D-lightful sunshine disrupted: Vitamin D deficiency as a method for the reconstruction of changes in sociocultural practices due to industrialisation in 17th - 19th century Netherlands

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5 Micro-CT assessment of dental mineralization defects indicative of vitamin D deficiency in two 17th - 19th century Dutch communities

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ABSTRACT

Objectives: This study investigates vitamin D deficiency patterns in individuals from birth to the beginning of adolescence. Microscopic computed tomography (micro-CT) evaluation of interglobular dentine (IGD) in the teeth provides information on the age of disease onset and the number of deficient periods per individual, which will increase our understanding of factors influencing vitamin D deficiency prevalence, including sociocultural practices and latitude.

Materials and Methods: Beemster and Hattem, two Dutch 17th to 19th century communities, yielded with relatively high prevalences of rickets (15 – 24%) and residual rickets (14 – 24%). From the affected individuals, a subsample of thirty teeth were selected for micro-CT scanning. Thin sections were made of 17 teeth, consisting of 11 teeth without observable IGD on micro-CT and six teeth that were included for method comparison.

Results: Nineteen out of 29 (65.5%) individuals (one tooth was deemed unobservable) presented with IGD. Eight of the 11 (72.7%) individuals without IGD on micro-CT demonstrated histologically visible IGD. In 40.7% (11/27) of the affected individuals, vitamin D deficiency was recurrent, and in four individuals, these episodes were chronologically successive suggesting vitamin D deficiency was seasonal. In three individuals, IGD occurred in the dentine formed around birth, suggesting maternal vitamin D deficiency.

Discussion: Micro-CT analysis of IGD is found to be a valuable non-destructive method that can improve our understanding of the influence of sociocultural practices and latitude on disease development within age and sex groups in past communities.

INTRODUCTION

Vitamin D is important for calcium homeostasis needed for mineralization of osteoid, newly formed bone tissue (Holick, 2006). Mineralization ensures structural integrity of the skeleton enabling it to withstand gravity and muscular tension. Cutaneous production of vitamin D under the influence of ultraviolet B (UVB) radiation in sunlight is the most effective way of obtaining an adequate amount of vitamin D, with foods such as oily fish providing supplemental amounts (Brickley, Moffat & Watamaniuk, 2014; Holick, 2003, 2006). Insufficient sunlight exposure will lead to vitamin D deficiency and bending deformities of the skeleton may develop that are visible in archaeological human remains (Brickley, Mays & Ives, 2010; Brickley et al., 2014; Brickley, Mays, George & Prowse, 2018; Mays, Brickley & Ives 2006; Ortner & Mays, 1998).

Recent research has shown that skeletal evidence of vitamin D deficiency provides important information on sociocultural practices related to sunlight exposure and diet, and can aid in the reconstruction of past lifeways (Brickley et al., 2014; Giuffra et al., 2015; Palkovich, 2012; Veselka, Van der Merwe, Hoogland & Waters-Rist, 2017; Watts & Valme, 2018). Vitamin D deficiency in archaeological remains is traditionally assessed via macroscopic examination of various lesions. This approach does not offer information about the number of separate episodes of vitamin D deficiency nor the age at which deficiency occurred. A recently developed method by D'Ortenzio et al. (2016) demonstrated the use of histological examination of dental mineralization defects called interglobular dentine (IGD) as a marker of vitamin D deficiency. A recent clinical investigation has shown that IGD is visible on microscopic computed tomography (micro-CT) scans (Ribeiro, Costa, Soares, Williams Jr. & Fonteles, 2015) and Brickley et al. (2017a) have shown IGD can also be observed in archaeological teeth. IGD analysis via non-destructive micro-CT should enable the determination of the age of vitamin D deficiency onset and the assessment of the frequency of vitamin D deficient periods within an individual (Brickley et al., 2017a). This study set out to apply micro-CT analysis of IGD that can aid in the diagnosis of vitamin D deficiency. This paper will use micro-CT IGD analysis to investigate parameters of vitamin D deficiency in a subset (n = 30) of two previously studied 17th - 19th century

Dutch communities, Beemster (MB11) and Hattem (HT15) that were shown to have high levels of rickets (between 15% and 24%) and residual rickets (between 14% and 24%) (Veselka, Hoogland & Waters-Rist, 2015; Veselka et al., 2017; Veselka, Brickley, Hoogland & Waters-Rist, submitted). Although researchers have started to differentiate between cases of healing and active rickets (Brickley et al., 2018; Mays et al., 2006), the development of vitamin D deficiency in various age groups is still poorly understood, whereby the influence of sex and gender roles in nonadults is rarely investigated (Veselka et al., 2015). Although this can be examined in adults with residual rickets (Veselka et al., 2017), the information that can be obtained via macroscopic analysis is limited due to growth and the process of remodeling which may have obliterated certain features (Hess, 1930).

The objectives of this paper are, firstly, to investigate vitamin D deficiency patterns in individuals from birth to the beginning of adolescence via the assessment of the age of disease onset and the number of deficient periods per individual in two post-Medieval Dutch communities. Information on the age of disease onset improves our understanding of vitamin D deficiency development and may provide information on sociocultural practices that possibly increased the risk of deficiency or that exacerbated the condition. The influence of seasonality will be assessed by examining the age of the consecutive IGD formation periods. Furthermore, comparison of IGD severity and the number of IGD episodes between males and females may improve our understanding of the gendered division of activities that influenced sunlight exposure. Our second aim is to investigate the potential of micro-CT analysis of vitamin D deficiency by comparing macroscopic, micro-CT, and histological assessment of this condition. This study is the first to apply a wide-scale assessment of macroscopic, micro-CT, and histological methods of vitamin D deficiency diagnosis.

MATERIALS AND METHODS

Two communities were assessed for this paper: 1) Beemster (MB11), a rural settlement dating to the 19th century in the province of North-Holland with a skeletal sample consisting of 59 nonadults and 200 adults and 2) Hattem (HT15), a small urban center dating from 17th to 19th centuries in the province of Gelderland with a skeletal sample consisting of 21 nonadults and 88 adult individuals. Figure 1 shows the location of these sites.

Macroscopic analysis of both skeletal collections was undertaken prior to this study (Veselka et al., 2015, 2017, submitted). Rickets was assessed scoring various skeletal lesions, including flaring and/ or cupping of the long-bone metaphyses, porosity underlying the growth plate, and bending deformities of the long bones (Brickley & Ives, 2008; Mays et al., 2006; Ortner & Mays, 1998). Residual rickets was scored using the criteria described by Brickley and Ives (2008) and Brickley et al. (2010). Rickets

Figure 1. Location of Beemster and Hattem in the Netherlands.

prevalence in the Beemster community was 15.3% (9/59) and 23.8% (5/21) in Hattem. Residual rickets prevalence was observed to be 14.5% (29/200) in Beemster and 23.9% (21/88) in Hattem (Veselka et al., 2015, 2017, submitted). A subset of 30 affected individuals (out of 64) was selected for micro-CT IGD analysis, 15 from each site, including nonadults, and both sexes. For Beemster, none of the affected nonadults and only two males (out of the eight affected) had suitable teeth for micro-CT scanning. The selected individuals with their basic demographic information are presented in Table 1 in the Results section.

The first permanent molar was selected for micro-CT assessment if available and if dental wear did not exceed stage H (Lovejoy, 1985), indicating the dentine underlying the enamel was still intact. If the first permanent molar was not available (worn or absent), the permanent canine was sampled if available and dental wear did not exceed stage D (Lovejoy, 1985), ensuring the dentine would be observable. In the first permanent molar, dentine formation starts in utero and will enable IGD assessment up to the first 10 years of age, while in the permanent canine, dentine formation starts around 3 months of age and ends at about 14 years of age (Gustafson & Koch 1974; Massler, Schour, & Poncher, 1941; Moorrees, Fanning & Hunt, 1963).

Dentine is formed is two phases, whereby odontoblasts secrete predentine which then is mineralized (Beaumont, Gledhill, Lee Thorp & Montgommery, 2013; Bevelander & Nakahara, 1966; Hillson, 2002). If there are adequate nutritional conditions during the process of formation and calcification, the matrix will appear homogenous and fusion of calcospherites, spheres containing calcium salts, will be complete (D'Ortenzio et al. 2016; Hillson, 2002). When vitamin D levels are inadequate, some of the calcospherites fail to fuse, visible as poorly mineralized patches of dentine with a bubble-like appearance, referred to as IGD (Isokawa, Kosakai & Kajiyama, 1963; D'Ortenzio et al., 2016; Vital et al., 2012). The presence of IGD is clinically associated with conditions disrupting mineralization by affecting vitamin D, calcium or phosphate levels, and vitamin D deficiency is considered to be pathognomonic for this mineralization defect (Chaussain-Miller et al., 2003; McDonnell, Derkson, Zhang & Hlady, 1997; Souza, Soares, Alves dos Santos & Viasbisch, 2010; Vital et al., 2012).

D'Ortenzio et al. (2016) divide IGD into three grades of severity from 1 to 3. However, grading of IGD severity on micro-CT was not undertaken in this study since this grading method was based on histological assessment and features of IGD are less clear on a micro CT scan (Brickley et al., 2017a). Instead, in this study IGD is scored as 'present', 'absent', or 'unobservable'. If present, IGD will be visible as bands of dispersed micro-defects that can be distinguished from taphonomic degradation, because bands of IGD will follow the incremental lines found in dentine. Micro-CT detects differences in mineralization density and since the bubble-like spaces in grade 1 are relatively small, is it suggested that grade 1 is not visible on micro CT scan (Brickley et al., 2017a). Therefore, isolated micro-defects are more likely to be taphonomic or developmental defects. If patches of degradation or cracks are present and mimic bands, these are easily distinguished from IGD by switching to other views, for instance craniocaudal vs. transverse. Dentine grows in concentric cones (Eerkens, Berget & Bartelink, 2011; Hillson, 2002) at rate of about 4 – 6 µm a day in permanent teeth and the first mm of the dentine below the crown in the first permanent molar represents the period around birth to about 1.5 years of age (Beaumont et al., 2013; Massler et al., 1941; Moorrees et al., 1963). In this study, the age at which an episode of IGD occurred is estimated using the criteria of Massler et al. (1941) and Moorrees et al. (1963).

Micro CT scans were performed using a SkyScan 1272 (100 kV, 100 uA, Cu 0.11 mm, 10.0 µm). The scan was reconstructed using NRecon© software. The 3D reconstruction was assessed and when IGD was present, a section image from each plane (buccal – lingual, mesial – distal, and transverse) was made. The occurrence and number of IGD bands were assessed by all researchers independently and only cases which were separately classified as having IGD were included to limit individual bias.

Since IGD grade 1 is not expected to be detectable on micro-CT, a total of 17 thin sections for histological assessment were made from all individuals that did not display IGD on micro-CT (n = 10), in cases where observation for IGD was not possible (n = 1), and six individuals that display IGD on micro-CT for comparison (n = 6). The teeth were embedded in resin (EpoThin©) after which a thin section was made with an IsoMet® 1000 precision saw. After further grinding and polishing as described by De Boer, Aarents and Maat (2013), the thin section was mounted on a glass slide for histological analysis using a Leica® DM500 compound microscope with 40x magnification. The number and the severity of IGD periods were scored for each tooth, whereby grading of IGD was evaluated according to D'Ortenzio et al. (2016).

A chi-square test was performed to test the difference in the number of IGD periods per individual, the difference in the occurrence of the first episode of IGD, and to assess the statistical

Table 1. Overview of analyzed individuals with their sex, age, sampled tooth, macroscopic vitamin D deficiency, number of IGD periods, and the age of IGD formation.

Individual				Macro-scopy*	Micro-CT		Histology		
	Sex	Age-at-death (years)	Tooth sampled		N IGD periods	Age (period of deficiency)†	N IGD periods	Grade	Age (period of deficiency)†
HT15S020	U	15 ± 2	RM1	Possible	0	–	1	1	6 - 12 mths.
HT15S042	M	36 - 49	LM1	Present	1	2.5 yrs.	1	2	2.5 yrs.
HT15S062	F	36 - 49	LM1	Present	0	–	0	–	–
HT15S066	M	36 - 49	LM1	Present	1	6 - 12 mths.	NA	–	–
HT15S067	U	6.5 ± 0.5	LM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	–	–
HT15S071	M	18 - 25	RM1	Present	0	–	0	–	–
HT15S075	F	36 - 49	LC1	Present	U	U	3	2 and 3	2.5 yrs. 5 yrs. 6 yrs.
HT15S080	M	26 - 35	RM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	–	–
HT15S094	F	18 - 25	RM1	Present	2	6 - 12 mths. 3 yrs.	2	2	6 - 12 mths. 2.5 yrs.
HT15S099	F	18 - 25	LM1	Present	0	–	1	1 - 2	6 - 12 mths.
HT15S106	M	36 - 49	RM1	Present	1	6 - 12 mths.	NA	–	–
HT15S109	F	36 - 49	RM1	Present	0	–	1	1 - 2	2.5 yrs.
HT15S123	U	2 ± 0.5	RM1	Present	1	6 - 12 mths.	NA	–	–
HT15S127	U	9 ± 1	RM1	Present	2	Birth 6 - 12 mths.	NA	–	–
HT15S130	M	18 - 25	RC1	Present	4	12 mths. 2.5 yrs. 3 yrs. 5 yrs.	6	2 and 3	1.5 yrs. 2 yrs. 2.5 yrs. 3.5 yrs. 6.5 yrs. 7.5 yrs.
MB11S101	F	26 - 35	RM1	Present	2	6 -12 mths. 2.5 yrs.	3	2 and 3	6 - 12 mths. 2 yrs. 3 yrs.
MB11S126	F	36 - 49	RM1	Possible	0	–	1	1 - 2	Birth - 12 mths.
MB11S183	F	26 - 35	LM1	Present	0	–	1	1 - 2	6 - 12 mths.
MB11S234	F	18 - 25	RM1	Present	0	–	1	2 - 3	6 - 12 mths.
MB11S307	F	18 - 25	RM1	Present	4	Birth 6 - 12 mths. 2.5 yrs. 5 yrs.	4	2 and 3	1 yr. 2 yrs. 3 yrs. 5 yrs.
MB11S321	M	50+	RM1	Present	0	–	0	–	–
MB11S327	F	26 - 35	RM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	–	–
MB11S401	F	26 - 35	LC1	Present	2	2.5 yrs. 3 yrs.	NA	–	–
MB11S413	F	36 - 49	LM1	Possible	1	6 - 12 mths.	NA	–	–
MB11S420	F	26 - 35	RM1	Present	1	2.5 yrs.	2	2 - 3	6 - 12 mths. 2.5 yrs.
MB11S422	F	36 - 49	RM1	Present	1	Birth - 12 mths.	NA	–	–
MB11S427	M	26 - 35	RM1	Present	1	6 - 12 mths.	NA	–	–
MB11S437	F	26 - 35	LM1	Present	2	6 - 12 mths. 2.5 yrs.	NA	–	–
MB11S488	F	36 - 49	RM1	Present	1	2.5 yrs.	NA	–	–
MB11S498	F	50+	LC1	Present	0	–	1	1 - 2	2.5 yrs.

* = data from Veselka et al. (2015; 2017; submitted). † = Age is based on Moorrees et al. (1963) and Massler et al. (1941). U = unobservable, M = male, F = female, RM1 = right first permanent maxillary molar, LM1 = left first permanent maxillary molar, RM1 = right first permanent mandibular molar, LM1 = left first permanent mandibular molar, RC1 = right permanent maxillary canine, LC1 = left permanent maxillary canine, LC1 = left permanent mandibular canine, mths. = months, yrs. = years, HT15 = Hattem, MB11 = Beemster, IGD = interglobular dentine, NA = not assessed.

significance of the difference in the occurrence of IGD bands in specific age periods per individual. A Mann-Whitney U test was performed to assess the statistical significance of the number of IGD episodes per site and the difference in the number of IGD episodes between males and females.

RESULTS

Table 1 provides an overview of all individuals whose teeth were selected for micro-CT scanning. For each individual basic demographic data is noted along with macroscopic vitamin D deficiency diagnosis, the number of IGD episodes, and the approximate age of formation of each IGD episode from both micro-CT and histological analyses. Grading of IGD is noted for the thin sections. Appendix A provides details of the macroscopic lesions attributed to vitamin D deficiency for each individual.

Nineteen individuals displayed bands of IGD on micro-CT, with the majority (89.5%; 17/19) displaying one or two periods of vitamin D deficiency. Figure 2 displays a first right permanent maxillary molar (RM1) of individual HT15S067 showing the location of two episodes of IGD.

As mentioned, the majority of individuals with observable IGD display one or two episodes and this difference is statistically significant ($\chi^2 = 13.586$, $p = 0.009$). There is no statistically significant difference between the number of vitamin D deficient periods per individual between sites (Hattem: mean number of IGD periods = 1.20; Beemster: mean number of IGD periods = 1.21; $U = 104.500$, $p = 0.983$). In 63.2 % of the affected individuals (12/19), the first observable episode of vitamin D deficiency is present in the layer of dentine secreted between 6 and 12 months of age, which is statistically significant ($\chi^2 = 8.103$, $p = 0.044$). The difference in IGD prevalence in the layer of dentine formed between 6 and 12

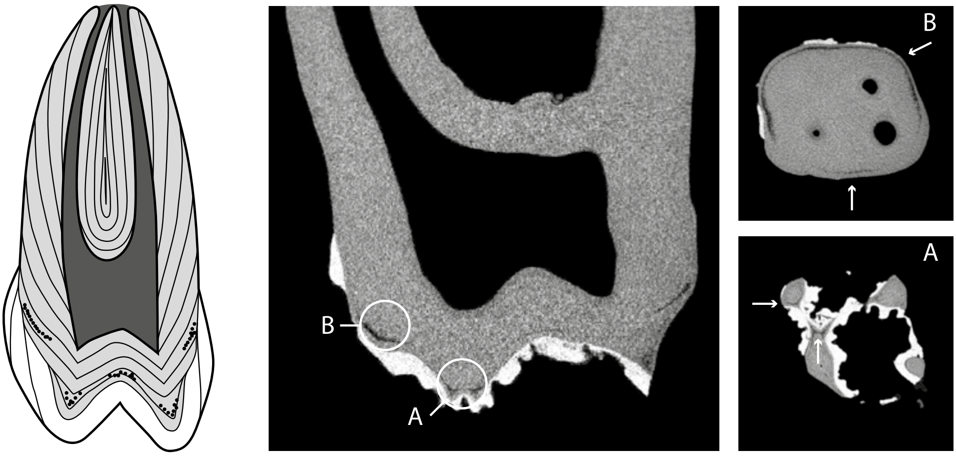

Figure 2. HT15S067 shows 2 episodes of IGD (A and B), visible in the schematic drawing, the micro-CT scan in craniocaudal view, and both episodes in transverse view marked by white arrows.

months of age is statistically significant (χ^2 = 10.862, p = 0.012, and most individuals demonstrated IGD in this layer of dentine (78.9%; 15/19). The majority of individuals (89.5%; 17/19) did not display IGD bands after about 2.5 – 3 years of age.

Eleven out of 17 individuals (64.7%; 11/17) displayed more IGD episodes on thin section than on micro-CT (including HT15S075 which was considered unobservable on micro-CT). Figure 3 shows a craniocaudal micro-CT image of the right permanent maxillary canine (RC1) of individual HT15S130 demonstrating 4 possibly 5 episodes of IGD (A to E). It was difficult to determine on micro-CT if episode A and B occurred in the same age period. Therefore, the fifth episode of IGD was considered to be possibly present. On the histological image of the same tooth, the same episodes are visible (A to E) and one additional period (F) that is not visible on micro-CT. In episode A of the histological image, two bands of IGD are clearly visible within the same layer of dentin, which could be the result of differences in appositional growth of the dentin throughout the layer (D'Ortenzio, Kahlon, Peacock, Salahuddin & Brickley, 2018).

Three out of the 30 individuals (10%) that displayed macroscopic lesions that were (possibly) attributed to vitamin D deficiency, did not present with IGD episodes after micro-CT and histological assessment. As described in the Appendix, these individuals (HT15S062, HT15S071, and MB11S321) have bending deformities of both tibiae. Individual HT15S062 also displayed bowing of the left femur, and HT15S071 presented with coxa vara of both femora.

Three different individuals were previously macroscopically diagnosed as having possible residual rickets due to only partially meeting the criteria described by Veselka et al. (2017). HT15S020 displayed anterolateral bending of both femora, MB11S126

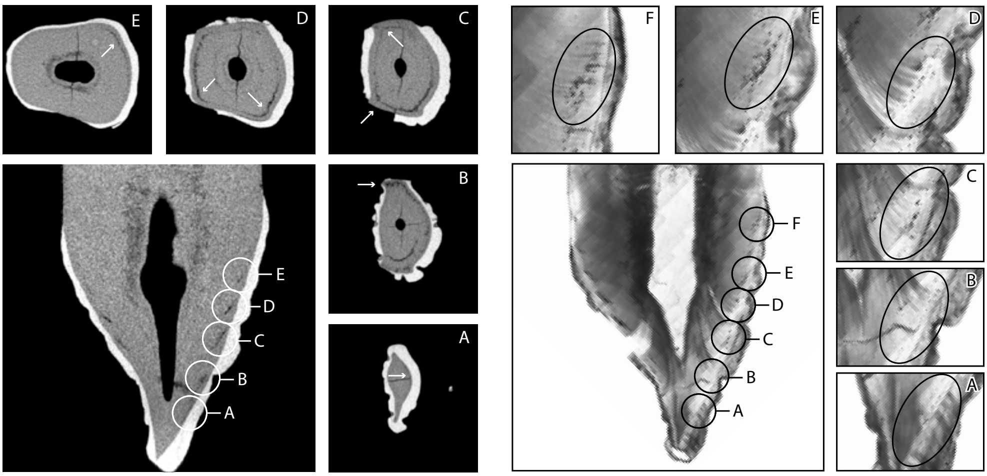

Figure 3. Craniocaudal micro-CT image of the right permanent maxillary canine (RC1) of individual HT15S130 with corresponding transverse image of each IGD episode marked by white arrows, and a thin section image at 40x magnification of the same tooth with each of the IGD episodes shown separately.

showed bending deformities of both radii and ulnae, and MB11S413 presented with bending deformities of the left femur and the left radius. All three presented with episodes of IGD; dentine defects were visible after micro-CT in MB11S413 and histological analysis in HT15S020 and MB11S126.

The difference in IGD prevalence on micro-CT between Hattem females (1/4) and males (5/6) is not statistically significant (Fisher's exact test: $p = 0.190$), which may partially be attributed to small sample size. It is worth noting that the majority of Hattem females (75.0%; 3/4) did not display visible bands of IGD on micro CT, whereas the majority of the males displayed one or more IGD episodes (83.3%; 5/6). It was not possible to assess differences between the prevalence of IGD in Beemster females and males, because only two male individuals had observable teeth.

DISCUSSION

Macroscopy, micro-CT and histology

Dentine and bone are mineralized tissues that display similarities in their formation and composition, and will react similarly to pathological conditions affecting mineralization (D'Ortenzio et al., 2016; Foster, Nociti & Somerman, 2014; Vital et al., 2012). Vitamin D plays a vital role in the mineralization process of both teeth and bone, and a vitamin D deficiency will disrupt this process. Vitamin D deficiency may lead to bending deformities of the long bones, changes to the metaphyses (flaring, cupping, and/or thickening) and sternal rib ends (thickening, and/or increased porosity), and other skeletal deformities in the growing skeleton (Brickley et al., 2014, 2018; Mays et al., 2006; Ortner & Mays, 1998). However, bone is a dynamic tissue that undergoes continuous remodeling, and if vitamin D deficiency is overcome, less severe bending deformities and skeletal lesions will remodel and may be completely obliterated (Brickley et al., 2017b; Hess, 1930), whereas dentine shows no turnover and poorly mineralized dentine will remain visible after vitamin D deficiency was overcome (D’Ortenzio et al. 2016; Foster et al., 2014; Vital et al., 2012).

Most individuals with macroscopic lesions due to rickets and residual rickets, with teeth available for scanning, presented with one or more vitamin D deficient periods visible as episodes of IGD on micro-CT (65.5%; 19/29). Histological assessment of 17 individuals demonstrated additional bands of IGD in 64.7% (11/17) of them. The combined results of micro-CT and histological analysis demonstrated that 90.0% (27/30) of the individuals with macroscopic lesions attributed to past episodes of vitamin D deficiency, displayed one or more episodes of IGD. This suggests that macroscopic assessment of vitamin D deficiency is relatively reliable. However, three individuals with macroscopic lesions attributed to vitamin D deficiency, did not display IGD on micro-CT or thin section. Bowing deformities can be produced by a wide range of pathological conditions (Brickley and Ives 2008: Table 5.8) and as discussed by Brickley and co-workers (2018), lesion development is complex and depends on several factors including nutrition, co-occurrence, and aspects of lifeways. It is possible that these individuals did suffer from vitamin D deficiency but that the condition may have developed after apical closure of the root of the permanent first molars and canines. The rate of appositional dentine growth in the roots of the teeth differs from the growth rate of the dentine formed near the crown which may result in the absence of IGD formation in the roots of the first permanent molar and permanent canine despite vitamin D deficiency (D'Ortenzio et al., 2018). However, the bending deformities observed in these individuals may simply represent more pronounced expression of human variation, or relate to an alternate cause; differential diagnosis undertaken produced no clear alternate diagnosis (Veselka et al. 2017; submitted), but this remains a possibility.

The use of the non-destructive micro-CT method to detect vitamin D deficiency can aid in the identification of the disease in individuals who lack clear lesions, as was the case with MB11S413. This individual was diagnosed with possible residual rickets due to partially meeting the criteria as described by Veselka et al. (2017), but displayed IGD on micro-CT supporting residual rickets diagnosis.

Previous research found that not all individuals that experience an episode of vitamin D deficiency develop lesions that could be identified via macroscopic examination (D'Ortenzio et al., 2018), and it is possible that some of the episodes of deficiency identified in the current investigation were not linked to skeletal lesions identified. Of those that do develop visible bending deformities of the long bones during childhood, only 10 – 15% are estimated to remain visible in the adult skeleton due to growth and the process of remodeling (Brickley et al., 2017b; Hess, 1930). Micro-CT assessment of IGD can also be applied to individuals with missing skeletal elements. Poor skeletal preservation and incompleteness of the skeleton have been shown to affect diagnosis in nonadults, and assessment of past vitamin D deficiency in adults may be hindered when long bones and ribs are unobservable since bending deformities of these skeletal elements are the most frequently observed (Brickley et al., 2018).

Extensive diagenetic change prevented assessment using micro-CT of one individual (HT15S075). In this case, assessment via thin section revealed that IGD was present and relatively easy to assess, demonstrating three episodes of IGD with grade 2 and 3 that should have been visible on micro-CT.

It was previously reported by Brickley et al. (2017a) that grade 1 IGD would not be detected using micro-CT assessment, but the current study analyzed a much larger number of individuals and results from this investigation demonstrate that depending on the micro-CT set-up, some individuals at the lower end of grade 2 IGD may also be missed. Most individuals without IGD on micro-CT and the individuals that displayed additional bands of IGD on thin section, presented with IGD grade 1 and a lower grade 2 on histological examination. Our results seem to support the limitation of IGD observation on micro-CT scan to grade 2 (excluding the lower end of the range) and 3 as noted by Brickley et al. 2017a).

Although histological analysis appears to provide the most accurate results and the process of producing thin sections is relatively fast and inexpensive, it requires the destruction of archaeological human remains that may not be desirable or possible in all skeletal collections. Furthermore, various other features not attributed to vitamin D deficiency are visible on thin sections, such as developmental IGD (a possible result of difference in the dentine formation rate in various parts of the tooth) and marbling or dappling (variation in normal dentine) (D'Ortenzio et al. 2018) that may hinder pathological IGD identification. If histological examination is not possible, micro-CT analysis of more severe IGD may aid in the identification of vitamin D deficiency in affected individuals without clear macroscopic lesions. More importantly, the 3D nature of a micro CT scan enables better comparison of various IGD periods than on thin section, whereby IGD can be evaluated in different planes of the tooth.

Number of deficient periods and age of onset

The results of micro-CT assessment demonstrated that three individuals (10.3%; 3/29) displayed their first period of vitamin D deficiency around birth. During pregnancy, the fetus is dependent on vitamin D levels of the mother (Dror & Allen, 2010; Mulligan, Felton, Riek & Bernal-Mizrachi, 2010; Thandrayen & Pettifor, 2010). If the mother is vitamin D deficient, the fetus will not be able to obtain sufficient vitamin D and may present with IGD in the first layers of dentine deposited around birth. It is possible that the mothers of HT15S127, MB11S307, and MB11S422, who show IGD in the first layers of dentine, may have suffered from adult vitamin D deficiency. The prevalence of IGD in the dentine just below the enamel may be used as an indication of adult vitamin D deficiency within a population.

The majority of individuals display the first vitamin D deficient episode before the age of about 2.5 years (78.9%; 15/19) whereby most affected individuals (89.5%; 17/19) do not present with IGD after the age of about 3 years. However, the possibility exists that individuals experienced periods of vitamin D deficiency during formation of the dentine in the roots (D'Ortenzio et al., 2018) or in adolescence which would be unobservable in the first permanent molar and permanent canine. Future analysis of the second and third permanent molars of the affected individuals will provide more information on periods of vitamin D deficiency in adolescence.

Recent macroscopic analysis of Beemster and Hattem nonadults yielded active and healing cases of rickets (Veselka et al., 2015, submitted), whereby active cases presented with underlying porosity of the growth plates (Brickley et al., 2018; Mays et al., 2006). Almost all of the active cases of rickets were observed in individuals younger than 3 years of age, whereas most healing cases (in which porosity of the growth plates was absent) were observed in older individuals. It was postulated that an increase of sunlight exposure occurred in individuals older than 3 years of age thereby decreasing the number of active cases after this age (Veselka et al., submitted). Although additional periods of vitamin D deficiency may be evident in the second and third molars, the results of micro-CT analysis appear to indicate an increase in vitamin D levels after the age of about 3 years, since the majority of affected individuals (89.5%; 17/19) do not present with periods of IGD after that age. However, this does not seem to have been the case for HT15S075, HT15S130, and MB11S307. These individuals experienced their first episode of vitamin D deficiency in the first year of their lives and on thin section presented with another 2, 3 and 5 consecutive periods respectively. The combined results of micro-CT and histological analysis demonstrate that 40.7% (11/27) of the individuals with IGD display two or more bands of IGD indicating vitamin D deficiency to have been recurrent in both communities. Since the Netherlands has a latitude of 35°N, no dermal synthesis of vitamin D takes place in the winter months (Jablonski & Chaplin, 2013; Webb, Kline & Holick, 1988), and vitamin D deficiency may have been seasonal, as suggested by the number of chronologically successive IGD periods in HT15S075, MB11S101, MB11S307, and HT15S130. Seasonal vitamin D deficiency, especially in more northern latitudes, has previously been proposed to be observable in the archaeological collection from St. Martin's Birmingham from the UK (Mays, Brickley & Ives, 2009), and the recurrent episodes of vitamin D deficiency that were observed in individuals from 15 Roman settlements across Western Europe may have been indicative of seasonality (Brickley et al., 2018). Our results provide the first clear evidence of vitamin D deficiency occurring in recurrent episodes that would fit seasonal vitamin D deficiency.

Differences in macroscopically visible rickets and residual rickets prevalence between Beemster and Hattem were not statistically significant (Veselka et al., submitted), and the prevalence of IGD (first episode and the number of episodes) seems to support the notion that both communities experienced a similar risk of developing vitamin D deficiency. Recent research suggested a gendered risk for developing vitamin D deficiency in the Beemster community, whereby significantly more females (21/100) than males (8/100) were affected (Veselka et al., 2017). Due to the small number of males with suitable teeth for micro-CT assessment, comparison of IGD between Beemster females and males was not undertaken. For Hattem, no significant difference ($\chi^2 = 2.191$, $p = 0.139$) between affected males and females was observed (Veselka et al., submitted) and the difference in IGD prevalence between Hattem males and females was not statistically significant (Fisher's exact test: $p = 0.190$), partially due to small sample size. It is, however, worth noting that the majority of males (83.3%; 5/6) displayed one or more IGD episode on micro-CT, whereas the majority of females (60.0%; 3/5) did not display IGD on micro-CT but did so on thin section. This suggests that males experienced more severe mineralization defects than females, which may be indicative of more severe periods of vitamin D deficiency in males. However, the linkages between the severity of IGD, the degree and presence of macroscopic bending deformities, and the severity of vitamin D deficiency, are complex and need further study.

CONCLUSION

The combined results of macroscopic, radiographic, and histological assessment of vitamin D deficiency suggested histological analysis of IGD provides the most accurate results. However, this study demonstrates that non-destructive micro-CT analysis of IGD is a valuable method that may aid in the identification of vitamin D deficient individuals without no observable, or subtle, macroscopic bending deformities. This method provides information on the age of onset and the number of deficient periods, allowing a more nuanced understanding of vitamin D deficiency development in both males and females during growth and development.

Results of micro-CT analysis support previous findings, that nonadults after the age of 3 years were likely to have experienced an increase in sunlight exposure. In three individuals, episodes of IGD were observed in the dentine formed around birth that suggests maternal vitamin D deficiency. Further examination of the number of IGD episodes and the attributed age periods showed vitamin D deficiency to be a recurrent condition in 40.7% (11/27) of the affected individuals and seasonal deficiency appears to have occurred in at least four individuals. This is an important finding that supports the notion that vitamin D deficiency is seasonal in more northern latitudes.

Micro-CT and histological analysis of IGD enables comparison of IGD severity between males and females. Although Hattem males and females are suggested to have experienced similar risks of developing vitamin D deficiency, the males display more severe IGD than females suggesting they experienced more severe periods of vitamin D deficiency. The comparison of IGD severity between males and females may provide valuable information on gendered differences. Moreover, it aids in better understanding the linkage between macroscopic lesions and IGD prevalence, and improves our knowledge of the influence of vitamin D deficiency on certain groups in past communities. Future research on the influence of the interplay of various biophysical variables (e.g. latitude, season), sociocultural factors (e.g. clothing, socioeconomic status, gendered division of labor), and aspects of diet on vitamin D deficiency prevalence using macroscopic examination, micro-CT and histological analysis of IGD will enhance our understanding of this relationship.

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REFERENCES

Beaumont, J., Gledhill, A., Lee Thorp, J., Montgomery, J., 2013. Childhood diet: a closer examination of the evidence from dental tissues using stable isotope analysis of incremental human dentine. Archaeometry 55, 277-295. doi: 10.1111/j.1475-4754.2012.00682.x

Bevelander, G., Nakahara, H., 1966. The Formation and Mineralization of Dentin. Anat. Rec. 156, 303-323. doi: 10.1002/ar.1091560306

Brickley, M., Ives, R., 2008. The Bioarchaeology of Metabolic Bone Disease. Second ed. Academic Press, San Diego, pp. 75-134.

Brickley, M., Mays, S., Ives, R., 2010. Evaluation and Interpretation of Residual Rickets Deformities in Adults. Int. J. Osteoarcheol. 20, 54-66. doi: 10.1002/oa.1007.

Brickley, M., Moffat, T., Watamaniuk, L., 2014. Biocultural perspectives of vitamin D deficiency in the past. J. Anthropol. Archaeol. 23, 48-59. doi: 10.1016/j.jaa.2014.08.002.

Brickley, M., D'Ortenzio, L., Kahlon, B., Colombo, A., Coqueugniot H., Knusel C.J., Betrand B., 2017a. Micro-CT analysis of Dental Structures to Detect Vitamin D deficiency. Poster presentation on the annual BABAO conference in Liverpool. doi: 10.13140/RG.2.2.24372.09603

Brickley, M., D'Ortenzio, L., Kahlon, B., Schattmann, A., Ribot, I., Raguin, E., Betrand, B., 2017b. Ancient Vitamin D Deficiency: Long-Term Trends. Curr. Anthropol. 38, 420-427. doi: 10.1086/691683

Brickley, M., Mays, S., George, M., Prowse, T.L., 2018. Analysis of patterning in the occurrence of skeletal lesions used as indicators of vitamin D deficiency in subadult and adult skeletal remains. Int. J. Paleopathol. in press. doi: 10.1016/j.ijpp.2018.01.001

Chaussain-Miller, C., Sinding, C., Wolikow, M., Lasfargues, J., Godeau, G., Garabédian, M., 2003. Dental abnormalities in patients with familial hypophosphatemic vitamin D-resistent rickets: prevention by early treatment with 1-hydroxyvitamin D. J. Pediatr. 142, 324-331. doi: 10.1067/mpd.2003.119

De Boer, H.H., Aarents, M.J., Maat, G.J.R., 2013. Manual for the Preparation and Staining of Embedded Natural Dry Bone Tissue Sections for Microscopy. Int. J. Osteoarchaeol. 23, 83-93. doi: 10.1002/oa.1242

D'Ortenzio, L., Ribot, I., Raguin, E., Schattmann, A., Bertrand, B., Kahlon, B., Brickley, M., 2016. The rachitic tooth: A histological examination. J. Archaeol. Sci. 74, 152-163. doi: 10.1016/j.jas.2016.06.006

D'Ortenzio, L., Kahlon, B., Peacock, T., Salahuddin, H., Brickley, M., 2018. The rachitic tooth: Refining the use of interglobular dentine in diagnosing vitamin D deficiency. Int. J. Paleopathol. 22; 101-108. doi: 10.1016/j.ijpp.2018.07.001

Dror, D.K., Allen, L.H., 2010. Vitamin D inadequacy in pregnancy: biology, outomces, and interventions. Nutr. Rev. 68, 465-477. doi: 10.1111/j.1753-4887.2010.00306.x

Eerkens, J.W., Berget, A.G., Bartelink, E.J., 2011. Estimating weaning and early childhood diet from serial micro-samples of dentin collagen. J. Archaeol. Sci. 38, 3101-3111. doi: 10.1016/j.jas.2011.07.010

Foster, B.L., Nociti, F.H., Somerman, M.J., 2014. The Rachitic Tooth. Endocr. Rev. 35, 1-34. doi: 10.1210/er.2013-1009

Giuffra, V., Vitiello, A., Caramella, D., Fornaciari, A., Giustini, D., Fornaciari, G., 2015. Rickets in a High Social Class of Renaissance Italy: The Medici Children. Int. J. Osteoarchaeol. 25, 608-624. doi: 10.1002/oa.2324.

Gustafson, G., Koch, G., 1974. Age estimation up to 16 years of age based on dental development. Odont. Rev. 25: 297-306.

Hess, A.F., 1930. Rickets. Including osteomalacia and tetany. London: Kimpton.

Hillson, S., 2002. Dental Anthropology. Cambridge University Press, Cambridge, UK.

Holick, M.F., 2003. Vitamin D: a Millennium Perspective. J. of Cell. Biochem. 88, 296-307. doi: 10.1002/jcb.10338.

Holick, M.F., 2006. Resurrection of vitamin D deficiency and rickets. J. Clin. Invest. 116, 2062-2072. doi: 10.1172/JCI29449.

Isokawa, S., Kosakai, T., Kajiyama, S., 1963. Interglobular dentine in the Deciduous Tooth. J. Dent. Res. 42, 831-834. doi: 10.1177/00220345630420031301

Jablonski, N.G., Chaplin, G., 2013. Epidermal pigmentation in the human lineage is an adaptation to ultraviolet radiation. J. Hum. Evol. 65, 671-675. doi: 10.1016/j.jhevol.2013.06.004

Liversigde, H.M., Molleson, T., 2004. Variation in Crown and Root formation and Eruption of Human Deciduous Teeth. Am. J. Phys. Anthropol. 123, 172-180. doi: 10.1002/ajpa.10318

Lovejoy, C.O., 1985. Dental wear in the Libben population: Its functional pattern and role in the determination of adult skeletal age at death. Am. J. Phys. Anthropol. 68, 47-56. doi: 10.1002/ajpa.1330680105

Massler, M., Schour, L., Poncher, H.G., 1941. Developmental pattern of the child as reflected in the calcification pattern of the teeth. Am. J. Dis. Child. 62, 33-67.

doi: 10.1001/archpedi.1941.02000130042004

Mays, S., Brickley, M., Ives, R., 2006. Skeletal Manifestations of Rickets in Infants and Young Children in a Historic Population From England. Am. J. Phys. Anthropol. 129,362-374. doi: 10.1002/ajpa. 20292

Mays, S., Brickley, M., Ives, R., 2009. Growth and Vitamin D Deficiency in a Population from 19th Century Birmingham, England. Int. J. of Osteoarchaeol. 19, 406-415. doi: 10.1002/oa.976.

McDonnell, D., Derkson, G., Zhang, L., Hlady, J., 1997. Nutirional rickets in a 2-year-old child: case report. Pediatr. Dent. 19, 127-130.

Moorrees, C., Fanning, E., Hunt, E., 1963. Age Variation of Formation Stage for Ten Permanent Teeth, J. Dent. Res. 42, 1490-1502. doi: abs/10.1177/00220345630420062701

Mulligan, M.L., Felton, S.K., Riek, A.E., Bernal-Mizrachi, C., 2010. Implications of vitamin D deficiency in pregnancy and lactation. Am. J. Obstet. Gynecol, 429, e1-e9. doi: 10.1016/j.ajog.2009.09.002.

Ortner, D.J., Mays, S., 1998. Dry-bone Manifestations of Rickets in Infancy and Early Childhood. Int. J. Osteoarchaeol. 8,45-55. doi:10.1002/(SICI)1099-1212(199801/02)8:1<45::AID-OA405>3.0.CO;2-D

Palkovich, A.M., 2012. Reading a Life: a Fourteenth-Century Ancestral Puebloan

Woman. In: Stodder ALW, Palkovich AM, (Eds). Bioarchaeology of Individuals. Florida: University Press of Florida, pp. 242– 254. doi:10.5744/florida/9780813038070.003.0016.

Ribeiro, T.R., Costa, F.W.G., Soares, E.C.S., Williams Jr., J.R., Fonteles, C.S.R., 2015. Enamel and dentin mineralization in familial hypophosphatemic rickets: a micro-CT study. Dent. Radiol. 44, 20140347. doi: 10.1259/dmfr.20140347

Schatmann, A., Betrand, B., Vatteoni, S., Brickley, M., 2016. Approaches to co-occurrence: Scurvy and rickets in infants and young children of 16–18th century Douai, France. Int. J. Paleopathol. 12, 63 – 75. doi: 10.1016/j.ijpp.2015.12.002

Souza, M.A., Soares Jr., L.A.V., Alves dos Santos, M., Vaisbisch, M.H., 2010. Dental abnormalities and oral health in patients with hypophosphatemic rickets. Clin. 65, 1023-1026. doi: 10.1590/S1807-59322919991999917

Thandrayen, K., Pettifor, J.M., 2010. Maternal Vitamin D Status: Implications for the Development of Infantile Nutritional Rickets. Endocrinol. Metab. Clin. North Am. 39, 303-320. doi: 10.1016/j.ecl. 2010.02.006.

Veselka, B., Hoogland, M.L.P., Waters-Rist, A.L., 2015. Rural Rickets: Vitamin D deficiency in a post-Medieval farming community from the Netherlands. Int. J. Osteoarchaeol. 25, 665-675. doi: 10.1002/oa.2329

Veselka, B., Hoogland, M.L.P., Waters-Rist, A.L., 2017. Gender-related vitamin D deficiency in a Dutch 19th century farming community. Int. J. of Paleopathol., in press. doi: 10.1016/j.ijpp.2017.11.001

Veselka, B., Brickley, M., Hoogland, M.L.P., Waters-Rist, A.L., The impact on sociocultural practices on vitamin D deficiency in five post-Medieval populations from the Netherlands. Unpublished results.

Vital, S.O., Gaucher, C., Bardet, C., Rowe, P.S., George, A., Linglart, A., Chaussain, C., 2012. Tooth dentin defects reflect genetic disorders affecting bone mineralization. Bone 50, 989-997. doi: 10.1016/j.bone.2012.01.010

Watts, R., Valme, S.R., Osteological evidence for juvenile vitamin D deficiency in a 19th century suburban population from Surrey, England. Int. J. Paleopathol. in press. doi: 10.1016/j.ijpp.2018.01.007

Webb, A.R., Kline, L., Holick, M.F., Influence of season and latitude on the cutaneous synthesis of vitamin D3: Exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J. Clin. Endocrinol. Metab. 1988, 67: 373-378. doi: 10.1210/jcem-67-2-373

APPENDIX.

Overview of macroscopic lesions per individual that may be attributed to vitamin D deficiency.

Individual						Bending deformities*							
	Age	Sex	DCP	ORP	EH	Femur	Tibia	Fibula	Humerus	Radius	Ulna	Clavicle	Ribs
HT15S020	15 ± 2 years	U				X							
HT15S042	36 – 49 years	M	X			X (L)	X						
HT15S062	36 – 49 years	F				X (L)	X						
HT15S066	36 – 49 years	M		X			X	X (L)					
HT15S067	6.5 ± 0.5 years	U					X	X					
HT15S071	18 – 25 years	M				X	X						
HT15S075	36 – 49 years	F			X	X			X			X	X
HT15S080	26 – 35 years	M	X			X		X					
HT15S094	18 – 25 years	F				X	X		X	X	X		
HT15S099	18 – 25 years	F		X		X	X						
HT15S106	36 – 49 years	M		X	X		X						
HT15S109	36 – 49 years	F				X	X						
HT15S123	2 ± 0.5 years	U		X		X	X	X	X	X	X		X
HT15S127	9 ± 1 years	U			X	X	X			X			
HT15S130	18 – 25 years	M	X		X	X	X	X		X	X		
MB11S101	26 – 35 years	F				X	X						
MB11S126	36 – 49 years	F								X	X		
MB11S183	26 – 35 years	F						X	X				
MB11S234	18 – 25 years	F				X							
MB11S307	18 – 25 years	F				X	X	X					
MB11S321	50+ years	M					X						
MB11S327	26 – 35 years	F				X	X		X				
MB11S401	26 – 35 years	F				X	X	X					
MB11S413	36 – 49 years	F				X (L)				X (L)			
MB11S420	26 – 35 years	F				X	X						
MB11S422	36 – 49 years	F					X			X			
MB11S427	26 – 35 years	M				X	X	X					
MB11S437	26 – 35 years	F				X							
MB11S488	36 – 49 years	F				X	X	X					
MB11S498	50+ years	F				X	X						

* = bending deformities are bilateral unless indicated otherwise, HT = Hattem, MB = Beemster, DCP = diffuse cranial vault porosity, ORP = orbital roof porosity, EH = enamel hypoplasia, L = left.