandibular molars as well (Feeney, 2009; Sorenti et al., 2019). It is not clear why enamel and dentin distinguish between males and females in these ways, or if factors thought to contribute to dental sexual dimorphism, such as intra-uterine testosterone and/or Y-chromosome genes, manifest differently in the upper and lower third molars (e.g., Alvesalo et al., 1991; Dempsey et al., 1999; Guatelli-Steinberg et al., 2008; Ribeiro et al., 2013).

1.1. Aims and hypotheses

Third molars, available in large numbers through dental extractions provide an

opportunity to test whether the biorhythm underlying RP is sexually dimorphic. Given that sex differences in enamel thickness have previously been detected in upper but not lower third molars, this tooth type is useful for assessing whether females have both higher periodicities and thicker enamel in M^3 but not M_3 . By focusing on a single tooth type we avoid potential variation in RP along the tooth row (McFarlane et al., 2020).

Consistent with average differences in height between the sexes, we test the hypothesis (1) that with a sufficient sample size we will detect a greater average RP in females than males in both upper and lower M_3 . Also in this study, we hypothesise that if RP has a detectable relationship with enamel thickness, then (2) greater AET should be found in females, especially in upper third molars, in association with their higher average periodicities. More specifically, we predict that if daily enamel secretion rates do not differ between sexes, then sex differences in RP and AET should be present in upper third molars. Such a result would suggest that RP plays a role in sex differences in enamel thickness consistent with the positive association of RP with enamel thickness. (3) Finally, we hypothesise a weaker correlation between RP and AET in lower third molars because of their tighter range of enamel thickness values. Variation is needed for correlation (Bland and Altman, 2001). Based upon prior research we do not expect lower third molars to exhibit sex differences in AET.

2. Material and methods

2.1. Study sample and Ethics

Ninety-four permanent third molars were selected from the UCL-Kent dental collection that is composed of permanent teeth collected from dental surgeries in northern Britain between 1964 and 1973. Each tooth had been extracted because of an impending impaction, or an impaction that was current at the time of the extraction. There was no evidence to indicate that enamel growth was abnormal in these molars due to impaction

(see comparisons of DSRs and RP to previous studies in Results and Discussion). Molars that displayed developmental pathology in the form of hypoplasia were excluded, as RP can change within a single crown in response to periods of non-specific stress (Mahoney et al., 2017). The sex of each individual was known but otherwise all teeth were anonymous. Ethical approval for histology research on this sample of teeth was obtained from the UK National Health Service research ethics committee (REC reference: 16/SC/0166; project ID: 203541).

- RP was calculated from the lateral enamel of the entire sample of molars. This larger sample was used to compare RP between females ($n=48$) and males ($n=46$). We did not have measurements of adult height for each individual that provided an M3. Instead, we relied upon the typical difference in sexual dimorphism whereby adult males are on average taller than adult females, which has been reported previously in population standards for adults of northern Britain (Miller et al., 1972).
- We separated out thin sections from females ($n=18$) and males ($n=17$) that had clearly preserved and unworn enamel to calculate AET. This smaller sample was used to determine if the biorhythm related to sex differences in enamel thickness.

2.2. Histology

Thin-sections of M3 were prepared in the Human Osteology Lab, University of Kent, using standard methods (Reid et al., 1998). Teeth were embedded in resin (Buehler EpoxiCure®) and sectioned through the tip of the cusp and dentin horn using a Buehler

Fig. 3. (a) Enamel incremental cross striations. (b) Black arrows point at daily cross striations. Prism direction is indicated by the black line

Isomet 1000 precision saw. Sections were fixed to glass microscope slides (Evo Stick® resin), ground with a series of grinding pads (P400, P600, P1200) (Buehler® EcoMet 300), and polished with a 0.3µm aluminium oxide powder to a 60 – 90 µm-thick section (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®). The thin sections of the teeth were examined using a high-powered microscope (Olympus® BX53) with a mounted microscope camera (Olympus® DP25). Images of the thin sections were obtained and examined in CELL® Live Biology imaging software.

Before calculating RP or AET all oblique thin sections were identified and removed from the study. Oblique sections can be easily identified from the morphology of the dentin horn together with the slope of the enamel buccal and lingual surfaces of the functional and guiding cusps. The sample size of $n=94$ is the final sample after these sections had been removed.

Retzius periodicity was calculated in lateral enamel from daily cross-striations (**Fig. 3**). Lateral enamel commences as the first Retzius line emerges on the outer enamel surface as a perikymata (Shellis, 1998). Cross-striations appear as light and dark markings along the length of enamel prisms when thin sections of human lateral crown enamel are viewed with transmitted light under a microscope (Boyde, 1989; Nanci, 2013). Cross-striations relate to a circadian rhythm in primate enamel (Schour and Poncher, 1937; Okada and Mimura, 1940; Bromage, 1991; Smith, 2006; Zheng et al., 2011, 2013).

Retzius periodicity was calculated in two ways. The number of daily cross-striations was counted along a prism between two adjacent Retzius lines in lateral enamel at 200-400x magnifications (includes the ocular magnification). When consecutive cross-striations were not clearly visible between two Retzius lines, RP was

calculated from local daily enamel secretion rates (DSR) divided by prism lengths (e.g., Dean, 1993; Schwartz et al., 2001; Mahoney et al., 2007; Smith, 2008). DSRs were calculated by measuring along a prism across the span of six cross striations, which corresponds to five days of enamel formation (two adjacent cross striations = 24hrs of enamel secretion), and dividing this measurement by five to get a daily mean DSR. This was repeated six times within the local enamel so that a grand mean DSR could be calculated. Following this, the distance between four to six adjacent Retzius lines was measured, corresponding to three to five repeat intervals respectively, and divided by three or five. This distance between two adjacent Retzius lines was then divided by the grand mean DSR to yield an RP value.

2.2.1. Inter-observer error test

Three observers recorded Retzius periodicity values in the present study (RP, MO, PM). Inter-observer error tests were conducted by RP, GM, and PM. Values from $n=4$ slides differed in the error test, and these slides were removed from the study giving the final sample size of $n=94$ reported in Section 2.1 above.

2.3. Enamel thickness measurement

The 2D AET in mm was calculated from thin sections of each crown by dividing the area of the enamel cap by the length of the dentin–enamel junction (DEJ), which provides the average straight-line distance between the DEJ and outer enamel surface (Martin, 1983, 1985). None of the thin sections displayed a complex topography of the DEJ, which could artificially increase the AET.

2.4. Statistical analyses

Retzius periodicity and AET were compared between the sexes with a Mann Whitney U

and Kruskal Wallis test. Associations between log-transformed AET and log-transformed RP were explored with Pearson's *r*.

3. Results

3.1. Retzius periodicity compared between males and females

Mean RP values subdivided by tooth type and sex are shown in Table 1. Corresponding raw data for the entire sample of RP's for this study are available in supplementary Table S1. The median and modal RP for the entire sample was eight days. Retzius periodicity ranged between 7 to 11 days in females (modal of 8 days) and between 6 to 10 days in males (modal of 7 days; **Fig. 4**). The mean RP of 8.63 for the entire sample of females was significantly higher when compared to the mean RP of 7.65 for the entire sample of males ($U= 621.50$; $p<0.001$). When the tooth types were subdivided and analysed separately RP was significantly higher in the M^3 of females compared to males ($U= 89.50$; $p<0.001$). There was a weaker but still significant difference when M_3 was compared between the sexes ($U= 229.00$; $p= 0.025$).

Table 1
Retzius periodicity in male and female third molars in days.

Sex	Tooth	Mean (sd)	95% CI	min	max	mode	median
Combined ($n=94$)	All M3s	8.15 (1.20)	7.90-8.39	6	11	8	8
Females ($n=48$)	All M3s	8.63 (1.17)	8.28-8.97	7	11	8	8
Males ($n=46$)	All M3s	7.65 (1.01)	7.35-7.95	6	10	7	8
Female ($n=22$)	M^3	8.68 (0.99)	8.24-9.12	7	11	8	9
Male ($n=19$)	M^3	7.58 (1.01)	7.09-8.07	6	10	7	7
Female ($n=26$)	M_3	8.58 (1.33)	8.04-9.11	7	11	8	8
Male ($n=27$)	M_3	7.75 (1.03)	7.30-8.11	6	9	7	8

Fig. 4. Plot of raw Retzius periodicity values for male and female third molars.

3.2. Enamel thickness compared between males and females

Mean AET subdivided by tooth type and sex are shown in Table 2. Corresponding raw data are available in supplementary Table S2. Sex differences in enamel thickness were apparent in M³ but not M₃, which is consistent with previous research for these tooth types (Schwartz and Dean, 2005; Smith et al., 2006). The mean female AET of 1.28 mm for M³ was significantly greater compared to the male AET of 1.15 mm ($U=11.00$; $p=0.019$; **Fig. 5a**). The AET of M₃ did not differ significantly between the sexes (**Fig. 5b**), which had a reduced range of enamel thickness values compared to the upper molars (M³ AET range of 0.43; M₃ AET range of 0.32: see Table S2). Thicker enamel of M³ compared to M₃ has been reported previously (Table 2).

Table 2
Average (sd) coronal enamel thickness of human third molars in mm.

Sex	M3	AET	Source
All	M ³ (n=18)	1.23 (0.11)	This study
All	M ₃ (n=18)	1.11 (0.07)	This study
All	M ³ (n=53)	1.38	Smith et al., 2008
All	M ₃ (n=45)	1.25	Smith et al., 2008
Female	M ³ (n=12)	1.28 (0.10)	This study
Male	M ³ (n=6)	1.15 (0.08)	This study
Female	M ³ (n=33)	1.43 (0.15)	^a Smith et al. 2006
Male	M ³ (n=11)	1.32 (0.08)	^a Smith et al. 2006
Female	M ₃ (n=7)	1.10 (0.07)	This study
Male	M ₃ (n=11)	1.12 (0.08)	This study
Female	M ₃ (n=111)	1.20 (0.15)	^b Schwartz and Dean, 2005
Male	M ₃ (n=33)	1.20 (0.15)	^b Schwartz and Dean, 2005
Female	M ₃ (n=14)	1.32 (0.19)	^a Smith et al. 2006
Male	M ₃ (n=4)	1.32 (0.04)	^a Smith et al. 2006

a= Mixed sample of South African, North American, and medieval Denmark, their Appendix B
b=Mixed sample from the UK

Fig. 5. Combined box and scatter plot of average enamel thickness for upper (a) and lower third molars (b). Box plots show the median, inter-quartile and min-max range.

3.3. Enamel thickness compared to Retzius periodicity

There was a significant correlation between log-AET and log-RP when all molar types were combined ($r=0.571$; $p<0.001$; **Fig. 6a**), and a slightly stronger correlation when just M³ was analyzed ($r=0.624$, $p=0.006$; **Fig. 6b**). The relationship between log-AET and log-RP of M₃ was much weaker and not significant (**Fig. 6c**).

Fig. 6. Scatter plot of log-Retzius periodicity against log-average enamel thickness for (a) all permanent third molars. (b) Permanent maxillary third molars. (c) Permanent mandibular third molars.

3.4. Sex differences in enamel thickness compared to periodicity and secretion rates

Retzius periodicity related to sex differences in AET of M³ (**Fig. 6b**). The association between RP and AET was explored further in males and females. When log-AET was compared between periodicities of 7, 8 and 9 days (the largest sample size), enamel thickness differed significantly amongst females (χ^2 (2)= 7.436; p = 0.024; **Fig. 7a**). Amongst males, log-AET increased from molars that had RP's of 7 days to those that had RPs of 8 or 9 days, but the difference was not significant. The greater increase in enamel thickness for each 'step-up' in RP suggests the link between the biorhythm and AET is stronger for females compared to males.

Comparisons of DSRs from the mid cuspal enamel region of permanent maxillary molars indicates that the distribution of DSRs from male and female molars overlaps but does not change greatly between the sexes (**Fig. 7b**). Males having a slightly lowered mean DSR of 4.08 μ m/day compared to the mean DSRs of 4.23 μ m/day of females and the difference in these mean values was not significant. These mean DSRs are similar to those reported previously for mid enamel regions of permanent molars (mean of 4.30 μ m $\pm$ 0.50 and 4.50 μ m $\pm$ 0.55; Beynon et al., 1991; Lacruz and Bromage, 2006). Our finding that DSRs do not differ between males and females is consistent with previous research that has reported no sex related differences in DSRs of human permanent canines (Schwartz et al., 2001), incisors (Aris et al., 2020a), or first molars (Aris et al., 2020b).

Fig. 7. Retzius periodicity compared to enamel thickness and DSRs. **(A)** Plot of the variable log-Retzius periodicity against log-average enamel thickness for female and male molars with RPs of 7, 8 and 9 days. **(B)** Plot of DSRs from the mid-cuspal enamel region of female and male maxillary molars. **(C)** A composite illustrating that as the biorhythm slows down there is a greater increase in the average enamel thickness of maxillary molars for females compared to males. The line for females is in dark blue; that for males in light blue. Daily enamel secretion rates from maxillary molars change only slightly when compared between the sexes. Box plots show the median, inter-quartile and min-max range.

4. Discussion

We build upon our previous work by showing that RP of human permanent third molars differed between a sample of adult males and females from northern Britain. Adult females had a slower biorhythm as well as thicker maxillary molar enamel when compared to adult males. That the sex with higher average enamel thickness also has a higher average RP is consistent with our finding that within the sexes, RP correlates with average enamel thickness. Our findings provide the first evidence that Retzius periodicity may be associated with sexual dimorphism in human enamel thickness.

4.1. The timing of Retzius lines

The median and modal RP of eight days for the entire sample of M3's from northern Britain is consistent with previous studies of human RP (e.g., Schwartz et al., 2001; Reid and Dean, 2006). Seventy-eight percent of all RP's from adults in northern Britain lay between seven to nine days, which is similar to the distribution of RPs reported by Beynon and Reid (1987), and Smith et al. (2007). We observed a lowermost RP of six days that was present in five males out of the entire sample of 94 adults. We found no evidence of the five-day periodicity observed by Hudda and Bowman (1994) and Hogg et al. (2018).

4.2. Sex differences in Retzius periodicity

Females had a higher average RP compared to males (**Fig. 4**). This finding supports the previously reported relationship between RP and final attained adult stature (Bromage et al., 2016a; Mahoney et al., 2018). There was however a large degree of overlap between the sexes in our data, and the distribution of these values was positively skewed. The overlap by itself is not surprising. Many of the males and females in our sample would likely have been of similar height, which could have contributed to the overlap.

Differences in RP were greater when M^3 was compared between the sexes and weaker in comparisons of M_3 . These different relationships with the biorhythm are also evident when smaller sub-sets of each tooth type are analysed. Sex differences in RP are apparent in both the larger (Table 1) and smaller sample of M^3 s (**Fig. 6b**), where this relationship is stronger. Sex differences are comparatively weaker in the larger sample of the lower molar (Table 1) and this link disappears in the smaller sample of this tooth type (**Fig. 6c**). This means that fundamental methodological choices are a factor when searching for dimorphism in RP. Sample size is one factor because of the large degree of overlap between males and females (**Fig. 4**). Tooth type is clearly another. Perhaps it is only possible to detect consistent sex differences in RP in teeth that also display sex differences in AET, as it may be these teeth that are most responsive to the underlying oscillation (see Sections 4.4-5 below).

4.3. Retzius periodicity related to enamel thickness

Correlation between AET and RP is consistent with our previously reported findings for human molars (Mahoney et al., 2016, 2018). The correlation was strongest when just M^3 s were considered and was weaker and non-significant for M_3 . Our data reveal a greater range of enamel thickness values (0.43mm) and a higher standard deviation (0.11) for the upper molar compared to the range of AETs (0.32mm) and standard deviation (0.07) for the lower molar. A greater range in M^3 AET has been reported previously (Smith et al., 2006), and also suggests a reason for the difference in the strength of the correlation coefficients for upper and lower M^3 's. Lower third molars display a weaker correlation between RP and AET in part because of their reduced range of enamel thickness values. Limited variation in male AET values (range=0.26; sd=0.08) compared to females (range=0.49; sd=0.12) may also explain why a 'step-up' in periodicity led to a greater effect on the AET of female molars (**Fig. 7a**).

Both upper and lower third molars develop at around the same time, but M^3 had a much stronger link to AET with higher periodicities. This makes sense as upper molars have thicker enamel that takes longer to form compared to M_3 (Table 2; Reid and Dean, 2006). Thus, an upper M^3 with a high RP of 9 or 10 days will have a greater AET because the thickened enamel layers will have more time to accumulate compared to a lower molar (see **Fig. 2**). By comparison, an M_3 with an RP of 9 or 10 days will have a lower AET because the same thickened enamel layers will have had relatively less time to accumulate producing a smaller change in AET. The effect of an increased periodicity is therefore more pronounced on the enamel thickness of the upper molar because of the relatively extended growth period, leading to a stronger correlation between RP and AET for M^3 . This may explain why the lower molars with a shorter formation time have a more restricted range of AET values. Others have also noted developmental differences between maxillary and mandibular teeth (McCollum and Sharpe 2001; Modesto-Mata et al. 2020).

Figures 6a and 6b illustrate that *within* the sexes, females with higher RPs and thicker enamel occupy the upper right side of the graph, though with overlap with males in the middle. It is clear that RP relates to enamel thickness in a predictable way for M^3 s and this relationship is strongest for females. We do *not* suggest RP is the only factor relating to thicker enamel. A reduction in the overall size of the dentin core along the molar row leads to greater AET and relatively thicker enamel on M_3 compared to the first molar (Grine, 2005; Smith et al., 2008). This was not the case for our samples, as there was only a weak non-significant correlation between AET and the size of the dentin core within maxillary third molars ($r=0.297$, $p=0.099$) or when just the female M_3 s are considered ($r=0.494$, $p=0.176$). Third molars with thicker enamel did not have a smaller dentin core. Thus, the relationship between these dental tissues for M_3 of

females differs to the mechanism that produces a gradient of enamel thickness along the molar row.

4.5. Sex differences in enamel thickness related to RP and DSRs

Retzius periodicity was associated with a sex difference in enamel thickness of M³ (**Fig. 6b**). There was a weaker link between RP and AET in the lower third molar that did not distinguish between males and females (**Fig. 6c**). The fact that RP co-varies with sex differences in enamel thickness suggests the ‘slowed oscillation’ that produces ‘thickened layers’ is also integrated into formation processes that lead to sexual dimorphism in enamel thickness. The greater AET of female M³’s is not achieved by altering the daily amount of enamel produced by secretory ameloblasts (**Fig. 7b**), which is the same as human permanent canines (Schwartz et al., 2001). There is reason to think therefore that if males and females have similar DSRs, and females with higher periodicities are taking longer to form their M³, they will build up more enamel in their molar crown than males. Retzius periodicity relates to formation time (Mahoney et al., 2016) and formation time relates to enamel thickness (Dean et al., 2001; Mahoney, 2011; Pitfield et al., 2018). Given that our data show sex differences in both periodicity and enamel thickness of M³’s it would seem likely that, on average, female molars will form over a longer period of time. Smaller dentin cores and shorter EDJ lengths do not explain the sex difference in AET in our samples (M³ female EDJ length= 20.77mm, male= 20.99mm), which is consistent with prior research for this tooth type (Smith et al, 2006: Appendix B; Feeney, 2009: Table 3.2-3.3). Future studies might explore dimorphism in formation time related to RP.

There is now a growing body of evidence that suggests circadian and infradian biorhythms do not connect to human hard tissue formation in the same way. First, we have shown that the infradian rhythm does not relate to the amount of enamel secreted

by ameloblasts over a 24-hour period in humans (Mahoney et al., 2017). As molar long period repeat intervals increase enamel layers became wider but ameloblasts do not secrete more or less enamel, which suggest the two underlying biorhythms relate to incremental enamel formation processes in different ways. Second, data in the present study are consistent with others that have reported the circadian rhythm underlying the production of daily cross-striations in human enamel is not sexually dimorphic (Schwartz et al., 2010; Ariz et al., 2020a,b). Our data provide evidence that the infradian rhythm underlying Retzius lines is sexually dimorphic. Third, there are now clear indications in the literature that one individual can have a sequence of infradian repeat intervals. The fact that one individual can have multiple RPs in different teeth that form at the same time is unlike the timing of the circadian rhythm in human enamel. The circadian rhythm is entrained by the 24-hour light-dark cycle, and remains synchronised to this rhythm unless it is removed from this environment and is free running. The effect of environment on RP is currently unknown. Taken together, these data point towards fundamental differences in the infradian and circadian biorhythms within humans, and the ways in which they connect to hard tissue formation.

5. Conclusions

Long period markings in enamel have fascinated dental morphologists for more than a century as they have eluded explanation. Our study explored their relationship to aspects of human skeletal dimorphism. We have shown that in a sample of $n=94$ permanent third molars from Britain there was a sex difference in the average timing of these markings that related to the thicker maxillary molar enamel of females. Our findings lend support to the growing body of evidence that points towards a biorhythm(s) that underpins these markings in enamel and relates to human skeletal growth and morphology.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary data

Tables S1 and S2 contains the raw RP and AET data.

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